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Evaluation of foraminiferal trace element cleaning protocols on the Mg/Ca of marine ostracod genus *Krithe*



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ABSTRACT

The Mg/Ca of calcite from the marine ostracod genus *Krithe* may be an important tool for reconstructing past changes in oceanic bottom water temperature (150–4000 m water depth). Rigorous cleaning procedures, routinely used to remove clays, organic matter and Fe–Mn oxyhydroxide coatings in trace element studies of foraminifera, are not regularly applied to marine ostracods despite the potential for Mg contamination. Here we apply standard oxidative and reductive foraminiferal cleaning procedures to core top *Krithe pernoideis* valves from boxcore OCE205-50BC (26.23°N, 77.7°W, 817 m water depth) to evaluate the effects of contamination on Mg/Ca ratios and assess the impact of cleaning techniques on contaminant removal and ostracod valve chemistry. Our results show that clays and Fe–Mn oxyhydroxides influence the Mg/Ca of *Krithe*. Following sonication in methanol/ultrapure water, there is a 1.6 mmol/mol (11%) decrease in Mg/Ca (equivalent to a reduction in reconstructed temperature of 1.5 °C), indicating that this is a critical step in the preparation of *Krithe* valves for Mg/Ca analyses. Oxidation with buffered hydrogen peroxide has little effect on the Mg/Ca of valves from our site. Reductive cleaning reduces inter-valve variability from 12% to 5%, resulting in an equivalent temperature precision of ± 0.6 °C. However, reductive cleaning also decreases Mg/Ca ratios due to the partial dissolution of the valve surface. Reductive cleaning offers the potential to improve *Krithe* Mg/Ca paleotemperature reconstructions and should be utilised in future *Krithe* Mg/Ca studies. Future work should also aim to constrain the effects of partial dissolution of the valve surface.

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1. Introduction

The oxygen isotope ($\delta^{18}\text{O}$) composition of marine calcite (CaCO_3) is a standard proxy for past climate and oceanographic change. However, the ratio of $^{18}\text{O}/^{16}\text{O}$ in the CaCO_3 of marine calcifiers is controlled by both calcification temperature and the $\delta^{18}\text{O}$ of the seawater ($\delta^{18}\text{O}_{\text{sw}}$) in which calcification occurred (see review in Bemis et al., 1998). For decades, paleoceanographers have focused on deconvolving the temperature and $\delta^{18}\text{O}_{\text{sw}}$ signals preserved in marine CaCO_3 to better understand past climate forcings and feedbacks (e.g. global ice volume, surface and deep ocean temperatures, and local salinity) (Shackleton, 1967; Schrag et al., 1996; Elderfield and Ganssen, 2000). The Mg/Ca of marine CaCO_3 is a robust and commonly employed paleotemperature proxy (Dwyer et al., 1995; Nürnberg et al., 1996; Rosenthal et al., 1997; Lea et al., 1999; Elderfield and Ganssen, 2000; Lear et al., 2000), with the thermal dependence of Mg uptake into marine CaCO_3 first demonstrated by Chave (1954). Importantly, Mg/Ca and $\delta^{18}\text{O}$ can be measured on the same marine CaCO_3 , allowing the simultaneous reconstruction of temperature and seawater $\delta^{18}\text{O}$ (Lea et al., 2000; Lear et al., 2000). Mg/Ca

ratios in marine carbonates are also influenced by the activity of Mg and Ca in seawater. Due to the long residence times of Ca and Mg in the ocean (1 and 12 Myr, respectively), seawater Mg/Ca may be considered constant on timescales of ~1 Myr. Over longer timescales past ocean temperatures may be reconstructed with knowledge of past seawater [Ca] and [Mg] (Lear et al., 2000; Shevenell et al., 2004; Coggon et al., 2010).

Bottom water temperature (BWT) reconstructions based on Mg/Ca ratios in marine carbonates may provide important insights into past ocean circulation changes, ocean heat transport, ice volume, and seawater carbonate chemistry. While benthic foraminiferal Mg/Ca is most often employed as a BWT proxy (Lear et al., 2000, 2010; Martin et al., 2002; Elderfield et al., 2006; Marchitto et al., 2007), the Mg/Ca of marine ostracod calcite has also been established as a BWT proxy at temperatures between -1.5 and 16 °C (e.g., Chave, 1954; Dwyer et al., 2002; Elmore et al., 2012; Farmer et al., 2012). Ostracods are benthic crustaceans that molt their CaCO_3 valves every 1–3 days and are often recovered where suitable benthic foraminifera taxa are limited or absent. They have a higher overall Mg concentration than benthic foraminifera, and also a generally higher Mg/Ca-temperature sensitivity, as well as exhibiting species-specific Mg/Ca-temperature relationships (Turpen and Angell, 1971; Dwyer et al., 1995; Farmer et al., 2012).

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To date, marine ostracod paleotemperature research has focused on the genus *Krithe*, due to its relatively smooth carapace structure, which makes it less prone to contamination, its ubiquitous geographic and depth (150–4000 m) distribution, resistance to dissolution (Swanson and van der Lingen, 1994), and long duration in the marine stratigraphic record (97.5 Ma to present) (Cadot and Kaesler, 1977; Coles et al., 1994; Cronin et al., 1996; Dwyer et al., 1995, 2002; Elmore et al., 2012). Core-top calibration studies of Mg/Ca in *Krithe* versus calcification temperature document both linear and exponential relationships (Dwyer et al., 2002; Elmore et al., 2012; Farmer et al., 2012), and *Krithe* Mg/Ca has been applied downcore in numerous orbital and millennial scale BWT reconstructions in the North Atlantic and Arctic Oceans (e.g. Dwyer et al., 1995; Dwyer et al., 2000; Cronin et al., 2012). While temperature is thought to be the primary driver of Mg incorporation in *Krithe*, the influence of seawater carbonate ion saturation (ΔCO_3^{2-}) on *Krithe* Mg/Ca is debated. Elmore et al. (2012) proposed a ΔCO_3^{2-} effect at low temperatures (<3 °C), while Farmer et al. (2012) concluded that ΔCO_3^{2-} state does not influence Mg uptake in *Krithe*.

1.1. Mg contaminants and cleaning procedures

Sedimentary contaminants, including clays, organic matter, and iron- and manganese-rich oxyhydroxide coatings (Mn–Fe oxyhydroxides), have the potential to bias elemental ratios in ostracod CaCO_3 . Yet, rigorous reductive-oxidative trace element cleaning protocols developed for foraminifera (Boyle, 1981; Boyle and Keigwin, 1985/1986; Lea and Boyle, 1991; Hastings et al., 1996) are not traditionally employed in marine ostracod-based paleotemperature studies because ostracod valves are not composed of enclosed chambers and thus, are thought to be less prone to contamination (Cronin et al., 2005). However, as in foraminifera, the morphological features of *Krithe* (e.g., lips, canals, and pores) may also trap clays and organic material, introducing the potential for contamination.

