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Effects of cleaning methods upon preservation of stable isotopes and trace elements in shells of *Cyprideis torosa* (Crustacea, Ostracoda): Implications for palaeoenvironmental reconstruction

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ABSTRACT

The trace element (Sr/Ca and Mg/Ca) and stable isotope ($\delta^{18}O$ and $\delta^{13}C$) geochemistry of fossil ostracod valves provide valuable information, particularly in lacustrine settings, on palaeo-water composition and palaeotemperature. The removal of sedimentary and organic contamination prior to geochemical analysis is essential to avoid bias of the results. Previous stable isotope and trace element work on ostracod shells has, however, employed different treatments for the removal of contamination beyond simple 'manual' cleaning using a paint brush and methanol under a low-power binocular microscope. For isotopic work pre-treatments include chemical oxidation, vacuum roasting and plasma ashing, and for trace element work sonication, chemical oxidation and reductive cleaning. The impact of different treatments on the geochemical composition of the valve calcite has not been evaluated in full, and a universal protocol has not been established. Here, a systematic investigation of the cleaning methods is undertaken using specimens of the ubiquitous euryhaline species, *Cyprideis torosa*. Cleaning methods are evaluated by undertaking paired analyses on a single carapace (comprising two valves); in modern ostracods, whose valves are assumed to be unaltered, the two valves should have identical geochemical and isotopic composition. Hence, when one valve is subjected to the chosen treatment and the other to simple manual cleaning any difference in composition can confidently be assigned to the treatment method. We show that certain cleaning methods have the potential to cause alteration to the geochemical signal, particularly Mg/Ca and $\delta^{18}O$, and hence have implications for palaeoenvironmental reconstructions. For trace–element determinations we recommend cleaning by sonication and for stable isotope analysis, oxidation by hydrogen peroxide. These methods remove contamination, yet do not significantly alter the geochemical signal.

1. Introduction

Ostracods are small (generally 0.5–3 mm long) bivalved crustaceans that occur in almost all aquatic environments. Their low Mg-calcite carapaces calcify using elements taken solely from the host water (Turpen and Angell, 1971), and are secreted within a short period providing a snapshot of conditions at the time of calcification. Often abundant and well preserved in Quaternary sediments, ostracods have become a popular proxy for palaeoenvironmental studies. The trace element (Sr/Ca and Mg/Ca) and stable isotope ($\delta^{18}O$ and $\delta^{13}C$) geochemistry of fossil ostracod valves (throughout the paper, the term 'valve' is used to discuss an individual valve (i.e. left or right), and the term 'shell' is used more generally when referring to a valve or carapace) provide valuable information, particularly in lacustrine settings, on palaeo-water composition and palaeotemperature. Typically, the Mg and Sr content of ostracod valves is positively correlated with Mg and Sr in lake water, which in some circumstances correlates with salinity and temperature (Chivas et al., 1985; De Deckker and Forester, 1988; Holmes and Chivas, 2002). The oxygen isotope ratio of the calcite is controlled by the temperature and oxygen isotopic

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composition of the water, along with any vital effects, which are species-specific for ostracod shells. Carbon isotope ratios predominantly reflect the isotope composition of the total dissolved inorganic carbon (TDIC) within the water.

An underlying assumption of the use of ostracod valve geochemistry in palaeoenvironmental reconstruction is that the original signal of the host water at the time of valve secretion is preserved in the ostracod calcite. However, the presence of adhering organic material or inorganic sediment may affect the measurements. It has therefore become common practice, especially prior to trace element analysis, to pre-treat valves in order to remove this potential contamination from both marine (e.g. Elmore et al., 2012; Gray et al., 2014) and non-marine (e.g. Leng et al., 1999; Hodell et al., 2005; Holmes et al., 2007; Li et al., 2007; Dixit et al., 2015) material.

For stable isotope analyses, pre–treatment aims to remove organic matter, fine-grained detrital or authigenic carbonate. Some organic material can fragment and have similar molecular weights to the carbon dioxide molecules that are measured by the mass spectrometer. For trace element analysis, Mg and Sr contamination is present in adhering organic matter (Ito, 2001), adhering clay particles (Hastings et al., 1996; Barker et al., 2003), and organic material from the chitin matrix of ostracod valves (Chivas et al., 1983; Ito, 2001). However, in contrast to foraminifera analyses, a consistent inter-laboratory protocol for sample preparation does not exist. Simple cleaning using a paint brush, needles, and methanol under a low-power binocular microscope (hereafter termed 'manual cleaning') is the least invasive, and most commonly used method. Generally, this method is efficient in removing visible contamination. However, Mg, Ba, Mn, Fe and U contamination can remain after manual cleaning (Börner et al., 2017), and in some sediment matrices or where species contain deep, difficult to clean canals, lips and pores, further pre-treatment may be required.

Previous studies involving isotopic and trace element analysis of ostracod shells have, therefore, employed different treatments for the removal of contamination. For stable isotopes, such treatments include chemical oxidation (using hydrogen peroxide or sodium hypochlorite) (Curtis and Hodell, 1993; Durazzi, 1977), vacuum roasting (Lister, 1988) and low-temperature 'ashing' in an oxygen plasma (Boomer, 1993) alongside manual cleaning. For trace element analysis, treatments include sonication (in ultra-pure deionised water and methanol), chemical oxidation (using hydrogen peroxide) and reductive cleaning (using a hydrous hydrazine/ammonium citrate solution) (Keatings et al., 2006; Gray et al., 2014). Börner et al. (2017) have recently demonstrated the potential of a sequential dissolution of flow-through time-resolved analysis (FT-TRA) as a means of distinguishing contaminant phases from uncontaminated shell calcite, although the impact of heterogeneity in trace-element distribution within the shell could not be assessed quantitatively using this approach and, moreover, appears to differ between taxa (Ito et al., 2003).

Some previous studies have suggested that the impact of different treatments may vary depending on species and sedimentary settings, yet this has not previously been fully evaluated or understood. Furthermore, the aggressive nature of some pre-treatments designed to remove contamination may cause alteration to the shell. Relatively few studies have focused on the effect on shell trace element chemistry, with the majority having only looked at the effect on isotopic composition. Durazzi (1977) was the first to examine the effect of pre-treatment on ostracod valves; he found that minimal exposure to sodium hypochlorite of up to 1 day to a 5% solution had little effect on both $\delta^{18}O$ and $\delta^{13}C$, but exposure for five days had the potential to decrease $\delta^{13}C$ values up to −0.42‰. Frogley (1997) suggested that both hydrogen peroxide and vacuum roasting have the potential to decrease $\delta^{18}O$ and $\delta^{13}C$. Hydrogen peroxide decreased the isotopic signal by 0.6‰ and 0.12‰ respectively, and vacuum roasting decreased it by 0.88‰ and 0.07‰ (Frogley, 1997). Li et al. (2007; personal communication, 2017), however, found that soaking for two hours in 5% buffered hydrogen peroxide did not have a significant effect on $\delta^{18}O$ or $\delta^{13}C$. For trace elements Jin et al. (2006) suggested the primary source of contamination is Mg in clays, and therefore recommend sonication in methanol and deionised water, which improved the precision of Mg/Ca measures by ~1.5 mmol/mol: oxidation in hydrogen peroxide and reduction in hydroxylamine hydrochloride solution had little effect on either Mg/Ca or Sr/Ca. Since the major source of contamination is additional Mg, most authors have found the Sr/Ca ratio to be less affected by pre-treatments (Keatings et al., 2006; Jin et al., 2006; Li et al., 2007).

Only one previous study (Keatings et al., 2006) has reviewed the effects of some pre-treatments on ostracod valve geochemical composition by comparing treated and untreated valves. The authors found that all pre-treatments for isotope analysis had a greater effect on $\delta^{13}C$ than $\delta^{18}O$, although mean values for both $\delta^{18}O$ and $\delta^{13}C$ were reduced after cleaning by vacuum roasting. Soaking in hydrogen peroxide or sodium hypochlorite caused greatest deviations in $\delta^{18}O$ between treated and untreated valves, of ~0.8‰ and deviations of up to 2.3‰ for $\delta^{13}C$. For trace–elements, Mg/Ca was most affected by hydrogen peroxide with changes of up to 31%. Sr/Ca was less affected with difference between treated and untreated valves of 2.1%. There was no significant change in Mg/Ca or Sr/Ca following treatment with reducing agents, but differences in valve weight were noted, indicating that the treatment removed some of the shell calcite.

