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# Effects of increased $p\mathrm{CO}_2$ and geographic origin on purple sea urchin (*Strongylocentrotus purpuratus*) calcite elemental composition

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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 (such as Mg and Sr) into the crystal lattice of these skeletal structures, a process that is sensitive to a variety of biological and environmental factors. Here we explore the effects of life stage, oceanographic region of origin, and changes in the partial pressure of carbon dioxide in seawater ($p\mathrm{CO}_2$) 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 magnesium 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 along the US west coast (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 ppm; $\mathrm{pH} = 8.02 \pm 0.03$ 1 SD; $\Omega_{\mathrm{calcite}} = 3.3 \pm 0.2$ 1 SD). However, when reared under elevated $\mathrm{CO}_2$ (900 ppm; $\mathrm{pH} = 7.72 \pm 0.03$; $\Omega_{\mathrm{calcite}} = 1.8 \pm 0.1$), 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 $\mathrm{CO}_2$ ($\mathrm{Sr/Ca} = 2.09 \pm 0.06\ \mathrm{mmol\,mol^{-1}}$; $\mathrm{Mg/Ca} = 66.9 \pm 4.1\ \mathrm{mmol\,mol^{-1}}$), juveniles of Southern California origin partitioned ~ 8 % more Sr into their skeletons when exposed to higher $\mathrm{CO}_2$ ($\mathrm{Sr/Ca} = 2.26 \pm 0.05$ vs. $2.10 \pm 0.03\ \mathrm{mmol\,mol^{-1}}$ 1 SD). Together these results suggest that the diversity of carbonate minerologies present across different skeletal structures and life stages in purple sea urchins does not translate into an equivalent 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 changes in seawater carbonate chemistry for 2100. 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 $CO_2$ threshold. The degree to which this latter geochemical plasticity might affect mineral stability and solubility in a future, altered ocean requires additional study.

## 1 Introduction

Rising levels of atmospheric carbon dioxide ($CO_2$) have resulted in increased dissolution of $CO_2$ 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 ($CaCO_3$) 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 $CO_2$ 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 because 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).

The utilization of different calcium carbonate polymorphs by different life stages may have implications for how species respond to ocean acidification. Biomineral solubility – a measure of how robust $CaCO_3$ 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 microstructure and surface area, the substitution of "foreign ions", such as magnesium (Mg) and strontium (Sr), for calcium (Ca) into the calcite crystal lattice is known to substantially increase biomineral solubility within a single polymorph (Chave et al., 1962; Walter and Morse, 1983; Morse et al., 2006, 2007). The degree to which ion substitution occurs is sensitive to environmental variables such as temperature (Chave, 1954), seawater composition (Ries, 2010), skeletal growth rate (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 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). For example, effects of environmental variation on Mg or Sr substitution rates can be large, with recent work by Ries (2011) showing that some echinoderm species exposed to elevated $p\mathrm{CO}_2$ incorporate ~ 30 % less Mg into their skeleton than under control conditions, whereas others incorporate ~ 20 % more Mg. Portions of these differences may stem from variation in the sensitivity of species-specific mineralization pathways to $p\mathrm{CO}_2$ (Ries, 2011).

Carbon dioxide levels 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

(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 $CO_2$ 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 $CO_2$, including how trends vary both across and within species, and as a function of geography, will provide key insight to 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), 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) 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 $CO_2$ remains unknown.

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 ($\delta^{18}$O and $\delta^{13}$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 magnesium 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 adult *Eucidaris tribuloides* and *Arbacia punctulata* sea urchin growth and geochemical composition will be resistant to future acidification (Reis et al., 2009, 2011). However, no such studies have investigated differences in trace element incorporation between life stages, or between 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 natural environmental gradients. 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 set-up

Purple sea urchin (*Strongylocentrotus purpuratus*) larvae were cultured through their entire larval duration (~ 50 days) under modern global-mean atmospheric $p\mathrm{CO}_2$ levels (400 ppm) and a "fossil-fuel intensive" scenario predicted for 2100 (900 ppm; Solomon et al., 2007; Pespeni et al., 2012). 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$^{-1}$) 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 $p\mathrm{CO}_2$ for 24 h before hatched blastulae were transferred into culture jars ($n = 3$–4 jars per population × $\mathrm{CO}_2$ level; 0.66 larvae $\mathrm{mL}^{-1}$) containing 3 L of filtered seawater (FSW) pre-equilibrated with NIST-certified, pre-mixed treatment gases (Airgas, Inc.). Jars fit into sealed boxes (3 per $\mathrm{CO}_2$ level), which received $\mathrm{CO}_2$ air mixtures to minimize degassing of culture jars. The jars were held in seawater tables maintained at 14 °C (±0.2 °C) and stirred using oscillating paddles. Every 2 days, 90 % of the culture water was removed via reverse filtration through a 60-µm 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).

