Manuscript for special issue of Journal of Micropalaeontology on *Cyprideis torosa*

Version 3 (revision, based on referees' comments): last edited 12 January 2016

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

Jonathan A. Holmes[1] & Patrick De Deckker[2]

[1]Environmental Change Research Centre, Department of Geography, University College London, Pearson Building, Gower Street, London, WC1E 6BT, UK

[2]Research School of Earth Sciences, The Australian National University, Canberra ACT2600, Australia

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 the *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.

Supplementary material: descriptions of sites, materials and methods for previously unpublished studies are available at http://www.geolsoc.org.uk/SUP…..

Keywords Magnesium/calcium, strontium/calcium, strontium isotopes, oxygen isotopes, carbon isotopes

INTRODUCTION

The use of ostracod shells for geochemical analyses is now well established (Holmes and 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 shell reflects that of strontium-isotope composition 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 pCO_2. 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 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., 2011; Decrouy, 2012).

Clearly, despite understanding having moved a long way since the techniques were first proposed in the 1950s (e.g. Chave, 1954) for trace elements and 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, this issue). *Cyprideis torosa* in particular is extremely eurytopic and found in a wide range water types from near fresh to hypersaline (Sandberg, 1964; De Deckker, 1981; Wouters & Martens, 1994, 2001; Athersuch et al., 1989: Meisch, 2000). Second, 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 & Lohman, 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 ~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 (Vesper, 1972a,b, 1975; Kilenyi, 1972; Van Harten, 2000; Keyser, 2005) and the morphology of sieve pores (Rosenfeld & Vesper, 1977). Noded morphotypes, sometimes referred to *Cyprideis torosa* forma *torosa,* are commonly found in fresher water whereas smooth morphotypes (*Cyprideis torosa* forma littoralis) 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., 1998; De Deckker et al., 1999; Keatings et al., 2007; Marco-Barba et al., 2012: for stable isotopes; Keatings et al., 2007; Bodergat et al., 2014) and analyses of fossil specimens from sediments (for trace elements; Anadón et al., 1987; Gasse et al., 1987; De Deckker et al., 1988a,b; Gibert et al., 1990; De Deckker and Williams, 1993; Wansard, 1996; Ingram et al., 1998; Holmes et al., 2010; Marco-Barba et al., 2013; Grossi et al., 2015; Wansard et al., this issue: for stable isotopes; Ingram et al., 1998; Keatings et al., 2007: for strontium isotopes; McCullogh et al., 1989, McCullogh & De Deckker, 1989; Holmes et al., 2007; 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 (Dettman & Dwyer, 2012; De Deckker et al., 1999).

The aim is 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 palaeoeonvironment. 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 ecophenotypy 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 euryotopic. It is found in virtually freshwater, brackish and estuarine waters and hypersaline environments, with maximum reported salinity tolerance of 140 gL^{-1} or more (Carbonel & Peypouquet, 1983). It is also tolerant of hypoxia (Jahn et al., 1996), high sulfide 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 less than 1. It occurs in modified (i.e. diluted or evaporated) seawater as well as continental water that is saline as a result of evaporate-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 metres 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 spring and late summer/autumn. Spring adults produce offspring that mature in late summer/autumn, whereas the latter adults produce eggs that over-winter and that moult to adults the following spring. Heip's study was based on a brackish-water pond (Dievegat) 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*, 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, this issue). 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 with calcite of ostracod shells and that trace-element content, especially that 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_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 $K_D[M] = M/Ca_{shell}/M/Ca_{water}$ 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_D 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_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. 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.

Chivas et al. (1986) undertook analyses of shells of *C. australiensis* that had calcified in water of marine composition but of varying salinity (from dilution or evaporation of seawater) and reported $K_D[Sr]$ value of 0.471 ± 0.061 for specimens collected from natural settings, and 0.475 ± 0.057 for specimens from laboratory cultures. De Deckker et al. (1999) undertook further experimental cultures in marine-type water and noted, in contrast to their previous experiments, a small temperature dependence of strontium uptake of about 2% $^{\circ}C^{-1}$, along with a strong dependence of strontium in the shell on that in the host water. They found that $K_D[Sr] = 0.223 + 0.0086T$, with $K_D[Sr]$ at 25˚C of 0.438, which is similar to that reported in earlier studies. Keatings et al. (2007) reported a $K_D[Sr]$ value of 0.470 ± 0.053 from *C. torosa* material collected from Lake Qarun, Egypt, a $Na\text{-}SO_4\text{-}Cl$-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 $K_D[Sr]$ values of between 0.602 and 0.830 from Lake Banyoles, an oligohaline lake (TDS = 0.83 – 1.01 gL^{-1}) in northern Spain. Although these authors speculated that variation could potentially reflect unaccounted-for differences in Sr/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 $K_D[Sr]$ values. The authors suggested, rather, that the higher $K_D[Sr]$ 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 $K_D[Sr]$ values, of

0.67 ± 0.09 from the brackish-water end (TDS = 6 gL^{-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_D[Sr] value from this site. Finally, Marco-Barba et al. (2012) reported K_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_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 moult 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_D[Sr] values from this study fall between values reported by Chivas and others (Chivas et al., 1986; De Deckker et al., 1999) and those from Wansard (1986) 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_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_D[Sr] value of 0.475 yields 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_D[Sr] value). This value is close to the commonly-accepted Sr/Ca ratio of seawater of 0.0089 (Chester, 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 $K_D[M]$ values to back-calculate water M/Ca values for several reasons, including variation in $K_D[M]$ 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 $K_D[Sr]$ 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 $K_D[Mg]$ 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 (~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 $K_D[Mg] = -0.000514 + 0.00019T$ and if the Mg/Ca of the water is known, temperature can be calculated using the equation $T^\circ C = 2.69 + 5230(Mg/Ca_{shell}/Mg/Ca_{water})$ i.e. a 1˚C rise in temperature leads to ~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 Fig. 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_{water} of 5.1 and 20.5 ± 3.9 ˚C for Mg/Ca_{water} of 4.3. However, Fig. 1 shows that the distribution of Mg/Ca is slightly bimodal, the first (lowest) mode equating to a temperature of 16.0 to 18.3˚C (for Mg/Ca_{water} of 5.1 and 4.3, respectively) and the second to 20.1 to 23.4˚C (for Mg/Ca_{water} 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/Ca_{water} value would likely have been close to that of seawater, i.e. ~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 (~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/Ca_{water} 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. (this issue).