However, the work of Keatings et al. (2006) is limited by only evaluating the effect of oxidation and reduction on trace elements; moreover, the effect of different exposure times and reagent concentrations used in various studies was not taken into account. More recently, new cleaning methods for trace element analysis have since been suggested based on cleaning protocols for foraminifera (e.g. Gray et al., 2014). The proposed method of a four–step sonication, oxidative and reductive cleaning protocol specifically removes high–Mg marine clays and Fe-Mn oxyhydroxide coatings, which form on marine biogenic calcite. Mg/Ca ratios, derived from foraminifera tests and the valves of the deep–marine ostracod genus *Krithe*, which are used as a proxy for palaeothermometry of oceanic bottom waters, are subject to bias from these coatings. During the removal of contamination by reductive cleaning, partial dissolution of calcite has been observed in both foraminifera and ostracods (Yu et al., 2007; Gray et al., 2014). Despite this, the removal of coatings appears to improve the accuracy of Mg/Ca thermometry calculation of up to 1.5 °C and thus is thought to outweigh the effect that any calcite removal may have. The effect on Sr/Ca, however has not yet been fully explored. Since contamination of lacustrine material has received less attention, the efficacy and impact of the individual steps has yet to be fully evaluated on non–marine taxa.

Although all treatments appear to be effective in removing sediment contamination, there is a concern that some pre-treatments may alter the isotopic and/or trace element composition of the ostracod valve itself during the cleaning procedure. Studies of marine and non–marine ostracods and foraminifera have suggested that there is potential for preferential dissolution of surface calcite during some cleaning procedures (Holmes, 1992; Keatings et al., 2006; Yu et al., 2007; Gray et al., 2014). An effective pre-treatment must be efficient in removing contamination, yet preserve the geochemical signal of the valve calcite; however conflicting results and lack of systematic review have hindered a universal protocol from being developed. In this study, we have systematically evaluated the effect of pre–treatment upon the

preservation of the isotopic and trace element signature within ostracod valves.

Cyprideis torosa is an excellent species for testing the preservation of the original trace element and stable isotope composition after cleaning treatments as it is widely used in palaeoenvironmental studies (McCulloch et al., 1989; Anadón et al., 1994, 2002; Mazzini et al., 1999; Marco-Barba et al., 2012, 2013; Holmes and De Deckker, 2017). It is geographically widespread (Wouters, 2017) and tolerant of a wide range of ecological conditions. Most notably, it is extremely euryhaline, and found in waters from almost fresh to hypersaline (De Deckker and Lord, 2017; Pint and Frenzel, 2017; Scharf et al., 2017) although restricted to saline waters with a marine–like chemistry and absent, for example, from saline water with a carbonate–bicarbonate composition. The species is benthic, associated with a range of substrates and tolerant of widely varying dissolved oxygen levels, including hypoxic conditions although is absent from ephemeral water bodies as it shows brood care and cannot therefore tolerate desiccation (De Deckker and Lord, 2017). The adult shell is relatively large and well calcified (up to 100 μg per valve – Marco-Barba et al., 2012). Ecological monitoring has shown that the species produces a single generation per year in temperate environments with stable salinity, but with two peaks of adults, in spring and late summer to early autumn (Heip, 1976). In contrast, a different life cycle was observed in Mediterranean waters, with two of more generations per year (Mezquita et al., 2000). Due to the widespread nature of the *C. torosa*, both living and fossil, an evaluation of cleaning impacts on the species is applicable to a wide range of studies and, therefore, able to suggest and establish a pre-treatment for a large number of palaeoenvironmental studies.

Magnesium partitioning into *C. australiensis* (now considered a separate species to *C. torosa*; Schön et al., 2017: cf. Holmes and De Deckker, 2017) is temperature-dependent and, if the Mg/Ca ratio of the host water is known, calcification temperature is given by $T°C = 2.69 + 5230(Mg/Ca_{shell}/Mg/Ca_{water})$ (De Deckker et al., 1999): this equation has been successfully applied to living and fossil specimens of *C.torosa* in marine–type waters (Holmes and De Deckker, 2017) although the relationship differs for this species in waters with contrasting chemistry (Wansard et al., 2017). Despite evidence for a small temperature dependence of Sr partitioning in *C. australiensis*, the Sr content of the shells is determined primarily by the Sr/Ca of the water (De Deckker et al., 1999).

Determining the vital offset for oxygen isotopes has proven problematical for *C. torosa*: because it often inhabits waters that vary on short timescales, establishing the temperature and isotope composition of the water at the exact time of shell calcification (cf. the time of shell collection) is difficult (Marco-Barba et al., 2012; Bodergat et al., 2014) and no culturing studies have been published to date. However, Keatings et al. (2007) proposed an offset of about +0.8‰ based on field collections from a site with relatively stable conditions, which can be regarded as the best estimate at the time of writing. For carbon isotopes, it is possible that the species calcifies infaunally, and so carries a carbon isotope signature that relates to that of sediment porewaters rather than the open water body (Marco-Barba et al., 2012; De Deckker and Lord, 2017).

2. Materials and methods

2.1. Ostracods

Living specimens of *C. torosa* were collected in August 2016 from a shallow coastal pond in Pegwell Bay Nature Reserve, Kent, where the species is known to be abundant, using a 250 μm zooplankton net to collect the top 1 cm of sediment. Sediment was washed through a 250 μm sieve and dried in an oven at 50 °C. Adult or A-1 carapaces with soft tissue (indicating that the individuals were alive at the time of collection) were selected for analyses. The decision to use modern specimens in the study was twofold. Firstly, uncertainty of whether the cleaning method is causing shell alteration or removing contamination is minimised since significant sediment contamination generally occurs post mortem, and modern shells are less likely to be influenced by diagenesis. The use of live material also allows cleaning methods to be evaluated by undertaking paired analyses on a single carapace (comprising two valves). In unaltered individuals, the two valves have near identical geochemical and isotopic composition (Fig. 1). Hence, when one valve is subjected to the chosen treatment and the other to simple manual cleaning, any differences in composition can confidently be assigned to the treatment method. Articulated carapaces are however less commonly found in fossil contexts.

2.2. Cleaning treatments

For stable isotope analyses, the pre–treatment methods were designed to remove organic matter, which can interfere with the calcite CO_2 signal during analysis. For trace–element analysis, pre-treatments are designed to remove Mg contamination from organic matter (within the shell matrix itself and adhering material) and adhering clay particles.

30 carapaces were selected for each cleaning method. Prior to all analyses, soft tissue and any adhering dried sediment were removed from all valves using needles, a fine paint brush wetted with methanol and ultra-pure 18.2Ω Milli Q deionised water under a binocular microscope. One valve from each carapace then underwent further treatment prior to analysis. All methods are summarised in Table 1.

2.2.1. Cleaning methods for stable isotope analysis

Stable isotope pre-treatment methods were derived from a web of science literature search. The range of methods within each treatment was considered, and a more universal and more extreme method was selected for each.

Single valves were placed into 600 μl micro-centrifuge tubes. For chemical oxidation, valves treated as HP1 were soaked in 500 μl of 15% H_2O_2 for 15 min at room temperature. HP2 valves were soaked in 500 μl of 30% H_2O_2 for 30 min at room temperature. Valves treated as SH1 were soaked in 500 μl of 5% NaClO for 24 h at room temperature. SH2 valves were soaked in 500 μl of 5% NaClO for 4 h at room temperature. For both hydrogen peroxide and sodium hypochlorite treatments, the micro-centrifuge tubes were tapped firmly to ensure the valve had settled to the bottom and was fully submerged during the treatment. Following treatment, the solution was removed with a syringe and needle, and the samples rinsed once with 500 μl of ultra-pure 18.2Ω Milli Q deionised water. The valves were dried in an oven at 50 °C overnight before analysis. For vacuum roasting, VR1 single valves were placed in individual glass vials and placed in a vacuum roaster for 3 h at 80 °C. VR2 valves were individually placed in ceramic crucibles and roasted for 1 h at 250 °C. During plasma ashing, valves were loaded into individual glass vials and placed in to a plasma asher at 125 °C for 2 h (PA1) or 6 h (PA2).

2.2.2. Cleaning methods for trace element analysis

For trace element analysis, individual valves were placed into 600 μl micro-centrifuge tubes for the following treatments, transferred to new tubes, and allowed to dry in a laminar–flow hood before analysis.