### 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 ($n$ = 15–20) 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 deionized water, dried at room temperature, and ashed in a muffle furnace to oxidize all organic tissue to $\mathrm{CO}_2$ (500 °C; 4 h). The ashed metamorph skeleton was transferred to acid-cleaned microcentrifuge tubes 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 diet of diatoms in a common flow-through seawater table for an additional 5.5 months post-settlement before the tests were chemically cleaned and analyzed for Mg/Ca and Sr/Ca.

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### 2.2.3 Seawater chemistry

#### Carbonate chemistry

Samples of culture jar water were collected during each water change. Seawater pH 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 (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 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 $CO_2$Sys (Lewis and Wallace 1998) using $K_1$ and $K_2$ equilibrium constants from (Mehrbach et al., 1973) refit by (Dickson and Millero, 1987), $K_{SO_4}$ from (Dickson, 1990), and employing $pH_{total}$ and alkalinity as the primary input variables (Table 2). Experimental $pH_{total}$, $pCO_2$, and carbonate ion concentration data were analyzed using separate one-way ANOVAs. Salinity and temperature (mean ± 1SD) were 32.17 ± 0.66 psu and 14.11 ± 0.14 °C, respectively. During all culturing, we verified that seawater chemistries of the rearing jars from the two $CO_2$ treatments were significantly different ($n = 12$–14 culture jars per $CO_2$ level in each of the two trials) (ANOVA, "$pCO_2$", $F_{1,161} = 4897$; $p < 0.0001$; "pH total," $F_{1,665} = 17116$; $p < 0.0001$; "$[CO_3^{2-}]$", $F_{1,161} = 5372$; $p < 0.0001$; "$\Omega_{calcite}$", $F_{1,161} = 5372$; $p < 0.0001$).

#### 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 $CO_2$ 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 µm poly-sulfone syringe filters, acidified to pH < 2 by addition of 25 µL of OPTIMA grade $HNO_3$, and diluted 10× 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 ($Mg/Ca_{SW}$ and $Sr/Ca_{SW}$) in each of the 8 jars sampled was stable within < 2 % throughout the 50-day experiment and there were no statistically significant differences in $Mg/Ca_{SW}$ or $Sr/Ca_{SW}$ 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 MΩ-cm Milli-Q water) at 60 °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 nebulizer for 10 mL seawater samples (Meinhard, CO, USA), and a PFA MicroFlow 100 (100 $\mu L\,min^{-1}$) nebulizer for 250–500 µL 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 279Mg 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 $MgCO_3$. 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.

## 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 strong 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 = $2.8 \pm 0.2\,\%\,MgCO_3$ $n = 31$; tests = $6.4 \pm 0.6\,\%\,MgCO_3$ $n = 16$; Table 3; Weber, 1969; Davies et al., 1972; Ries, 2011). Similar to other taxa, ~ 2.4× 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 ~ 40 % less $MgCO_3$ than previously studied species, such that the range of *S. purpuratus* Mg values spans the transition between high and low Mg calcite (4 % $MgCO_3$). According to typical definitions, the adult and 5.5-month post-settlement juvenile *S. purpuratus* tests are composed of high Mg calcite (6–7 % $MgCO_3$), 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). The composition of newly metamorphosed juvenile skeleton falls between these spine and test endmembers (5.3 ± 0.3 % $MgCO_3$; $n = 22$; 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), 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 $CO_2$ (Ebert, 2007). Carbon isotopic data from sea urchin metamorph skeleton could validate this trace elemental evidence.

