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# Alteration of micromilled carbonate $\delta^{18}\text{O}$ during Kiel Device analysis

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Isotopic analysis of carbonate material has been greatly facilitated by the development of autosampling devices such as the Kiel III Carbonate Device, allowing rapid automated analysis of small sample sizes. This analysis is facilitated by holding samples and acid at temperatures around 70 °C prior to reaction. In most situations this has no measurable effect on sample powders on practical timeframes, but, when analyzing exceptionally fine-grained material produced by micromilling, the  $\delta^{18}\text{O}$  of both aragonite and calcite is altered by  $-0.1\%$ /day. Laboratories that use this technique should thoroughly test and correct for this phenomenon and avoid storing pre-weighed materials within the Kiel Device or similar drying oven prior to analysis. Copyright © 2011 John Wiley & Sons, Ltd.

The stable isotopic composition of carbonate has been used as record of paleotemperature and other paleoclimatic variables for over 50 years.<sup>[1]</sup> This analysis is typically done by phosphoric acid digestion of solid carbonate material to release  $\text{H}_2\text{O}$  and  $\text{CO}_2$  with subsequent purification of the  $\text{CO}_2$  gas for analysis in an isotope ratio mass spectrometer capable of measuring  $m/z$  44–46.<sup>[2,3]</sup> Advances in this technique have allowed for the analysis of sample sizes as low as 10  $\mu\text{g}$ , with a throughput approaching 100 analyses per day, using an autosampling instrument such as the Kiel III Carbonate Device (Thermo Scientific, Bremen, Germany, and hereafter Kiel Device). The Kiel Device holds samples, standards and acid at above ambient temperatures (Kiel Device default temperature is 70 °C) to facilitate acid digestion.

As required sample sizes have decreased, numerous studies[e.g. 4–6] have employed computer-guided, fine-tipped, high-speed drills, for example, the Merchantek Micromill (New Wave, Portland, OR, USA). These tools have allowed high-resolution sampling in fossil and modern shell material, often generating temporal resolution at the monthly, or finer, scale, an impossible task using coarser sampling methods. Previous studies have debated the effect of high-speed drilling on aragonite material. Gill *et al.*<sup>[7]</sup> argue for a significant direct effect of drilling on  $\delta^{18}\text{O}$ , although Swart *et al.*<sup>[8]</sup> were concerned about the homogeneity of their material. Foster *et al.*<sup>[9]</sup> later confirmed that high-speed drilling has no detectable influence on the measurable isotopic value of the aragonite. While micromilling probably does not induce direct alteration of carbonate material, we present evidence below that it may compromise the stability of  $\delta^{18}\text{O}$  in both aragonite and calcite when analyzed using a Kiel III Carbonate Device.

## EXPERIMENTAL

Aragonite and calcite, confirmed by X-ray diffraction, were sampled via two methods: crushing with mortar and pestle (hereafter crushed), and drilling with a fine-tipped, high-speed Merchantek Micromill drill (hereafter micromilled). The drill was set to 50% speed (approximately 18 500 rpm). Aragonite was derived from a Late Cretaceous molluscan shell which was crushed and sieved through a set of standard sized sieves, generating a sub-32- $\mu\text{m}$  fraction. Crushed and micromilled samples from the same shell were homogenized and then weighed ( $0.049 \pm 0.017$  mg;  $1\sigma$ ) and interspersed into two full 46-analysis, 20-h runs for analysis in a Kiel Device attached to a Delta Plus isotope ratio mass spectrometer (Thermo Scientific). The first run was put directly into the carousel for immediate analysis while the second run sat in a sample tray within the Kiel Device oven. The samples were interspersed within a run with internal lab standards calibrated to NBS-18 ( $-3.01\%$  VPBD) (IAEA, Vienna, Austria) and NBS-19 ( $-2.20\%$  VPDB) for  $\delta^{18}\text{O}$  and NBS-19 ( $+1.95\%$  VPBD) and LSVEC ( $-46.6\%$  VPBD) for  $\delta^{13}\text{C}$  (NIST, Gaithersburg, MD, USA). The internal lab standards are pure calcite, and they experience a different oxygen isotope fractionation from aragonite, as demonstrated by Kim *et al.*<sup>[10]</sup> The aragonite  $\delta^{18}\text{O}$  values were therefore corrected for this fractionation, adjusted to 70 °C. This procedure was repeated with a crushed internal lab marble calcite standard and a micromilled Late Cretaceous molluscan shell preparation. In addition to focused testing as outlined above, a variety of aragonite materials were run as duplicates, one placed at the beginning and one placed at the end of a 46-analysis, 20-h run, over a period of 8 months to determine whether any alteration was a product of seasonal variations in humidity or other factors.

