

Supplemental Material:

EFFECTS OF INCREASED pCO₂ AND GEOGRAPHIC ORIGIN ON PURPLE SEA URCHIN (*STRONGYLOCENTROTUS PURPURATUS*) CALCITE ELEMENTAL COMPOSITION

Michèle LaVigne, Tessa M. Hill, Eric Sanford, Brian Gaylord, Ann D. Russell, Elizabeth A. Lenz, Jessica D. Hosfelt, Megan K. Young

Chemical Cleaning of *S. purpuratus* Calcite

We cleaned *S. purpuratus* test, spine, and newly settled metamorph calcite prior to dissolution for ICP-OES analysis in order to remove Mg and Sr associated with non-skeletally bound organic matter. Peroxide (H₂O₂) based methods have been utilized for cleaning live foraminiferal calcite and surface coral aragonite for Mg/Ca and Sr/Ca analysis, respectively (e.g. Pak et al., 2004; Shen and Boyle, 1988). Prior to adopting the H₂O₂ method for preparing our samples, however, we compared several methods for chemically cleaning *S. purpuratus* test calcite powders (similar to the methods tested by Pak et al., 2004 for cleaning live planktonic foraminifera). Because our cultured urchin metamorph samples had been heated in a muffle furnace for ash free dry weight analysis prior to elemental analysis (Sanford et al., in prep), we also tested whether this heating affected *S. purpuratus* calcite Mg/Ca and Sr/Ca.

Cleaning Treatments following Pak et al., 2004:

Several calcium carbonate plates from an adult sea urchin test collected from Bodega Marine Reserve were homogenized to a coarse powder with an acid-cleaned ceramic mortar and pestle. Approximately 100 mg of homogenized calcite powder was transferred to two aluminum weigh boats and heated in a muffle furnace for 4 hours at 500°C. The remainder of the homogenized test calcite was not heated. The heated powders were further split into aliquots of 1-2mg of calcite powder in acid cleaned 0.5 mL microcentrifuge vials. The unheated powder was split into

~1-2 mg aliquots in acid cleaned 0.5 mL microcentrifuge vials. The unheated aliquots were then subjected to the following treatments: Unheated group 1 was rinsed once with Milli-Q water (sonicated for 2 minutes) and received three treatments of buffered peroxide (500 μ L of: 1 part 50% H_2O_2 and 2.3 parts 0.1N NaOH at room temperature for 1 hour, sonicated for 2 minutes every 15 minutes), followed by 5 rinses with Milli-Q water and transferred to acid-leached vials (Pak et al., 2004, Shen and Boyle, 1988). Unheated group 2 was bleached (500 μ L of NaOCl at room temperature: 2 hours; sonicated for 2 minutes every 15 minutes), rinsed with Milli-Q water (5 rinses allowing powders to settle to bottom of tube for 5 minutes between siphoning water off), and transferred to acid-leached vials (after Pak et al., 2004). Unheated group 3 was minimally cleaned with three seven-minute rinses with Milli-Q water (sonicated for first two minutes) before being transferred to acid-leached vials. Unheated group 4 received no cleaning or rinsing. Following heating in the muffle furnace, the heated powder aliquots group 1 and 2 were subjected to the same chemical treatments as unheated groups 1 and 2, respectively, in order to determine whether heating in the muffle furnace influenced calcite Mg/Ca and Sr/Ca composition. The samples were dissolved and analyzed for Mg/Ca and Sr/Ca following the technique described in Section 2.3 of the main text.

Results:

Mean Mg/Ca and Sr/Ca values from all six cleaning methods tested agreed within +/- 1SD (Fig. S1; with the exception of the Mg/Ca values from heated group 1 (muffled + bleached treatment), which only overlapped with 3 of the other 5 treatments tested). Therefore, we employed the peroxide method used for cleaning live foraminiferal calcite described by Pak et al., 2004 for cleaning all of our urchin calcite samples (equivalent of the method applied to Heated Group 2 described above).

