Trace-element and stable-isotope composition of the *Cyprideis torosa* (Crustacea, Ostracoda) shell

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Abstract: Shells of *Cyprideis*, a widespread euryhaline ostracod, have commonly been used in geochemical investigations involving determinations of trace elements (especially magnesium and strontium) and isotopes (of oxygen, carbon and strontium). In this paper, we evaluate geochemical signatures in *Cyprideis* based on new and previously published data. Mg/Ca and Sr/Ca determinations of fossil shells that calcified in marine-type water have potential to reconstruct palaeotemperature and past water composition using empirical relationships derived from living ostracods recovered from *in vitro* cultures or natural settings. For shells that calcified in non-marine waters of contrasting composition, partitioning of trace metals from water into ostracod shells may differ, meaning that relationships developed for marine waters do not apply. However, variations in Mg/Ca and Sr/Ca in *Cyprideis* in continental settings may still provide valuable palaeohydrological information. Determinations of oxygen isotopes in *Cyprideis* shells are consistent with positive offsets from equilibrium, in common with other ostracod taxa: carbon-isotope values reflect the fact that *Cyprideis* is a detritivore. Oxygen-isotope analyses of *Cyprideis* shells from continental settings provide important palaeohydrological information. Strontium-isotope analyses of *Cyprideis* shells provide valuable records of mixing of marine and continental water in marginal-marine settings. Geochemical analyses of different morphotypes of *Cyprideis* lend support to suggestions that ecophenotypic variations are controlled by factors other than, or additional to, salinity.

Keywords: magnesium/calcium, strontium/calcium, strontium isotopes, oxygen isotopes, carbon isotopes

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The use of ostracod shells for geochemical analyses is now well established (Holmes & De Deckker 2012) provide a recent review), with applications to marine and non-marine material. Most trace-element analyses have used magnesium and strontium and there now exists a considerable understanding of the partitioning of these elements from water into the ostracod calcite (Dettman & Dwyer 2012). Ostracod shells are also much-used sources of calcite for isotope analyses, mainly oxygen and carbon isotopes in non-marine settings (Decrouy 2012), although there have been a few studies based on marine material (Didié & Bauch 2002) and investigations using other isotopes, such as those of strontium (McCulloch & De Deckker 1989; McCulloch et al. 1989; Ingram et al. 1998; Holmes et al. 2007a). Previous studies have demonstrated the following:

1. magnesium and strontium are strongly partitioned between ostracod shell and water (e.g. Dettman & Dwyer 2012), with the amount of partitioning related to taxonomy and environment (especially water Sr/Ca and Mg/Ca and, for magnesium, temperature);
2. the oxygen-isotope composition of ostracod calcite is determined by water temperature and water isotope composition, with taxonomically controlled (generally) positive offset from oxygen-isotope equilibrium (Xia et al. 1997; von Grafenstein et al. 1999; Chivas et al. 2002; Decrouy 2012);
3. carbon-isotope composition of ostracod shells is determined by the carbon-isotope composition of dissolved inorganic carbon (DIC) (Keatings et al. 2002) and, at times, ambient pore water if the ostracod is endobenthic (Decrouy et al. 2011a, b, 2012);
4. strontium-isotope composition of ostracod shells reflects that of strontium in water, which is determined by water source and water–rock interaction (Holmes et al. 2007b). There is no temperature effect and no control by precipitation of minerals, such as aragonite, from the water that could significantly alter the water’s Sr/Ca.

However, there are a number of important areas of incomplete understanding, including the following:

1. controls on trace-element partitioning. Although there is strong evidence that water composition and water temperature are significant controls, it is increasingly clear that other factors play a role, such as water ionic activity, bicarbonate content and pCO2. Moreover, variation in partitioning within taxa is probably important (Wansard et al. 1998; Dettman & Dwyer 2012); (2) the magnitude of vital offsets from oxygen-isotope equilibrium has only been established for a relatively small number of taxa (von Grafenstein et al. 1999; Keatings et al. 2002). Moreover, the possibility exists that these offsets may not be constant within a single species or genus, contrary to what has previously been suggested (Decrouy et al. 2011b; Decrouy 2012). Accurate knowledge of offsets from oxygen-isotope equilibrium is important if oxygen isotopes are to be used in quantitative reconstructions of calcification temperature or water
Composition of the Cyprideis torosa shell

isotope composition (as demonstrated by von Grafenstein 2002);

(3) there is poor understanding of factors that control the carbon-isotope composition, arising from the fact that ostracod shells ‘sample’ DIC at the micro-scale; variations in the carbon-isotope composition of DIC are poorly understood and generally difficult to measure (Decrouy et al. 2011a; Decrouy 2012).

Clearly, despite understanding having moved a long way since the techniques were first proposed in the 1950s (e.g. Cheve 1954) for trace elements and the 1970s (Fritz et al. 1975) for isotopes (Holmes & De Deckker 2012) provide an historical overview, there are still areas that require clarification.

