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PRETREATMENT FOR REMOVAL OF ORGANIC MATERIAL IS NOT NECESSARY FOR X-RAY-DIFFRACTION DETERMINATION OF MINERALOGY IN TEMPERATE SKELETAL CARBONATE

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ABSTRACT: **Quantifying the effects of ocean acidification requires understanding the skeletal carbonate mineralogy in living marine organisms. X-ray diffractometry (XRD) is the simplest and most commonly used technique for determining this. Samples being analyzed by XRD are typically pretreated to remove organic material prior to grinding to a crystallite powder. This pretreatment was traditionally performed as organic material may obscure the mineral peaks on XRD traces. This study compared controls with no pretreatment with the most common pretreatment methods: roasting, immersion in chlorine bleach, and immersion in hydrogen peroxide. The latter two methods were performed at two strengths and two durations. We test the hypothesis that bleaching and/or roasting of skeletal carbonate to remove organic material does not affect the mineralogy of temperate skeletal carbonate at a scale detectable by XRD. This was done with biogenic skeletal carbonate from temperate marine environments around southern New Zealand. Specimens included 5 species of bivalve mollusks, 4 species of bryozoans, 2 species of barnacles, as well as 1 species each of serpulid worm, echinoid, gastropod mollusk, brachiopod, and coralline algae. Comparison to the untreated control showed that all pretreatments removed some organic matter and that the presence of organic matter in temperate skeletal carbonate does not affect the ability to qualitatively interpret mineralogy or semiquantitatively measure mineralogy using XRD. Given that pretreatment does not appear to be necessary and that some methods at least can cause unacceptable changes in mineralogy, we recommend that pretreatment for the removal of organic material be abandoned.**

INTRODUCTION

Determination of skeletal carbonate mineralogy is contributing to the developing understanding of the effects of ocean acidification on calcification in living organisms, including solubility, dissolution, and sequestration in carbonate sediments (e.g., Tynan and Opkyke 2011; Haese et al. 2014). X-ray diffractometry (XRD) is the simplest and most commonly used technique for determining calcite:aragonite ratio and the degree of substitution of Mg in calcite (e.g., Loxton et al. 2013; Smith et al. 2013). Samples for XRD analysis are routinely cleaned using chlorine bleach (usually sodium hypochlorite), peroxide (usually hydrogen peroxide), or heat (roasting or "calcining") to remove organic material (which may obscure the mineral peaks on XRD traces) by oxidation prior to grinding to a crystallite powder (e.g., Poppe et al. 2001).

Studies heretofore have noted that roasting changes mineral composition, especially with water present (Dickson 2001). Though some authors recommend roasting at low temperatures (Gaffey et al. 1991), others suggest avoiding it altogether (Boiseau and Juillet-Leclerc 1997). Oxidation of organic matter, often called "bleaching" (with NaOCl, NaOH, or H_2O_2) is often used and/or recommended (e.g., Gaffey and Bronnimann 1993; Boiseau and Juillet-Leclerc 1997; Krause-Nehring et al. 2011) at a wide variety of concentrations. As a result of the confusion, some authors have chosen to avoid all chemical pretreatment (Barker et al. 2003). Most studies examining the effects of pretreatment (Table 1) have been carried out on tropical corals, foraminifera, and echinoids; there is no similar study that focuses on temperate carbonate organisms that dominate in cool-to-cold waters (i.e., mollusks, bryozoans, echinoids, barnacles, and serpulid worms; James and Clarke 1997).

Here we test the hypothesis that bleaching and/or roasting of skeletal carbonate to remove organic material does not affect the mineralogy of temperate skeletal carbonate at a scale detectable by X-ray diffractometry. If the null hypothesis is not proved, possible variations in post-treatment composition could include loss of an entire mineralogical component, systematic change regardless of original concentration, or composition-dependent change (Fig. 1).

MATERIALS AND METHODS

Biogenic skeletal carbonate was collected from a range of temperate marine environments around southern New Zealand, particularly the Otago shelf and Stewart Island. Un-encrusted and un-bored specimens were collected (by hand or by dredge) either living or apparently freshly dead, and not exposed to chemicals or preservatives of any kind after collection. Where possible, the dead organism was scraped out without damaging the shells, which were then rinsed in distilled water at room temperature.

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TABLE 1.—*Results of some recent studies on effectiveness of pretreatments for removal of organic material. HMC, high-magnesium calcite (> 8 wt% $MgCO_3$); IMC, intermediate-magnesium calcite (4–8 wt% $MgCO_3$); LMC, low-magnesium calcite (< 4 wt% $MgCO_3$); OM, organic material.*