In addition, based on a comparison between unheated and heated group 1 samples, we found no significant effect of muffle furnace heating (500°C) on skeletal Mg/Ca and Sr/Ca when using the peroxide cleaning method (H₂O₂ treatment only vs. muffle furnace heating + H₂O₂ treatment, respectively: Mg/Ca ANOVA, $F_{1,5} = 2.3698$, $p = 0.1843$; Sr/Ca ANOVA, $F_{1,5} = 0.2469$, $p = 0.6404$). This indicates that the muffle furnace heating of newly settled metamorphs had no significant impact on skeletal Mg/Ca and Sr/Ca measured.

The overall agreement between chemically cleaned and the uncleaned samples demonstrates that Mg and Sr associated with *S. purpuratus* calcite are resistant to oxidative cleaning procedures. Given that the cleaning procedures described above are designed to remove any non-“intracrystalline” organic material (organics that are not bound between or within individual calcite crystals), these data suggest Mg and Sr are bound within the skeletal calcite lattice, most likely via cationic substitution for Ca. Thus, the chemical cleaning methods tested are appropriate for trace elemental measurements in this study.

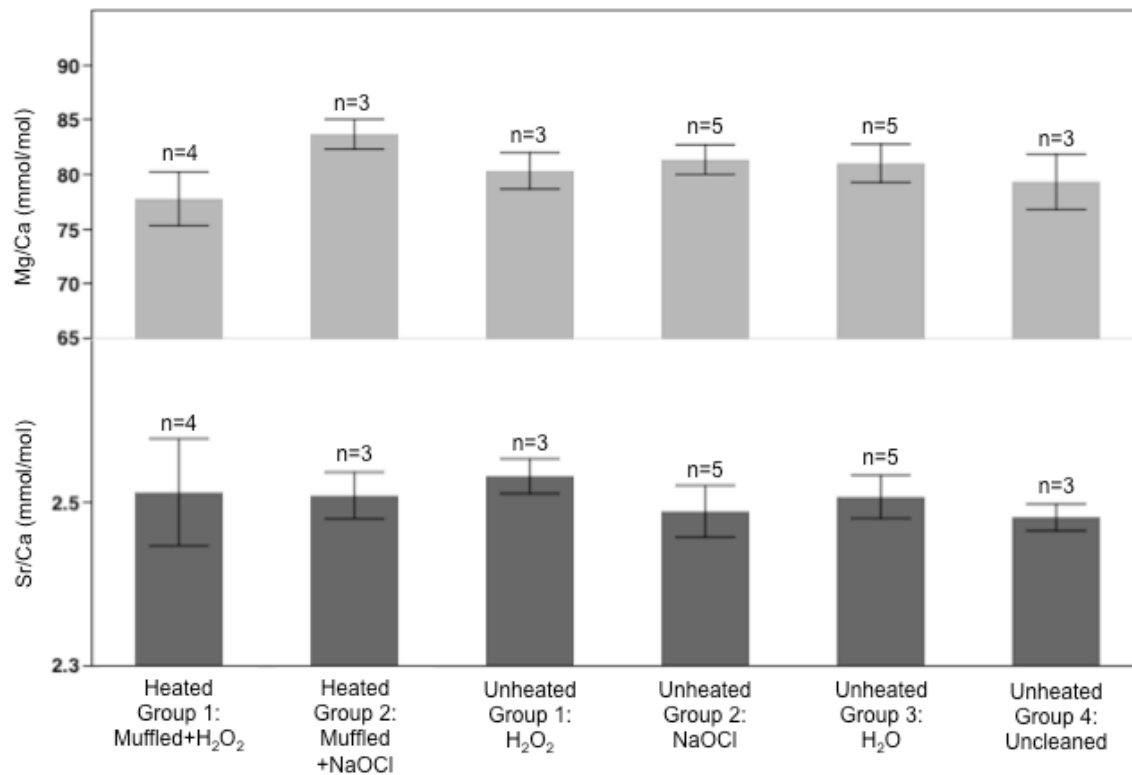


Figure S1:

Mean Mg/Ca (top) and Sr/Ca (bottom panel) for cleaning methods tested. Error bars represent +/- 1SD (n = 3-5 as indicated).