Wansard (1996) and Wansard et al. (this issue) 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˚C = 3.3 + 1971 Mg/Ca. This equation assumes that peaks in *C. torosa* calcification in this lake occur in late spring and autumn, following Heip's (1976) 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., this issue). 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 is low-salinity water (Mg/Ca in Lake Banyoles is 0.64 and TDS = 0.83 – 1.01 gL^{-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-M/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 gL^{-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 water body 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/Ca_{water} = 1.9799 $Sr/Ca_{Cyprideis}$ + 0.00144 to give a mean Sr/Ca_{water} value of 0.00496. If we assume, as a rough approximation, that Allhallows water was a mixture of chalk-derived groundwater (TDS = 0.5 mgL^{-1}, Sr content = 0.35 mgL^{-1} and Sr/Ca = 0.0012) and seawater (Sr content = 7.7 mgL^{-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 gL^{-1} (Fig. 2). This is broadly consistent with the ostracod assemblages, which indicate a salinity of no more than 5 gL^{-1} (Bates et al., 2002). A similar mixing model for magnesium (groundwater Mg/Ca = 0.03 and Mg content = 15 mgL^{-1}; seawater Mg content = 1290 $mgL^{-1}$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 of uncertainties in the Mg/Ca-temperature equation, and so are unrealistically small. We can, however, evaluate the reconstructed temperatures using 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 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 holds 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 water and M/Ca shell, and between Mg/Ca shell 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; Decrouy, 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}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 (étangs) 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. McCullogh 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 $^{87}Sr/^{86}Sr$ 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 $^{87}Sr/^{86}Sr$ will not change. The papers by McCulloch et al. (1989) and McCulloch & De Deckker (1989) have demonstrated very clearly that $^{87}Sr/^{86}Sr$ analyses of *C. torosa* and associate 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-metre-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 sea-water 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., 2007b), 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 sea water, 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 gL^{-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 gL^{-1}. Salinity values only rise to near present-day values after about 1000 years BP (Holmes et al., 2007). 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 previously been suggested 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 of no significant difference between the chemical composition of noded and smooth shells where these co-exist (Wansard et al., 1998; Marco-Barba et al., 2012; Wansard et al. this issue). Moreover, Grossi et al. (2015) cast similar doubt over the use of ecophenotypic variations in the congener *C. agrigentina*, 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 confirm 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 for 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 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 effective moisture. Such an approach has been used to good effect for Neogene and Quaternary sequences (e.g. Gasse et al., 1988; Anadón and Julià, 1990; Gibert et al., 1990; Anadón et al., 1987, 1994; 2002b; 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., in press), 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 environment 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 are clear needs 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 reconstitutions as has been done for other species (e.g. von Grafenstein 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 interannual) fluctuations in the past environment (Holmes, 2008; Dixit et al., 2015). Finally, it is important to carry out palaeoecological investigations of ostracod assemblage 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.

ACKNOWLEDGEMENTS

We thank Dave Horne and John Whittaker for providing material from Kyleakin and Allhallows, respectively, and Gary Eisenhauer for allowing us to quote unpublished data from the Fleet from his PhD thesis. We are grateful to Steffen Mischke and Jessica Reeves for their detailed and thought-provoking comments on an earlier version of the paper, and to Alan Lord for editorial handling.

REFERENCES

Anadón, P. & Julià, R. 1990. Hydrochemistry from Sr and Mg contents of ostracodes in Pleistocene lacustrine deposits, Baza Basin (SE Spain). *Hydrobiologia*, **197**: 291-303.

Anadón, P., Gliozzi, E. & Mazzini, I. 2002a. Palaeoenvironmental reconstruction of marginal marine environments from combined palaeoecological and geochemical analyses on ostracods. *In*: Holmes, J.A. & Chivas, A.R. (eds.) *The Ostracoda: Applications in Quaternary Research*. American Geophysical Union, Washington DC, Geophysical Monograph, 227-247.

Anadón, P., Ghetti, P. & Gliozzi, E. 2002b. Sr/Ca, Mg/Ca ratios and Sr and stable isotopes of biogenic carbonates from the Late Miocene Velona Basin (central Apennines, Italy) provide evidence of unusual non-marine Messinian conditions. *Chemical Geology*, **187**: 213-230.

Anadón, P., Julià, R., De Deckker, P., Rosso, J.C. & Soulié-Marsche, I. 1987. Contribución a la paleolimnologia del Pleistoceno inferior de la cuenca da Baza (Sector Orce-Venta Micena). *Paleontologia i Evolució. Memoria Especial,* **1:** 35-72.

Anadón, P., Utrilla, R. & Julià, R. 1994. Paleoenvironmental reconstruction of a Pleistocene lacustrine sequence from faunal assemblages and ostracode shell geochemistry, Baza Basin, SE Spain. *Palaeogeography Palaeoclimatology Palaeoecology*, **111**: 191-205.