Treatment	Sample Material	Measured Parameters		Comments	Recommendation	Reference
		Calcite: Aragonite Ratio	Mg content in calcite			
30% H_2O_2 (≈ 200 µg of coral powder into 1000 µl of 30% hydrogen peroxide, dark, room temp, 12 h, rinsed through 0.45 µm filter, dried for 2 h at 40°C)	Recent coral aragonite from Moorea lagoon (17°30′ S, 149°50′ W, French Polynesia)	no change, minor superficial dissolution of coral aragonite		H_2O_2 removes carbonate formed after skeleton formation as well as organic matter	30% H_2O_2 recommended for corals	Boiseau and Juillet-Leclerc 1997
Roasting (partial vacuum, 350°C, 2 h)	Recent coral aragonite from Moorea lagoon (17°30′ S, 149°50′ W, French Polynesia)	mineralogical inversion from aragonite to calcite			should not use roasting on skeletal carbonate	Boiseau and Juillet-Leclerc 1997
Roasting (urchin plates in uncovered fused-quartz crucibles and welded pressurized gold capsules, some with water added; 300°C; 1–620 h)	Tropical echinoid *Heterocentrotus trigonarius* interambulacral plates	HMC became IMC and dolomite (> 10 h)	HMC (≈ 14 mol% $MgCO_3$) > dolomite (43.5 mol% $MgCO_3$) and IMC (4–7 mol% $MgCO_3$) (> 10 h); dolomite ≈ 47 mol% $MgCO_3$ and calcite to ≈ 2 mol% $MgCO_3$ at 120 h) .	Cleaned using sodium hypochlorite before the experiment; changes occur through dissolution–precipitation reaction, therefore needs some water	roasting at 300°C especially with water present changes mineral composition	Dickson 2001
Five chemical treatments: 30% H_2O_2 buffered and not, 2.5% and 5% NaOCl, and 1N NaOH	Green alga *Halimeda incrassata;* echinoid *Lytechinus variegatus* (San Salvador Island, Bahamas)			H_2O_2 does not remove polysaccharides, causes dissolution; NaOCl is most effective at removing OM	Full-strength NaOCl (5%) recommended	Gaffey and Bronnimann 1993
Roasting (105, 150, 200 and 300°C, 2–24 h)	Tropical corals *Acropora cervicornis*, *Diploria strigosa*, echinoids *Clypeaster rosacea*, *Leodia sexiesperforata*, and *Encope emarginata*, red alga *Neogoniolithon*, foraminifera *Homotrema rubrum* and *Quinqueloculina* sp. (San Salvador, Bahamas)	No significant changes at these temperatures (400°C is the limit)	No significant changes at these temperatures	All bleached in 5% NaOCl or 30% H_2O_2 14 days, ultrasonically cleaned	roasting at less than 400°C is okay	Gaffey et al. 1991
Eight different bleaching regimes incorporating NaOH, NaOCl, H_2O_2, and acetone in various combinations, applied to fine powder	Inorganic calcite and aragonite from bivalve *Arctica islandica*, North Sea (40 m)	No changes noted	30% H_2O_2 lowered Mg content	Sonicated and powdered, treated warm and mixed, dried before experiment. NaOH reacted with the very fine powder to make new compounds. H_2O_2 dissolved some of the carbonate.	NaOCl and NaOH most effective at removing OM	Krause-Nehring et al. 2011
Roasting (200, 340, 450°C)	*Hibolithes* and *Pachyteurhis* spp (Late Jurassic fossils from Poland and Scotland); Modern *Ostrea* spp from Cuba and *Cardium edule* from Baltic Sea; synthetic calcite and aragonite; marble	Aragonitic samples converted to calcite at higher temperatures		Mostly this study was looking at effects on stable isotope composition.		Wierzbowski 2007
Hot buffered H_2O_2 solution	Foram *Globigerina ruber*, Arabian Sea		Mg/Ca ratio (in mmol/mol) decreases with removal of OM	Treatment here is to remove contaminants and OM	Avoid treatment of forams, just use washing	Barker et al. 2003

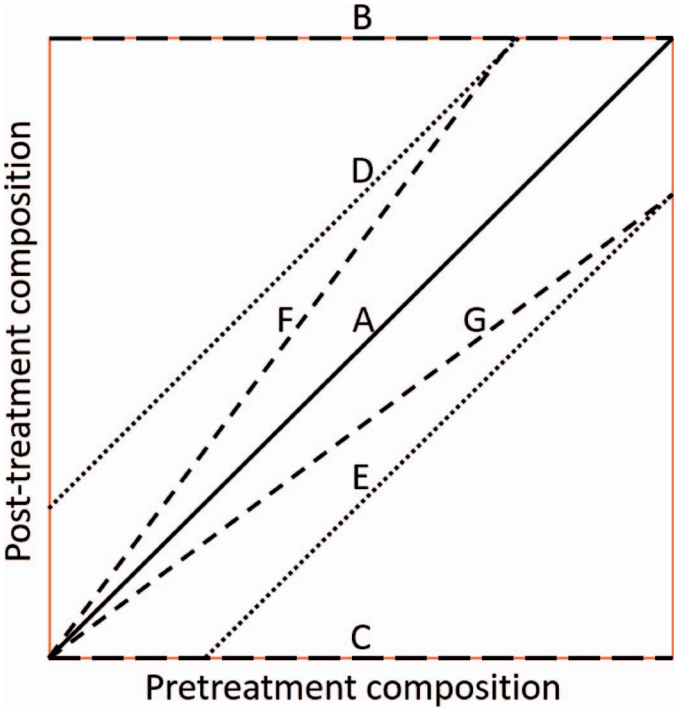


FIG. 1.—Conceptual model of compositional change caused by treatment. Line A shows a lack of effect (pretreatment composition is the same as post-treatment composition). Lines B and C show that composition changes to one end member regardless of original composition. Lines D and E show intercept shift caused by a constant increase or decrease in a component regardless of original composition. Lines F and G show the slope change caused by composition-related change.

Specimens included important producers of carbonate sediment in temperate regions (Nelson 1988): bivalve mollusks (5 species), bryozoans (4 species), barnacles (2 species), as well as serpulid worms, gastropod mollusks, brachiopods, and coralline algae (1 species each). Spines and test plates (which have different Mg contents) from a single echinoid species were also examined (Table 2).

For each species, three clean individual specimens (or colonies, in the case of bryozoans) were used. Each specimen was cut (using a cold hacksaw) or snapped into six pieces weighing about 5 g each. Each piece was given a unique identifier and assigned to one of six treatments; each species was thus represented in each treatment. Three replicate blanks (thoroughly bleached aragonite cockle shell) were prepared for each treatment, to test the that pretreatment methods removed only organic material. Specimens and blanks were kept at 15°C in a desiccator until ready for treatment. Immediately prior to treatment, specimens (except *Arthrocardia* and the *Evechinus* spines) and blanks were weighed and photographed.

Six treatments were selected (Table 3) from the commonly used methods to clean and/or oxidize marine carbonate samples (Table 1). They were: a control with no pretreatment, roasting in a muffle furnace at 550°C for 1.5 hours, immersion in 30% household chlorine bleach for three hours ("light bleach"), and in 100% household chlorine bleach for three days ("hard bleach") (representing the endpoints of the range normally used in published studies), immersion in 5% hydrogen peroxide for 3 hours ("light H_2O_2"), and immersion in 30% hydrogen peroxide for three days ("hard H_2O_2") (also representing the endpoints of the range normally used). After treatment, specimens and blanks were rinsed and dried at 15°C, then kept in a desiccator until weighed. Each specimen was then subsampled: a 2 g piece was kept for archive, and a 3 g piece was hand-ground to a crystallite powder in an agate mortar, and spiked with 0.2 g halite as an internal standard for X-ray analysis.