The shells of Cyprideis torosa and of other members of the genus Cyprideis have been used extensively for trace-element and, to a lesser degree, stable-isotope and strontium-isotope determinations. There are a number of reasons why this genus is a good candidate for geochemical investigations. First, the species C. torosa and, more generally, the genus Cyprideis are widely distributed both geographically and ecologically (Wouters 2016). Cyprideis torosa, in particular, is extremely eurytopic and found in a wide range of water types from near fresh to hypersaline (Sandberg 1964; De Deckker 1981; Athersuch et al. 1989; Wouters & Martens 1994, 2001; Meisch 2000). Secondly, because C. torosa has been the subject of a number of detailed ecological studies (Vesper 1972a,b; 1975; Heip 1976a,b; Herman & Heip 1982; Herman et al. 1983; Schweitzer & Lohmann 1990; Mezquita et al. 2000), much is known about its ecology and life cycle, both of which are important in the interpretation of geochemical records. Third, it produces large, robust shells (up to c. 100 μg per valve for well-calcified adults: Marco-Barba et al. 2012) that are often well preserved in sediments and well suited to geochemical determinations. Finally, the shells of C. torosa show ecophenotypic variability, notably in the presence and strength of noding (Kilenyi 1972; Vesper 1972a,b, 1975; Van Harten 2000; Keyser 2005) and the morphology of sieve pores (Rosenfeld & Vesper 1977). Noded morphotypes, sometimes referred to Cyprideis torosa formosa torosa, are commonly found in fresher water whereas smooth morphotypes (Cyprideis torosa formosa litoralis) are associated with more saline water. However, the relationship between noding and salinity is not simple and some authors, including Van Harten (2000) and Keyser (2005), have suggested that factors other than salinity may also be important controls on ecophenotypic variations in this species. Notwithstanding the fact that the underlying mechanism and environmental significance of these variations are not fully understood, shell morphology is a valuable complementary line of evidence to geochemistry. Moreover, geochemical composition of the shell has some potential to place constraints on morphological variability.

Given the above, it is perhaps not surprising that there have been a good number of studies that have utilized the trace-element and isotope composition of Cyprideis shells. These include investigations into modern populations in natural settings and cultures (for trace elements: Chivas et al. 1986a,b; Wansard et al. 1996; De Deckker et al. 1999; Keatings et al. 2007; Marco-Barba et al. 2012; for stable isotopes: Keatings et al. 2007; Boderget et al. 2014) and analyses of fossil specimens from sediments (for trace elements: Anadon et al. 1987; Gasse et al. 1987; De Deckker et al. 1988a,b; Gibert et al. 1990; De Deckker & Williams 1993; Wansard 1996; Ingram et al. 1998; Holmes et al. 2010; Marco-Barba et al. 2013; Grossi et al. 2015; Wansard et al. 2016; for stable isotopes: Ingram et al. 1998; Keatings et al. 2007; for strontium isotopes: McCulloch & De Deckker 1989; McCulloch et al. 1989; Holmes et al. 2007b; Grossi et al. 2015).

Despite the attraction of Cyprideis as a target for geochemical analyses, quantifying trace-element partitioning and stable-isotope fractionation has proven especially difficult owing to uncertainties surrounding environmental conditions, especially water temperature and composition, at the time of calcification. Although this problem is certainly not unique to Cyprideis torosa, it is exacerbated by the fact that the species is extremely eurytopic. Thus, the temperature and composition of the host water at the time of ostracod collection may differ markedly from those at the time of shell calcification. This means that even with detailed monitoring of the environment and for ostracods that were definitely living at the time of sampling, the exact timing of shell calcification may not be known. Laboratory cultures, in which conditions are controlled, can circumvent this problem, but ostracods grown in the laboratory are often under-calcified and may not be representative of fully calcified individuals (De Deckker et al. 1999; Dettman & Dwyer 2012).

The aim of this paper is to present a critical evaluation of trace-element partitioning and stable-isotope fractionation in Cyprideis and their application to reconstruction of Quaternary palaeoenvironment. Particular focus is placed on C. torosa. We begin with a brief overview of the ecology and life history of Cyprideis torosa, since knowledge of these is vital to our understanding of geochemical signatures. Next, we go on to examine trace-element partitioning: particular emphasis is placed on magnesium and strontium, since these elements have received most attention in the literature. We then examine isotope fractionation: the main focus is on oxygen isotopes, but with additional reference to the more limited body of work on carbon and strontium. After that, we consider the extent to which geochemistry and ecophenotype can provide complementary information. Finally, we conclude by summarizing the application of geochemical signatures to environmental reconstruction in marginal-marine and continental settings and suggest avenues for future research that might allow such reconstructions to be refined.

Ecology and life history of Cyprideis torosa

Cyprideis torosa is found in a wide range of environments and is considered highly eurytopic. It is found in virtually freshwater, brackish and estuarine waters and hypersaline environments, with maximum reported salinity tolerance of 140 g l$^{-1}$ or more (Carbonel & Peypouquet 1983). It is also tolerant of hypoxia (Jahn et al. 1996), high sulphide concentration (Jahn et al. 1996) and high water temperature (Mezquita et al. 2000). The species is commonly associated with waters that are Cl–SO$_4$-dominated and with an alkalinity/Ca ratio of <1. It occurs in modified (i.e. diluted or evaporated) seawater as well as continental water that is saline as a result of evaporative-mineral dissolution or evaporative concentration under dry climate. It is found in a wide range of continental and marginal-marine waters down to depths of 10 m or more (Meisch 2000; Pint et al. 2012) on mud, sandy-mud or algal substrates (Meisch 2000). Population densities can be very high in favourable environments, in which the species is the dominant, or sometimes the sole, constituent of ostracod assemblages (Heip 1976a; Frenzel et al. 2012).

Life cycle appears to vary with environment. Heip (1976a,b) showed that C. torosa has one generation per year but with adults appearing in spring and late summer/autumn. Spring adults produce offspring that mature in late summer/autumn, whereas the late summer/autumn adults produce eggs that overwinter and that moult to adults the following spring. Heip’s study was based on a brackish-water pond (Drevet) in Belgium that had stable salinity, and Heip questioned how applicable his findings would be in other environments. This comment is borne out by studies in Mediterranean waterbodies with highly variable salinity and ionic composition, and high summer temperatures. Here, two or more generations may occur (Mezquita et al. 2000; Marco-Barba et al. 2012), with maximum densities of adults in early summer and mid-autumn to early spring.
Much of our understanding of Cyprideis ecology, life cycle and geochemistry is derived from studies of Cyprideis torosa or the likely conspecific taxon *C. australiensis* Hartmann, which is found in Australia. Although yet to be confirmed by genetic studies, morphological evidence points to *C. australiensis* being a synonym of *C. torosa* (Chivas et al. 1986a; but see Wouters 2016). Within *C. torosa* itself, there is evidence of genetic diversity (Sywula et al. 1995), which may have some relevance to our understanding of differences in calcification and geochemical signatures that have been observed in other species (Ito & Forester 2009; Dettman & Dwyer 2012). We consider this possibility in further detail later in the paper.