Athersuch, J., Horne, D.J. & Whittaker, J. 1989. Marine and Brackish Water Ostracods. Linnean Society. Brackish-water Science Association. E. J. Brill, Leiden.

Bates, M.R., Keen, D.H., Whittaker, J.E., Merry, J.S. & Wenban-Smith, F.F. 2002. Middle Pleistocene molluscan and ostracod faunas from Allhallows, Kent, UK. *Proceedings of the Geologists Association*, **113**: 223-236.

Bodergat, A.-M., Rio, M. & Andréani, A.-M. 1991. Composition chimique et ornementation de *Cyprideis torosa* (Crustacea, Ostracoda) dans la domaine paralique. *Oceanologica Acta*, **14**: 505-514.

Bodergat, A.M., Lecuyer, C., Martineau, F., Nazik, A., Gurbuz, K. & Legendre, S. 2014. Oxygen isotope variability in calcite shells of the ostracod *Cyprideis torosa* in Akyatan Lagoon, Turkey. *Journal of Paleolimnology*, **52**: 43-59.

Bridgland, D.R., Harding, P., Allen, P., Candy, I., Cherry, C., George, W., Horne, D.J., Keen, D.H., Penkman, K.E.H., Preece, R.C., Rhodes, E.J., Scaife, R., Schreve, D.C., Schwenninger, J-L., Slipper, I., Ward, G.R., White, M.J. White, T.S. & Whittaker, J.E. 2013. An enhanced record of MIS 9 environments, geochronology and geoarchaeology: data from construction of the High Speed 1 (London–Channel Tunnel) rail-link and other recent investigations at Purfleet, Essex, UK. *Proceedings of the Geologists' Association,* **124:** 417–476

Carbonel, P. & Peypouquet, J.P. 1983. Ostracoda as indicators of ionic concentrations and dynamic variations: methodology (Lake Bogoria, Kenya). *In*: Maddocks, R.F. (ed.) *Applications of Ostracoda*, 264-276.

Chave, K.E. 1954. Aspects of the biogeochemistry of magnesium, I. calcareous marine organisms. *Journal of Geology*, **62**: 266-283.

Chester, R. 2000. *Marine Geochemistry.* Blackwell, Malden MA (2nd Ed.).

Chivas, A.R., De Deckker, P. & Shelley, J.M.G. 1983. Magnesium, strontium and barium partioning in non marine ostracod shells and their use in paleoenvironment reconstructions - a preliminary study. *In*: Maddocks, R.F. (ed.) *Applications of Ostracoda*, 238-249.

Chivas, A.R., De Deckker, P. & Shelley, J.M.G. 1986a. Magnesium and strontium in non-marine ostracod shells as indicators of palaeosalinity and palaeotemperature. *Hydrobiologia*, **143**: 135-142.

Chivas, A.R., De Deckker, P. & Shelley, J.M.G. 1986b. Magnesium content of non-marine ostracod shells: a new palaeosalinometer and palaeothermometer. *Palaeogeography Palaeoclimatology Palaeoecology*, **54**: 43-61.

Chivas, A.R., De Deckker, P., Wang, S.X. & Cali, J.A. 2002. Oxygen-isotope systematics of the nektic ostracod *Australocypris robusta*. *In*: Holmes, J.A. & Chivas, A.R. (eds.) The Ostracoda: Applications in Quaternary Research. AGU, Washingtson D.C., *American Geophysical Union, Geophysical Monograph,* **131:** 301-313.

De Deckker, P. 1981. Ostracods of athalassic saline lakes - a review. *Hydrobiologia*, **81**: 131-144.

De Deckker, P. & Williams, M.A.J. 1993. Lacustrine paleoenvironments of the area of Bir Tarfawi-Bir Sahara East reconstructed from fossil ostracods and the chemistry of their shells. *In:* Wendorf, F., Schild, R., Close, A.E, and associates (eds.) *Egypt during the Last Interglacial. The Middle Paleolithic of Bir Tarfawi and Bir Sahara East.* (eds.) 115-119. Plenum Press, New York.

De Deckker, P., Chivas, A.R. & Shelley, J.M.G. 1988a. Paleoenvironment of the Messinian Mediterranean "Largo Mare" from strontium and magnesium in ostracode shells. *Palaios*, **3**: 352-358.

De Deckker, P., Chivas, A.R., Shelley, J.M.G. & Torersen, T. 1988b. Ostracod shell chemistry: a new palaeoenvironmental indicator applied to a regressive/transgressive record from the Gulf of Carpentaria, Australia. *Palaeogeography Palaeoclimatology Palaeoecology*, **66**: 231-241.

De Deckker, P., Chivas, A.R. & Shelley, J.M.G. 1999. Uptake of Mg and Sr in the euryhaline ostracod *Cyprideis* determined from in vitro experiments. *Palaeogeography Palaeoclimatology Palaeoecology*, **148**: 105-116.

Decrouy, L., 2012. Biological and environmental controls on isotopes in ostracod shells. *In:* Horne, D.J., Holmes, J.A., Rodriguez-Lazaro, J. & Viehberg, F. (eds), Ostracoda as Proxies for Quaternary Climate Change. *Developments in Quaternary Science* **17:** 165–181.

Decrouy, L., Vennemann, T.W. & Ariztegui, D., 2011a. Controls on ostracod valve geochemistry, Part 1: Variations of environmental parameters in ostracod (micro-) habitats. *Geochimica et Cosmochimica Acta*, **75**: 7364–7379.