Determination of mineral content was carried out using a PAN Analytical X'Pert PRO X-Ray Diffractometer, scanning over the range of 26 to 32.5°2θ at a scan speed of 0.02571°2θ/s. Spectra were processed using HighScore data processing software. Mg content in calcite was calculated from the location of the calcite peak at 29.4–29.8 °2θ relative to the halite peak at 31.7°2θ, as the addition of Mg causes displacement of the peak to higher °2θ values (see e.g., Gray and Smith 2004). The calcite:aragonite ratio was quantified and the ratio between different Mg-calcites was also calculated using peak-height ratios from previously developed in-house instrument-specific calibration curves (Gray and Smith 2004; Smith and Lawton 2010).

We used linear models to compare the effect of each treatment on mineralogical measurements. To incorporate the nested nature of our study design, in which both controls and the effect of each treatment were measured for each specimen, we used the R-library package Linear and Non-Linear Mixed Effects Models (NLME, Pinheiro et al. 2007) to create random-effects models that estimate the difference between non-treated and treated subsamples for each mineralogical measurement. This statistical approach is equivalent to a repeated measures analysis of variance (ANOVA) in which each treatment is considered a separate categorical variable. We fitted one model for each species for the calcite:aragonite ratio (expressed in wt% calcite) and Mg content in calcite (expressed in wt% $MgCO_3$) data.

Initial examination of results showed that two species were problematic. *Atrina zelandica* (the horse mussel) and *Cardita distorta* (the nesting cockle) showed particularly wide variations in mineralogy among control specimens. We carried out a second full replicate set of analyses for these two species, but variability among controls was as large as or larger than variation in treated specimens, which rendered these two species useless for the study, and consequently they were removed from detailed statistical analysis.

RESULTS

Heavily bleached cockles, used as a standard, should have contained no organic material; indeed they lost less than 1% of their original weight in all treatments except roasting (mean wt loss 2.5%, $n = 3$, $sd = 0.1\%$). In all species (Table 4, Fig. 2) the greatest weight loss was found in the roasting treatment. The least weight loss was after the light H_2O_2 treatment (Table 4, Fig. 2). In every treatment, total weight loss was less than 10% of the original weight of the shell.

In total, 252 specimens from 13 species were analyzed for skeletal carbonate mineralogy. In all cases the XRD traces were sharp and readable, with no extraneous peaks or distortions. There were three species with mixed mineralogy, which were thus able to be analyzed for changes in the calcite:aragonite ratio (Fig. 3A–C). Roasting showed the strongest effect (Fig. 3A), with both strengths of chlorine bleach and H_2O_2 making little difference overall in the calcite:aragonite ratio (Fig. 3B, C).

Roasting also affected Mg content, reducing the $MgCO_3$ content in specimens made of Mg-calcite ($>$ 6 wt% $MgCO_3$, Fig. 3D). Bleaching with either chlorine bleach or H_2O_2 had no significant effect (Fig. 3E, F).

DISCUSSION

Weight change in blanks (thoroughly bleached cockle shells) after treatment suggests that drying samples in an oven and then storing in a desiccator may not have removed all the water present. Water trapped in the mollusk shell structure could account for the weight loss that occurred in the roasting treatment. Alternatively, roasting may have removed a small proportion of inorganic carbonate. While removal of organic material is the main purpose of pretreatment of samples, the removal of carbonate is not a

TABLE 2.—*Species used in this study. In each case, specimens were collected by hand or dredge from the* RV Polaris II *in Nov–Dec 2011 from around southern New Zealand.*

Species	Taxonomy (Phylum: Class: Order: Family)	Carbonate Mineralogy	Sample Location (Station ID)	Latitude	Longitude	Water Depth (m)
Arthrocardia corymbosa (Lamarck) Decaisne, 1842	Rhodophyta: Florideophyceae: Corallinales: Corallinaceae	High-Mg Calcite (11–12 wt% $MgCO_3$)	Paterson Inlet, Stewart Island	–46.87	168.15	intertidal
Galeolaria hystrix Mörch, 1863	Annelida: Polychaeta: Sabellida: Serpulidae	Mainly High-Mg Calcite (9–12 wt% $MgCO_3$), some Aragonite (up to 5 wt%)	Paterson Inlet (PI3)	–46.96	168.13	8
Elminius modestus Darwin, 1854	Arthropoda: Maxillopoda: Sessilia: Austrobalanidae	Calcite (< 1 wt% $MgCO_3$)	Port Pegasus (PP2)	–47.24	167.62	intertidal
Austromegabalanus psittacus Molina, 1782	Arthropoda: Maxillopoda: Sessilia: Balanidae	Calcite (< 1 wt% $MgCO_3$)	Sailors Rest, Paterson Inlet (PI2)	–46.97	168.14	15
Calloria inconspicua Sowerby, 1846	Brachiopoda: Rhynchonellata: Terebratulida: Terebratellidae	Calcite (< 1 wt% $MgCO_3$)	Bench Island, Paterson Inlet (SS3)	–46.87	168.21	43
Hippomenella vellicata Hutton, 1873	Bryozoa: Gymnolaemata: Cheilostomata: Schizoporellidae	Intermediate-Mg Calcite (6–8 wt% $MgCO_3$)	Otago Shelf	–45.67	170.96	70
Celleporaria emancipata Gordon, 1989	Bryozoa: Gymnolaemata: Cheilostomata: Lepraliellidae	Intermediate-Mg Calcite (6–8 wt% $MgCO_3$)	NW of Snares Islands (SS7)	–47.52	166.71	168
Hornera robusta MacGillivray, 1883	Bryozoa: Stenolaemata: Cyclostomata: Horneridae	Low-Mg Calcite (1–3 wt% $MgCO_3$)	Otago Shelf	–45.67	170.96	70
Hornera foliacea MacGillivray, 1869	Bryozoa: Stenolaemata: Cyclostomata: Horneridae	Low-Mg Calcite (1–4 wt% $MgCO_3$)	Otago Shelf	–45.67	170.96	70
Evechinus chloroticus Valenciennes, 1846 TEST	Echinodermata: Echinoidea: Camarodonta: Echinometridae	High-Mg Calcite (8–10 wt% $MgCO_3$)	Lords River	–47.12	168.15	3
Evechinus chloroticus Valenciennes, 1846 SPINES	Echinodermata: Echinoidea: Camarodonta: Echinometridae	Intermediate-Mg Calcite (4–5 wt% $MgCO_3$)	Lords River	–47.12	168.15	3
Barbatia novaezealandiae E.A. Smith, 1915	Mollusca: Bivalvia: Arcida: Arcidae	Aragonite	Bench Island, Paterson Inlet (SS1)	–46.90	168.24	47
Mytilus galloprovincialis Lamarck, 1819	Mollusca: Bivalvia: Mytilida: Mytilidae	Mostly Low-Mg Calcite (1–4 wt% $MgCO_3$), some Aragonite (20–40 wt%)	Port Pegasus (PP2)	–47.24	167.62	intertidal
Atrina zelandica Gray, 1835	Mollusca: Bivalvia: Pteriida: Pinnidae	Low-Mg Calcite (1–4 wt% $MgCO_3$)	Otago Shelf	–45.67	170.96	70
Pecten novaezelandiae Reeve, 1852	Mollusca: Bivalvia: Pectenida: Pectenidae	Very Low-Mg Calcite (< 1 wt% $MgCO_3$)	Port Pegasus (PP4)	–47.21	167.62	17
Cardita distorta Reeve, 1843	Mollusca: Bivalvia: Carditida: Carditidae	Aragonite with trace Calcite of widely varying Mg content	Bench Island, Paterson Inlet (SS1)	–46.90	168.24	47