For sonication (S), 500 μl of methanol was added to the tubes and firmly tapped to ensure the valve had settled to the bottom before placing in a Cole-Parmer 8892-06 ultrasonic bath at 100 W and 42 kHz ± 6% for 60 s (30 s for small or less well-preserved

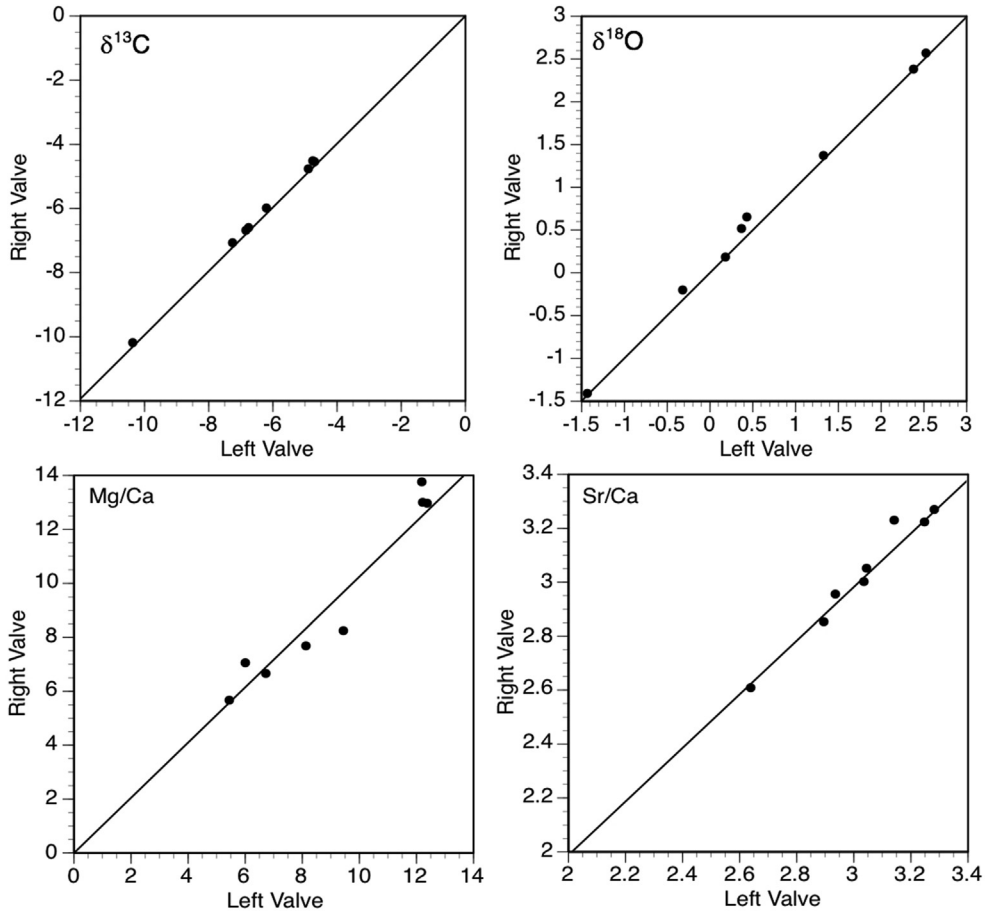

Fig. 1. Isotopic and trace element composition of paired left and right valves of single *Cyprideis torosa* collected from Pegwell Bay.

Table 1
Summary of abbreviations and pre-treatment methods used in the study for isotope and trace element analysis.

	Abbreviation	Method	Details
Isotope Analysis	HP1	Soaking in Hydrogen peroxide	500 μl of 15% H_2O_2 for 15 min at room temperature in a 600 μl micro-centrifuge tube
	HP2	Soaking in Hydrogen peroxide	500 μl of 30% H_2O_2 for 30 min at room temperature in a 600 μl micro-centrifuge tube
	PA1	Low-temperature 'ashing' in an oxygen plasma	Ashed at 125 °C for 2 h in individual glass vials
	PA2	Low-temperature 'ashing' in an oxygen plasma	Ashed at 125 °C for 6 h in individual glass vials
	SH1	Soaking in sodium hypochlorite	500 μl of 5% NaClO for 24 h at room temperature in a 600 μl micro-centrifuge tube
	SH2	Soaking in sodium hypochlorite	500 μl of 5% NaClO for 4 h at room temperature in a 600 μl micro-centrifuge tube
	VR1	Vacuum roasting	For 3 h at 80 °C in individual glass vials
	VR2	Vacuum roasting	For 1 h at 250 °C in individual ceramic crucibles
Trace Element analysis	R	Reductive cleaning	50 μl of hydrous hydrazine/ammonium citrate solution in a 600 μl micro-centrifuge tube in a hot water bath at 80 °C for 10 min
	S	Sonication in an ultrasonic bath	500 μl of methanol in a 600 μl micro-centrifuge tube for 60s, syringed off, repeated with 500 μl of ultra−pure 18.2Ω Milli Q deionised water for 30 s, and rinsed with 500 μl of Milli Q
	O	Chemical oxidation	100 μl of buffered hydrogen peroxide (prepared with 10 ml of 0.1 M NaOH and 100 μl of 30% H_2O_2) in a 600 μl micro-centrifuge tube in a hot water bath at 90 °C for 5 min.

valves). Following sonication, the methanol was removed with a syringe and needle. The process was repeated with 500 μl of ultra-pure 18.2Ω Milli Q deionised water for 30 s, and the valves finally rinsed once with 500 μl of Milli Q. For reductive cleaning (R), 50 μl of hydrous hydrazine/ammonium citrate solution was added and the tubes placed in a hot water bath at 80 °C for 10 min. The solution was removed with a syringe and needle and rinsed three times with 500 μl of Milli Q water. Oxidation (O) was performed using 100 μl of buffered hydrogen peroxide (prepared with 10 ml of 0.1 M NaOH and 100 μl of 30% H_2O_2) added to the tubes, and placed in a hot water bath at 90 °C for 5 min. The solution was removed with a syringe and needle and rinsed three times with 500 μl of

Milli Q water.

2.3. Analytical methods

2.3.1. Stable isotopes

Stable isotope analysis was undertaken on single valves, which were composed of <30 μg of calcite, using an IsoPrime dual inlet mass spectrometer plus Multiprep at the British Geological Survey, Keyworth. Samples are loaded into glass vials and sealed with septa. The automated system evacuates vials and delivers anhydrous phosphoric acid to the carbonate at 90 °C. The evolved CO_2 is collected for 15 min, cryogenically cleaned and passed to the mass spectrometer. Isotope values ($\delta^{13}C$, $\delta^{18}O$) are reported as per mille (‰) deviations of the isotope ratios ($^{13}C/^{12}C$, $^{18}O/^{16}O$) calculated to the VPDB scale using a within-run laboratory standard calibrated against NBS−19. The calcite-acid fractionation factor applied to the gas values is 1.00798. Due to the long run time of 21 h a drift correction is applied across the run, calculated using the standards that bracket the samples. The Craig correction is also applied to account for $\delta^{17}O$. The average analytical reproducibility of the standard calcite (KCM) across the period of analysis was ±0.04‰ for $\delta^{18}O$ and $\delta^{13}C$.

2.3.2. Trace elements

For trace element analyses, single ostracod valves were dissolved in 500 μl of 1.07 M HNO_3 (trace metal grade) in an acid−leached (48 h in 80 °C 10% HNO_3) 600 μl micro−centrifuge tube. The Mg/Ca, Sr/Ca, Fe/Ca, Mn/Ca and Al/Ca ratios of valves were determined using the intensity ratio calibration of de Villiers et al. (2002) using a Varian 720 ES ICP−OES at University College London (UCL). The results were corrected for blank intensity. Analysis of the carbonate standard BCS-CRM 393 gave an Mg/Ca of 3.4 ± 0.07 mmol/mol and Sr/Ca of 0.16 ± 0.003 mmol/mol in good agreement with the mean values of 3.9 mmol/mol and 0.19 mmol/mol quoted in Greaves et al. (2008). Within run reproducibility at 25 ppm Ca was ±1.7% (RSD) and ±2.9% (RSD) for Mg/Ca and Sr/Ca respectively based on 21 determinations across five runs.

3. Results

3.1. Cleaning for isotope analysis

All treatments caused some deviation between treated and untreated valves, which is exclusively negative for $\delta^{18}O$ and negative for five out of eight treatments for $\delta^{13}C$ (Table 2). Three pre−treatments (HP1, HP2 and VR1) caused mean change to the $\delta^{18}O$ signal that was within the analytical error of ±0.04‰. Furthermore, these changes were within the error associated with the natural variation between valves of ±0.03‰ and thus cannot be regarded as significant. The remaining five treatments (PA1, PA2, SH1, SH2 and VR2) caused alteration of more than twice the analytical error (>±0.09‰) in all cases. For $\delta^{13}C$, sodium hypochlorite (SH1 and SH2) was the only treatment to cause alteration greater than the analytical error *and* natural variation of carapaces; both of which were ±0.04‰. Six pre−treatments (HP1, HP2, PA1, PA2, VR1 and VR2) caused alteration to the shell that was within analytical error. The maximum changes associated with all pre-treatments for both $\delta^{18}O$ and $\delta^{13}C$ were all significantly outside of the analytical and natural variation error.

For chemical oxidation, treatment with sodium hypochlorite caused largest deviation from the untreated valve of −0.29‰ for $\delta^{18}O$ (Table 2). Valves treated with hydrogen peroxide all fell within 0.17‰ (although not systematically positive or negative) of the untreated valve for $\delta^{18}O$ and −0.44 for $\delta^{13}C$. Valves treated with HP1 produced a larger spread of data for $\delta^{13}C$ (Fig. 2), but the smallest mean change for all the treatments (−0.005 for $\delta^{18}O$ and −0.003 for $\delta^{13}C$) (Table 2). HP1 is the only method to have caused a positive maximum change for $\delta^{18}O$ of +0.12‰. The smallest changes in mean values, the range of values and the maximum change all resulted from treatment with chemical oxidation.