### 3.1.2 Strontium content

We also found that ~ 1.4× more Sr is incorporated into the tests of adult and 5.5-month post settlement urchins (2.72 ± 0.1 $\text{mmol mol}^{-1}$; $n = 16$ and 2.85 ± 0.1 $\text{mmol mol}^{-1}$; $n = 4$, respectively) than into the low-Mg calcite of adult spines (1.9 ± 0.05 $\text{mmol mol}^{-1}$; $n = 31$), with newly settled juveniles falling between the spine and test end-members (2.1 ± 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 $MgCO_3$ 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 ($D_{\mathrm{Sr}}$-values) for each of the sea urchin carbonate samples.

$$D_{\mathrm{Sr}} = (\mathrm{Sr}/\mathrm{Ca}_{\mathrm{calcite}})/(\mathrm{Sr}/\mathrm{Ca}_{\mathrm{SW}}) \quad (1)$$

*S. purpuratus* $D_{\mathrm{Sr}}$-values calculated based on the mean Sr/Ca ratio measured in the experimental culture waters (derived from a local coastal seawater supply; $8.62 \pm 0.17\,\mathrm{mMol\,mol^{-1}}$; Table 5) were regressed against skeletal $MgCO_3$ concentration (Fig. 2). In general, the positive relationship between *S. purpuratus* $MgCO_3$ content and $D_{\mathrm{Sr}}$ 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 mechanisms (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 for the adult and 5.5 mo 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 with faster and more variable growth rates (Carpenter and Lohmann, 1992). This finding is in agreement with early isotopic evidence of adult urchin ossicles (Weber and Raup, 1966; Weber, 1969; Ebert, 2007). This result furthermore suggests that 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; Huari et al., 2009). The strength of coastal upwelling in the CCS generally follows a latitudinal gradient, where Southern Oregon and Northern California experience more intense upwelling and lower pH conditions than Central and Southern California (Feely et al., 2008; Huari et al., 2009). Based on these upwelling trends, we therefore expect more northern sites across our latitudinal gradient to experience cooler more acidic conditions, whereas our Southern CA site would be the warmest and most buffered. Adult spine Mg/Ca was not statistically different among populations spanning this natural environmental mosaic 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 gradient (which mirrors temperature gradients) along the US west coast does not impact *S. purpuratus* spine Mg composition or Sr incorporation via growth rate variability (Carpenter and Lohmann, 1992). This natural solubility gradient will continue to shift in the next several decades (Andersson et al., 2008). As a consequence, although adult *S. purpuratus* skeleton precipitated in northern high upwelling and variable pH regions is geochemically similar to skeleton precipitated under warmer, more buffered conditions, the higher latitude regions will likely become undersaturated with respect to high Mg calcites (> 12 % $MgCO_3$) before the lower latitude sites (perhaps by the end of this century; Andersson et al., 2008).

### 3.3 Impact of $CO_2$ on skeletal composition during early life history stages

Although we found little evidence of effects of oceanographic regime on adult spine geochemistry, the utilization by larvae of 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 ($Mg/Ca_{SW}$ and $Sr/Ca_{SW}$) 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 $Mg/Ca_{SW}$ or $Sr/Ca_{SW}$ 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 can be attributed to biological processes rather than differences in culture water composition.

We found that elevated $CO_2$ did not affect the incorporation of Mg or Sr in Oregon, Northern California, or Central California populations (Fig. 5; Tables 6, 7), which is consistent with a lack of response of adult Atlantic urchins to acidification (Ries, 2011). Elevated $CO_2$ 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 $CO_2$ ($F_{1,3} = 18.961$; $p = 0.0224$; Table 7). Similarly, the Mg composition of newly settled metamorph skeleton from the Southern California population appears to have increased under elevated $CO_2$ (Fig. 5). Although this Mg trend is not statistically significant, it is consistent with the strontium evidence for increased skeletal "foreign ion" incorporation under elevated $CO_2$ conditions during early life history stages (Table 6). The increase in Sr incorporation under elevated $CO_2$ conditions most likely resulted from faster calcite precipitation rates at the time of calcification (Carpenter and Lohmann, 1992). The cluster of metamorph data in Fig. 3 reveal that across populations, Sr incorporation ($D_{Sr}$) in the skeleton precipitated by urchins reared under elevated $CO_2$ levels was considerably more variable than those reared under modern $CO_2$ levels. This would suggest that the rate of skeletal precipitation during early stages of *S. purpuratus* development may be more variable under future ocean acidification scenarios. This pattern provides evidence for a physiological stress 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 demonstrate that elevated $CO_2$ and reduced carbonate saturation state can affect mineral composition, which could reflect the rate at which biominerals are precipitated during early life history stages. The varied response among populations suggests that larval calcification and skeletal composition may depend upon a threshold response to elevated $pCO_2$. If so, then beyond 2100, the precipitation rate and composition of skeleton calcified during purple sea urchin early life history stages in the more northern sites may also begin to shift as $pCO_2$ levels exceed 900 ppm.