TABLE 3.—*Sample treatments used in this study*.

Control	Rinse three times in distilled water, air dry, and place in desiccator at room temperature.
Roast	Roast in muffle furnace at 550°C for 1.5 hours, rinse three times with distilled water, air dry, and place in desiccator at room temperature.
Light bleach	Immerse in 30% household bleach (7 g/l sodium hypochlorite) for 3 hours. Rinse three times with distilled water, air dry, and place in desiccator all at room temperature.
Hard bleach	Immerse in 100% household bleach (21 g/l sodium hypochlorite) for 3 days, stirring several times. Rinse three times with distilled water, air dry, and place in desiccator all at room temperature.
Light hydrogen peroxide	Immerse in hydrogen peroxide (5% H_2O_2) for 3 hours. Rinse three times with distilled water, air dry, and place in desiccator all at room temperature.
Hard hydrogen peroxide	Immerse in hydrogen peroxide (30% H_2O_2) for 3 days, stirring several times. Rinse three times with distilled water, air dry, and place in desiccator all at room temperature.

desirable outcome. Other studies have found that hydrogen peroxide, while an effective bleach, can also cause dissolution of carbonate (Gaffey and Bronniman 1993; Boiseau and Juillet-Leclerc 1997). Our study, the first to include many important nontropical taxa, supports this finding and suggests that chlorine bleach at high concentrations can also remove some carbonate. We could have understood more about the removal of organic material under these treatments had we been able to include a "standard" material that closely resembled the material found in our samples but was entirely organic. It is, however, difficult to imagine what material would replicate internal organic layers in taxa as diverse as mollusks, worms, bryozoans, echinoids, barnacles, and algae.

The 550°C temperature used in this study is the sedimentological standard for removing organic matter from sediments and soils (Lewis and McConchie 1994; Poppe et al. 2001; Rayment and Lyons 2011), but it appears to be too high for marine carbonates. Roasting marine shells at lower temperatures could avoid decomposition of carbonate but might not remove all the organic material. Of the immersion pretreatments, we found that the hard H_2O_2 and chlorine bleach methods removed more weight compared to the light bleaching in most species (Table 4). The effect of pretreatment on specimen weight does not appear to be consistent among mineralogy types or taxon.

TABLE 4.—*Mean percentage weight loss for specimens (n = 3) in six treatments to remove organic material: control, roasting, light and hard bleaching, and light and hard treatment in hydrogen peroxide.*

	Control		Roasting		Light Bleach		Hard Bleach		Light H_2O_2		Hard H_2O_2	
Samples	**Mean**	**Stdev**	**Mean**	**Stdev**	**Mean**	**Stdev**	**Mean**	**Stdev**	**Mean**	**Stdev**	**Mean**	**Stdev**
Blank (cockles)	0.434	0.091	2.489	0.119	0.274	0.069	0.282	0.127	0.435	0.117	0.794	0.121
Standard (raisins)	33.800	2.415	89.253	0.357	68.164	1.351	78.929	0.995	66.835	12.088	87.566	0.423
Barbatia novaezealandiae	0.029	0.029	2.895	0.218	0.948	0.141	1.766	0.505	0.198	0.081	1.206	0.086
Calloria inconspicua	0.035	0.006	1.635	0.092	0.314	0.039	0.445	0.066	0.129	0.017	0.590	0.038
Celleporaria emancipata	3.759	2.532	6.519	0.340	2.065	0.288	2.443	0.339	2.639	0.433	4.378	0.133
Evechinus chloroticus (test)	0.267	0.029	6.542	0.356	1.386	0.060	1.349	0.222	0.969	0.122	2.288	0.249
Austromegabalanus psittacus	0.191	0.077	2.632	0.035	0.683	0.059	1.086	0.067	0.407	0.085	3.330	2.880
Galeolaria hystrix	0.416	0.095	8.129	0.108	2.225	0.198	3.464	0.364	1.769	0.136	6.111	1.140
Hornera foliacea	0.511	0.065	5.429	0.240	2.362	0.275	3.466	0.343	1.671	0.536	3.716	0.060
Hippomenella vellicata	1.481	0.300	8.705	0.209	3.975	0.357	4.354	0.083	2.485	0.231	4.827	0.072
Hornera robusta	0.371	0.064	4.825	0.191	1.568	0.069	2.712	0.576	0.777	0.017	2.887	0.245
Mytilus galloprovincialis	0.047	0.024	2.810	0.120	1.141	0.111	2.208	0.144	0.158	0.023	0.805	0.120
Pecten novaezelandiae	0.056	0.030	7.026	8.339	2.110	2.716	0.555	0.052	0.211	0.042	0.734	0.138
Elminius modestus	0.533	0.094	5.201	0.054	2.858	0.116	4.194	0.233	1.354	0.377	2.521	0.137
Mean of all treated samples (excluding blank and standard)	0.641	0.279	5.196	0.859	1.803	0.369	2.337	0.250	1.064	0.175	2.783	0.442