The smallest maximum change in $\delta^{13}C$ of −0.24‰ was associated with vacuum roasting. Similarly, for $\delta^{18}O$, vacuum roasting, particularly VR1, led to small changes between treated and untreated valves (mean change of +0.02‰ and maximum change of −0.28‰). The range of results for $\delta^{18}O$ was, however, much higher (+0.43‰ and +0.46‰) than the range of results for five of the pre-treatments (0.12‰ for HP1). The range of results for $\delta^{13}C$ was more similar to that associated with other pre-treatments (+0.66‰ for HP1, +0.69‰ for PA1 and +0.60‰ for PA2). PA1 was associated with the largest range of values of +0.69‰ for $\delta^{13}C$ with a maximum change of −0.48‰. Plasma ashing is the only method to produce a maximum positive change for $\delta^{13}C$ (+0.30‰ for PA2). For $\delta^{18}O$, PA1, along with SH2, was associated with the second largest mean change of −0.12‰. The maximum change, on the other hand, is the second smallest after the changes associated with hydrogen peroxide treatment (−0.25‰ compared with −0.17‰ for HP2 and +0.12‰ for HP1). PA2 is associated with a mean change lower than PA1 (−0.01 and −0.12), but produced a larger range of values and larger maximum difference between treated and

Table 2
Summary of the effect of pre-treatment on $\delta^{18}O$, $\delta^{13}C$, Mg/Ca and Sr/Ca.

Isotope analysis	Mean change:	Range:	Max. change:
		(‰)	
HP1			
$\delta^{18}O$	−0.005	+0.12	+0.12
$\delta^{13}C$	−0.003	+0.66	−0.44
HP2			
$\delta^{18}O$	−0.02	+0.27	−0.17
$\delta^{13}C$	+0.02	+0.41	−0.25
PA1			
$\delta^{18}O$	−0.12	+0.37	−0.25
$\delta^{13}C$	−0.04	+0.69	−0.48
PA2			
$\delta^{18}O$	−0.01	+0.41	−0.27
$\delta^{13}C$	+0.02	+0.60	+0.30
SH1			
$\delta^{18}O$	−0.17	+0.34	−0.29
$\delta^{13}C$	−0.13	+0.37	−0.28
SH2			
$\delta^{18}O$	−0.12	+0.34	−0.29
$\delta^{13}C$	−0.07	+0.37	−0.25
VR1			
$\delta^{18}O$	−0.03	+0.43	−0.28
$\delta^{13}C$	+0.02	+0.46	−0.24
VR2			
$\delta^{18}O$	−0.09	+0.46	−0.38
$\delta^{13}C$	−0.04	+0.65	−0.45
Trace Element analysis		(mmol/mol)	
R			
Mg/Ca	−1.77	+11.75	−6.30
Sr/Ca	+0.08	+0.41	+0.29
S			
Mg/Ca	−0.09	+3.97	+2.11
Sr/Ca	−0.05	+0.76	+0.11
O			
Mg/Ca	−0.26	+11.08	+5.84
Sr/Ca	+0.01	+0.83	+0.69

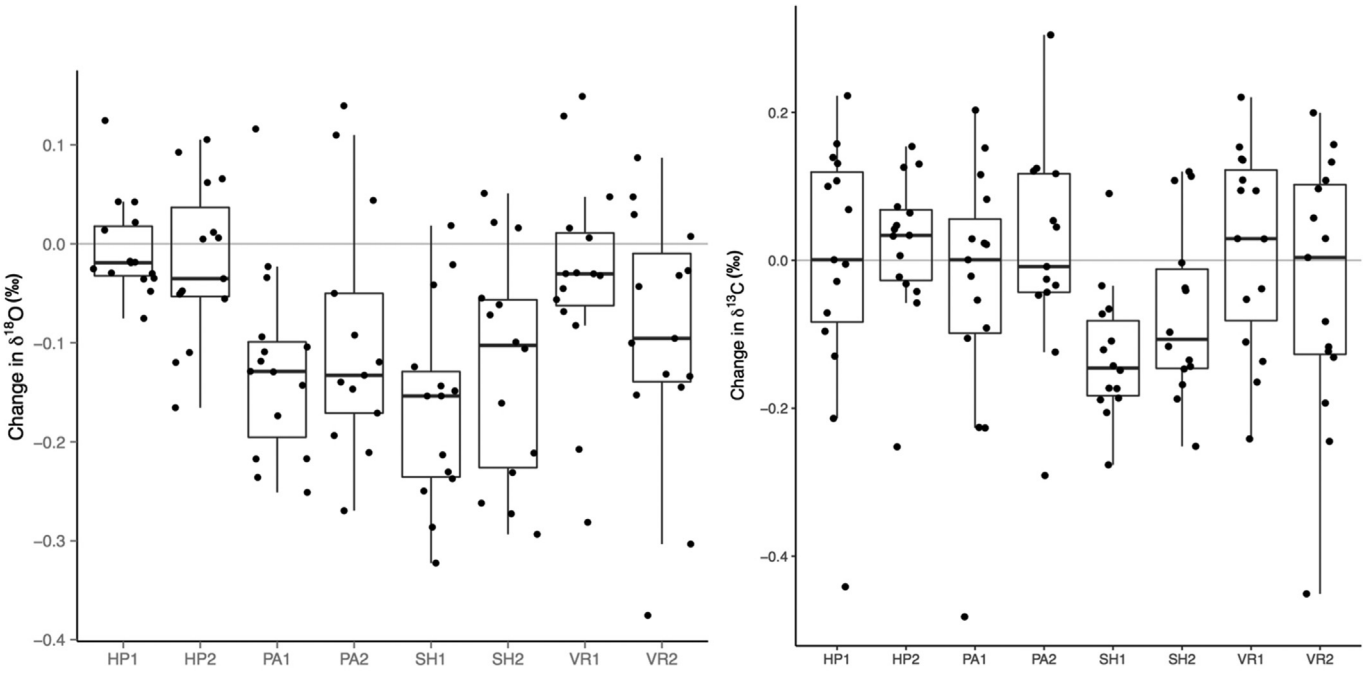

Fig. 2. Change in isotope ratios between treated and untreated valves. Filled circles indicate individual analyses (random x-axis jitter has been applied to prevent sample overlay. The individual data points plotted above may therefore not accurately represent the actual values listed in Table 2). The lower and upper hinges correspond to the 25th and 75th percentiles. The upper/lower whisker extends to the largest/smallest value no further than 1.5* the inter-quartile range.

untreated valves.

There is evidence to suggest that increasing the concentrations/temperatures and/or exposure lengths of certain methods can affect the maximum and mean change in both $\delta^{18}O$ and $\delta^{13}C$. There is a weak ($R^2 = 0.12$, $p = <0.0002$) relationship between the change in $\delta^{18}O$ and $\delta^{13}C$ for most pre-treatments (Fig. 3). The largest increase in mean change is associated with the increase in vacuum roasting time and temperature, with a difference in $\delta^{18}O$ of −0.057 between VR1 and VR2. Increasing the concentration and exposure of hydrogen peroxide produced the largest increase in the range of results for $\delta^{18}O$ (+0.12‰ to +0.27‰). Most other methods showed little change in the overall range of values between methods 1 and 2.

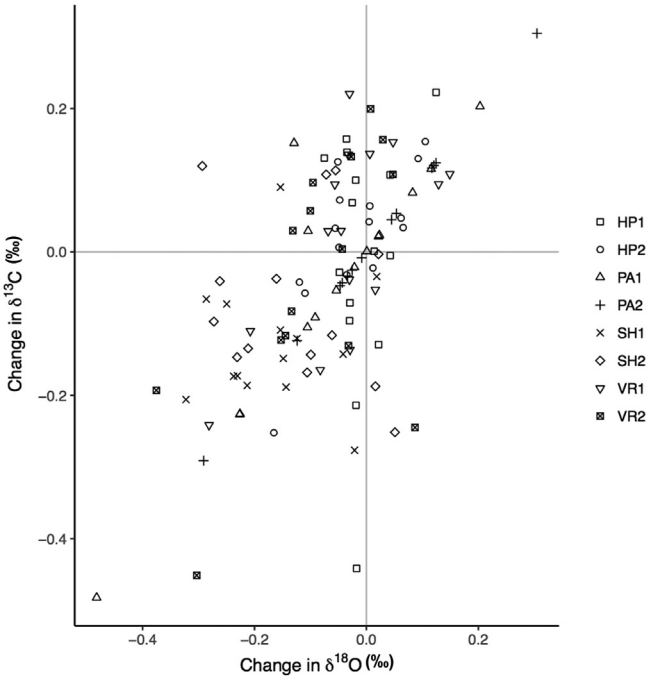

Fig. 3. The relationship between the change in $\delta^{18}O$ and $\delta^{13}C$ for each valve and each treatment method.