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

### Mg/Ca

The Mg content of *S. purpuratus* skeleton varies widely among skeletal ossicles and across life history stages. Such differences likely derive from strong biological controls on calcification and the use of multiple biomineralization pathways by *S. purpuratus* in different $CaCO_3$ 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 acidification. Although not statistically significant, Mg incorporation into the calcite of newly metamorphosed Southern California sea urchins does appear to be sensitive to elevated $CO_2$ conditions. Further work in purple sea urchins on multiple stressors and threshold responses will be required to fully understand this response and distinctions from other species (Ries, 2011).

### 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, the Southern California population revealed a trend that would suggest faster mineral precipitation rates at the site of calcification at the time of metamorphosis. This varied response could indicate a possible biological threshold, which varies by regional upwelling, oceanography, and carbonate chemistry. If so then, Central, Northern California and Oregon larval purple sea urchin skeletal calcification and composition may eventually be affected at $p\mathrm{CO}_2$ levels beyond the range tested in this study. Further study of the relationship between Sr incorporation and *S. purpuratus* calcite precipitation rates would validate and quantify this adaptive response of biologically mediated calcification to future acidification.

## 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 $CO_2$-induced ocean acidification. However, elevated $CO_2$ resulted in greater incorporation of "foreign ions" and thus, faster calcification rates, in newly settled juvenile calcite from the warmest and most-buffered site (Southern California). While this study was designed to represent $CO_2$ levels projected for 2100, a similar response may be evident in central and northern California and Oregon larval purple sea urchins when $CO_2$ concentrations exceed the levels studied here (beyond 2100, 900 ppm). Geochemical plasticity including increased mineral precipitation rates resulting in greater incorporation of "foreign ions" such as Sr and Mg during early life history stages would likely have negative effects on 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-discuss.net/9/17939/2012/bgd-9-17939-2012-supplement.pdf.**

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**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 |
|---|---|---|---|
| Adult Spines | | | |
| Purisima 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 |
| Cultured Metamorphs | | | |
| 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 |

**Table 2.** Mean (±s.d.) culture conditions during the study.

| | | |
|---|---|---|
| Supplied or Measured Parameters | | |
| Treatment gas $pCO_2$ (ppm) | 385 ± 8 | 1000 ± 19 |
| pH total | 8.02 ± 0.03 ($n = 335$) | 7.73 ± 0.03 ($n = 332$) |
| Total Alkalinity ($\mu$mol kg$_{SW}^{-1}$) | 2212 ± 12 ($n = 81$) | 2213 ± 14 ($n = 82$) |
| DIC ($\mu$mol kg$_{SW}^{-1}$) | 2043 ± 42 ($n = 67$) | 2140 ± 39 ($n = 66$) |
| Mg/Ca (mol mol$^{-1}$) | 4.79 ± 0.06 ($n = 23$) | 4.80 ± 0.05 ($n = 20$) |
| Sr/Ca (mMol mol$^{-1}$) | 8.61 ± 0.17 ($n = 23$) | 8.62 ± 0.17 ($n = 20$) |
| Temperature | 14.1 ± 0.3 ($n = 335$) | 14.1 ± 0.3 ($n = 332$) |
| Calculated parameters based on $CO_2$Sys estimation program | | |
| $pCO_2$ from pH$_{tot}$/TA | 410 ± 27 | 871 ± 53 |
| $pCO_2$ from TA/DIC | 490 ± 138 | 1001 ± 291 |
| [$CO_3^{2-}$] ($\mu$mol kg$_{SW}^{-1}$) | 137 ± 7 | 76 ± 4 |
| $\Omega_{calcite}$ | 3.3 ± 0.2 | 1.8 ± 0.1 |