The micromilled and the smallest crushed size fractions of aragonite were also examined at high magnification to assess grain size as sieves were unavailable to provide the size details in the sub-32- $\mu\text{m}$  range (Fig. 1). The grains were

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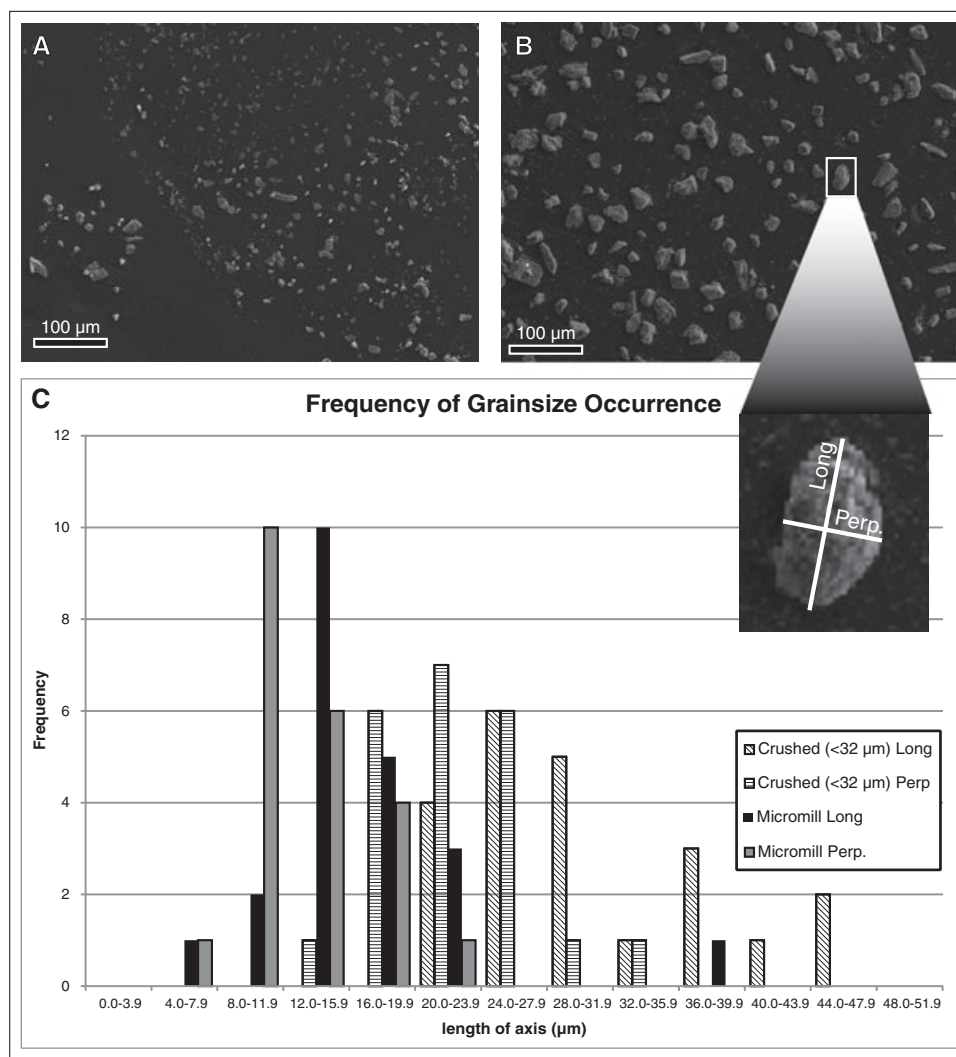
sonicated on non-contaminating weighing paper (Fisher Scientific, Hampton, NH, USA) for 5 s to disperse clumps, then pressed onto an adhesive carbon stub and carbon-coated for analysis and imaged using a JEOL 733 electron microprobe equipped with an Everhart-Thornley secondary electron detector (JEOL USA, Peabody, MA, USA) and the Geller dPict imaging system (Geller MicroAnalytical Laboratory Inc., Topsfield, MA, USA). Size measurements on each size fraction were performed by randomly selecting pixels via a random number generator. If a grain was selected, its longest visible dimension was measured, as was the widest distance perpendicular to the long axis.