### Trace-element partitioning

It has long been known that trace metals co-precipitate in calcite of ostracod shells and that trace-element content, especially of magnesium and strontium, is related to both the chemical composition and temperature of the water in which calcification took place. It has further been suggested that the trace element content of fossil ostracod shells can yield information about past environments (Chave 1954, and reviews in Holmes 1996; Holmes & Chivas 2002; Ito & Forester 2009; Dettman & Dwyer 2012; Holmes & De Deckker 2012). Following the early studies on ostracods grown in vitro by Chivas et al. (1983), trace-metal precipitation in ostracod shell calcite is often described using partition coefficients (K\textsubscript{D}[M]) values, which are empirically derived relationships between the molar M/Ca in the ostracod shell and that in the water in which the ostracod calcified, whereby K\textsubscript{D}[M] = M/C\textsubscript{Ca shell}/M/C\textsubscript{Ca water} and M is a trace element, in this case either magnesium or strontium. Partitioning is classically thought to be governed by the M/Ca of the host water and, for magnesium, water temperature, and to be invariant within species or genera. In some studies, K\textsubscript{D}[M] values have been used to back-calculate the host water’s ionic composition using analyses of ostracods from fossil sequences. Notwithstanding criticism of this approach (Dettman et al. 2002; Dettman & Dwyer 2012) and evidence that trace-element partitioning is more complex than is often thought (e.g. Wansard et al. 1998; Ito & Forester 2009), a survey of K\textsubscript{D}[M] values in *Cyprideis torosa* in different hydrochemical and hydrological settings provides a valuable insight into the complexities of trace-element partitioning in this species (Table 1). For the purposes of this discussion, although *C. torosa* and *C. australiensis* are reported as separate species, we regard them as conspecific in the absence of firm evidence to the contrary.

### Table 1. Published and unpublished K\textsubscript{D} values for *Cyprideis*

<table>
<thead>
<tr>
<th>K\textsubscript{D}[Sr]</th>
<th>K\textsubscript{D}[Mg]</th>
<th>Species</th>
<th>Site details</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.471 ± 0.061</td>
<td></td>
<td><em>C. australiensis</em></td>
<td>Diluted or evaporated seawater. Natural waters, Australia.</td>
<td>Chivas et al. (1986a)</td>
</tr>
<tr>
<td>0.475 ± 0.057</td>
<td>0.0046 ± 0.001</td>
<td><em>C. australiensis</em></td>
<td>Diluted or evaporated seawater. Laboratory cultures. T = 25°C</td>
<td>Chivas et al. (1986a)</td>
</tr>
<tr>
<td>0.223 ± 0.00086</td>
<td>−0.000514 ± 0.00019</td>
<td><em>C. australiensis</em></td>
<td>Marine-type water. Laboratory cultures</td>
<td>De Deckker et al. (1999)</td>
</tr>
<tr>
<td>0.470 ± 0.053</td>
<td>0.0030 ± 0.0012</td>
<td><em>C. torosa</em></td>
<td>Lake Qarun, Egypt. T = 19°C</td>
<td>Keatings et al. (2007)</td>
</tr>
<tr>
<td>0.602 – 0.830</td>
<td>0.0169</td>
<td><em>C. torosa</em></td>
<td>Lake Banyoles, NE Spain. K\textsubscript{D}[Mg] quoted for 25°C</td>
<td>Wansard et al. (1998)</td>
</tr>
<tr>
<td>0.67 ± 0.09</td>
<td>0.002124 ± 0.0037</td>
<td><em>C. torosa</em></td>
<td>The Fleet Lagoon, S. England. T = 19 – 16°C</td>
<td>G. Eisenhauer, unpublished data</td>
</tr>
</tbody>
</table>

*\textsuperscript{T} = \text{temperature (°C)}.*

They found that K\textsubscript{D}[Sr] = 0.223 ± 0.0086 *T*, with K\textsubscript{D}[Sr] at 25°C of 0.438, which is similar to that reported in earlier studies. Keatings et al. (2007) reported a K\textsubscript{D}[Sr] value of 0.470 ± 0.053 from *C. torosa* material collected from Lake Qarun, Egypt, a Na–SO\textsubscript{4}–Cl-type lake with total dissolved solids (TDS) similar to that of seawater.