Marine clays (e.g., illite, kaolinite, montmorillonite, and chlorite) contain 1–10% Mg by weight (Riley and Chester, 1971; Barker et al., 2003). In foraminiferal-based trace element cleaning protocols, clays are removed using multiple methanol/deionised-water sonication steps (Boyle and Keigwin, 1985/1986; Barker et al., 2003; Lea et al., 2005). Four approaches have been used to remove clays in ostracod-based Mg/Ca studies; (i) rinsing with ethanol using a fine-haired brush (Chivas et al., 1983); (ii) sonication in ethanol (Corrège, 1993); (iii) sonication in deionised water (Dwyer et al., 1995; Farmer et al., 2012); and (iv) sonication in deionised water and methanol (Elmore et al., 2012). Incomplete removal of clays has the potential to introduce significant errors in analyses of deep-sea benthic ostracods, where BWTs are cold and Mg/Ca ratios are typically low. For example, the presence of 0.05 μg of clay (~10% Mg w/w) could increase the Mg/Ca-derived temperature of a typical adult *Krithe* valve (average CaCO_3 weight: ~20 μg ; average Mg/Ca of ~12 mmol/mol) by ~1 °C, which is significant given the low range in BWTs typical of deep-sea locations and the *Krithe* paleotemperature calibration error of ± 0.3 °C (Elmore et al., 2012).

Organic matter may also provide a source of Mg, from both the organic matter itself, and from adhering clay particles (Hastings et al., 1996; Barker et al., 2003). The chitin matrix of ostracod valves, in which the calcite is embedded, also contains significant levels of Mg (Chivas et al., 1983). To oxidise organic matter, foraminiferal trace element cleaning protocols include an alkali-buffered hydrogen peroxide step (Boyle, 1981; Boyle and Keigwin, 1985/1986). Marine ostracod studies have oxidised organic matter by; (i) a multi-day soak of bulk sediment in 3% hydrogen peroxide (Corrège, 1993); (ii) soaking valves overnight in a 5% NaClO solution (Dwyer et al., 1995; Farmer et al., 2012), which has been shown to have no effect on the Mg/Ca ratio of inorganic spar calcite (Dwyer et al., 2002), and effectively removes organic matter without causing CaCO_3 dissolution (Gaffey and Bronnimann, 1993); and (iii) application of the alkali-buffered hydrogen peroxide

step (Elmore et al., 2012), originally developed for foraminifera (Boyle, 1981; Boyle and Keigwin, 1985/1986).

Fe–Mn oxyhydroxide coatings form on marine biogenic calcite under suboxic sedimentary conditions below the Mn redox boundary or when Mn^{2+} is remobilised in sediments (Lynn and Bonatti, 1965; Froelich et al., 1979). Boyle (1981, 1983) concluded that Fe–Mn oxyhydroxide coatings were a significant source of trace element contamination in foraminiferal CaCO_3 when Mn/Ca ratios were >0.1 mmol/mol. Thus, the standard foraminiferal trace element cleaning protocol employs a reductive (hydrazine) treatment step to remove Fe–Mn oxyhydroxide coatings from foraminifera (Boyle and Keigwin, 1985/86).

While the reductive step has been questioned for foraminiferal Mg/Ca studies due to the partial dissolution of CaCO_3 (Barker et al., 2003, 2005; Vetter et al., 2013), laser ablation of individual foraminifera has revealed both MnCO_3 coatings with Mg/Ca ratios up to 50 mmol/mol (Pena et al., 2005), and regions of high Mn, Fe and Mg on the calcite surface (thought to be Fe–Mn oxyhydroxides) (Pena et al., 2008). Moreover, a flow-through method developed to sequentially leach contaminant phases before dissolving foraminiferal calcite demonstrated the presence of Mn-oxide coatings containing high levels of Mg (Haley and Klinkhammer, 2002; Benway et al., 2003), further highlighting the importance of reductive cleaning in foraminiferal Mg/Ca studies. With the exception of one recent study of *Krithe* Mg/Ca (Elmore et al., 2012), the reductive cleaning step is not routinely employed to remove Fe–Mn oxyhydroxide coatings in ostracod Mg/Ca studies because the higher concentrations of Mg in ostracods compared with foraminifera suggests that the Mg associated with Fe–Mn oxyhydroxide coatings may not affect ostracod Mg/Ca as profoundly as in foraminiferal studies (Elmore et al., 2012). This hypothesis is supported for lacustrine ostracods (Jin et al., 2006) but has not been adequately assessed in the marine environment (Elmore et al., 2012), where the sedimentary redox conditions under which Fe–Mn oxyhydroxide coatings form are widespread.

Reconstructions of BWTs using the Mg/Ca of ostracod valves are typically based on multiple, single-shell trace element determinations at each individual stratigraphic horizon. Not unexpectedly, inter-valve variability at each horizon is pronounced (Dwyer et al., 2002). Along with physiological, environmental and taphonomic factors, contamination resulting from incomplete or improper removal of Mg-rich sedimentary contaminants may cause the observed variability (Holmes, 2008). The existing global *Krithe* Mg/Ca-temperature calibration comprises datasets cleaned by three different cleaning protocols, as outlined above. To improve the utility of the *Krithe* Mg/Ca paleothermometer a systematic study of the sequential cleaning steps on ostracod calcite is required.

The most common Mg-bearing marine clay minerals also contain Al and Fe, and Fe–Mn oxyhydroxide coatings comprise Fe and Mn. Al/Ca, Fe/Ca and Mn/Ca ratios are routinely measured in foraminiferal trace element studies as indicators of clay and Fe–Mn oxyhydroxide contamination (Boyle, 1981, 1983; Barker et al., 2003). In this study we apply foraminiferal cleaning procedures to individual *Krithe* valves, and use Al/Ca, Fe/Ca and Mn/Ca ratios to monitor contamination from clays and Fe–Mn oxyhydroxide coatings in order to (1) evaluate the effects of contamination as a bias on Mg/Ca ratios in *Krithe*, and (2) assess the efficacy of foraminiferal trace-element cleaning procedures on the removal of contamination, and the effects of these procedures on valve chemistry.

2. Study location and methodology

2.1. Site selection and sample preparation

This study is based on core top (0–2 cm) sediments from a box core (OCE205-50BC; 26.23°N, 77.7°W) raised from 817 m of water, west of Great Abaco, Bahamas. The site has a BWT of 8.4 °C (Slowey and Curry, 1995), and bottom-waters have a dissolved oxygen content of ~150 $\mu\text{mol/kg}$ (Key et al., 2004). The core was chosen to give bottom-

water temperature and redox conditions representative of deep-sea paleoceanographic studies, while ensuring sufficient numbers of well-preserved *Krithe* were present for the study. Benthic foraminifera in the coretop have $\delta^{18}\text{O}$ values expected from the measured seawater $\delta^{18}\text{O}$ value and BWT at the site, indicating the sediment is of late-Holocene age (Slowey and Curry, 1995). Furthermore, the presence of complete *Krithe* carapaces and other ostracod species with remaining setae suggests that the ostracods were living close to the time of collection.

Sediments were washed through a 63 μm sieve and the residues were oven dried. To eliminate potential Mg-variability associated with species and gender, only male adult and A-1 juvenile (the final molt stage before adult) *Krithe pernoidea* valves were selected for analysis (Coles et al., 1994) (Fig. 1). Valve length and height were measured using a stage micrometer and valves were classified as adult or A-1 juvenile according to the criteria of McKenzie et al. (1989). Valve preservation was assessed via the visual preservation index (VPI) of Dwyer et al. (1995), in which a value of one (1) indicates a translucent carapace and seven (7) indicates an opaque carapace. To minimise potential dissolution effects, only valves with a VPI between one and three were selected for our Mg cleaning tests.