## RESULTS

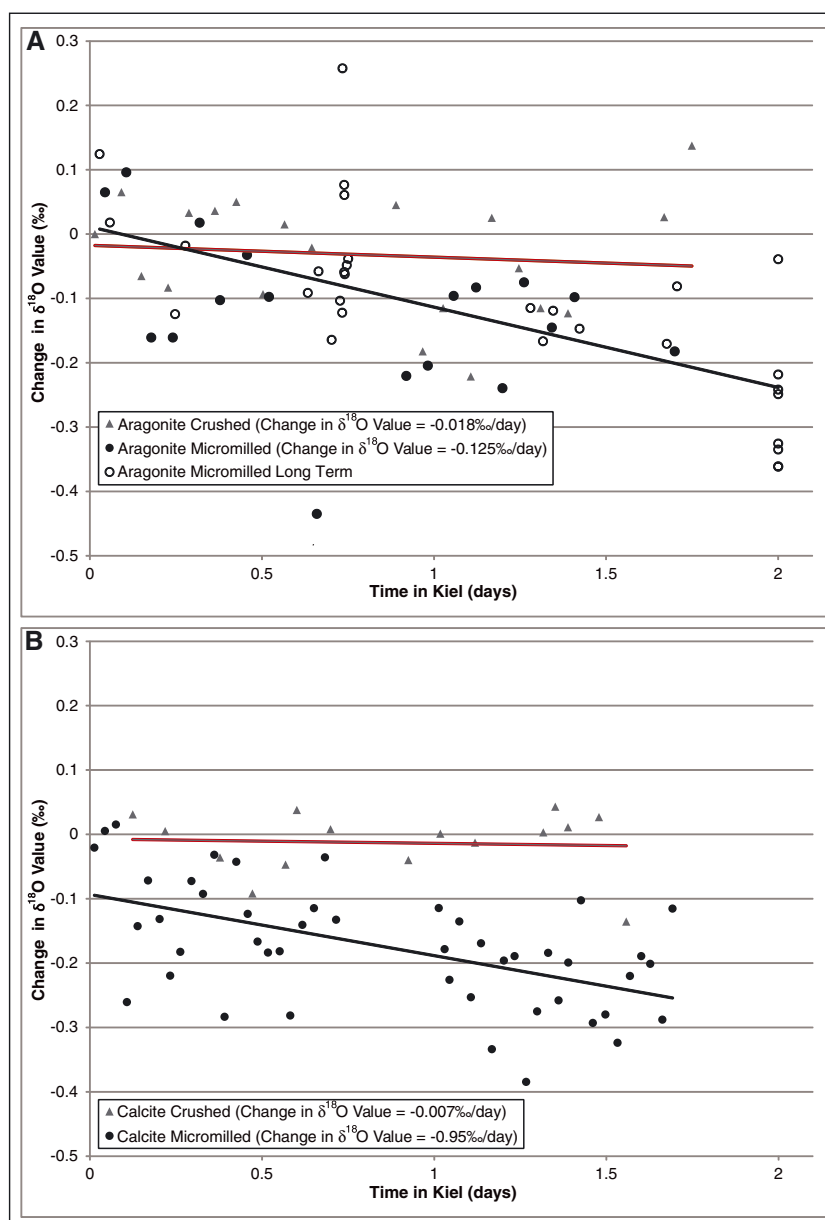
The mean ( $\pm 1 \sigma$ ) size of micromilled aragonite is  $13 \pm 6 \times 11 \pm 5 \mu\text{m}$  (Fig. 1). Figure 1(c) summarizes the measurement statistics for the long and perpendicular axes of the micromilled and crushed ( $<32 \mu\text{m}$ ) size fractions examined with the microprobe.