Decrouy, L., Vennemann, T.W. & Ariztegui, D., 2011b. Controls on ostracod shell geochemistry: Part 2. Carbon and oxygen isotope composition. *Geochimica et Cosmochimica Acta,* **75**: 7380–7399.

Decrouy, L., Vennemann, T.W.& Ariztegui, D., 2012. Sediment penetration depths of epi- and infaunal ostracods from Lake Geneva (Switzerland). Hydrobiologia **688**: 5–23.

Dettman, D.L. & Dwyer, G. S. 2012. The calibration of environmental controls on elemental ratios in ostracod shell calcite: a critical assessment. *In:* Horne, D.J., Holmes, J.A., Rodriguez-Lazaro, J. & Viehberg, F. (eds), Ostracoda as Proxies for Quaternary Climate Change. *Developments in Quaternary Science,* **17:** 145-163.

Dettman, D.L., Palacios-Fest, M. & Cohen, A.S. 2002. Comment on G. Wansard & F. Mezquita, The response of ostracode shell chemistry to seasonal change in a Mediterranean freshwater spring environment. *Journal of Paleolimnology*, **27**: 487-491.

Didié, C. & Bauch, H.A. 2002. Implications of upper Quaternary stable isotope records of marine ostracodes and foraminifers for paleoecological and paleoceanographical investigations. *In*: Holmes, J.A. & Chivas, A.R. (eds.) The Ostracoda: Applications in Quaternary Research. AGU, Washington D.C. *American Geophysical Union, Geophysical Monograph,* **131:** 279-299.

Dixit, Y., Hodell, D.A. & Petrie, C.A. 2014a. Abrupt weakening of the summer monsoon in northwest India ~ 4100 yr ago. *Geology*, **42**: 339-342.

Dixit, Y., Hodell, D.A., Sinha, R. & Petrie, C.A. 2014b. Abrupt weakening of the Indian summer monsoon at 8.2 kyr BP. *Earth and Planetary Science Letters*, **391**: 16-23.

Dixit, Y., Hodell, D.A., Sinha, R., Petrie, C.A., 2015. Oxygen isotope analysis of multiple, single ostracod valves as a proxy for combined variability in seasonal temperature and lake water oxygen isotopes. *Journal of Paleolimnology*, **53:** 35-45.

Frenzel, P., Schulze, I. & Pint, A. 2012. Noding of *Cyprideis torosa* valves (Ostracoda) - a proxy for salinity? New data from field observations and a long-term microcosm experiment. *International Review of Hydrobiology*, **97**: 314-329.

Fritz, P., Anderson, T.W. & Lewis, C.F.M. 1975. Late Quaternary climatic trends and history of Lake Erie from stable isotope studies. *Science*, **190**: 267-269.

Gasse, F., Fontes, J.C., Plaziat, J.C., Carbonel, P., Maczmarska, I., De Deckker, P., Soulié-Marsche, I., Callot, Y. & Dupeuple, P.A. 1987. Biological remains, geochemistry and stable isotopes for the reconstruction of environmental and hydrological changes in the Holocene Lakes from North Sahara. *Palaeogeography Palaeoclimatology Palaeoecology*, **60**: 1-46.

Ghetti, P., Anadón, P., Bertini, A., Esu, D., Gliozzi, E., Rook, L. & Soulié-Marsche, I. 2002. The Early Messinian Velona basin (Siena, central Italy): paleoenvironmental and paleobiogeographical reconstructions. *Palaeogeography Palaeoclimatology Palaeoecology*, **187**: 1-33.

Gibert, E., Arnold, M., Conrad, G., De Deckker, P., Fontes, J.-C., Gasse, F. & Kassir, A. 1990. Retour des conditions humides au tardiglaciaire au Sahara Septentrional (sebkha Mellala, Algérie. *Bulletin de la Société Géologique de France*, **8**: 497-504.

Grossi, F., Gliozzi, E., Anadón, P., Castorina, F. & Voltaggio, M. 2015. Is *Cyprideis agrigentina* Decima a good paleosalinometer for the Messinian Salinity Crisis? Morphometrical and geochemical analyses from the Eraclea Minoa section (Sicily). *Palaeogeography Palaeoclimatology Palaeoecology*, **419**: 75-89.

Heip, C. 1976a. The life-cycle of *Cyprideis torosa* (Crustacea, Ostracoda). *Oecologia*, **24**: 229-245.

Heip, C. 1976b. The spatial pattern of *Cyprideis torosa* (Jones, 1850) (Crustacea: Ostracoda). *Journal of Marine Biological Association of the United Kingdom*, **56**: 179-189.

Herman, P.M.J. & Heip, C. 1982. Growth and respiration of *Cyprideis torosa* Jones 1850 (Crustacea Ostracoda). *Oecologia*, **54**: 300-303.

Herman, P.M.J., Heip, C. & Vranken, G. 1983. The production of *Cyprideis torosa* Jones 1850 (Crustacea, Ostracoda). *Oecologia*, **58**: 326-331.

Holmes, J.A., Jones, R.L., Haas, J.N., McDermott, F., Molloy, K., & O'Connell, M. 2007a. Multi-proxy evidence for Holocene lake-level and salinity changes at An Loch Mór, a coastal lake on the Aran Islands, western Ireland. *Quaternary Science Reviews,* **26:** 2438-2462.

Holmes, J., Darbyshire, D. P. F., Heaton, T. H. E. 2007b. Palaeohydrological significance of late Quaternary strontium isotope ratios in a tropical lake. *Chemical Geology,* **236**: 281-290.

Holmes, J., Sayer, C.D., Liptrot, E. & Hoare, D.J. 2010. Complex controls on ostracod palaeoecology in a shallow coastal brackish-water lake: implications for palaeosalinity reconstruction. *Freshwater Biology*, **55**: 2484-2498.