3.2. Cleaning for trace element analysis

All pre-treatments caused alteration to the trace element content of the valves. For Mg/Ca all mean deviation was negative, while two out of three treatments (R and O) caused positive deviation in Sr/Ca. Mean change for all pre-treatments was greater than the analytical error of ±0.07 mmol/mol for Mg/Ca and ±0.003 mmol/mol for Sr/Ca (Table 2). Two out of the three pre-treatments were associated with Mg/Ca deviation that is outside both the analytical error of ±0.07 and the error associated with the natural variation of ±0.20 mmol/mol. For sonication, the mean change of −0.09 mmol/mol was greater than the analytical error, but less than the error that may be associated with the natural variation between the two valves of a carapace. For Sr/Ca all pre−treatments caused mean deviation, up to +0.08 mmol/mol, significantly greater than the analytical error (±0.003 mmol/mol) and natural variation (±0.004 mmol/mol). The maximum changes associated with all pre-treatments for both Mg/Ca and Sr/Ca were all significantly greater than the analytical and natural variation error.

Pre-treatment of valves had a greater effect on the deviation between treated and untreated valves for Mg/Ca than Sr/Ca (Fig. 4). All treatments led to a mean reduction in Mg/Ca ratios, with sonication giving the smallest change of −0.09 mmol/mol (Table 2). Sonication is the only pre-treatment to have reduced both Mg/Ca and Sr/Ca ratios. Furthermore, with sonication both Mg/Ca and Sr/Ca have smaller ranges of +3.97 mmol/mol for Mg/Ca and +0.76 mmol/mol for Sr/Ca with maximum changes of +2.11 mmol/mol and +0.11 mmol/mol; much lower than that observed with reductive cleaning (a range of +11.75 mmol/mol for

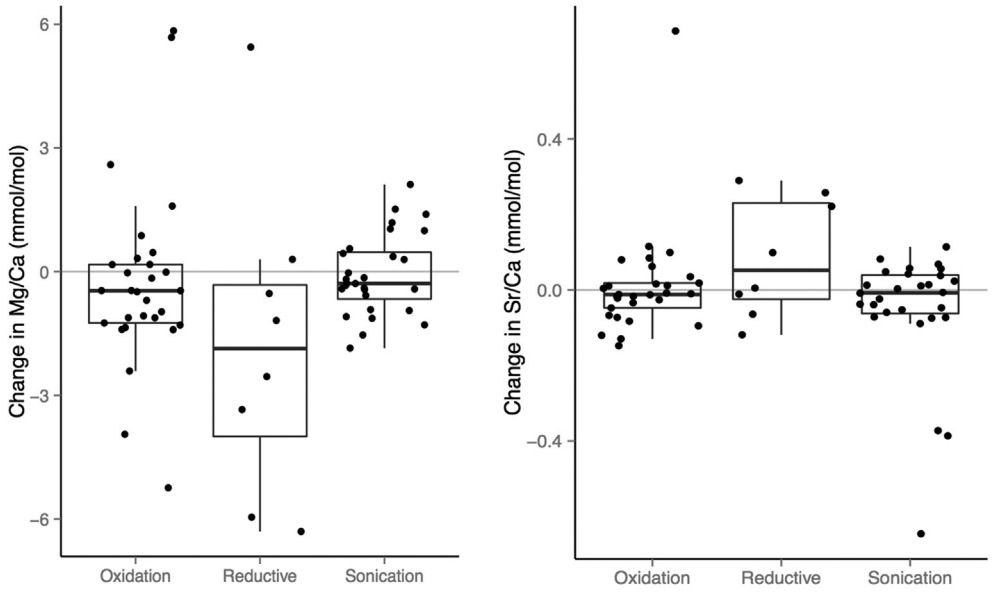

Fig. 4. Change in M/Ca ratio between treated and untreated valves. Filled circles indicate individual analyses (random x-axis jitter has been applied to prevent sample overlay. The individual data points plotted above may therefore not accurately represent the actual values listed in Table 2). The lower and upper hinges correspond to the 25th and 75th percentiles. The upper/lower whisker extends to the largest/smallest value no further than 1.5* the inter-quartile range.

Mg/Ca and a maximum change of +0.29 compared to +0.11 for Sr/Ca) and oxidation (a range of +11.08 mmol/mol for Mg/Ca and 0.83 mmol/mol for Sr/Ca). There is no systematic relationship ($R^2 = 0.09$, p = >0.1759) between changes in Mg/Ca and Sr/Ca (Fig. 5). For some treatments, there is therefore evidence that alteration can occur, particularly for Mg/Ca.

3.2.1. Contamination indicators

Typically to assess the efficacy of pre-treatments at removing contamination, Fe, Mn and Al are sometimes measured as contamination indicators (Jin et al., 2006; Gray et al., 2014). All were measured here to evaluate whether pre-treatment could bias the contamination indicator. The elements show a varied response to pre-treatment with some, primarily Fe/Ca, showing large changes of up to −33.67 mmol/mol during oxidation and in Al/Ca of −14.17 mmol/mol during sonication. However, there is no correlation between changes in Mg/Ca and Sr/Ca with Al/Ca or Fe/Ca (Fig. 6). The only significant correlation is between changes in Mg/Ca and Mn/Ca after sonication ($R^2 = 0.63$). The ability for some pre−treatments to vastly change the contamination indicators, in material that is assumed to be contamination free, suggests that some elements used as indicators for contamination removal may also be affected by the pre-treatment.

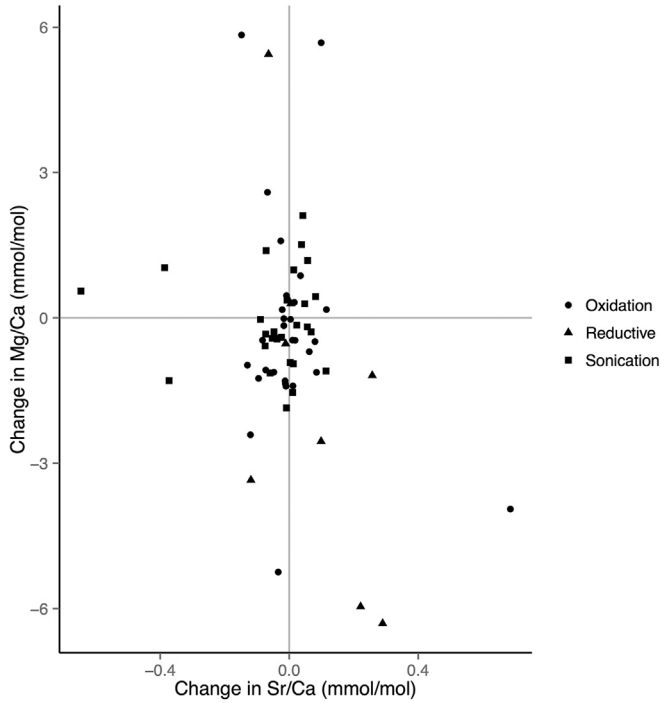

Fig. 5. The relationship between the change in Mg/Ca and Sr/Ca for each valve and each treatment method.

4. Discussion

4.1. Effect of cleaning on isotope composition

The cleaning experiments showed that all treatments have the potential to cause alteration to the isotopic composition of valves. In most cases the large changes in composition involved shifts to more negative values. Lower $\delta^{18}O$ and $\delta^{13}C$ may be related to 1) selective removal of calcite leaving a greater proportion of organic matter with lower $\delta^{18}O$, 2) selective removal of the heavy isotopes (^{13}C and ^{18}O) from the shell, and/or 3) isotope exchange with CO_2 in the atmosphere.

Gaffey and Bronnimann (1993), Ito (2001) and Keatings et al. (2006) have all suggested that hydrogen peroxide does little to remove organic material adhered to the shell, and instead weak organic acid produced during the oxidation process may cause partial dissolution of the calcite surface. Since organic material usually has lower $\delta^{18}O$ and $\delta^{13}C$ values than calcite, removal of calcite material could produce the observed decrease in $\delta^{18}O$ and $\delta^{13}C$. The +0.02‰ change in $\delta^{13}C$ during HP2 is within error suggesting no significant effect on the removal of organic material, but the range of $\delta^{18}O$ values suggests removal of calcite from the shell structure. Comparison of manually cleaned valves and those