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

| | | Mg content ($MgCO_3$ % ± 1 SD) | | Sr content (Sr/Ca mmol $mol^{-1}$ ± 1 SD) | |
|---|---|---|---|---|---|
| Species | Data Source | spine | test | spine | test |
| *Strongylocentrotus franciscanus* (CA, USA) | Weber (1969) | 5.8 ± 0.9 | 8.8 ± 0.7 | – | – |
| *Strongylocentrotus drobachiensis* (ME, USA) | Weber (1969) | 4.5 | 7.4 | – | – |
| *Lytechinus varieqatus* (FL, USA) | Carpenter and Lohmann (1992) | 6.8 | – | 2.1 | – |
| *Echinus sp.* | Carpenter and Lohmann (1992) | – | 12.9 ± 0.6 | – | 2.5 ± 0.1 |
| *Echinometra lacunter* (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 |
| *Eucidaris tribuloides* (FL, USA) | Carpenter and Lohmann (1992) | 5.8 | – | 2.2 | – |
| *Eucidaris tribuloides* (FL, USA) | Ries (2011) | 4.4 ± 0.1 | 9.4 ± 0.1 | – | – |
| *Arbacia punctulata* (FL, USA) | Ries (2011) | 4.9 ± 0.1 | 6.9 ± 0.2 | – | – |
| *Arbacia punctulata* (NC, USA) | Davies et al. (1972) | 5.3–6.7 | – | 2.0–2.5 | – |
| *Strongylocentrotus purpuratus* (CA-OR, USA) | this study | 2.8 ± 0.2 | 6.4 ± 0.6 | 1.9 ± 0.05 | 2.72 ± 0.09 |

**Table 4.** Trace elemental ratios of *S. purpuratus* life history stages.

| *Strongylocentrotus purpuratus* | Mg/Ca ($\text{mmol mol}^{-1}$) | D Mg ($\text{mmol mol}^{-1}$ calcite / $\text{mmol mol}^{-1}$ SW) | Sr/Ca ($\text{mmol mol}^{-1}$) | D Sr ($\text{mmol mol}^{-1}$ calcite / $\text{mmol mol}^{-1}$ SW) |
|---|---|---|---|---|
| Adult spines | 33.5 ± 2.4 | 0.007 ± 0.0006 | 1.92 ± 0.04 | 0.22 ± 0.006 |
| Adult tests | 82.5 ± 7.0 | 0.017 ± 0.002 | 2.72 ± 0.09 | 0.32 ± 0.012 |
| 5.5 month old juvenile tests | 92.4 ± 2.5 | 0.019 ± 0.0005 | 2.85 ± 0.07 | 0.33 ± 0.008 |
| 50 day old cultured metamorphs | 67.0 ± 4.1 | 0.014 ± 0.0008 | 2.11 ± 0.07 | 0.24 ± 0.009 |

**Table 5.** Trace elemental ratios in culture jars.

| $CO_2$ treatment | Site | Jar # | Mg/Ca ($\mathrm{mol\,mol^{-1}}$) | Sr/Ca ($\mathrm{mmol\,mol^{-1}}$) | *n* |
|---|---|---|---|---|---|
| 900 | B | 3 | 4.79 ± 0.06 | 8.64 ± 0.18 | 6 |
| 900 | B | 2 | 4.80 ± 0.03 | 8.62 ± 0.21 | 5 |
| 900 | SB | 3 | 4.81 ± 0.03 | 8.59 ± 0.15 | 5 |
| 900 | SB | 2 | 4.80 ± 0.08 | 8.62 ± 0.20 | 4 |
| 400 | B | 3 | 4.79 ± 0.11 | 8.59 ± 0.16 | 6 |
| 400 | B | 2 | 4.78 ± 0.03 | 8.57 ± 0.17 | 6 |
| 400 | SB | 3 | 4.77 ± 0.04 | 8.54 ± 0.12 | 6 |
| 400 | SB | 1 | 4.81 ± 0.06 | 8.76 ± 0.19 | 5 |
| MEAN (all samples) | | | 4.79 ± 0.06 | 8.61 ± 0.17 | 43 |