Scientists have known for decades that pretreatment can cause mineralogical change. As aragonite is a metastable polymorph of calcium carbonate, it is prone to inversion to the more stable form, low-Mg calcite. This neomorphic inversion of aragonite to calcite has been long known (Johnston et al. 1916), driven by increased temperature and/or pressure at temperatures as low as 100°C through heating with water for a few days, while dry samples invert in a few hours at 400°C (Spry 1969) and in as little as a few minutes at 470°C (Johnston et al. 1916; Yoshioka and Kitano 1985). The rate varies with the number of lattice defects and fluid inclusions, which varies with the type of aragonite (e.g., coral, mollusk, synthetic, etc.) (Yoshioka and Kitano 1985). Among entirely aragonitic corals, for example, roasting above 350°C results in at least some conversion to calcite (Boiseau and Juillet-Leclerc 1997), whereas roasting at 200°C does not (Wierzbowski 2007). Gaffey et al. (1991) suggested that 400°C was the upper limit for roasting that does not remove aragonite, but Dickson (2001) noted that roasting at 300°C with water present does result in mineralogical change. The aragonitic species in this study (bivalve *Barbatia novaezelandiae*) and mixed-mineralogy species (serpulid *Galeolaria hystrix*, mussel *Mytilus galloprovincialis,* and also the bivalve *Cardita distorta*), showed no significant change in the calcite:aragonite ratio except under the treatment of roasting, and the calcitic specimens showed no change at all (Table 5, Fig. 4).

Mg content, too, was most strongly affected by roasting, especially in the high-Mg carbonate produced by the red alga *Arthrocardia corymbosa* and the serpulid *Galeolaria hystrix*, and to a lesser extent in intermediate-Mg bryozoan calcite (*Hippomenella vellicata* and *Celleporaria emancipata*). Interestingly, echinoid calcite with similar original high-Mg content lost no significant Mg under any treatment, including roasting, which may

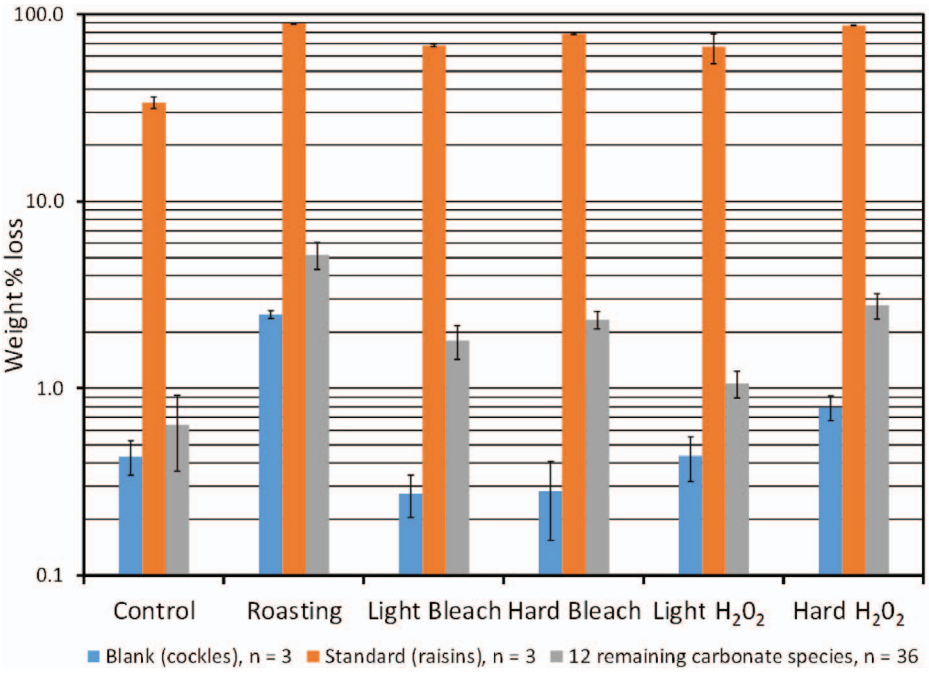
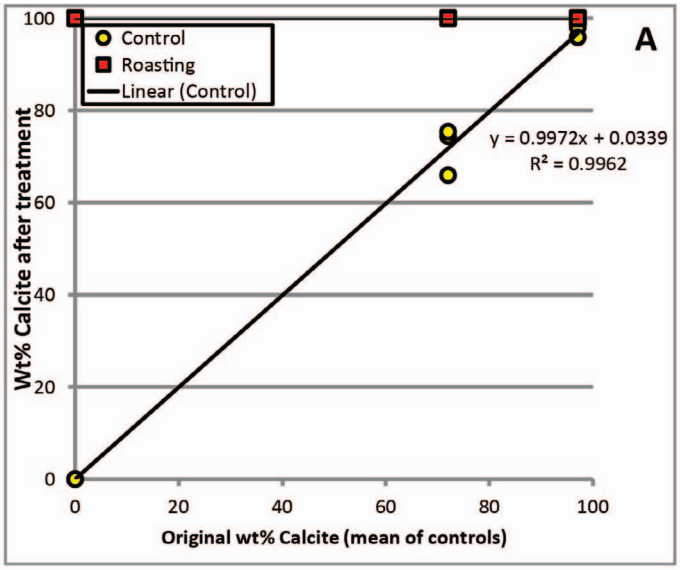
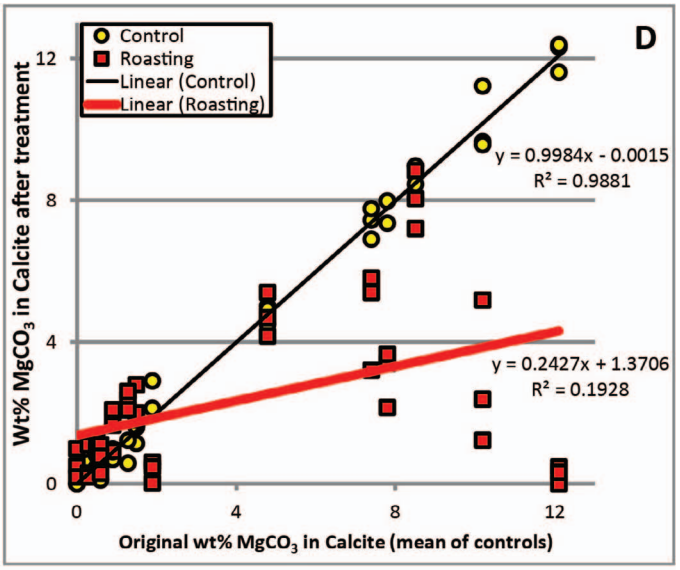
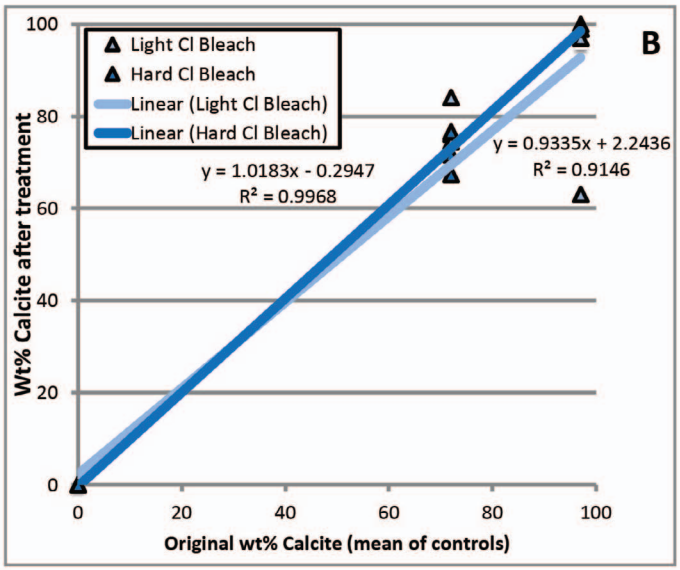
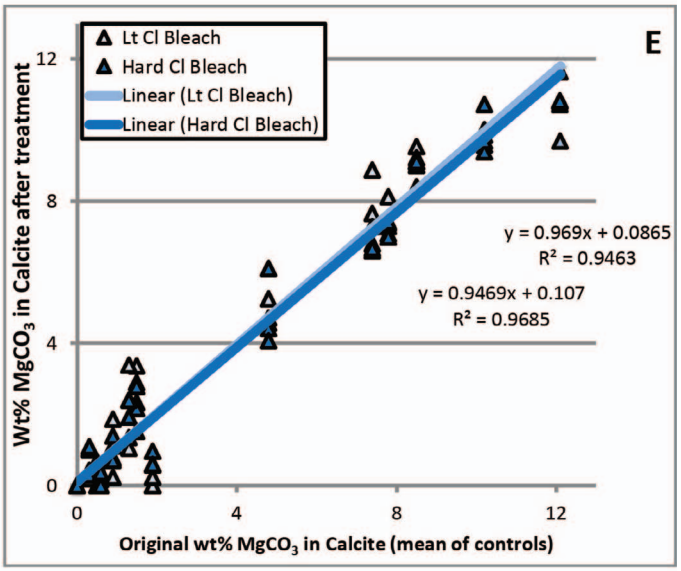