A Welch's t-test confirms, at greater than 99.999% likelihood, the visual evidence from the micrographs and histogram that the two size fractions are significantly different.

An 'accepted' isotopic value (‰) for each different preparation and mineralogy was calculated by taking the mean of the first three measurements (shortest time spent in the Kiel Device) from each sample group. Each subsequent value was subtracted from this accepted value to determine the amount of change due to alteration. Figure 2 shows the pattern of alteration in  $\delta^{18}\text{O}$  with total time spent in the Kiel Device at  $70^\circ\text{C}$ . Micromilled aragonite (Fig. 2(a)) and calcite (Fig. 2(b)) demonstrate a significant trend toward more negative  $\delta^{18}\text{O}$  values with longer exposure time:  $-0.12 \pm 0.03\text{‰}/\text{day}$  for aragonite ( $p < 0.001$ ) and  $-0.10 \pm 0.02\text{‰}/\text{day}$  for calcite ( $p < 0.001$ ). This trend is not present in either of the crushed sample size fractions or in the  $\delta^{13}\text{C}$  of any sample (data not shown). In some cases the best-fit line intercept is non-zero, as would be initially expected for unaltered samples. The calculated intercept is an artifact of the assigned 'accepted' value for each sample group, a value that does not affect the



**Figure 1.** Representative scanning electron micrographs of the micromilled aragonite (A) and ( $<32 \mu\text{m}$ ) crushed aragonite size fraction (B) with a histogram of the distribution of long and perpendicular axes lengths (C).



**Figure 2.** Alteration of  $\delta^{18}\text{O}$  for crushed (triangles) and micromilled (circles) size fractions for aragonite (A) and calcite (B). Open symbols are data generated over a longer (months) time frame. The slope for micromilled data in (a) is generated using all micromilled data, including the long term analyses. Data presented as Supporting Information (Supplementary Table 1).

slope that describes the rate of alteration. In addition, data collected during long-term analysis had a consistent trend with the short-term experiment ( $-0.15 \pm 0.03\text{‰/day}$ ,  $p < 0.001$ ).

## DISCUSSION

The  $\delta^{18}\text{O}$  of micromilled carbonate is detectably altered ( $-0.1\text{‰/day}$ ) over a 24-h period when stored at  $70\text{ }^\circ\text{C}$ . Only micromilled carbonate, with grain size  $13 \times 11\ \mu\text{m}$ , showed alteration in isotopic values, and only in  $\delta^{18}\text{O}$ , while carbonate above  $27 \times 19\ \mu\text{m}$  grain size, did not show alteration. The authors attribute the alteration to exchange with atmospheric water facilitated by the high temperature inside the Kiel Device.

Exchange with gaseous  $\text{CO}_2$  may also occur, although the lack of alteration in  $\delta^{13}\text{C}$  values suggests that this is a relatively minor factor. This alteration probably occurs in every sample and standard, but only affects the surficial layer and, consequently, only has a measurable isotopic effect on very small grain sizes with high surface area to volume ratios. This effect is just measurable over the course of a single day run, as the regularly reported precision for  $\delta^{18}\text{O}$  in Kiel Device systems is  $\pm 0.1\text{‰}$ , suggesting a correction factor should be applied to micromilled, or similarly prepared, carbonate samples based on the total time spent in the Kiel Device. Ideally, standards of similar grain size would be run in tandem with micromilled samples. Due to the limitations of the available sieves, creating a sufficiently small grain size by conventional crushing and

sieving might be problematic. The practice of placing pre-weighed samples inside the Kiel Device or similar low-temperature oven for storage or drying should be avoided. The authors recommend storing pre-weighed samples in a room temperature desiccator, optionally with soda lime to scrub CO<sub>2</sub>.<sup>[11]</sup> Furthermore, similar alteration tests should be performed for other carbonate-processing procedures.

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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