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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