The above studies lend support to the commonly held view that trace-element partitioning is constant within a species (Table 1). However, other studies have challenged this for *Cyprideis*. Wansard et al. (1998) reported K\textsubscript{D}[Sr] values of between 0.602 and 0.830 from Lake Banyoles, an oligohaline lake (TDS = 0.83 – 1.01 g l\textsuperscript{-1}) in northern Spain. Although these authors speculated that variation could potentially reflect unaccounted-for differences in Sr/C\textsubscript{Ca water}, it seemed unlikely that uncertainties in water chemistry at the time of shell calcification can explain these high values, since the range does not overlap with previously reported K\textsubscript{D}[Sr] values. These authors suggested, rather, that the higher K\textsubscript{D}[Sr] value was more likely to be an outcome of the species’ response to calcification in lower-salinity water. Unpublished data from G. Eisenhauer also indicated higher K\textsubscript{D}[Sr] values, of 0.67 ± 0.09 from the brackish-water end (TDS = 6 g l\textsuperscript{-1}) of the Fleet lagoon in southern England. However, salinity and, presumably, the water Sr/Ca value were variable and the precise value at the time of calcification was not well constrained. This will lead to inevitable uncertainties in the calculated K\textsubscript{D}[Sr] value from this site. Finally, Marco-Barba et al. (2012) reported K\textsubscript{D}[Sr] values of 0.57 ± 0.25 across waters of widely varying TDS in eastern Spain, although all of the waters were derived ultimately from meteoric sources. The wider variability in the K\textsubscript{D}[Sr] values reflects large seasonal variations in Sr/Ca ratio in the water. Interestingly, the Sr/Ca ratios of A-1 shells (A-1 = penultimate moulting stage before adulthood) better tracks the Sr/Ca ratio of the water, which the authors attribute to the short timespan represented by the juvenile stage compared to the adult. Despite this, the K\textsubscript{D}[Sr] values from this study fall between values reported by Chivas and others (Chivas et al. 1986a; De Deckker et al. 1999) and those from Wansard (1996) noted above.

The limited evidence above suggests that for *Cyprideis* shells that have calcified in water of marine-derived composition, it may be possible to back-calculate water composition using the K\textsubscript{D}[Sr] value. We test this assumption using data from a living population of *C. torosa* from a small, shallow tidal inlet at Kyleakin on the Isle of Skye, Scotland (this and other previously unpublished studies presented in this paper are described in detail in the accompanying online supplement). Here, use of the K\textsubscript{D}[Sr] value of 0.475 yields a water Sr/Ca ratio of 0.0079 ± 0.0007 (note that the error term here is derived solely from the variation in Sr/Ca ratios from the single shells from this site and does not take into account the additional uncertainties in the published K\textsubscript{D}[Sr] value). This value is close to the commonly accepted Sr/Ca ratio of seawater of 0.0089 (Chester et al. 2000), when allowance is made for freshwater seepage into the tidal inlet.

Dettman et al. (2002) and Dettman & Dwyer (2012) raised serious concerns over the use of K\textsubscript{D}[M] values to back-calculate water M/Ca values for several reasons, including variation in K\textsubscript{D}[M] values within a species and non-systematic variation of trace metals.
in the ostracod shell with trace metals in the water. However, when dealing with waters of marine origin within certain salinity limits, it may be valid to use a $K_{D}[\text{Sr}]$ value of 0.475 to back-calculate the composition of palaeo-waters, using a similar approach to that described above. The challenge remains to determine what those limits are through further experimental work, noting also the importance of assessing the ionic activities, as well as concentrations, in the ambient water.

Temperature is a significant control on magnesium partitioning into ostracod shells in many circumstances, leading to the possibility that the magnesium content of fossil ostracod shells can be used as a palaeothermometer. Magnesium is generally more strongly partitioned from water into ostracod shells than strontium, reflecting the fact that many natural waters contain relatively high concentrations of magnesium, and borne out by the observation that ostracods appear always to produce low-Mg calcite shells, even in Mg-rich water (De Deckker et al. 1999), and thus not following the thermodynamically driven partition coefficient.

Chivas et al. (1986a) established a $K_{D}[\text{Mg}]$ value of 0.0046 ± 0.001 for C. australiensis calcifying in evolved seawater under experimental conditions at 25°C. Values of 0.0030 ± 0.0012 for Lake Qarun, Egypt (Keatings et al. 2007) and 0.00214 ± 0.0037 for the Fleet lagoon, southern England (G. Eisenhauer, unpublished data) are consistent with lower temperatures at the time of likely calcification at these two sites (c. 19°C and 9–16°C, respectively). De Deckker et al. (1999) quantified the temperature-dependence of magnesium uptake in C. australiensis using culture experiments at 20°C and 25°C and they proposed that $K_{D}[\text{Mg}] = -0.000514 + 0.00019 T$ and if the Mg/Ca of the water is known, temperature can be calculated using the equation $T^\circ C = 2.69 + 5230(\text{Mg/Ca}_{\text{shell}}/\text{Mg/Ca}_{\text{water}})$ i.e. a 1°C rise in temperature leads to c. 5% increase in shell Mg/Ca content.

Here, we test the De Deckker et al. (1999) equation by applying it to two modern datasets, from Kyleakin, Isle of Skye and the Fleet, Dorset. In Figure 1, we show magnesium-inferred temperature to include the range of Mg/Ca values encountered in the Kyleakin material and for a range of water Mg/Ca values from fully marine (Mg/Ca = 5.1) to slightly brackish (Mg/Ca = 4.3) to cover the likely range of water composition at this site. We then show the measured Mg/Ca ratios of the ostracod specimens in the form of a frequency histogram. The results suggest that the mean Mg/Ca-inferred water temperature at Kyleakin is 17.7 ± 3.3°C for Mg/Ca of 5.1 and 20.5 ± 3.9°C for Mg/Ca of 4.3. However, Figure 1 shows that the distribution of Mg/Ca is slightly bimodal, the first (lowest) mode equating to a temperature of 16.0–18.3°C (for Mg/Ca of 5.1 and 4.3, respectively) and the second to 20.1–23.4°C (for Mg/Ca of 5.1 and 4.3, respectively). It is possible that the two modal classes represent calcification in late spring and late summer, as suggested
by life-cycle studies reviewed above. Spot measurements of June temperature in the waterbody were up to 18°C. Given that the ostracods may have calcified in late spring (i.e. before this time, when temperature would have been cooler) and late summer (when warmer), the values are not unreasonable. However, we do not have water chemistry determinations for this site, although measured salinity values are consistent with near-marine salinity, for which the Mg/Cawater value would likely have been close to that of seawater, i.e. c. 5.1. Greater certainty in these temperature estimates would require detailed monitoring of water conditions and of ostracod populations. However, these results suggest that the equation of De Deckker et al. (1999) does have the potential to allow realistic water temperatures to be reconstructed by Mg/Ca ratios in *Cyprideis*. Interestingly, lack of correlation between Sr/Ca and Mg/Ca in this dataset suggests that temperature is not a significant control on strontium partitioning, if we are correct in our interpretation of the Mg/Ca data (Fig. 1).