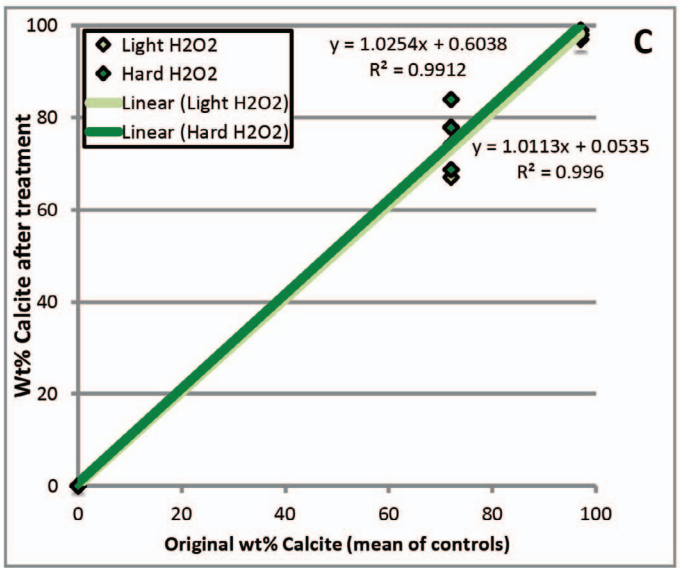
FIG. 2.—Percent weight loss by three different materials in six different treatments. Error bars are ± 1 st dev.

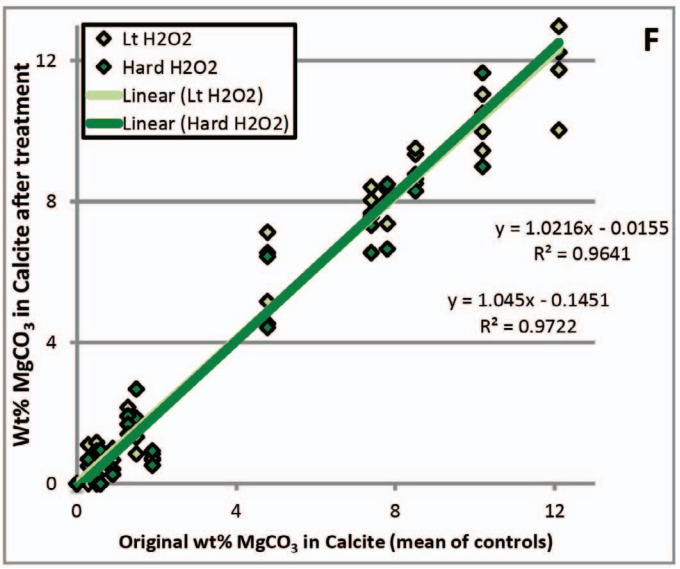

A
Control
Roasting
Linear (Control)
y = 0.9972x + 0.0339
R² = 0.9962
Wt% Calcite after treatment
Original wt% Calcite (mean of controls)
0
20
40
60
80
100


D
Control
Roasting
Linear (Control)
Linear (Roasting)
y = 0.9984x - 0.0015
R² = 0.9881
y = 0.2427x + 1.3706
R² = 0.1928
Wt% MgCO3 in Calcite after treatment
Original wt% MgCO3 in Calcite (mean of controls)
0
4
8
12