Holmes, J.A. 1996. Trace-element and stable-isotope geochemistry of non-marine ostracod shells in Quaternary palaeoenvironmental reconstruction. *Journal of Paleolimnology*, **15**: 223-235.

Holmes, J.A. 2008. Sample-size implications of the trace-element variability of ostracod shells. *Geochimica et Cosmochimica Acta,* **72:** 2934–2945

Holmes, J. A. & De Deckker (2012) Introduction to ostracod shell chemistry and its application to Quaternary palaeoclimate studies. *In:* Horne, D.J., Holmes, J.A., Rodriguez-Lazaro, J. & Viehberg, F. (eds), Ostracoda as Proxies for Quaternary Climate Change. *Developments in Quaternary Science,* **17:**131-143.

Horne, D.J. 2007. A Mutual Temperature Range method for Quaternary palaeoclimatic analysis using European nonmarine Ostracoda. *Quaternary Science Reviews*, **26**: 1398-1415.

Ingram, B.L., De Deckker, P., Chivas, A.R., Conrad, M.E. & Byrne, A.R. 1998. Stable isotopes, Sr/Ca, and Mg/Ca in biogenic carbonates from Petaluma Marsh, northern California, USA. *Geochimica et Cosmochimica Acta*, **62**: 3229-3237.

Ito, E. & Forester, R.M. 2009. Changes in continental ostracode shell chemistry; uncertainty of cause. *Hydrobiologia*, **620**: 1-15.

Jahn, A., Gamenick, I. & Theede, H. 1996. Physiological adaptations of *Cyprideis torosa* (Crustacea, Ostracoda) to hydrogen sulphide. *Marine Ecology Progress Series*, **142**: 215-223.

Keatings, K.W., Hawkes, I., Holmes, J.A., Flower, R.J., Leng, M.J., Abu-Zied, R.H. & Lord, A.R. 2007. Evaluation of ostracod-based palaeoenvironmental reconstruction with instrumental data from the arid Faiyum Depression, Egypt. *Journal of Paleolimnology,* **38:** 261-283.

Keatings, K.W., Heaton, T.H.E. & Holmes, J.A. 2002. Carbon and oxygen isotope fractionation in non-marine ostracods: results from a 'natural culture' environment. *Geochimica et Cosmochimica Acta*, **66**: 1701-1711.

Keyser, D. 2005. Histological peculiarities of the noding process in *Cyprideis torosa* (Jones) (Crustacea, Ostracoda). *Hydrobiologia*, **538**: 95-106.

Kilenyi, T.I. 1972. Transient and balanced genetic polymorphism as an explanation of variable noding in the ostracode *Cyprideis torosa*. *Micropaleontology*, **18**: 47-63.

Marco-Barba, J., Ito, E., Carbonell, E., & Mesquita-Joanes, F., 2012. Empirical shell chemistry calibration of *Cyprideis torosa* (Jones, 1850) (Crustacea: Ostracoda). *Geochimica et Cosmochimica Acta,* **93**: 143–163.

Marco-Barba, J., Holmes, J.A., Mesquita-Joanes, F. & Miracle, M. R. 2013. The influence of climate and sea-level change on the Holocene evolution of a Mediterranean coastal lagoon: evidence from ostracod palaeoecology and geochemistry. *Geobios,* **46**: 409-421.

Mazzini, I., Anadón, P., Barbieri, M., Castorina, F., Ferreli, L., Gliozzi, E., Mola, M. & Vittori, E. 1999. Late Quaternary sea-level changes along the Tyrrhenian coast near Orbetello (Tuscany, central Italy): palaeoenvironmental reconstruction using ostracods. *Marine Micropaleontology*, **37**: 289-311.

McCulloch, M.T. & De Deckker, P. 1989. Sr isotopic constraints on the evolution of the Mediterranean Basin during the Messinian "salinity crisis". *Nature,* **342**: 62-65.

McCulloch, M.T., De Deckker, P. & Chivas, A.R. 1989. Strontium isotope variations in single ostracod valves from the Gulf of Carpentaria, Australia: a palaeoenvironmental indicator. *Geochimica et Cosmochimica Acta*, **53**: 1703-1710.

Meisch, C., 2000. Freshwater Ostracoda of western and central Europe. Süßwasserfauna von Mitteleuropa 8/3. Gustav Fischer, Stuttgart.

Mezquita, F., Olmos, V. & Oltra, R. 2000. Population ecology of *Cyprideis torosa* (Jones, 1850) in a hypersaline environment of the Western Mediterranean (Santa Pola, Alacant) (Crustacea: Ostracoda). *Ophelia*, **53**: 119-130.

Pint, A., Frenzel, P., Fuhrmann, R., Scharf, B. & Wennrich, V. 2012. Distribution of *Cyprideis torosa* (Ostracoda) in Quaternary athalassic sediments in Germany and its application for palaeoecological reconstructions. *International Review of Hydrobiology*, **97**: 330-355.

Rosenfeld, A. & Vesper, B. 1977. The variability of the sieve-pores in recent and fossil species of Cyprideis torosa (Jones, 1850) as an indicator for salinity and palaeosalinity. *In*: Löffler, H. & Danielpol, D. (eds.) *Aspects of Ecology and Zoogeography of Recent and Fossil Ostracoda*, 55-67.

Sandberg, P.A. 1964. The ostracod genus *Cyprideis* in the Americas. *Acta Universitatis Stockholmiensis, Stockholm Contributions in Geology*, **12**: 1-178.