We further use the equation of De Deckker et al. (1999) to reconstruct water temperature using Mg/Ca from *C. torosa* collected from the Fleet, using unpublished data from G. Eisenhauer (Fig. 2). We note that the sampling locality has a Mg/Ca (c. 3.45) that falls below the range (5 < Mg/Ca < 20) within which De Deckker et al. (1999) suggest the equation is applicable. Despite this, reconstructed temperature values for ambient Mg/Ca (12.7 ± 2.3°C) are reasonable, although small variations in water Mg/Ca could have led to significant changes in ostracod Mg/Ca without any temperature change. Observed, short-term changes in salinity in the brackish water end of the Fleet, coupled with quite wide variation in the Sr/Ca of the measured ostracod specimens (Fig. 2), imply that shifts in Mg/Ca might indeed have influenced the ostracod shell magnesium content. Figure 2 illustrates the relatively high sensitivity of temperature reconstructions to water Mg/Ca at levels <5, suggesting that our reconstructions probably have quite large uncertainties.

Other studies have shown a less obvious, or different, relationship between Mg/Ca and water temperature. Marco-Barba et al. (2012), in a detailed study of *C. torosa* from coastal lagoons in eastern Spain, found lack of a clear control on Mg/Ca in ostracod shells either by Mg/Cawater or temperature, although this may have arisen from seasonal variability of water conditions and lack of close coupling between water conditions at the time of shell sampling with those at the time of calcification. In any case, the apparent discrepancy between the findings of this Spanish study and other studies remains unresolved, as noted by Wansard et al. (2016).

Wansard (1996) and Wansard et al. (2016) proposed a different equation relating Mg/Ca and temperature for Lake Banyoles, northeastern Spain. Here, the maximum and minimum Mg/Ca ratios in the shells of a population of living ostracods were regarded as representing the minimum calcification temperature for *C. torosa* (derived from Heip 1976a) and the maximum annual water temperature at the site, respectively. The mean Mg/Ca was deemed to represent mean water temperature at the time of calcification. These three data points are described by an equation

\[ T^\circ C = 3.3 + 1971 \frac{\text{Mg/Ca}_{\text{shelling}}}{(\text{Mg/Ca}_{\text{water}})} \]

(De Deckker et al., 1999)
following Heip’s (1976a) observations. However, given that the lake is deep and seasonally stable, this is probably reasonable. The equation also clearly differs from the one of De Deckker et al. (1999), although when applied to fossil ostracods from a sediment record from Lake Banyoles, it yielded reasonable values that compared very well with palaeotemperature reconstructions from other proxies and different archives within the western Mediterranean region (Wansard et al. 2016). The difference in the relationships between temperature and ostracod shell Mg/Ca relationship in Lake Banyoles compared to other settings may represent an adaptation to calcification in low-salinity water (Mg/Ca in Lake Banyoles is 0.64 and TDS = 0.83–1.01 g l$^{-1}$). Clearly, application of either the Wansard (1996) equation to water of marine-like composition, or the De Deckker et al. (1999) equation to dilute water with low Mg/Ca, would yield unreasonable results.

We further test palaeotemperature and palaeo-Mg/Ca reconstruction using fossil specimens of C. torosa from Allhallows, a site in eastern England (Fig. 3). The Allhallows sequence, which dates to Marine Isotope Stage 9, lies at the confluence of the Proto-Thames and Proto-Medway estuaries (Bates et al. 2002). The sediments under discussion here were laid down in a slightly brackish environment on a low-energy floodplain (Bates et al. 2002), with elevated salinity the result of mixing of marine and freshwater. Faunal evidence suggests that TDS was no more than about 5 g l$^{-1}$ and the ostracod assemblages, which comprise adults and a full range of juvenile valves, were likely in situ (Bates et al. 2002). Given that the former waterbody was a mixture of marine and meteoric water, it is appropriate to use the equations of De Deckker et al. (1999) in our reconstructions.