B
Light Cl Bleach
Hard Cl Bleach
Linear (Light Cl Bleach)
Linear (Hard Cl Bleach)
y = 1.0183x - 0.2947
R² = 0.9968
y = 0.9335x + 2.2436
R² = 0.9146
Wt% Calcite after treatment
Original wt% Calcite (mean of controls)
0
20
40
60
80
100


E
Lt Cl Bleach
Hard Cl Bleach
Linear (Lt Cl Bleach)
Linear (Hard Cl Bleach)
y = 0.969x + 0.0865
R² = 0.9463
y = 0.9469x + 0.107
R² = 0.9685
Wt% MgCO3 in Calcite after treatment
Original wt% MgCO3 in Calcite (mean of controls)
0
4
8
12


C
Light H2O2
Hard H2O2
Linear (Light H2O2)
Linear (Hard H2O2)
y = 1.0254x + 0.6038
R² = 0.9912
y = 1.0113x + 0.0535
R² = 0.996
Wt% Calcite after treatment
Original wt% Calcite (mean of controls)
0
20
40
60
80
100


F
Lt H2O2
Hard H2O2
Linear (Lt H2O2)
Linear (Hard H2O2)
y = 1.0216x - 0.0155
R² = 0.9641
y = 1.045x - 0.1451
R² = 0.9722
Wt% MgCO3 in Calcite after treatment
Original wt% MgCO3 in Calcite (mean of controls)
0
4
8
12

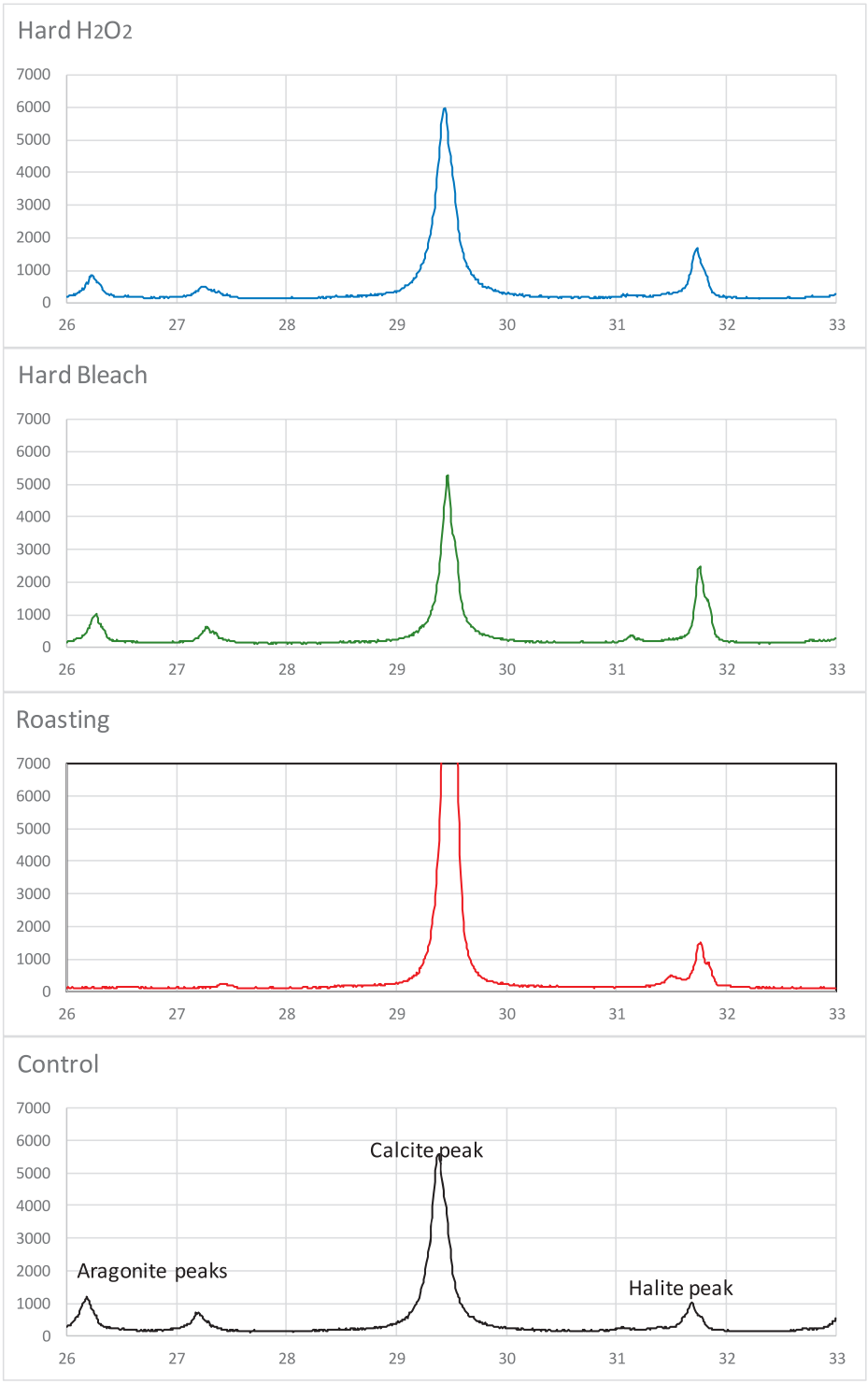


FIG. 4.—X-ray diffractograms showing carbonate mineralogy of bimineralic *Mytilus galloprovincialis* specimens exposed to various pretreatments to remove organic material. Major aragonite peaks occur at peak positions 26.2 and 27.2 °2θ (sometimes a minor peak appears at 31.1 °2θ); calcite is at 29.4 °2θ; halite internal standard is at 31.7 °2θ. Y axis is "counts."

←

FIG. 3.—Changes in **A–C)** calcite:aragonite ratio and **D–F)** $MgCO_3$ in calcite after pretreatment. Original mineralogy for each species is given by yellow circles in parts A and D, and is the mean of the three control replicates. Post-treatment mineralogy is shown as squares (roasting), triangles (bleaching), and diamonds (hydrogen peroxide). Linear regressions show that most of the data lie on a line with a slope close to one, meaning that there is little change in mineralogy post-treatment (see Supplemental Materials).