Cleaning experiments were carried out on individual ostracod valves ($n = 43$). Our preliminary cleaning tests indicated that the full trace metal cleaning procedure of Boyle and Keigwin (1985/86) (excluding the final acid leach) resulted in excessive sample loss in delicate *Krithe* valves. Therefore, we applied the Boyle and Keigwin (1985/86) methodology with modifications outlined in Appendix A, which included reducing the number of sonication steps and decreasing the volume of, and exposure time to the oxidising and reducing reagents. When applied, the reductive step preceded the oxidative step following Martin and Lea (2002). In order to assess the impact of each cleaning step on valve chemistry, between 8 and 16 valves were removed from the cleaning procedure after each cleaning step and the individual valves were analysed by ICP-OES for a suite of major and trace elements.

2.2. ICP-OES analysis

For analysis by ICP-OES, single ostracod valves were dissolved in 350 μl of 0.1 M HNO_3 (Optima Grade) in an acid-leached (48 h in 80 $^\circ\text{C}$ 2.2 M HNO_3) Molecule BioProducts 0.6 ml microcentrifuge tube and centrifuged at 5000 rpm for 5 min. After centrifuging, 300 μl of supernatant was transferred to an acid-leached 0.6 ml microcentrifuge tube for analysis. The Mg/Ca, Sr/Ca, Al/Ca, Fe/Ca and Mn/Ca ratios of acidified samples were analysed at University College London (UCL) by Varian 720 ES ICP-OES using the intensity ratio calibration method of de Villiers et al. (2002). Trace metal/Ca ratios were corrected for matrix effects relating to differences in Ca concentration using matrix-matched standards; all Mg/Ca matrix corrections were less than 1 mmol/mol (7%), with the majority of corrections <0.3 mmol/mol (2%). Analysis of carbonate standard PE3 during the run gave a Mg/Ca

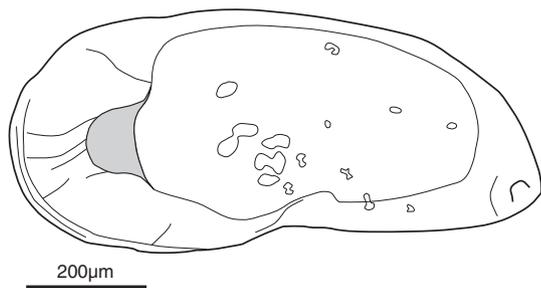


Fig. 1. External view of an adult male *Krithe pernoidea* valve from OCE205-50BC drawn under light microscope. *Krithe pernoidea* can be identified by the elongated valve shape, and 'pocket' shaped vestibule (shown in grey). See Coles et al. (1994) for further details.

value of 8.51 ± 0.06 mmol/mol ($n = 7$), in good agreement with the value of 8.50 ± 0.04 mmol/mol reported by Dwyer et al. (1995). Over the 6 month study interval, Mg/Ca reproducibility of the BSC-CRM 393 calcite standard on UCL's Varian ICP-OES was 3.74 ± 0.05 mmol/mol ($<1.4\%$), based on 92 determinations. The detection limit, calculated as $3 \times$ standard deviation of the blank (Harris, 2002), at average ostracod Ca concentrations after acidification is 0.01 mmol/mol (adults) and 0.04 mmol/mol (A-1 juveniles) for Fe, 0.14 and 0.55 mmol/mol for Al, and <0.01 and 0.01 mmol/mol for Mn. Analytical precision ($\pm 1\sigma$) for each element/Ca at typical concentrations is displayed in Fig. 2. The full dataset is presented in Table 1.

2.3. Statistical analyses

A Student's *t*-test was employed to evaluate the significance of differences in mean Mg/Ca ratios of valves cleaned with each subsequent cleaning treatment, and between adult and A-1 valves. All *t*-tests were one-tailed, and either a homoscedastic (equal-variance) or heteroscedastic (unequal-variance) *t*-test was chosen depending on the result of a preceding analysis of variance (ANOVA) F-test.

3. Results and discussion

3.1. Contamination indicators

Al and Fe concentrations are below detection limit in most samples (Fig. 2), and the proportion of samples with Al, Fe and Mn concentrations below detection generally increases with each subsequent cleaning step. Several valves have very high Al/Ca ratios, of up to 12.9 mmol/mol. As Al is more difficult to measure using ICP-OES and more abundant in contaminant phases than Mg (Kaolinite comprises $\sim 20\%$ Al (w/w) and no Mg; Riley and Chester, 1971), high Al/Ca values are not necessarily indicative of Mg-bearing contaminants (Elderfield et al., 2010). Fe/Ca is below 0.3 mmol/mol in all samples and Mn/Ca is below 0.4 mmol/mol in all but one outlying sample. Elevated Al/Ca, Fe/Ca and Mn/Ca values are generally not associated with higher Mg/Ca values, and no relationship is observed between Mg/Ca and Al/Ca, Fe/Ca or Mn/Ca (Fig. 3). These results suggest that levels of contamination in *Krithe* valves are generally low, supporting the assumption that the morphology of ostracod valves makes them less prone to contamination than foraminifera (Cronin et al., 2005). However, the general decrease in the proportion of samples with detectable Al, Fe and Mn concentrations with each subsequent cleaning step indicates that clay particles and Fe–Mn oxyhydroxides are being removed during the cleaning procedure.

3.2. Effects of cleaning

Following sonication, the mean Mg/Ca ratio decreases significantly from 14.8 ± 1.8 to 13.2 ± 1.9 mmol/mol (11%) ($p = <0.01$), however inter-valve variability remains high, with a relative standard deviation (RSD) of 14% (Fig. 2a). The significant decrease in Mg/Ca following sonication suggests the removal of Mg-bearing clays. Mean Mg/Ca ratios and inter-valve Mg/Ca variability show no significant differences following oxidation with buffered hydrogen peroxide, indicating that oxidative cleaning has little effect on Mg-contaminant removal or valve chemistry at the site. Rigorous testing across a wide range of bottom water oxygen, productivity, and water depth conditions is required to further assess the effects of oxidative cleaning on *Krithe* Mg/Ca.