We assume that the Sr/Ca ratio at Allhallows can be regarded as a sensitive tracer for marine input to the water for this low-salinity site. We use the regression equation of De Deckker et al. (1999) to calculate the past Sr/Ca ratio of Allhallows water i.e. Sr/Cawater = 1.9799 Sr/CaCyprideis + 0.00144 to give a mean Sr/Cawater value of 0.00496. If we assume, as a rough approximation, that Allhallows water was a mixture of chalk-derived groundwater (TDS = 0.5 mg l$^{-1}$, Sr content = 0.35 mg l$^{-1}$ and Sr/Ca = 0.0012) and seawater (Sr content = 7.7 mg l$^{-1}$ and Sr/Ca = 0.0089) in a two-component mixing model, a Sr/Ca ratio of 0.00496 would be equivalent to about 4% seawater in the mixture and a TDS of about 2 g l$^{-1}$ (Fig. 3). This is broadly consistent with the ostracod assemblages, which indicate a salinity of no more than 5 g l$^{-1}$ (Bates et al. 2002). A similar mixing model for magnesium (groundwater Mg/Ca = 0.03 and Mg content = 15 mg l$^{-1}$; seawater Mg content = 1290 mg l$^{-1}$ and Mg/Ca = 5.1) would suggest a Mg/Ca ratio for Allhallows water of 4.92. We then use this value and the Mg/Ca ratios for the ostracods to calculate water temperature, using the equation of De Deckker et al. (1999) quoted above, and derive a value of 10.8 ± 1.4 °C. The uncertainties expressed here are based on variations in ostracod Mg/Ca values alone and do not take into account uncertainty in the Sr/Ca-inferred Mg/Ca ratio of the water, or uncertainties in the Mg/Ca-temperature equation, and so are
unrealistically small. We can, however, evaluate the reconstructed temperatures using the MOTR (Mutual Ostracod Temperature Range) reconstruction method (Horne 2007) based on the ostracod faunal assemblages, which gives a mean July temperature range of 15–21 °C (Bridgland et al. 2013). Although higher than the Mg/Ca-inferred temperature, it is possible that the ostracods calcified earlier in the year, under cooler conditions. However, we note no evidence of bimodality in the Mg/Ca ratios at Allhallows, suggesting that the life cycle of C. torosa at this site may have differed from that described by Heip (1976a) and evident in the Kyleakin Mg/Ca data referred to above.

We can offer some additional observations about the temperature sensitivity of C. torosa and general comments about the use of Sr/Ca and Mg/Ca equations. For magnesium, there is no single relationship between Mg/Ca of shells and either water temperature or water Mg/Ca ratio if the water’s ionic composition differs significantly from that of seawater. For Sr/Ca, the same conclusions hold for water Sr/Ca ratio. However, for seawater or water that has a seawater-type composition (modified by dilution or evaporation), it would appear possible to derive reasonable estimates of water temperature and water Sr/Ca ratio using the equations of De Deckker et al. (1999), as shown above, especially since water temperature appears to have only minimal influence on strontium partitioning. However, the challenge remains to determine how far water can depart from marine composition for these equations to remain valid. Additional culture experiments and field collections under closely monitored conditions, coupled with palaeoenvironmental reconstructions that can be verified using other proxies, are clearly needed. For waters that depart markedly from marine composition, different relationships exist between M/Ca \(_{\text{water}}\) and M/Ca\(_{\text{shells}}\), and between Mg/Ca\(_{\text{shells}}\) and temperature. These require further investigation, as does the possible temperature dependence of strontium uptake that was suggested by De Deckker et al. (1999).

Overall, these findings confirm the suggestions of Dettman & Dwyer (2012) and Marco-Barba et al. (2012) that euryhaline ostracod species may employ contrasting calcification strategies in dilute and saline waters and, in particular, in waters with differing ionic composition.

Isotope fractionation

Non-marine ostracod shells have been used somewhat extensively for isotope determinations, mostly for oxygen and carbon isotopes, more rarely for strontium isotopes. They are a valuable source of material for isotope analyses given their abundance and good preservation in many lacustrine and estuarine sediments. The advantage also is that examination of the ostracod valves can assure us as to whether or not they are still in pristine condition. On the contrary, isotopic analysis of bulk sediment cannot be assured to relate to the original precipitation of carbonate crystals as some may be secondary or have precipitated interstitially.

Oxygen

Numerous studies have demonstrated that ostracod shells typically show positive offsets from oxygen-isotope equilibrium, and that the magnitude of the offsets tends to be taxon-specific (von Grafenstein et al. 1999; Decarosy 2012). Quantifying the magnitude of offsets from oxygen-isotope equilibrium is important if data from different species are compared or in cases where the oxygen-isotope values are used to calculate water temperature or water composition using equations that assume oxygen-isotope equilibrium between calcite and water. Moreover, the magnitude of offsets from equilibrium may provide insights into the calcification process (Keatings et al. 2002). Quantifying disequilibrium for individual ostracod species has relied on ostracods collected from natural settings in which the water temperature, water isotope composition, or both, are near constant (von Grafenstein et al. 1999; Keatings et al. 2002) or raised in controlled cultures (Chivas et al. 2002).

Defining the offset for C. torosa has proved difficult, however, because it tends to inhabit rapidly changing conditions, such as estuaries, where it abounds as discussed earlier. Despite this, there have been several attempts to quantify offsets from equilibrium for this species. Bodergat et al. (2014) undertook seasonal sampling of C. torosa from a Mediterranean coastal lagoon. They determined a weak negative correlation between temperature and oxygen-isotope fractionation, although noted large variation in oxygen-isotope values in ostracods collected at any one time, a response to rapidly changing conditions within the lagoon, which precluded the calculation of a vital offset value. Similar conclusions were reached by Marco-Barba et al. (2012). However, Keatings et al. (2007) calculated a vital offset of ≤0.8‰ based on analyses of multiple-shell samples of C. torosa from Lake Qarun, Egypt. Owing to greater seasonal stability of this site, coupled with a reasonable knowledge of the timing of calcification of C. torosa within this lake, the value is probably reasonable. Moreover, we note that it falls within the range of values quoted for other species (Holmes & Chivas 2002).

Carbon

There is limited information about carbon isotope fractionation in C. torosa. Its benthic habitat suggests that it will record a signal from the dissolved inorganic carbon (DIC) at the sediment–water interface. This was confirmed by Marco-Barba et al. (2012), who found that the carbon-isotope values in C. torosa in a Mediterranean coastal lagoon were typically up to 4‰ depleted in \(^{13}\text{C}\) compared with the bulk waterbody DIC, but also highly variable. This observation is also consistent with the fact that Cyprideis is a detritivore as seen by P. De Deckker (unpublished observations) for C. torosa in lakes (étagus) of the Carmargue (France) and at Divegat (Belgium) and for C. australiensis in Dip Lake and Salt Dip Lake (South Australia).