Schweitzer, P.N. & Lohmann, G.P. 1990. Life-history and the evolution of ontogeny in the ostracode genus *Cyprideis*. *Paleobiology*, **16**, 107-125.

Sywula, T., Glazewska, I., Whatley, R.C. & Moguilevsky, A. 1995. Genetic differentiation in the brackish-water ostracod *Cyprideis torosa*. *Marine Biology*, **121**: 647-653.

Van Harten, D. 2000. Variable noding in *Cyprideis torosa* (Ostracoda, Crustacea): an overview, experimental results and a model from Catastrophe Theory. *Hydrobiologia*, **419**: 131-139.

Vesper, B., 1972a. Zur Morphologie und Ökologie von *Cyprideis torosa* (Jones, 1850) (Crustacea, Ostracoda, Cytheridae) unter besonderer Berücksichtigung seiner Biometrie. *Mitteilungen Hamburger Zoologisches Museum und Institut*, **68:** 21–77.

Vesper, B., 1972b. Zum Problem der Buckelbildung bei *Cyprideis torosa* (Jones, 1850) (Crustacea, Ostracoda, Cytheridae). *Mitteilungen Hamburger Zoologisches Museum und Institut*, **68:** 79–94.

Vesper, B., 1975. Ein Beitrag zur Ostracodenfauna Schleswig- Holsteins. *Mitteilungen Hamburger Zoologisches Museum und Institut*, **72:** 97–108.

von Grafenstein, U. 2002. Oxygen-isotope studies of ostracods from deep lakes. *In:* Holmes, J.A. & Chivas, A.R. (eds.) The Ostracoda: Applications in Quaternary Research. AGU, Washingtson D.C. *American Geophysical Union, Geophysical Monograph,* **131:** 249-266.

von Grafenstein, U., Erlernkeuser, H. & Trimborn, P. 1999. Oxygen and carbon isotopes in modern fresh-water ostracod valves: assessing vital offsets and autecological effects of interest for palaeoclimate studies. *Palaeogeography Palaeoclimatology Palaeoecology*, **148:** 133-152.

Wansard, G. 1996. Quantification of paleotemperature changes during isotopic stage 2 in the La Draga continental sequence (NE Spain) based on the Mg/Ca ratio of freshwater ostracods. *Quaternary Science Reviews*, **15:** 237-245.

Wansard, G., De Deckker, P. & Julià, R. 1998. Variability in ostracod partition coefficients D(Sr) and D(Mg): Implications for lacustrine palaeoenvironmental reconstructions. *Chemical Geology*, **146:** 39-54.

Wansard, G., De Deckker, P. & Julià, R. In press. Combining the Mg/Ca of the ostracod *Cyprideis torosa* with its ontogenic development for reconstructing a 28 kyr temperature record for Lake Banyoles (NE Spain). *Journal of Micropalaeontology* (This issue).

Wouters, K. In press. Distribution patterns of *C. torosa*. *Journal of Micropalaeontology* (This issue)

Wouters, K. & Martens, K. 1994. Contribution to the knowledge of the *Cyprideis* species flock (Crustacea: Ostracoda) of Lake Tanganyika, with the desription of three new species. *Bulletin de l'Institut Royal des Sciences Naturelles de Belgiques*, **64:** 111-128.

Wouters, K. & Martens, K. 2001. On the Cyprideis species flock (Crustacea, Ostracoda) in Lake Tanganyika, with the description of four new species. *Hydrobiologia*, **450:** 111-127.

Xia, J., Ito, E. & Engstrom, D.R. 1997. Geochemistry of ostracode calcite: Part 1. An experimental determination of oxygen isotope fractionation. *Geochimica et Cosmochimica Acta*, **61:** 377-382.

LIST OF FIGURES

Fig. 1. Trace-element chemistry of single adult shells of *C. torosa* from a tidal lagoon at Kyleakin, Isle of Skye, Scotland. (a) Location of the sample site shonw by black dot. (b) Cross-plot of Mg/Ca and Sr/Ca. (c) Temperature inferences under conditions of contrasting water Mg/Ca.

Fig. 2. Trace-element chemistry of single shells of *C. torosa* from the Fleet lagoon, southern England. (a) Location of the study site (b) Cross-plot of Mg/Ca and Sr/Ca. (c) Temperature inferences under conditions of contrasting water Mg/Ca