Excluding the outlying valve with anomalously high Mn/Ca (>1 mmol/mol), there is a significant relationship between samples with above-detection Mn/Ca and Fe/Ca (including one reductively cleaned valve with a detectable Fe/Ca ratio of 0.08 mmol/mol) in a $\sim 1:1$ molar relationship suggesting a detectable presence of Fe–Mn oxyhydroxides in the samples (Fig. 3d). Under scanning electron microscope (SEM), secondary overgrowths are visible on the calcite surface of ostracods that have not been reductively cleaned (Fig. 4a). After

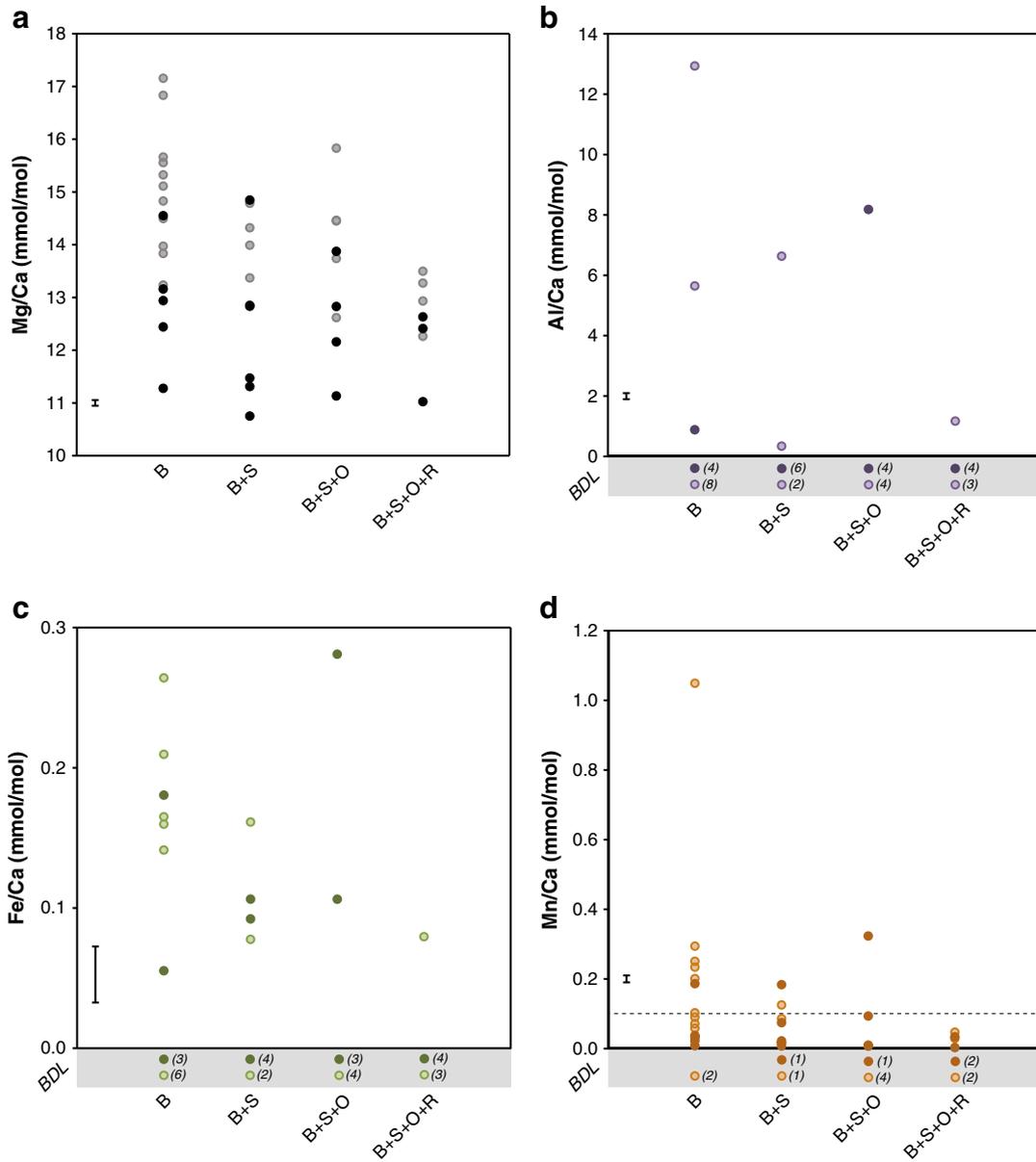


Fig. 2. Effect of foraminiferal trace element cleaning procedures on *Kriethe* (a) Mg/Ca (b) Al/Ca (c) Fe/Ca and (d) Mn/Ca (The dashed line indicates Mn/Ca ratios of 0.1 mmol/mol, the value above which Boyle (1981, 1983) concluded that foraminiferal calcite contained significant Fe–Mn oxyhydroxide contamination). A–1 juveniles are shown by the light symbols and adults by the dark symbols. The number of valves with Al, Fe, and Mn concentrations below detection limit (BDL) is given in parentheses within the grey shaded area of the graphs for each cleaning step. Any data points appearing in the un-shaded part of the graph have trace element concentrations above detection. The cleaning steps are: *B* brush with ethanol, *S* sonication in methanol, *O* oxidation with hydrogen peroxide, *R* reduction with hydrous hydrazine/ammonium citrate. A full description of the procedure is given in Appendix A. Analytical precision ($\pm 1\sigma$) for each element/Ca at typical concentrations is shown by the black error bar.

reductive cleaning, all Mn/Ca values fall below 0.1 mmol/mol (Fig. 2d), mean Mg/Ca values show a statistically significant decrease from 13.6 ± 1.6 to 12.7 ± 0.6 mmol/mol ($p < 0.05$), and inter-valve variability also decreases substantially, with a reduction in RSD from 12% to 5% (Fig. 2a). The decrease in Mn/Ca values and the reduction in Mg/Ca variability suggest that Mg-bearing Fe–Mn oxyhydroxide overgrowths are removed during reductive cleaning. This decrease in inter-valve variability delimits contamination from Fe–Mn oxyhydroxide overgrowths as one of the potential causes of Mg/Ca variability observed between *Kriethe* valves within the same stratigraphic interval (Dwyer et al., 2002). Foraminiferal cleaning studies have also demonstrated a reduction in Mg/Ca values and an improvement in intra-specimen variability following reductive cleaning (Martin and Lea, 2002; Barker et al., 2003; Yu et al., 2007).

Examination of the ostracod calcite surface under SEM also reveals that the reductive cleaning process partially removes the outer calcite layer (Fig. 4b and c). LA-ICP-MS studies have demonstrated that the outer 2 μm layer of calcite in adult *Kriethe* is enriched in Mg by ~ 8 mmol/mol, and the complete removal of this layer results in a net Mg/Ca reduction of ~ 1.2 mmol/mol (Ito et al., 2003). Our observed 0.9 mmol/mol decrease in Mg/Ca following reductive cleaning is therefore likely caused both by the removal of high-Mg overgrowths, and the partial removal of the Mg-enriched outer calcite layer. While the outer-layer of calcite is also enriched in Mn by ~ 0.02 mmol/mol and Fe by ~ 0.25 mmol/mol (as well as many other trace elements), the mass of Mn in the outer 2 μm of calcite is insufficient to account for the high Mn/Ca ratios observed before reductive cleaning. It is unlikely that the trace element-enriched outer layer of calcite is due to secondary

Table 1
Trace metal/Ca values of adult and A-1 juvenile male *Krithe pernoides* valves analysed in this study. BDL = below detection level. The cleaning steps are: B brush with ethanol, S sonication in methanol, O oxidation with hydrogen peroxide, R reduction with hydrous hydrazine/ammonium citrate. A full description of the procedure is given in Appendix A.