Strontium

A number of studies have utilized strontium-isotope analyses of C. torosa shells (e.g. McCulloch et al. 1989) for C. australiensis; McCulloch & De Deckker (1989) for C. torosa). Such analyses are especially applicable to this species in marginal-marine environments, which are characterized by the mixing of spatially and temporally variable proportions of marine and meteoric water, both components often having contrasting \(^{87}\text{Sr}/^{86}\text{Sr}\) ratios and strontium concentrations. Because of the small relative difference between the masses of the respective isotopes of strontium, it is generally assumed that there is no isotope fractionation between strontium in water and strontium in carbonate, an assumption that has been supported for ostracods (albeit not C. torosa: Holmes et al. 2007b). Moreover, even in cases where aragonite precipitates from the host water, its \(^{87}\text{Sr}/^{86}\text{Sr}\) will not change. The papers by McCulloch et al. (1989) and McCulloch & De Deckker (1989) have demonstrated very clearly that \(^{87}\text{Sr}/^{86}\text{Sr}\) analyses of C. torosa and associated species can be used to pinpoint changes in water composition associated with the mixing of marine and meteoric waters during, for example, marine transgression and regression.

Ecophenotypic variations

In situations where smooth and noded morphotypes of C. torosa are found together in modern environments or stratigraphic sequences, it might be expected that, if all other factors remain constant, the geochemical indicators in noded forms would be consistent with
lower salinity and in smooth forms with higher salinity. Here, we evaluate several studies of the different morphotypes to determine whether geochemistry can add anything to the noding debate for C. torosa.

An Loch Mór is a 23 m-deep, brackish (TDS = 4–6 gl⁻¹), karstic lake on the island of Iniss Oírr, Galway Bay, Ireland. It is fed by a mix of groundwater and seawater from subsurface seepage. Cyprideis torosa shells are preserved sporadically in the Holocene lake sediments and the species still lives in the present-day lake. Smooth or very weakly noded specimens are found in the early Holocene, whereas more recent individuals are weakly to moderately noded; modern specimens are generally strongly noded (Holmes et al. 2007a). Conventional interpretation of the stratigraphic distribution of the different morphotypes would envisage change from a more saline lake in the early Holocene to lower salinity at present. An inference of elevated salinity during the very early Holocene is not, however, consistent with much lower sea-level at this time, so if it is correct, may require sea-level models for this part of western Ireland to be re-evaluated. We present strontium-isotope analyses of ostracod shells from four levels within the core, as well as from the modern lake, in order to test this interpretation. Although other palaeosalinity indicators are available for the sediments from An Loch Mór (Holmes et al. 2007a), strontium-isotope analyses have the advantage that they provide a direct record of the strontium-isotope composition of the water in which the ostracod specimens calcified. We assume that the strontium-isotope composition of lake water at any time is a function of the relative contributions of strontium from limestone-derived...
groundwater and seawater, described by a two-component mixing model (Fig. 4), and that the strontium-isotope composition of the ostracod shells is a direct measure of the strontium-isotope composition of the water in which they calcified. Strontium-isotope analyses of the modern specimens suggest that they calcified in water with TDS between 5 and 7 g l$^{-1}$, which is consistent with direct measurements of TDS in the modern lake. Specimens from the early and mid-Holocene have low strontium-isotope values that differ markedly from modern seawater values, consistent with TDS between 1.3 and 2.5 g l$^{-1}$. Salinity values only rise to near present-day values after about 1 ka BP (Holmes et al. 2007a). These inferred salinity values are consistent with other palaeoecological information from the lake, all of which suggests that it was fresh or slightly brackish. This information is not consistent with the presence of mainly smooth morphotypes of C. torosa shell during the early Holocene. Lack of sensitivity in the strontium-isotope salinity proxy is an unlikely explanation (Fig. 4): more likely is that the morphology of specimens in this lake is a response to factors other than, or in addition to salinity, as has been suggested previously by Van Harten (2000).

We also report Mg/Ca and Sr/Ca in specimens of C. torosa from Allhallows, the site described in a previous section. Here, we analysed smooth and noded forms from level OC8 (Fig. 5), although these were lumped together in the previous discussion. Population structure suggests that the smooth and noded forms were in situ and had not been transported, although we cannot say whether they actually co-existed in a life assemblage, since level OC8 in the sequence would have accumulated over a lengthy time period – likely hundreds of years – and potentially different life assemblages could have been conflated. The Mg/Ca and Sr/Ca for the smooth forms were slightly higher than for the noded forms, although the differences are not statistically significant (Fig. 5), leading us to conclude that shell chemistry does not support the use of shell morphology in C. torosa as a sensitive salinity indicator.

Several other studies have found little or no significant difference between the chemical composition of noded and smooth shells where these co-exist (Wansard et al. 1998, 2016; Marco-Barba et al. 2012). Moreover, Grossi et al. (2015) cast similar doubt over the use of ecophenotypic variations in the congener C. agrigentina Decima, from the European Miocene, as an indicator of palaeosalinity based on morphological and geochemical signatures. Bodergat et al. (1991) analysed the trace-element content and noding of C. torosa valves for European waters of contrasting salinity and found variations in morphology and trace-element composition with environmental variables, although no simple relationship between shell composition and morphology. However, the balance of evidence from these studies confirms the long-held view that factors other than, or as well as, salinity must explain noding in C. torosa and other members of the genus.