References:

Pak DK, Lea DW, Kennett JP (2004) Seasonal and interannual variation in Santa Barbara Basin water temperatures observed in sediment trap foraminiferal Mg/Ca. *Geochemistry Geophysics Geosystems* 5:Q12008

Shen GT, Boyle EA (1988) Determination of lead, cadmium and other trace metals in annually-banded corals. *Chem Geol* 67:47-62

Supplemental Data Tables for LaVigne et al., 2013 Biogeosciences

Sample Type	Source Population	Experimental Treatment (μCO_2 μatm)	Mg/Ca (mmol/mol)	Sr/Ca (mmol/mol)	%MgCO ₃ (calculated from Mg/Ca; see text)	D Sr (calculated from Sr/Ca; see text)
5.5-month old Juveniles	Strawberry Hill	n/a	89.3	2.79	6.99	0.324
5.5-month old Juveniles	Santa Barbara	n/a	91.6	2.93	7.16	0.341
5.5-month old Juveniles	Strawberry Hill	n/a	94.2	2.87	7.35	0.333
5.5-month old Juveniles	Strawberry Hill	n/a	94.5	2.81	7.38	0.326
Adult Spines	Purisima Point	n/a	34.9	1.89	2.85	0.219
Adult Spines	Purisima Point	n/a	43.6	2.06	3.54	0.239
Adult Spines	Purisima Point	n/a	30.8	1.91	2.53	0.222
Adult Spines	Purisima Point	n/a	36.6	1.92	2.99	0.223
Adult Spines	Purisima Point	n/a	31.5	1.90	2.58	0.220
Adult Spines	Purisima Point	n/a	34.0	1.90	2.79	0.220
Adult Spines	Purisima Point	n/a	32.1	1.91	2.63	0.222
Adult Spines	Purisima Point	n/a	37.1	1.93	3.03	0.225
Adult Spines	Purisima Point	n/a	31.9	1.94	2.62	0.225
Adult Spines	Purisima Point	n/a	30.4	1.91	2.49	0.221
Adult Spines	Purisima Point	n/a	34.6	1.84	2.84	0.214
Adult Spines	Purisima Point	n/a	32.6	1.94	2.67	0.225
Adult Spines	Purisima Point	n/a	30.0	1.93	2.47	0.224
Adult Spines	Purisima Point	n/a	36.4	1.91	2.97	0.222
Adult Spines	Terrace Point	n/a	29.8	1.95	2.45	0.227
Adult Spines	Terrace Point	n/a	34.9	1.98	2.86	0.230
Adult Spines	Terrace Point	n/a	29.0	1.95	2.38	0.226
Adult Spines	Terrace Point	n/a	34.4	2.04	2.82	0.237
Adult Spines	Terrace Point	n/a	37.1	1.99	3.03	0.231
Adult Spines	Fogarty Creek	n/a	30.6	1.92	2.51	0.223
Adult Spines	Fogarty Creek	n/a	34.5	1.99	2.82	0.231
Adult Spines	Fogarty Creek	n/a	34.3	1.86	2.81	0.216
Adult Spines	Fogarty Creek	n/a	33.7	1.90	2.76	0.220
Adult Spines	Fogarty Creek	n/a	33.7	1.93	2.76	0.224
Adult Spines	Fogarty Creek	n/a	34.1	1.93	2.79	0.224
Adult Spines	Fogarty Creek	n/a	33.7	1.92	2.76	0.223
Adult Spines	Van Damme	n/a	34.9	1.89	2.85	0.219
Adult Spines	Van Damme	n/a	34.3	1.90	2.81	0.221
Adult Spines	Van Damme	n/a	30.4	1.85	2.50	0.215
Adult Spines	Van Damme	n/a	37.2	1.94	3.04	0.226
Adult Spines	Van Damme	n/a	34.9	1.91	2.85	0.222