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

| Factor | df | Error df | F-ratio | $p$ |
|---|---|---|---|---|
| $p\mathrm{CO}_2$ | 1 | 14 | 1.4232 | 0.2527 |
| Source | 3 | 14 | 0.0633 | 0.9784 |
| $p\mathrm{CO}_2$ × 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 |
| $p\text{CO}_2$ × Source | 3 | 14 | 6.1670 | 0.0068 |

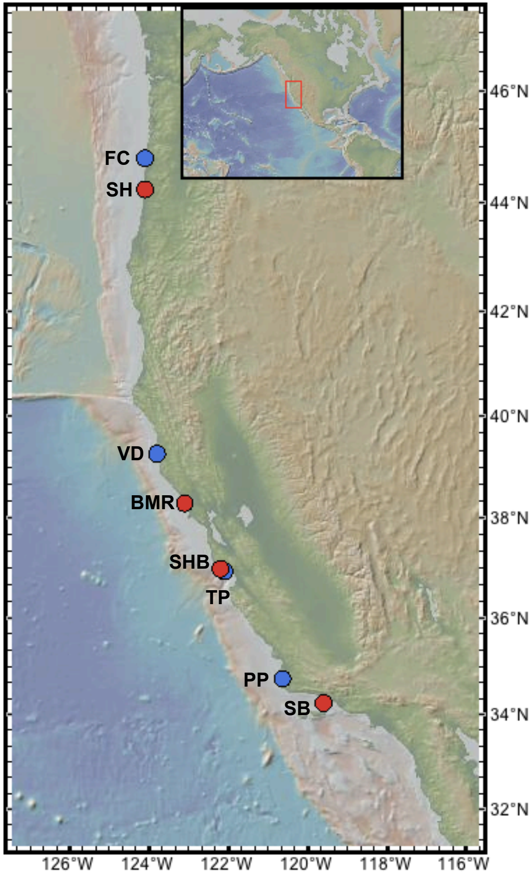

**Fig. 1.** Map of study area with collection sites of adult urchin spine samples (blue) and adults spawned for culturing experiment (red) noted (site abbreviations as in Table 1).

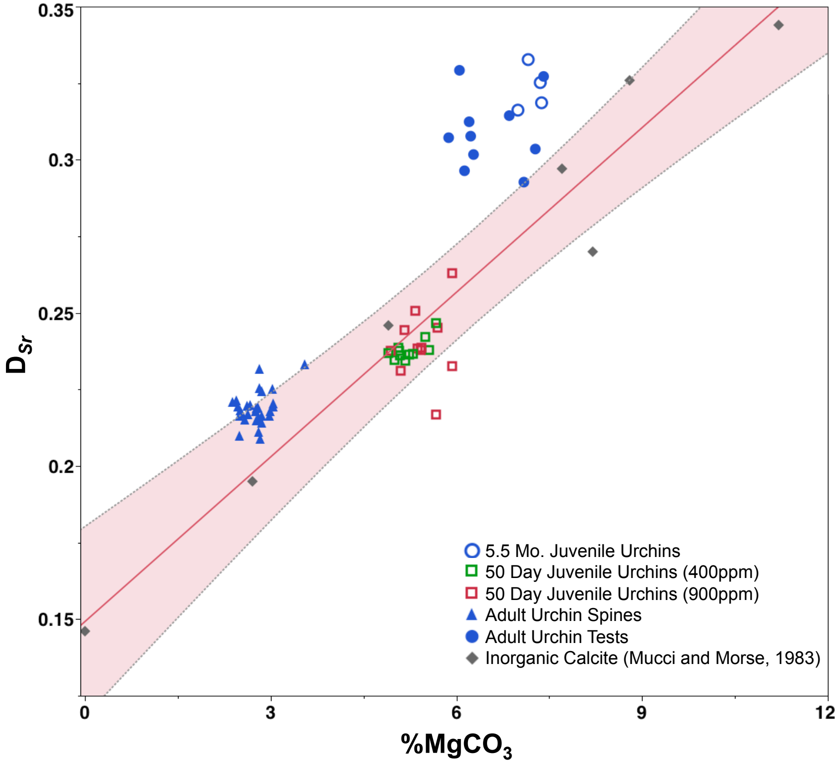

**Fig. 2.** The relationship between skeletal magnesium content (expressed as %MgCO$_3$; x-axis) and strontium partition coefficient ($D_{Sr}$; y-axis) for *S. purpuratus* adult spines (blue filled triangles), adult tests (blue filled circles), 5.5-month-old cultured juvenile tests (open blue circles), and newly metamorphosed juveniles settled after 48–49 days in culture at 400 (green open squares) and 900 (red open squares) ppm 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).