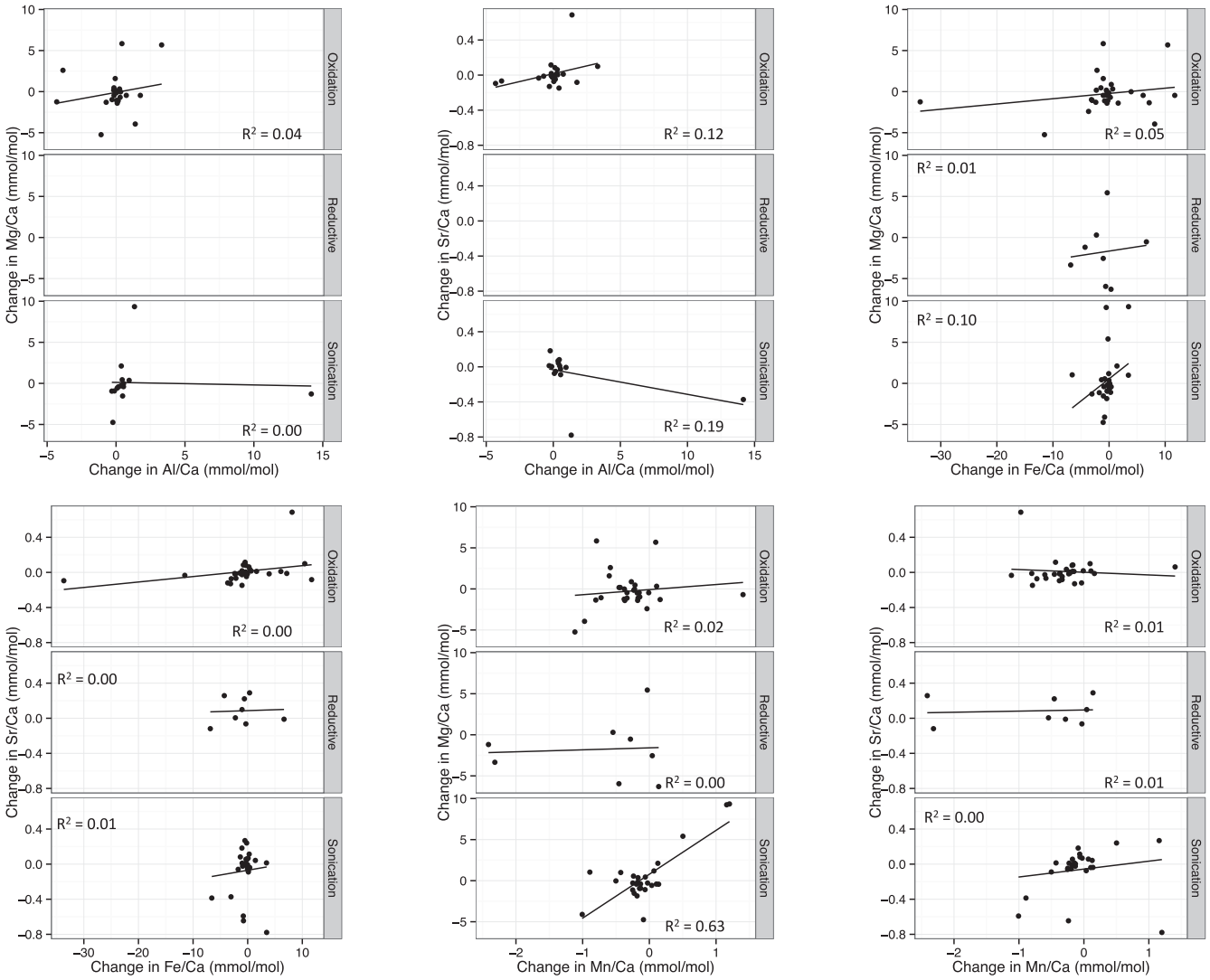

Fig. 6. Change in contamination indicators with changes in Mg/Ca and Sr/Ca after pre-treatment. Any values below detection limit have been removed; all Al/Ca values after reductive cleaning were below detection limit.

treated with hydrogen peroxide under SEM shows evidence of selective partial dissolution of the calcite surface when exposed to higher concentrations of the reagent for extended periods (Fig. 7). Where both $\delta^{18}O$ and $\delta^{13}C$ are of interest, it would therefore be recommended to limit exposure time and concentrations of hydrogen peroxide. It has previously been suggested that hydrogen peroxide is inefficient at removing organic material, from both within, and adhered to, the valve. This is however based on heating the hydrogen peroxide to 80 °C (Keatings et al., 2006), whilst the experiments presented here were conducted at room temperature; there is evidence to suggest that the decomposition of hydrogen peroxide occurs at high temperatures, and therefore the experiment conditions used by Keatings et al. (2006) may explain the reported inefficiency.

It has been argued that sodium hypochlorite is more effective at removing organic material contamination than hydrogen peroxide, with much improved reproducibility when valves are pre-treated with this reagent (Keatings et al., 2006). In the present study, we have shown that sodium hypochlorite has little effect on $\delta^{13}C$, but that $\delta^{18}O$ is affected substantially with some of the largest deviations from the untreated valve, which is in agreement with Durazzi (1977). The magnitude of deviation (~0.3‰) is unlikely to be purely from the removal of organic chitin from within the calcite, but more likely a result of preferential valve dissolution. Whilst at a lower concentration, the length of exposure to sodium hypochlorite is substantially longer than the exposure to hydrogen peroxide (maximum 24 h compared with 30 min). The effect of exposure to sodium hypochlorite for less than four hours has not been systematically evaluated. A reduction in exposure may however maintain the signal. It is also possible that isotopic exchange could take place during the treatment, producing the observed lowering of $\delta^{18}O$ values. Furthermore, partial dissolution of the shell, and resulting disruption to the calcite lattice could both promote isotopic exchange with atmospheric CO_2.

Similarly, isotopic exchange with atmospheric CO_2 may be responsible for the large reductions in $\delta^{18}O$ and $\delta^{13}C$ after plasma ashing or vacuum roasting due to disruptions to the calcite lattice associated with exposure to high temperatures. To improve the efficiency at removing organic material from within the calcite matrix, valves are sometimes crushed in ethanol prior to plasma ashing or vacuum roasting (Boomer, 1993; Keatings et al., 2006). The crushing increases the surface area of calcite exposed to the

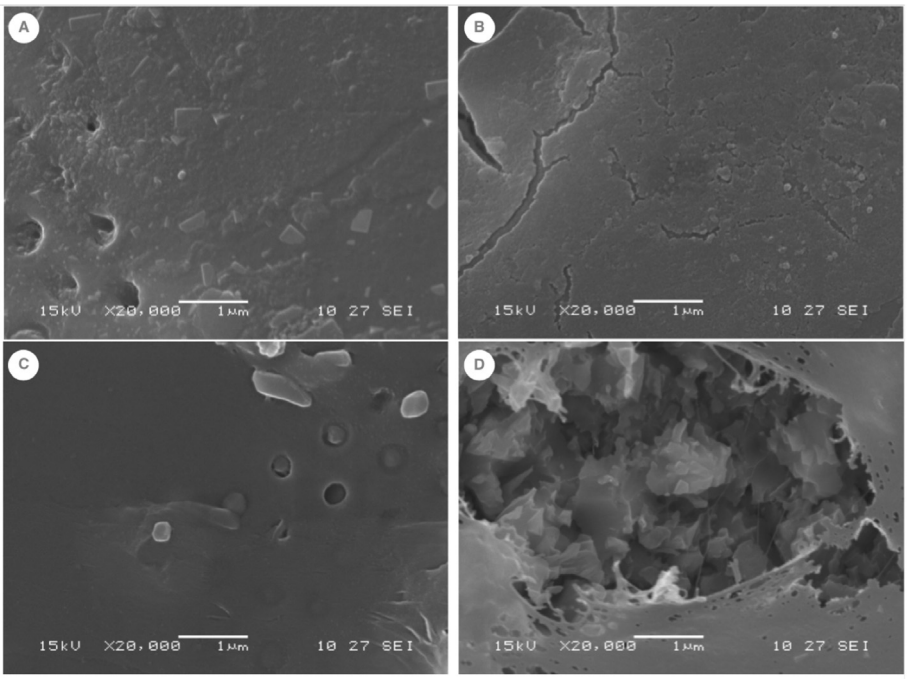

Fig. 7. SEM imagery of manually cleaned valves (A and C) and valves cleaned with hydrogen peroxide at HP1, 15% for 15 min, (B) and HP2, 30% for 30 min, (D). Cleaning at 30% shows evidence of selective partial dissolution of the calcite surface which is not evident after exposure to cleaning during treatment HP1. Valves A and B and C and D are from the same carapaces, and therefore any change in calcite structure can be attributed to cleaning methods.

elevated temperatures and increases potential areas for atmospheric isotopic exchange. However, when valves were crushed prior to plasma ashing a smaller range in values of ~0.2‰ for $\delta^{18}O$ and $\delta^{13}C$ was observed by Keatings et al. (2006). This is inconsistent with the range found here of ~0.4‰ when less removal of the organic matrix of the calcite would be expected given that the valves were not crushed. There is therefore potential for plasma ashing to produce inconsistent and easily misinterpreted effects on ostracod calcite. There may also still be an effect of crushing during vacuum roasting, with Frogley (1997) and Keatings et al. (2006) reporting a range of ~0.8‰ compared to the ~0.4‰ reported here. Furthermore, the experiments of Keatings et al. (2006) were undertaken at a higher temperature (380 °C), which could increase the potential for disruption to the calcite lattice. The increase from 80 °C to 250 °C between VR1 and VR2 supports this conclusion, with mean changes increasing from −0.03‰ to −0.09‰ and maximum changes from −0.28‰ to −0.38‰. The values reported in this study, however, agree with those presented by Grossman et al. (1986) for foraminifera tests roasted at 470 °C. Grossman et al. (1986) conclude that since organic matter had been previously removed, the results are related to isotopic exchange. Since Keatings et al. (2006) use fossil material, it is likely that their observations result from the removal of contamination, and not the direct effect of the pre-treatment on the valve calcite.