**Discussion and conclusions**

As a widely occurring taxon that is often present in large numbers, producing robust shells that are often well preserved in sediments, *Cyprideis torosa* is a promising target for geochemical analyses. Its common occurrence and abundance has led to a relatively large number of geochemical calibration studies having been undertaken, as reviewed above, meaning that we have reasonable understanding of trace-element partitioning and stable-isotope fractionation in this species. Furthermore, its eurytopic nature means it is commonly found throughout a sedimentary succession, even in cases where there have been major variations in inferred environmental conditions. Where such a situation occurs, it provides the added advantage that a single taxon can be analysed throughout the sequence.

Analyses of *Cyprideis* from natural waters and *in vitro* cultures has provided a wealth of information about trace-element partitioning. In waters of marine-type composition, there is strong evidence that strontium partitioning is constant. This provides the possibility of using the strontium content of *Cyprideis* shells to reconstruct the Sr/Ca of the host water under clearly defined conditions. Despite evidence for weak thermodependence of strontium uptake for *C. australiensis* (De Deckker et al. 1999), changes in the Sr/Ca of the host water have a greater influence on the strontium content of *Cyprideis* shells. The ability to reconstruct Sr/Ca of palaeo-waters is especially important in settings where there has been mixing of meteoric and marine water. Given that continental water often has contrasting strontium content and Sr/Ca to marine water, mixtures of two components have Sr/Ca values that relate to salinity (Anadón et al. 2002a), allowing past salinity to be estimated in some cases (Mazzini et al. 1999; Anadón et al. 2002a, b; Holmes et al. 2010; Marco-Barba et al. 2013).

For continental waters in which the ionic composition departs significantly from that of seawater, strontium partitioning into the shells of *Cyprideis* may differ, meaning that it may be difficult to back-calculate the Sr/Ca ratio of the water. However, if it can be shown that the Sr/Ca of waters varies from mineral precipitation as a result of evaporative concentration, Sr/Ca ratios in *Cyprideis* shells
can be used as a valuable tracer of evaporative history and, hence, change in effective moisture. Such an approach has been used to good effect for Neogene and Quaternary sequences (e.g. Anadón et al. 1987, 1994, 2002b; Gasse et al. 1987; Anadón & Julià 1990; Gibert et al. 1990; Ghetti et al. 2002).

For magnesium, there is strong thermodependence of partitioning, leading to the possibility that magnesium content of Cyprideis can be used to infer past temperature. In cases where water composition is, or was, marine-derived, this approach can yield reliable estimates of palaeotemperature using the equation of De Deckker et al. (1999). In dilute waters, different relationships exist between temperature and magnesium uptake, although if water Mg/Ca remains constant and the relationship between temperature and magnesium uptake can be well defined, as was the case for the Lake Banyoles study (Wansard 1996; Wansard et al. 2016), robust temperature reconstructions can be achieved although it is likely that specific equations will need to be produced for individual water types. Where water composition varies markedly, this can override the temperature signal. In continental lakes subject to evaporative enrichment, Mg/Ca values of Cyprideis may complement Sr/Ca values as indicators of evaporative history (see references cited above for Sr/Ca).

For oxygen isotopes, there is limited evidence to show that Cyprideis shells show positive offsets from oxygen-isotope equilibrium, as has been demonstrated for most other taxa investigated. However, accurate quantification of this offset has been confounded by the species’ eurytopic nature. Even without an accurate assessment of the vital offset, shells of Cyprideis have been used to reconstruct past environments for Pleistocene and Holocene sequences, especially from continental lakes (Anadón et al. 1994, 2002a; Dixit et al. 2014a, b, 2015) and marginal-marine sequences (Mazzini et al. 1999; Anadón et al. 2002b; Ghetti et al. 2002; Marco-Barba et al. 2013).

Differences in trace-element partitioning and isotope fractionation into Cyprideis shells under conditions of contrasting salinity and water ionic composition suggest that it may show different calcification mechanisms under different environmental conditions. Carbon-isotope values of Cyprideis are consistent with the fact that it is a detritivore. Finally, strontium-isotope analyses of Cyprideis shells provide excellent evidence for mixing of marine and meteoric waters in marginal-marine settings.

Coupling of geochemical analyses with ecophenotypic assessments suggests that morphological variations in Cyprideis, especially the occurrence of noding, are not a simple response to salinity, implying that noding is controlled by additional factors and not salinity alone. These observations further suggest that the use of noding in fossil Cyprideis shells as an indicator of salinity should be approached with caution.

Despite the value of Cyprideis shells in geochemical studies, there is a clear need for further investigations. More studies are needed of trace-element partitioning in waters of different ionic composition, especially in order to validate the use of existing palaeotemperature questions or to propose alternative relationships. Further work is required to confirm the offset from oxygen-isotope equilibrium in Cyprideis so that analyses can be used in quantitative reconstructions as has been done for other species (e.g. von Grafenstien et al. 1999). Additional investigations into the life cycle of the species are urgently needed, especially in different climatic regimes and in waters of contrasting composition. Genetic studies are needed to assess whether species such as C. australiensis are truly conspecific with C. torosa, as morphological evidence suggests is the case. The eurytopic character of Cyprideis torosa means that it is adapted to fluctuating conditions on short timescales. Analyses of multiple single shells from a stratigraphic level often reveal marked inter-shell geochemical variability as a result. Analyses of large numbers of individuals may, therefore, often be required in order to characterize ‘mean’ conditions. That said, the amount of inter-shell geochemical variability within a stratigraphic layer might provide valuable insights into short-term (seasonal or inter-annual) fluctuations in the past environment (Holmes 2008; Dixit et al. 2015). Finally, it is important to carry out palaeoecological investigations of ostracod assemblages in parallel with ostracod shell chemical analyses. A thorough assessment of an ostracod assemblage can inform whether or not this material has been reworked/transported. If the assemblage is mixed and includes, for example, several taxa with contrasting salinity ranges or specimens with varying preservation, the sample may relate to different periods of deposition.

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