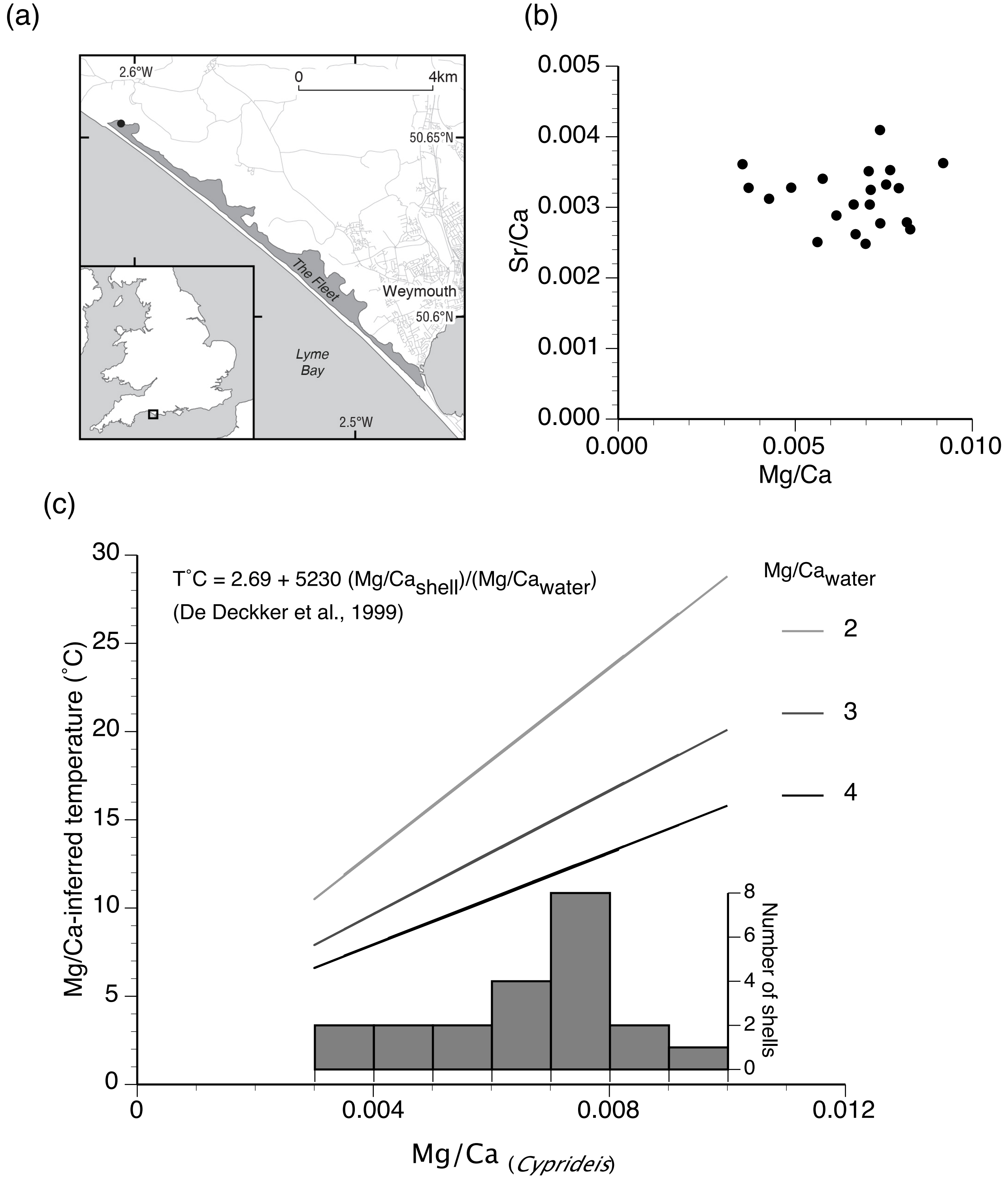
Fig. 3. Trace-element chemistry of single adult shells of *C. torosa* from the Allhallows site (Marine-isotope stage 9) (a) Location of the sampled section, marked by black dot. (b) Inferred TDS versus Sr/Ca in host water, calculated using a two-component mixing model based on the TDS and Sr/Ca of two end-members, namely groundwater and seawater. (c) Temperature inferences under conditions of contrasting water Mg/Ca.

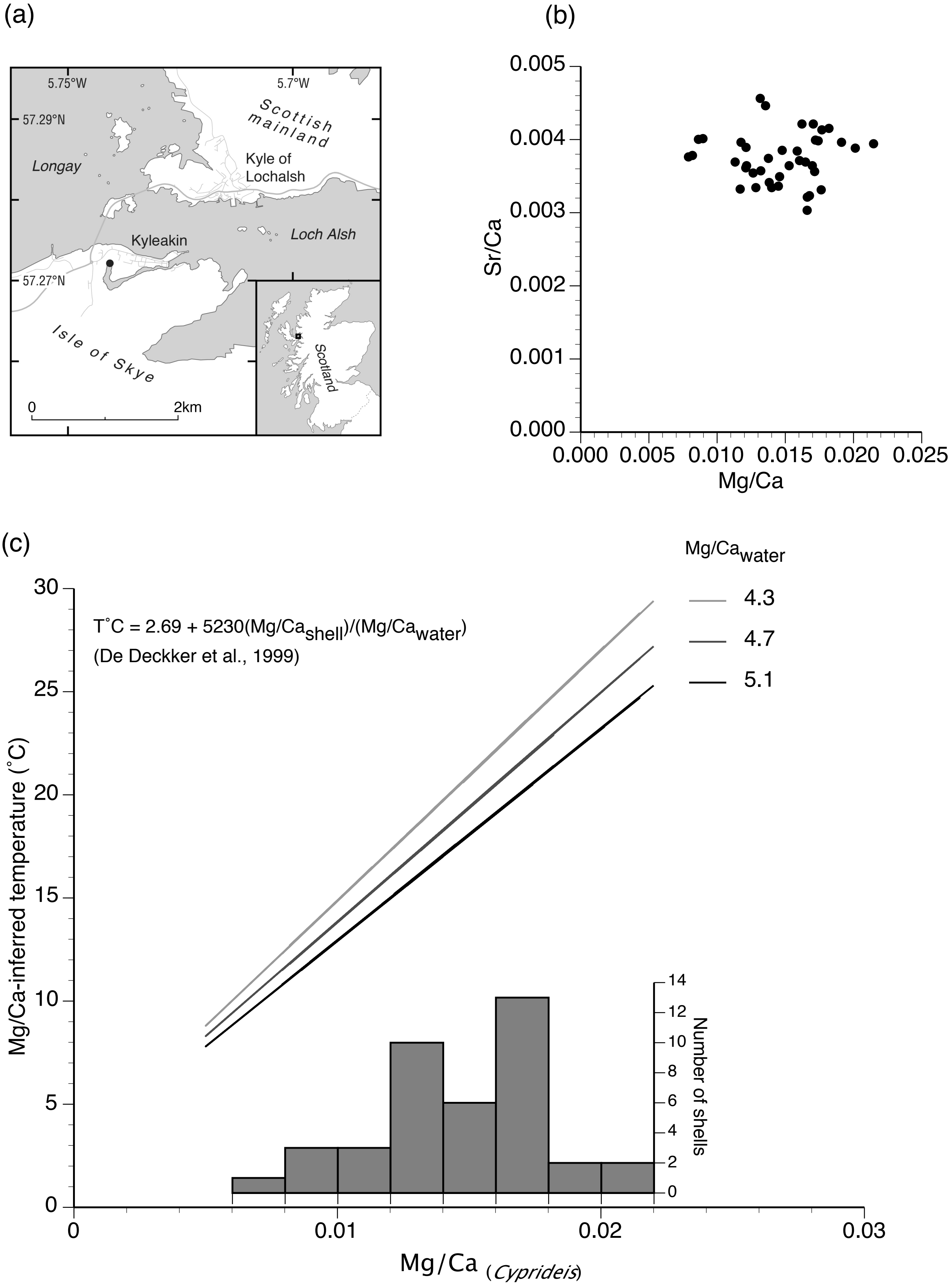
Fig. 4. Strontium-isotope ratios and morphology of shells of *Cyprideis torosa* from An Loch Mór, Ireland. (a) Location of study site. Black dot marks core location. (b) Variations in $^{87}Sr/^{86}Sr$ in Holocene sediments from the lake, plotted against inferred age (from information in Holmes et al., 2007). Dominant morphotype of shells in each analysed sample is also indicated. (c) Inferred TDS versus $^{87}Sr/^{86}Sr$ in lake water, calculated using a two-component mixing model based on the TDS and $^{87}Sr/^{86}Sr$ of two end-members, namely groundwater and seawater.