4.2. Effect of cleaning on trace element composition

All treatments were shown to cause alteration to the trace element composition of the shell. Sr/Ca was less affected by pre-treatments and, in most cases, showed an increase in deviation between valves. As expected average Mg/Ca ratios decreased after pre-treatment, but with large increases in some cases. Large decreases in Mg/Ca ratios may be attributed to 1) partial dissolution of Mg-rich calcite surface, and/or 2) removal of organic matter from within the calcite matrix, which is probably higher in Mg than the calcite. The increase in Sr/Ca is not fully understood, but could relate to depletion of Sr in the calcite surface.

Some pre-treatments show the potential to produce large changes in Al/Ca, Fe/Ca and Mn/Ca. These changes could be the result of partial dissolution of the shell during treatment, coupled with the heterogeneous nature of these elements in ostracod calcite (Fig. 8). Reductions in Mg/Ca without significant changes in trace-element/Ca (M/Ca) ratios, could be attributed to the removal of Mg-rich chitin. However, Chivas et al. (1983) and I. Boomer

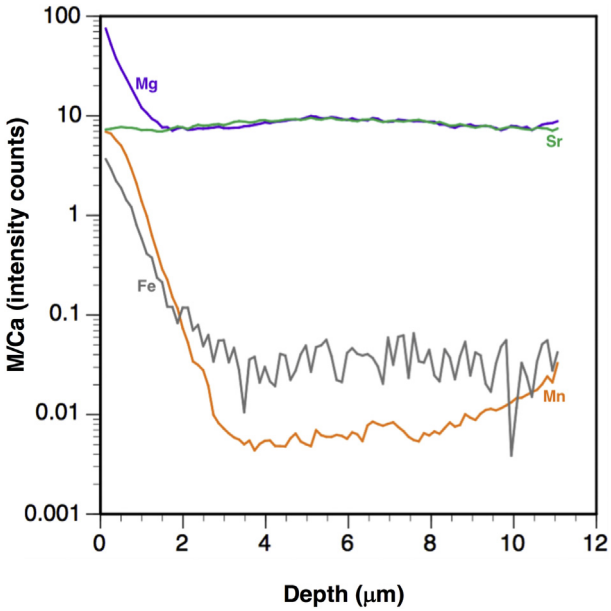

Fig. 8. LA-ICP-MS profile of a *Cyprideis australiensis* valve showing enrichment in Mg, Fe and Mn in the outer 2 μm. Data originally presented in Ito et al. (2003); raw data replotted here from Patrick De Deckker.

(personal communication, 2017) suggest that whilst chitin may be high in Mg (up to 910,00 ppm equivalent to $CaCO_3$), it does not contribute significantly to measured Mg in solution as a result of calcite dissolution in weak mineral acids. However, since ostracod valves are not composed of closed chambers, organic material, including chitinous soft parts, and sediment contamination are more readily removed via visual inspection. Based on this and since the modern valves are assumed to be free from contamination, large reductions reported in this study are unlikely to be due to removal of organic material. The large reductions (up to −6 mmol/mol) in Mg/Ca are therefore much more likely to be the result of partial dissolution of the ostracod calcite surface.

4.2.1. Evidence of partial dissolution

Citric acid, used in the reductive cleaning, exists mainly as citrate, which is a potential ligand to chelate metal. This may lead to dissolution of $CaCO_3$, thus decreasing M/Ca. Laser ablation of adult valves of *C. australiensis* have revealed that the outer 1 μm is enriched in both Mg and Mn (Ito et al., 2003) (Fig. 8). If shell structure is assumed to be similar within genus, removal of calcite material from *C. torosa* would account for the trends observed after reductive cleaning. Similarly, reductive cleaning may be less significant in the removal of Sr/Ca due to the relatively uniform Sr composition throughout the shell. There is slight depletion of Sr in the outer 2 μm of calcite which could explain some of the observed increases in Sr/Ca, but would assume a large amount of calcite removal.

Comparison of manually cleaned and reductively cleaned valves under SEM provide further evidence for dissolution of the calcite surface (Fig. 9). Yu et al. (2007) suggest that ~24 μg of solid $CaCO_3$ can be dissolved in 100 μl of reducing reagent containing 0.12 mol/L of citrate, which could account for the large sample loss experienced during this experiment, and reported by other authors (Li et al., 2007; Gray et al., 2014). The results here are consistent with those of Yu et al. (2007) for partial dissolution of foraminifera calcite, which showed that reductive cleaning had little effect on Sr/Ca composition of foraminifera shells, but caused a significant reduction in Mg/Ca and Mn/Ca.

4.3. Implications for palaeotemperature and palaeo-water composition reconstructions

For the use of shell geochemistry in palaeoenvironmental studies to be robust, the pre-treatment must limit the influence of contamination, but not bias the signal by altering the shell. Where shell alteration is greater than the analytical uncertainties in the trace element and stable isotope measurements, there is potential for incorrect palaeoenvironmental reconstructions. The analytical uncertainty for trace element measurements is ±0.07 mmol/mol for Mg/Ca and ±0.003 mmol/mol for Sr/Ca. For each treatment, the mean change between treated and untreated valves is greater than the analytical uncertainties in the trace element measurements, especially for Mg/Ca (Table 2). For both $\delta^{18}O$ and $\delta^{13}C$, the analytical uncertainty is ±0.04‰. However, unlike the trace elements, the mean changes in $\delta^{18}O$ and $\delta^{13}C$ during some treatments are less than analytical uncertainty and any change due to pre-treatment is therefore undetectable. The maximum changes in trace elements and stable isotope composition are greater than error for all treatments. The changes in the geochemical signature of the valve after pre-treatment are therefore to a degree that is detectable and of potential significance to palaeoenvironmental studies.

Since both $\delta^{18}O$ and Mg/Ca have a quantitative relationship with temperature, the implications of the alteration caused by pre-treatment can be quantified quite readily. For Mg/Ca, we evaluate the impact of shell alteration in palaeothermometry using the calibrations of De Deckker et al. (1999):

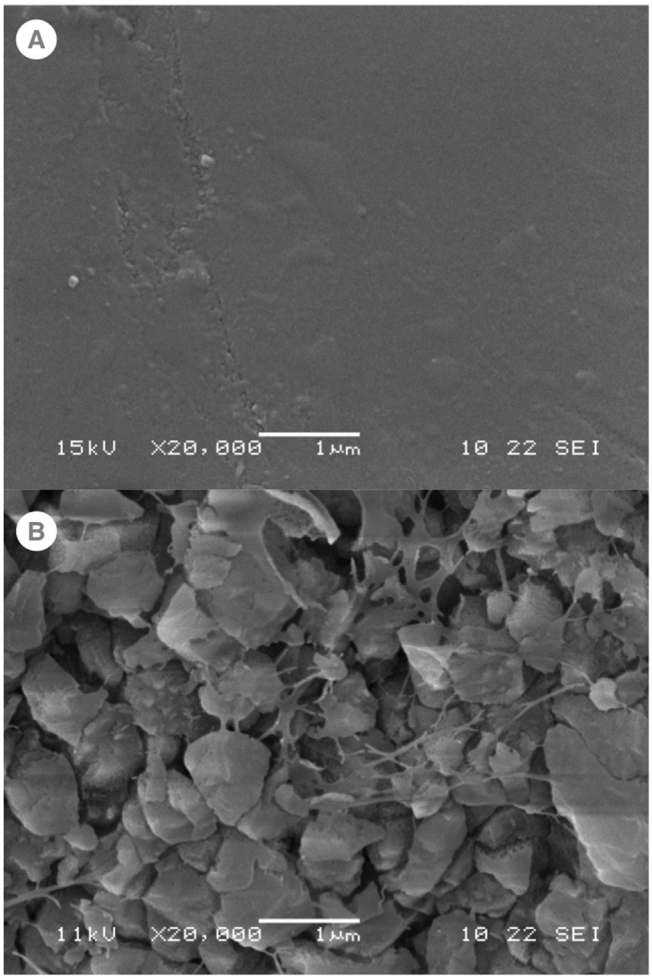

Fig. 9. SEM imagery of a manually cleaned valve (A) and a reductively cleaned valve (B). Clear evidence of calcite dissolution can be seen after reductive cleaning. The valves are from the same carapace, and therefore any change in calcite structure can be attributed to cleaning methods.

$$T\,(^{\circ}C) = 2.69 + (5230 * [Mg/Ca]_{ostracod}/[Mg/Ca]_{water})$$

And the equation of Wansard (1996), which is applicable to more dilute waters:

$$T\,(^{\circ}C) = 3.3 + (1971.0 * [Mg/Ca]_{ostracod})$$

Using the De Deckker et al. (1999) equation, and assuming a sea water Mg/Ca of 5 mol/mol, a 1 mmol/mol change in Mg/Ca equates to a 1.1 °C shift in inferred temperature, whilst the relationship in the equation of Wansard (1996) equates to a shift of 2 °C in inferred temperature. From the mean differences in Mg/Ca after pre-treatment, the reconstructed temperature could shift −1.85 °C using the calibration of De Deckker et al. (1999) and by −3.4 °C using the Wansard (1996) calibration (Table 3). Whilst the mean changes after sonication or oxidation do not shift temperatures by a significant margin, the maximum changes cause significant bias for all treatments, and could have a significant impact on inferred temperatures.