Cleaning method	Molt stage	Fe/Ca (mmol/mol)	Al/Ca (mmol/mol)	Mn/Ca (mmol/mol)	Sr/Ca (mmol/mol)	Mg/Ca (mmol/mol)
B	Adult	BDL	BDL	0.03	3.54	11.65
B	Adult	0.06	BDL	0.04	3.57	13.05
B	Adult	BDL	BDL	0.01	3.49	12.89
B	Adult	BDL	BDL	0.02	3.52	13.15
B	Adult	0.18	0.88	0.19	3.71	14.69
B	A-1	BDL	BDL	0.07	3.90	15.64
B	A-1	0.17	BDL	0.20	3.77	16.17
B	A-1	BDL	BDL	0.10	3.73	13.58
B	A-1	0.16	BDL	0.23	3.67	15.03
B	A-1	BDL	BDL	0.06	3.48	13.65
B	A-1	BDL	BDL	BDL	3.79	16.40
B	A-1	0.14	BDL	0.25	3.51	13.89
B	A-1	0.26	BDL	0.29	3.61	14.67
B	A-1	BDL	5.65	BDL	3.38	18.21
B	A-1	0.21	12.94	1.05	3.56	17.05
B	A-1	BDL	BDL	0.09	3.46	17.24
B + S	Adult	BDL	BDL	0.01	3.63	11.09
B + S	Adult	0.11	BDL	0.18	3.58	11.65
B + S	Adult	0.09	BDL	0.07	3.70	12.86
B + S	Adult	BDL	BDL	0.02	3.46	13.15
B + S	Adult	BDL	BDL	0.02	3.63	11.52
B + S	Adult	BDL	BDL	BDL	3.63	15.00
B + S	A-1	BDL	BDL	0.02	3.74	13.36
B + S	A-1	0.08	6.63	0.13	3.67	14.82
B + S	A-1	0.16	0.34	0.09	3.43	14.33
B + S	A-1	BDL	BDL	BDL	3.50	13.99
B + S + O	Adult	BDL	BDL	0.01	3.78	13.09
B + S + O	Adult	0.28	8.18	0.32	3.59	12.23
B + S + O	Adult	0.11	BDL	0.09	3.62	14.00
B + S + O	Adult	BDL	BDL	0.01	3.63	11.60
B + S + O	Adult	BDL	BDL	BDL	3.37	15.94
B + S + O	A-1	BDL	BDL	BDL	3.75	14.48
B + S + O	A-1	BDL	BDL	BDL	3.67	14.60
B + S + O	A-1	BDL	BDL	BDL	3.39	12.62
B + S + O	A-1	BDL	BDL	BDL	3.69	13.74
B + S + O + R	Adult	BDL	BDL	BDL	3.57	12.79
B + S + O + R	Adult	BDL	BDL	0.00	3.56	11.50
B + S + O + R	Adult	BDL	BDL	0.03	3.41	12.66
B + S + O + R	Adult	BDL	BDL	BDL	3.74	12.21
B + S + O + R	A-1	0.08	BDL	0.05	3.37	13.31
B + S + O + R	A-1	BDL	BDL	BDL	3.68	12.93
B + S + O + R	A-1	BDL	BDL	BDL	3.63	13.50
B + S + O + R	A-1	BDL	1.17	0.03	3.31	12.34

overgrowths as trace-element enrichment is also observed in the outer calcite layer of laboratory-cultured ostracods belonging to the same superfamily as *Krithe* (Ito et al., 2003).

3.3. Differential effects of cleaning on adult and A-1 juvenile valves

Adult and A-1 *Krithe* valves are often used interchangeably in Mg/Ca paleotemperature reconstructions because there is no significant difference in Mg/Ca ratios between these life stages (Dwyer et al., 2002). However, by assessing adult and A-1 valves separately, clear differences in the effects of cleaning on valves from the two molt stages emerge (Fig. 2a). The mean Mg/Ca ratio of brush cleaned A-1 juvenile valves is 2.5 mmol/mol higher than that of brush cleaned adult valves ($p = <0.01$). A-1 juveniles show a significant ($p = <0.05$) 1.5 mmol/mol (9%) decrease in Mg/Ca following sonication, whereas adult valves show no significant change ($p = 0.27$). It is only after oxidative cleaning that the Mg/Ca ratio of A-1 juvenile and adult valves becomes statistically indistinguishable. The greater Mg/Ca decrease in A-1 juvenile valves compared to adult valves following sonication indicates a greater influence of clay contamination in the former. Contamination would be expected to play a greater role in A-1 juveniles owing to their lower mass. In this study average adult valves weighed ~20 µg, while A-1 juveniles weighed just ~5 µg; any contaminating Mg in A-1 juvenile

valves therefore represents a much greater proportion of the Mg contained within the ostracod calcite (Fig. 5).

We acknowledge that by assessing adult and A-1 valves separately, the sample sizes become relatively small (the low abundance of well-preserved adult and A-1 juvenile single species *Krithe* valves limits our ability to further increase sample sizes). However the differences in the data on which we base our conclusion remain statistically robust. Our results confirm that adult and A-1 valves can be used interchangeably, but only after effective clay removal.

3.4. Implications for paleotemperature reconstructions

We convert *Krithe* Mg/Ca values to equivalent temperatures using the calibrations of Elmore et al. (2012) for BWTs >3 °C,