Adult Tests	Bodega Marine Reserve	n/a	74.1	2.71	5.88	0.315
Adult Tests	Bodega Marine Reserve	n/a	74.1	2.71	5.88	0.315
Adult Tests	Bodega Marine Reserve	n/a	76.4	2.90	6.05	0.337
Adult Tests	Bodega Marine Reserve	n/a	77.5	2.61	6.13	0.303
Adult Tests	Bodega Marine Reserve	n/a	78.5	2.76	6.21	0.320
Adult Tests	Bodega Marine Reserve	n/a	78.5	2.76	6.21	0.320
Adult Tests	Bodega Marine Reserve	n/a	78.9	2.71	6.23	0.315
Adult Tests	Bodega Marine Reserve	n/a	78.9	2.71	6.23	0.315
Adult Tests	Bodega Marine Reserve	n/a	79.5	2.66	6.28	0.309
Adult Tests	Bodega Marine Reserve	n/a	79.5	2.66	6.28	0.309
Adult Tests	Bodega Marine Reserve	n/a	87.3	2.77	6.85	0.322
Adult Tests	Bodega Marine Reserve	n/a	90.6	2.58	7.09	0.300
Adult Tests	Bodega Marine Reserve	n/a	90.6	2.58	7.09	0.300
Adult Tests	Bodega Marine Reserve	n/a	93.1	2.68	7.28	0.311
Adult Tests	Bodega Marine Reserve	n/a	95.0	2.89	7.41	0.335
Newly Metamorphosed Juveniles	Bodega Marine Reserve	900	68.0	2.10	5.42	0.244
Newly Metamorphosed Juveniles	Sand Hill Bluff	900	61.6	2.10	4.94	0.243
Newly Metamorphosed Juveniles	Sand Hill Bluff	900	68.3	2.10	5.44	0.244
Newly Metamorphosed Juveniles	Sand Hill Bluff	900	64.6	2.16	5.16	0.250
Newly Metamorphosed Juveniles	Sand Hill Bluff	900	71.8	2.16	5.70	0.251
Newly Metamorphosed Juveniles	Strawberry Hill	900	71.4	1.91	5.67	0.222
Newly Metamorphosed Juveniles	Strawberry Hill	900	63.8	2.04	5.10	0.237
Newly Metamorphosed Juveniles	Strawberry Hill	900	67.4	2.10	5.37	0.244
Newly Metamorphosed Juveniles	Strawberry Hill	900	74.8	2.05	5.93	0.238
Newly Metamorphosed Juveniles	Santa Barbara	900	66.9	2.21	5.34	0.257
Newly Metamorphosed Juveniles	Santa Barbara	900	74.9	2.32	5.94	0.269
Newly Metamorphosed Juveniles	Bodega Marine Reserve	400	63.6	2.08	5.09	0.242
Newly Metamorphosed Juveniles	Bodega Marine Reserve	400	65.7	2.08	5.24	0.242
Newly Metamorphosed Juveniles	Bodega Marine Reserve	400	69.1	2.14	5.50	0.248
Newly Metamorphosed Juveniles	Bodega Marine Reserve	400	64.8	2.07	5.18	0.240
Newly Metamorphosed Juveniles	Sand Hill Bluff	400	70.0	2.10	5.57	0.244
Newly Metamorphosed Juveniles	Strawberry Hill	400	62.5	2.07	5.00	0.240
Newly Metamorphosed Juveniles	Strawberry Hill	400	63.2	2.10	5.06	0.244
Newly Metamorphosed Juveniles	Strawberry Hill	400	71.3	2.18	5.67	0.253
Newly Metamorphosed Juveniles	Santa Barbara	400	63.4	2.10	5.07	0.243
Newly Metamorphosed Juveniles	Santa Barbara	400	66.4	2.09	5.30	0.242
Newly Metamorphosed Juveniles	Santa Barbara	400	61.1	2.09	4.90	0.243