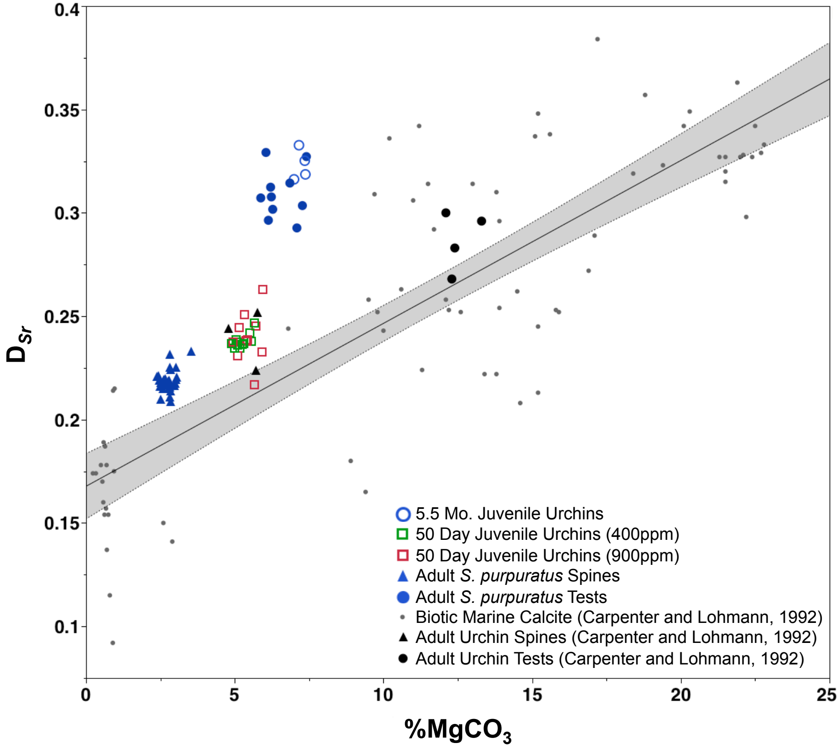

**Fig. 3.** The relationship between skeletal magnesium content (expressed as %MgCO$_3$; x-axis) and strontium partition coefficient ($D_{Sr}$; y-axis) for *S. purpuratus* adult spines (blue filled triangles), adult tests (blue filled circles), 5.5-month-old cultured juvenile tests (open blue circles), and newly metamorphosed juveniles settled after 48–49 days in culture at 400 (green open squares) and 900 (red open squares) ppm 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).