$$\text{Mg/Ca (mmol/mol)} = 0.97 * \text{BWT} + 7.95. \quad (1)$$

Farmer et al. (2012) for the North Atlantic,

$$\text{Mg/Ca (mmol/mol)} = 1.13 * \text{BWT} + 6.43 \quad (2)$$

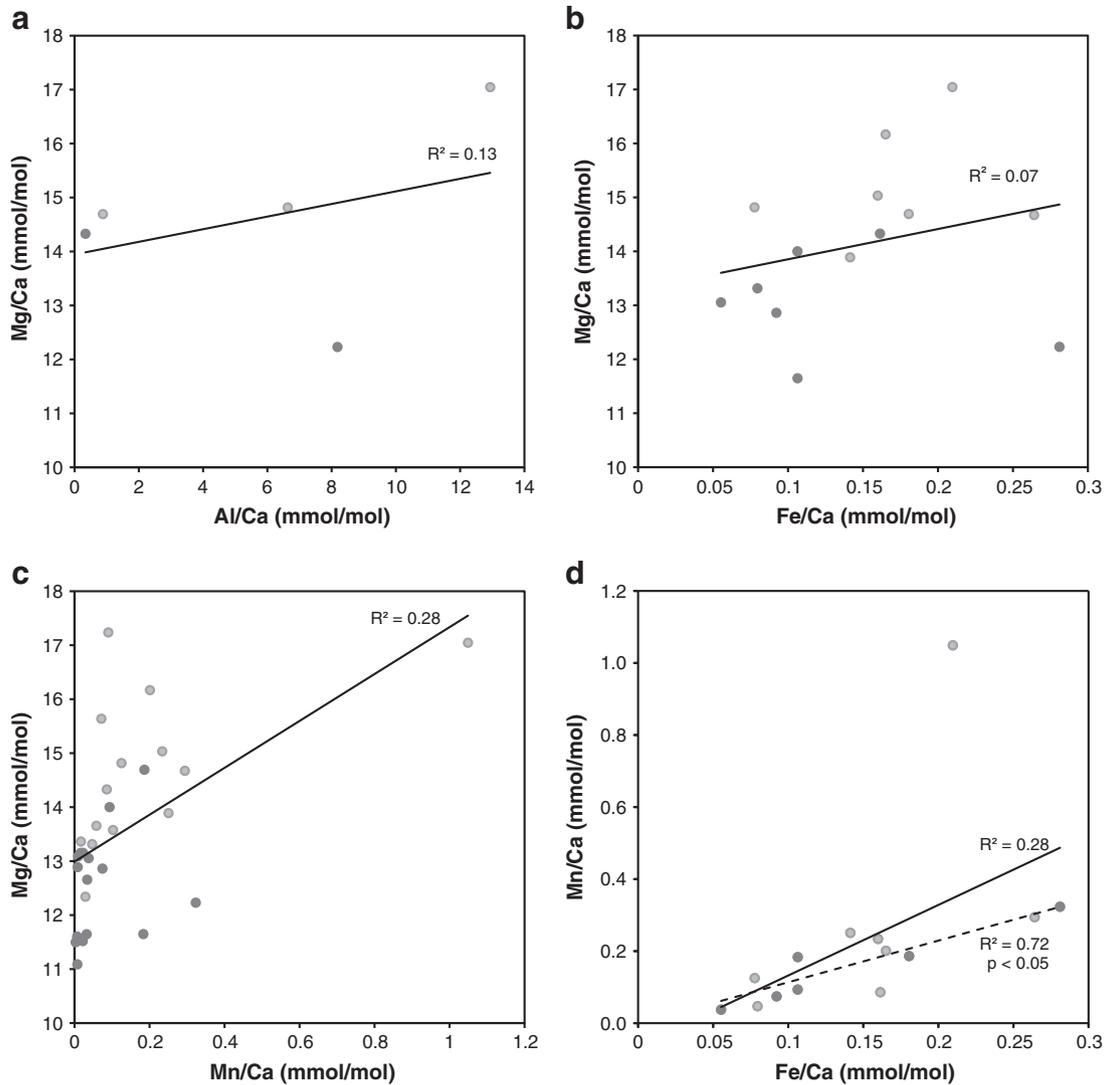


Fig. 3. (a) Regression of above-detection Al/Ca against Mg/Ca (b) above-detection Fe/Ca against Mg/Ca and (c) above-detection Mn/Ca against Mg/Ca. Mg/Ca is not significantly correlated to any of the contamination indicators. (d) Regression of above-detection Mn/Ca against Fe/Ca, including one reductively cleaned valve with a detectable Fe/Ca ratio of 0.08 mmol/mol. If the outlying valve with high (>1 mmol/mol) Mn/Ca is excluded (dashed line), the relationship between Mn/Ca and Fe/Ca is significant to 95%, indicating a detectable presence of Fe–Mn oxyhydroxides.

and the species-specific calibration of Farmer et al. (2012) for *K. pernoides*,

$$\text{Mg/Ca (mmol/mol)} = 1.12 * \text{BWT} + 7.06 \quad (3)$$

where BWT is in °C. Uncertainty in reconstructed BWT is given as the 1σ Mg/Ca inter-valve variability divided by the slope of the calibration equation (Eqs. (1)–(3)). The three paleotemperature equations show good agreement with each other, and reconstructed BWTs range between 7.4 ± 1.6 and 4.8 ± 0.7 °C (Fig. 6a). The reconstructed temperature of brush cleaned valves is between 1.0 ± 1.6 and 1.5 ± 1.6 °C below the measured BWT at our site. After sonication reconstructed temperatures fall to between 2.4 ± 1.6 and 3.0 ± 1.9 °C below the measured BWT, and the equivalent temperature of reductively cleaned valves is between 2.9 ± 0.6 and 3.6 ± 0.7 °C below measured BWT. Taking the mean reconstructed temperature from the three calibrations, the decrease in Mg/Ca following sonication is equivalent to a temperature decrease of 1.5 °C, and the decrease in Mg/Ca following reductive cleaning is equivalent to a temperature decrease of 0.9 °C. These apparent temperature decreases are significant given the calibration error of ± 0.3 °C for Elmore et al. (2012), and ± 0.7 °C for the North

Atlantic calibration of Farmer et al. (2012) (based on 10 replicate analyses).

Krithe valves used in the calibration of Farmer et al. (2012) were cleaned by sonication in deionised water and oxidation with 5% NaClO. While Elmore et al. (2012) used the reductive cleaning procedure of Rosenthal et al. (1999) (modified after Boyle and Keigwin, 1985/86), the data for reductively cleaned valves were combined with several previously-published datasets for non-reductively cleaned valves. As such, only 10% of the material comprising the Elmore et al. (2012) >3 °C calibration dataset was reductively cleaned, with the majority of the valves making up the dataset being cleaned by sonication in deionised water and oxidation with 5% NaClO.

Using the temperature calibrations of Elmore et al. (2012) and Farmer et al. (2012) reconstructed temperatures for brush cleaned valves are within error of measured BWT at the site. The data from valves cleaned by sonication should provide the most direct comparison to the calibrations of Elmore et al. (2012) and Farmer et al. (2012); however a large offset is observed between reconstructed and measured BWT for this dataset (Fig. 6a). We suggest that this large offset is the result of increased clay removal by sonication in methanol compared to sonication in deionised water only. Jin et al. (2006) demonstrated a 0.5–1 mmol/mol decrease in Mg/Ca following sonication in methanol,

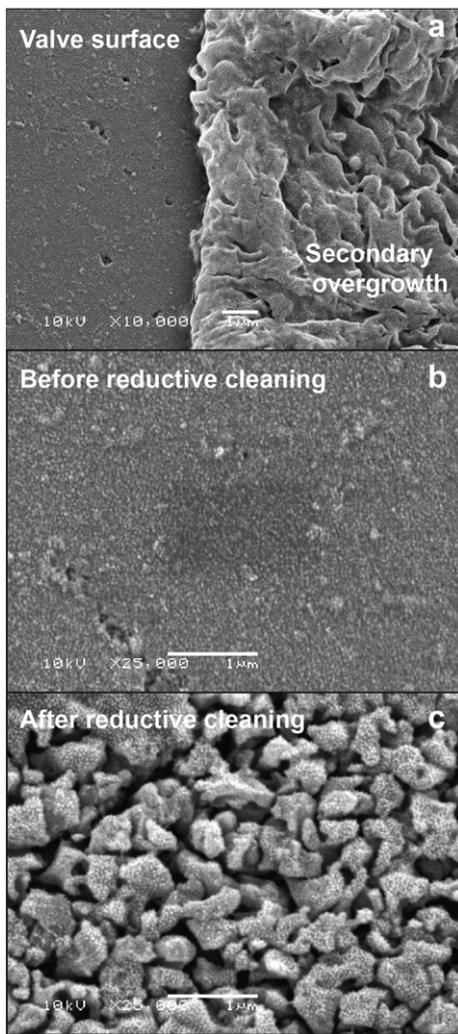


Fig. 4. SEM images of the calcite surface showing (a) an overgrowth on a valve that has not been reductively cleaned, (b) the surface calcite structure before reductive cleaning and (c) the surface calcite structure after reductive cleaning. Partial dissolution of the valve surface is clearly visible after reductive cleaning. While not from the same carapace, valves chosen for SEM imaging had similarly excellent preservation (VPI = 1) before any cleaning procedures took place. Scale bar indicates 1 μm .

even after multiple sonication steps in ultrapure water. The lower viscosity of methanol may more readily dislodge clay particles adhering to the valve surface, making it a more effective clay-removal agent. An alternative explanation is that methanol leaches Mg from ostracod calcite, and cleaning experiments performed on valves from cultured ostracods would provide a good test of any direct geochemical effects of methanol on ostracod calcite.