For $\delta^{18}O$, if water temperature were the sole control on the isotopic composition of the ostracod shell, a shift of 1 °C would

Table 3
Shifts in temperature based on the mean and maximum changes for each treatment using the calibrations of De Deckker et al. (1999) and Wansard (1996) for Mg/Ca.

	Mean change (°C)			Max. change (°C)		
	R	S	O	R	S	O
De Deckker et al. (1999)	−1.9	−0.1	−0.3	−6.4	+2.2	+6.1
Wansard (1996)	−3.5	−0.2	−0.5	−12.0	+4.2	+11.5

equate to a change of ~0.2‰ (Kim and O'Neil, 1997). Many of the maximum changes in $\delta^{18}O$ between untreated and treated valves are within 0.2‰ and therefore any alteration may be undetectable in the temperature reconstructions. However, water $\delta^{18}O$ is likely to be as important a control on ostracod $\delta^{18}O$, or in some cases more important. In non-marine and marginal marine environments, changes in water $\delta^{18}O$ can occur on short-term timescales of 12 h. Coexisting ostracods, which have probably calcified at different times, are therefore likely to have geochemical signals that may vary by more than 0.2‰. For instance, during late Spring and late Summer/early Autumn when *C.torosa* calcification is thought to occur (Heip, 1976), the $\delta^{18}O_{water}$ of the salt marsh pond at Pegwell Bay varies from −1.19‰ to +3.86‰. A change in the $\delta^{18}O$ of the ostracod shell to the magnitude caused by the cleaning method is therefore unlikely to be significant for palaeoenvironmental reconstructions in such cases. In open marine systems however, where even glacial − interglacial contrasts in $\delta^{18}O$ are small (Mashiotta et al., 1999) such a change resulting from the cleaning method may be significant. The impact of pre-treatments reported in this paper may however not be applicable to marine taxa since calcite composition varies with taxa (Ito et al., 2003).

Sr/Ca in the ostracod shell can be used to infer Sr/Ca in the host water, which in some circumstances co-varies with salinity. The partitioning of Sr into the ostracod shell from its host water can be described by a partition coefficient (K_D value):

$$K_D[Sr] = Sr/Ca_{(ostracod\ shell)} / Sr/Ca_{(water)}$$

KD[Sr] are typically constant for a given species and when combined with Sr/Ca values from ostracod shells, can be used to back calculate Sr/Ca_{water} and in some cases salinity. From the culture experiment of De Deckker et al. (1999), a change in Sr/Ca_{water} by 1 mmol/mol leads to a change in the ostracod of about 0.5 mmol/mol. Once the Sr/Ca_{water} is established from the analysed $Sr/Ca_{ostracod}$ using this relationship, in some instances where the mixing of meteoric and marine waters occurs a quantitate relationship with salinity can be established to estimate electrical conductivity (EC) of the water at the time of shell calcification. Here, we use the equation of Holmes et al. (2010), specific to the Thurne Broads catchment in Eastern England:

$$EC = ((4152717 * (Sr/Ca_{water}{}^2))) - (8883 * Sr/Ca_{water}) + 6.29$$

Using $Sr/Ca_{ostracod}$ data from the catchment and the mean differences in Sr/Ca after pre-treatment, the reconstructed electrical conductivity could shift by up to +4.5 mS cm^{-1} after reductive cleaning (Table 4). Sonication and oxidation are associated with reductions in reconstructed electrical conductivity, but to a lesser degree than the bias associated with reductive cleaning (a maximum change of −1.2 mS cm^{-1} associated with oxidation). In lacustrine environments, where increasing salinity may be small and gradual, the mean changes associated with all pre-treatments could cause significant bias and impact reconstructed electrical conductivity.

Table 4
Shifts in reconstructed EC based on the mean and maximum changes for each treatment using the equation of Holmes et al. (2010) for Sr/Ca.

Mean change (mS cm^{-1})			Max. change (mS cm^{-1})		
R	S	O	R	S	O
+2.9	−1.1	−1.6	+4.5	−0.7	−1.2

The $\delta^{13}C$ of the ostracod shell mainly reflects the $\delta^{13}C$ of DIC, but due to large changes in $\delta^{13}C$ of DIC at small spatial scales within a lake, and over time, accurate measures of $\delta^{13}C$ DIC at the time of shell secretion are unlikely. It is therefore difficult to assess the implications of changes in $\delta^{13}C$ caused by the cleaning treatments. Since the use of $\delta^{13}C$ as a proxy for productivity is associated with such assumptions already, it is likely that the uncertainties in measurements brought about by the cleaning methods have less serious implications for palaeoenvironmental reconstructions.

The majority of the trends resulting from the treatments involve systematic biases. However, this is not always the case; for instance changes as high as +5.84 mmol/mol in Mg/Ca were recorded after oxidation or −0.24‰ in $\delta^{13}C$ after vacuum roasting, despite the mean changes being in the opposite direction of change. Values cannot therefore be adjusted to account for the impact of pre-treatments. Furthermore, the unpredictability of changes in individual specimens to certain pre-treatments, despite average changes being small and one-directional, suggests the potential for bias that may be undetectable. Pre-treatments with the potential to cause these changes should therefore be avoided in palaeoenvironmental studies.

5. Conclusions

An effective pre-treatment of ostracod shells prior to geochemical analysis must maintain the original geochemical signal of the valve as authentically as possible whilst removing contamination. From the data presented here it is clear that some pre-treatments have the potential to alter the trace element signal beyond analytical error thus biasing palaeoenvironmental studies. The palaeoenvironmental implications of changes in stable isotope composition are less systematic. Instead the magnitude of the observed change may be insignificant due to the natural varying water isotopic composition of the host water. In some circumstances where water isotopic composition is relatively constant, such as marine or stable lake systems, a pre-treatment could have the potential to cause bias to the signal, albeit to a lesser degree than the bias in trace element ratios.

The average change in isotopic composition for carapaces where both valves were manually cleaned (i.e. the natural variation in a carapace) was ±0.03‰ for $\delta^{18}O$ and ±0.04‰ for $\delta^{13}C$, and the analytical error ±0.04‰ for both. All treatments can cause alteration greater than these values. Whilst some of the changes can be attributed to the removal of organic matter, the large changes produced by some methods are more likely to be caused by alteration to the signal through calcite dissolution or isotopic exchange with atmospheric CO_2. Treatment with hydrogen peroxide with limited exposure at room temperature (HP1) provides the most reproducible results with little alteration to the original signal. Whilst HP2 provides less variable results for $\delta^{13}C$, the $\delta^{13}C$ is generally more variable and the applications and requirements of $\delta^{18}O$ are more susceptible to bias from outliers. The experimental conditions (e.g. duration and temperature) for some treatments may be of greater importance than the selection of the treatment itself and so care must be taken to ensure that operating conditions do not increase bias into the geochemical results.

All methods for trace element pre-treatment showed the potential to cause alteration to the original signal. The average

'natural' variation between valves for Mg/Ca was ±0.20 mmol/mol and ±0.004 mmol/mol for Sr/Ca, and the analytical error ±0.07 mmol/mol for Mg/Ca and ±0.003 for Sr/Ca. Sonication provides the most reproducible results for Mg/Ca and was associated with the smallest change in both Mg/Ca and Sr/Ca. Whilst oxidation offers the most reproducible results for Sr/Ca, it is associated with a larger range of data for Mg/Ca due to calcite dissolution. Reductive cleaning would not be recommended for ostracod shells unless there is substantial evidence for extreme Fe-Mn oxyhydroxide contamination that also introduce contaminant Mg to the shell surface. The effect of calcite removal may cause significant bias in lacustrine ostracods, and pre-treatments that may promote calcite dissolution are not recommended. Furthermore, any intensive cleaning that is deemed unnecessary based on visual inspection of valves will lead to preventable sample loss. Where Fe-Mn oxyhydroxide contamination is visible, the method of analysis by flow-through time-resolved analysis (FT-TRA) proposed by Börner et al. (2017), which removes the need for aggressive pre-treatments by accounting for surface contamination during analysis, may be appropriate.

Where 'tandem' isotope and trace element analysis are being undertaken on the same valve (Chivas et al., 1993; Xia et al., 1997; Ito and Forester, 2017), the results presented here suggest that oxidation with hydrogen peroxide is the most effective method to employ as it provides reproducible results with limited outliers for both geochemical signals. However, an exposure time and temperature that suits both analyses will need to be established. Based on the results here, the method proposed by Xia et al. (1997), and subsequently used by Ito and Forester (2017), of 5% hydrogen peroxide for 10 min at 80 °C is unlikely to introduce any significant bias.

Recommendations for a universal inter-laboratory protocol that will effectively remove sediment contamination, retain the original geochemical signal, and reduce sample loss are therefore treatment with hydrogen peroxide for $\delta^{18}O$ and $\delta^{13}C$ and sonication in methanol and deionised water for trace element analysis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.quascirev.2018.03.030.

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