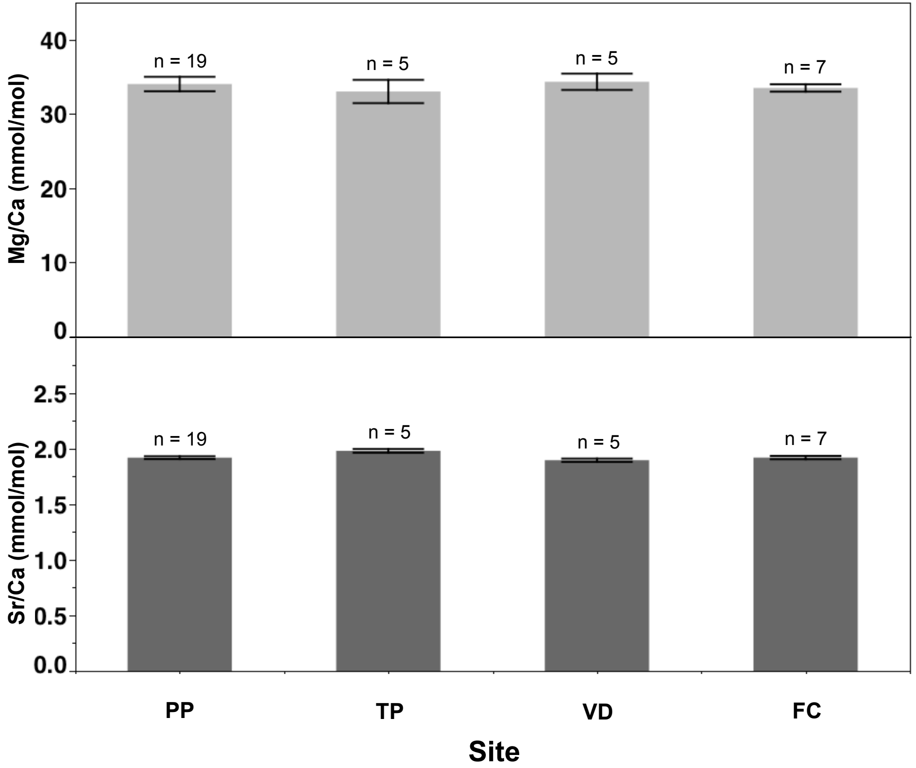

**Fig. 4.** Mean Mg/Ca (upper) and Sr/Ca (lower) ratios for spines of adult *S. purpuratus* populations spanning the West Coast US shown in Fig. 1. Error bars represent ±1 SE from mean.

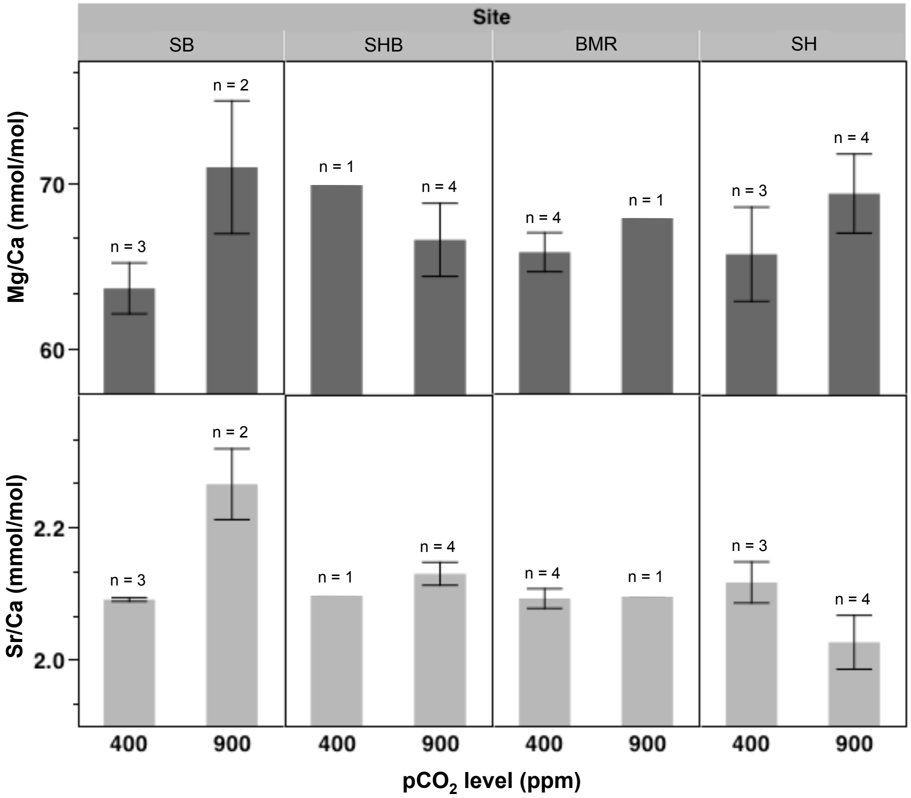

**Fig. 5.** Mean Mg/Ca (upper) and Sr/Ca (lower) ratios of newly metamorphosed juvenile *S. purpuratus* urchins, settled after 48–49 days in culture at control (400 ppm) or elevated $p\mathrm{CO_2}$ levels (900 ppm). Cultures initiated from sites indicated in Fig. 1. Error bars represent (±1 SE) of 2–4 samples (each consisting of ∼20 pooled metamorphs) from replicate culture jars from each population.

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