The large decrease in reconstructed temperature following sonication in methanol/ultrapure water highlights the necessity of effective clay removal in order to avoid significantly biasing paleotemperature reconstructions. This result suggests that previous *Krithe*-Mg/Ca paleotemperature reconstruction studies that have not utilised methanol could potentially be biased to warmer temperatures, particularly in ocean basins (or periods of the geological past) where clay input, or the Mg-content of clay, is elevated. Future Mg/Ca determinations of *Krithe* should include a methanol sonication step as outlined in Appendix A.

Using the temperature calibrations of Elmore et al. (2012) and Farmer et al. (2012), the reconstructed BWT from reductively cleaned valves is significantly below measured BWT at the site (Fig. 6a). However, the Mg/Ca from reductively cleaned valves in the Elmore et al. (2012) calibration is systematically lower than that for non-reductively cleaned

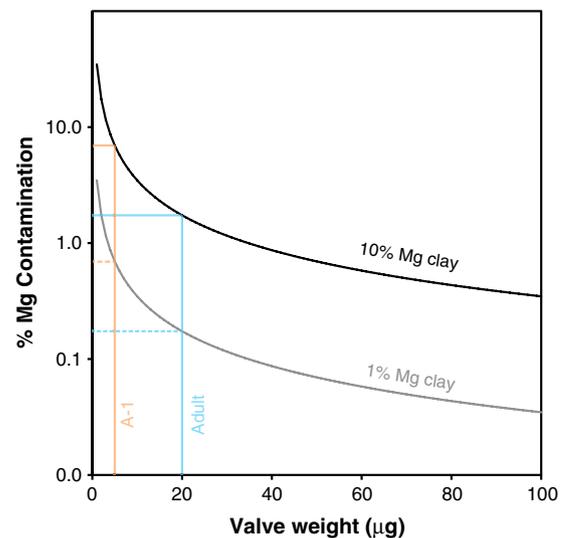


Fig. 5. The effect of 0.01 μg of clay (containing 1% Mg and 10% Mg by weight) as a percentage of the total Mg in a *Krithe* valve with a Mg/Ca of 12 mmol/mol across a range of valve weights. The average weight of adult (20 μg) and A-1 juvenile (5 μg) valves in this study is indicated.

valves. Regressing only the data for reductively cleaned valves from Elmore et al. (2012) for sites with BWT > 3 $^{\circ}\text{C}$, including the mean Mg/Ca value for reductively cleaned valves from this study, against BWT gives the relationship,

$$\text{Mg/Ca (mmol/mol)} = 1.00 * \text{BWT} + 4.02 \left(R^2 = 0.70, p = <0.0001, n = 17 \right) \quad (4)$$

with a standard error equivalent to ± 1.81 $^{\circ}\text{C}$ (Fig. 6b). Calibrating the mean Mg/Ca value for reductively cleaned valves from this study with Eq. (4) gives a reconstructed BWT of 8.64 ± 0.64 $^{\circ}\text{C}$, in good agreement with measured BWT at the site (Fig. 6a). It is important to note that removing the data point generated in this study from Eq. (4) results in no significant difference to the calibration equation ($\text{Mg/Ca (mmol/mol)} = 0.99 * \text{BWT} + 4.04$, $R^2 = 0.68$, $p = <0.0001$), or reconstructed BWT (8.68 ± 0.65 $^{\circ}\text{C}$).

The slope of the calibration in Eq. (4) above is very similar to the > 3 $^{\circ}\text{C}$ calibration of Elmore et al. (2012) (Eq. (1)) and the North Atlantic calibration of Farmer et al. (2012) (Eq. (2)), although the intercept is 3.93 mmol/mol lower than in Eq. (1) and 2.40 mmol/mol lower than in Eq. (2) (Fig. 6b). The similarity in slope, but reduction in intercept suggests that the calibrations based on non-reductively cleaned valves are systematically biased to higher Mg/Ca values due to the influence of clay and Fe–Mn oxyhydroxide contamination. Although some fraction of the difference in intercept is due to partial dissolution of the Mg-enriched valve surface, it is unlikely to account for more than ~ 1.2 mmol/mol, the resulting decrease following total removal of the Mg-enriched layer (Ito et al., 2003). As the difference between the calibrations is the intercept rather than the slope, previous BWT reconstructions based on valves that were not sonicated in methanol or reductively cleaned will be robust if clay/Mn–Fe oxyhydroxide contamination has remained temporally consistent; this assumption could be tested by reanalysing valves from the cores on which the published BWT records are based, however following the procedures outlined in this paper. Due to the relatively small dataset available, the calibration for reductively cleaned valves at sites with BWT > 3 $^{\circ}\text{C}$ presented here is tentative, and further coretop data from reductively cleaned *Krithe* are needed to confirm the Mg/Ca–BWT relationship.

Elmore et al. (2012) ascribed anomalously high Mg/Ca values from sites with BWTs < 3 $^{\circ}\text{C}$ to the influence of ΔCO_3^{2-} below this temperature. Much of the data for reductively cleaned valves from < 3 $^{\circ}\text{C}$ also lies

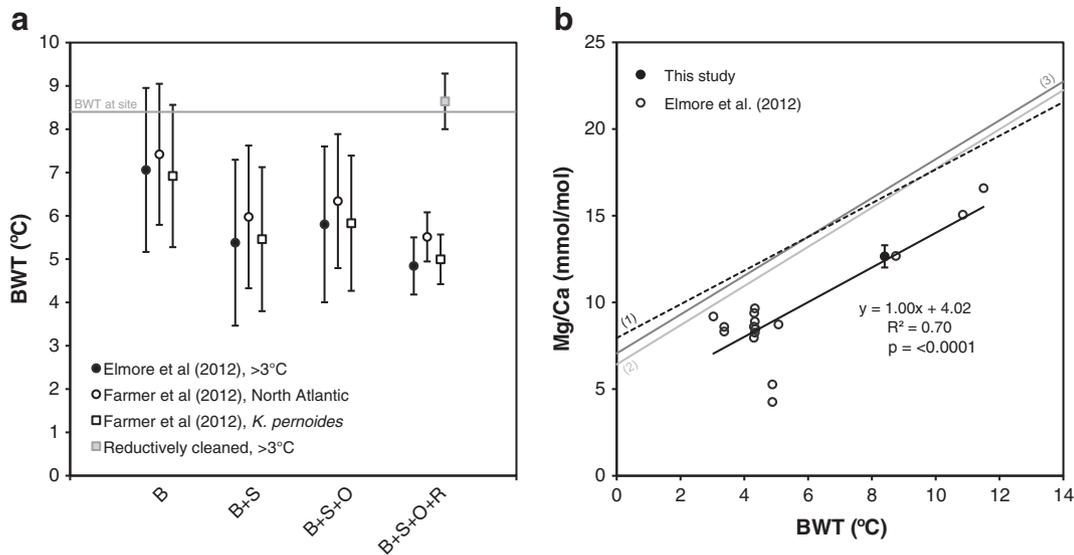


Fig. 6. (a) The effect of cleaning procedures on reconstructed BWT (see text for calibration details), error bars are $\pm 1\sigma$. The measured bottom-water temperature at the core site is indicated by the grey line. The cleaning steps are: B brush with ethanol, S sonication in methanol, O oxidation with hydrogen peroxide, R reduction with hydrous hydrazine/ammonium citrate. A full description of the procedure is given in Appendix A (b) Mg/Ca-temperature calibration of reductively cleaned valves for sites with BWT >3 °C. The calibration lines of (1) Elmore et al. (2012) for sites with BWT >3 °C, (2) Farmer et al. (2012) for the North Atlantic and (3) Farmer et al. (2012) for *Krithe pernoides* are also shown.