TABLE 5.—*Skeletal carbonate mineralogy of temperate marine organisms after treatment for the removal of organic material. Abbreviations as in Table 1; A, aragonite. Complete dataset is available as Supplementary Data.*

Species	Original Mineralogy	Mean Weight Percent Calcite in Carbonate (3 Replicates)						Mean Wt% $MgCO_3$ in Calcite (3 Replicates)					
		A Control	B Roasting	C Light Bleach	D Hard Bleach	E Light H_2O_2	F Hard H_2O_2	A Control	B Roasting	C Light Bleach	D Hard Bleach	E Light H_2O_2	F Hard H_2O_2
Arthrocardia corymbosa	HMC	100	100	100	96 ± 6.9	100	100	12.1 ± 0.4	0.3 ± 0.3	11.2 ± 1.8	11.1 ± 0.5	11.6 ± 1.5	13.2 ± 0.8
Austromegabalanus psittacus	LMC	100	100	100	100	100	100	0.9 ± 0.2	1.6 ± 0.6	1.0 ± 0.8	1.0 ± 0.4	0.8 ± 0.2	0.6 ± 0.4
Elminius modestus	LMC	100	100	100	100	100	100	0.5 ± 0.2	1.0 ± 0.2	0.3 ± 0.3	0.2 ± 0.4	0.4 ± 0.7	0.4 ± 0.5
Calloria inconspicua	LMC	100	100	100	100	100	100	0.3 ± 0.2	0.5 ± 0.5	0.5 ± 0.4	0.8 ± 0.4	0.5 ± 0.6	0.6 ± 0.1
Celleporaria emancipata	IMC	100	100	100	100	100	100	7.4 ± 0.5	4.8 ± 1.4	7.8 ± 1.1	6.8 ± 0.4	8.0 ± 0.4	7.2 ± 0.6
Hippomenella vellicata	IMC	100	100	100	100	100	100	7.8 ± 0.4	3.1 ± 0.8	7.6 ± 0.4	7.2 ± 0.2	7.9 ± 0.5	7.7 ± 0.9
Hornera foliacea	LMC	100	100	100	100	100	100	1.5 ± 0.3	2.2 ± 0.5	2.9 ± 0.6	2.2 ± 0.7	1.3 ± 0.5	2.0 ± 0.6
Hornera robusta	LMC	100	100	100	100	100	100	1.3 ± 0.8	2.4 ± 0.3	1.9 ± 1.3	2.2 ± 0.3	1.9 ± 0.3	1.7 ± 0.3
Evechinus chloroticus test plates	HMC	100	100	100	100	100	100	8.5 ± 0.5	8.0 ± 0.9	9.0 ± 0.6	9.1 ± 0.1	9.2 ± 0.5	8.5 ± 0.3
Evechinus chloroticus spines	IMC	100	100	100	100	100	100	4.8 ± 0.3	4.7 ± 0.6	4.8 ± 0.4	4.9 ± 1.1	6.3 ± 1.0	5.1 ± 1.1
Barbatia novaezealandiae	A	0	100	0	0	0	0	–	0.6 ± 0.4	–	–	–	–
Mytilus galloprovincialis	LMC and A	72 ± 4.9	100	78 ± 5.3	72 ± 5.0	73 ± 5.6	77 ± 7.5	1.9 ± 1.2	0.4 ± 0.3	0.1 ± 0.1	0.7 ± 0.2	0.7 ± 0.6	0.6 ± 0.2
Pecten novaezelandiae	LMC	100	100	100	100	100	100	0.6 ± 0.5	0.7 ± 0.4	0.1 ± 0.2	0.1 ± 0.2	0.0 ± 0.1	0.3 ± 2
Galeolaria hystrix	HMC with A	97 ± 1.2	100	86 ± 20.2	99 ± 0.6	98 ± 1.0	98 ±0.6	10.2 ± 0.9	2.9 ± 2.0	9.8 ± 0.2	9.9 ± 0.7	10.2 ± 0.8	10.4 ± 1.4

reflect the way that test plates and spines are formed of single crystals in that taxon (Table 5).

In the presence of heat (Dickson 2001), Mg was possibly reprecipitated elsewhere as microcrystalline dolomite (Hips et al. 2015). While we have no indication as to where that occurred, we can be sure from the X-ray diffractograms that it did not remain in the biogenic calcite skeleton. If it had, the dolomitization process would have destroyed the original skeletal fabric (Read et al. 2016). The original skeletal fabric would also be destroyed if the Mg was being precipitated *in situ* as the common salt magnesium sulfate as suggested by Balboni et al. (2011).

CONCLUSIONS

It is standard practice prior to XRD measurement to pretreat samples to remove organic matter (Mandile and Hutton 1995; McCarthy 2000; Poppe et al. 2001). Organic material, being amorphous, does not give a distinctive peak, but can produce an "organic hump" at 10–40 °2θ (illustrated by Mandile and Hutton 1995) which obscures or weakens the signal of the mineral peaks. In the limited scan length of this method (26–32 °2θ) on skeletal mineral carbonate, the amorphous hump appears to be undetectable and has no apparent effect on mineral peak strength or height (Fig. 4). Comparing the (untreated) control with the various pretreatments indicates that the presence of organic matter in temperate skeletal carbonate does not affect the ability to qualitatively interpret skeletal carbonate mineralogy or semiquantitatively measure that mineralogy using XRD.

Given that pretreatment does not appear to affect XRD traces in temperate biogenic carbonate such as studied here, and that some methods at least can cause unacceptable changes in mineralogy, we recommend that, whenever possible, pretreatment for the removal of organic material be abandoned. Temperate marine carbonate skeletons that have been rinsed and have had soft tissue removed by hand may require no further treatment prior to XRD analysis. It is recommended that acid-free paper be used for drying, and that they be stored in dry conditions until analyzed. If organic material is found to be difficult to remove, it appears that both chlorine bleach and hydrogen peroxide will remove it without causing appreciable mineralogical change. Under no circumstances should mineralogical samples be subjected to high temperatures; care should be taken even in drying ovens with specimens that contain aragonite or Mg in calcite.

SUPPLEMENTAL MATERIAL

Data is available from JSR's Data Archive: http://sepm.org/pages.aspx?pageid=229.

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