above the calibration line for reductively cleaned valves from sites >3 °C. This suggests that it is not an artefact of contamination, and thus likely results from a secondary influence on Mg uptake into ostracod calcite, such as ΔCO_3^{2-} as proposed by Elmore et al. (2012).

The reduction in inter-valve Mg/Ca variability following reductive cleaning represents a large increase in equivalent BWT precision, with reduction in error from ± 1.7 °C to ± 0.6 °C. Reductive cleaning therefore has the potential to improve *Krithe* Mg/Ca BWT reconstruction precision by >1 °C. While bottom waters bathing the core site are oxidic, the importance of removing Fe–Mn oxyhydroxide overgrowths will increase in regions (or during periods in the geological past) of higher productivity and/or reduced bottom water ventilation, where sedimentary redox conditions are more conducive to Fe–Mn oxyhydroxide formation. Given the prevalence of Fe–Mn oxyhydroxide formation on marine carbonates, the low range in BWTs expected at deep-sea locations, and the potential of reductive cleaning to improve *Krithe* Mg/Ca BWT reconstructions, future *Krithe* Mg/Ca paleotemperature reconstructions should include a reductive cleaning step. However, further experiments to better constrain the effects of partial dissolution of the valve surface and determine the optimum exposure time to the reducing reagents should also be conducted.

4. Conclusions

- While our data suggest generally low levels of Al, Fe and Mn in *Krithe*, Mg bearing clays and Fe–Mn oxyhydroxide overgrowths are present, detectable, and exert a significant influence on the Mg/Ca of *Krithe* valves.
- Sonication in methanol/ultrapure water lowers Mg/Ca by 1.6 mmol/mol (11%), most likely due to the removal of clays. This Mg/Ca reduction is equivalent to a decrease in reconstructed temperature of 1.5 °C, emphasising the importance of sonication as a critical step in the preparation of *Krithe* valves for Mg/Ca analysis.
- Clay contamination exerts a greater influence on the Mg/Ca of A-1 juvenile valves compared to adult valves, due to the lower mass of the former.
- Oxidation with buffered hydrogen peroxide does not influence Mg/Ca ratios in valves from our site. Future work should assess the

effects of contamination from organic matter and oxidative cleaning across different oceanographic and sedimentary settings.

- Reduction with hydrous hydrazine/ammonium citrate decreases Mg/Ca by 0.9 mmol/mol (7%), equivalent to a decrease in reconstructed temperature of 1 °C, due to both the removal of Fe–Mn oxyhydroxide overgrowths, and the partial dissolution of the valve surface.
- The lowest inter-valve Mg/Ca variability of ± 0.6 mmol/mol (5%) (± 0.6 °C) is achieved following reductive cleaning, indicating the high Mg/Ca variability previously observed in non-reductively cleaned *Krithe* valves is, in part, due to contamination. Our data demonstrate the potential of reductive cleaning to improve the precision of BWT reconstructions based on multiple, single-valve determinations of *Krithe* Mg/Ca per stratigraphic level. Future work should aim to better constrain the effects of partial dissolution of the valve surface and determine the optimum exposure time to the reducing reagents.
- Future *Krithe* Mg/Ca studies should employ the full cleaning procedure outlined in this study, namely sonication in methanol/ultrapure water, oxidation with alkali-buffered hydrogen peroxide and reduction with hydrous hydrazine/ammonium citrate. Further coretop data from *Krithe* that have been cleaned according to this protocol should be generated to improve the *Krithe* Mg/Ca-temperature calibration.

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Appendix A. Ostracod trace-element cleaning procedure

The cleaning procedure outlined below is a version of the foraminifera trace-element cleaning procedure developed by Boyle and Keigwin

(1985/86), modified to account for the lower weight and increased fragility of single ostracod valves, with the exception of step A1.1 which was developed for cleaning non-marine ostracod valves by Chivas et al. (1983).

A.1. Visible particulate removal (B)

Place valve on microscope slide and submerge in a few drops of trace metal grade ethanol. Remove all visible particulates using a soft hair paintbrush under a transmitted-light microscope. Unlike foraminifera, ostracods do not have closed chambers and it is not necessary to crush the valves during cleaning. Rinse with Milli-Q (or other ultra high quality) water and transfer to acid-washed 600 µl micro-centrifuge tubes.

A.2. Clay removal (S)

Add 500 µl of trace metal grade methanol into the micro-centrifuge tube. Tap micro-centrifuge tubes firmly to ensure ostracod valve settles to the bottom. Place in ultrasonic bath for 60 s (reduce to 30 s if valves are small/fragile). Using an acid leached pipette tip, re-circulate methanol within the micro-centrifuge tube to re-suspend clays before removing the methanol. Add 500 µl of Milli-Q water. Place in ultrasonic bath for 30 s. Remove Milli-Q water with an acid leached pipette. Rinse once with 500 µl of Milli-Q water. Increase number of methanol steps if sediments are clay-rich.

A.3. Fe–Mn oxyhydroxide coating removal (R)

Add 50 µl of hydrous hydrazine/ammonium citrate solution (150 µl of 20 M $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O} + 2$ ml of 15 M NH_4OH and 2 ml of $(\text{NH}_4)_2\text{C}_6\text{H}_8\text{O}_7$). The $(\text{NH}_4)_2\text{C}_6\text{H}_8\text{O}_7$ solution should be prepared prior to cleaning by adding 25 g of citric acid to 500 ml of 15 M ammonium hydroxide solution in an ice bath). Secure microcentrifuge caps tightly and place in hot water bath at 80 °C for 10 min, flipping once at 5 min. Remove micro-centrifuge tubes from the hot water bath and remove the hydrous hydrazine/ammonium citrate solution with an acid leached pipette tip. Rinse three times with 500 µl of Milli-Q water.

A.4. Organic matter removal (O)

Add 100 µl of buffered hydrogen peroxide (prepared with 10 ml of 0.1 M NaOH and 100 µl of 30% H_2O_2) to the micro-centrifuge tubes and secure caps tightly. Place in hot water bath at 90 °C for 5 min, flipping once at 2.5 min to ensure maximum contact between the sample and reagent. Remove micro-centrifuge tubes from the hot water bath and siphon off the buffered hydrogen peroxide with an acid leached pipette tip. Rinse three times with 500 µl of Milli-Q water.

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