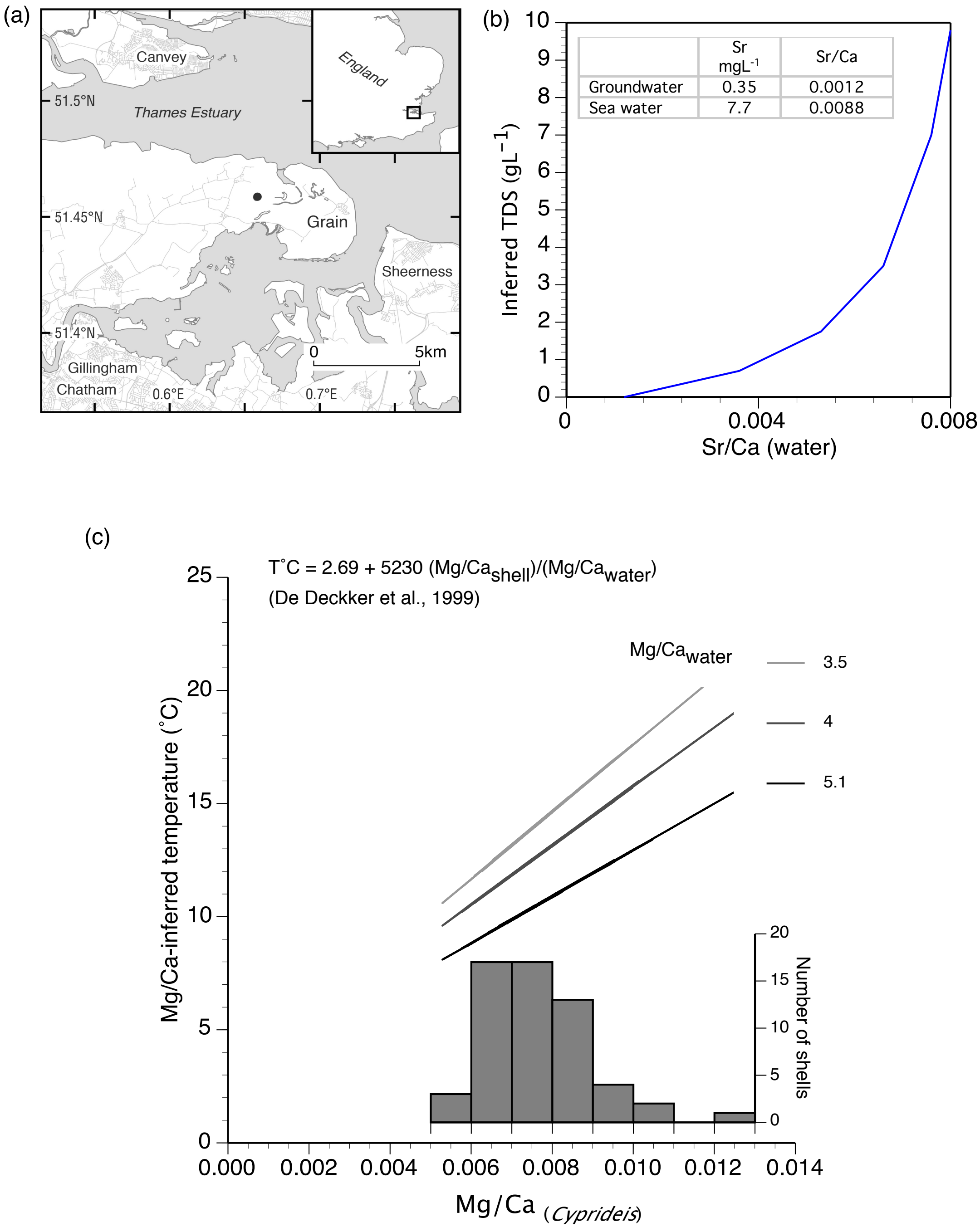
Fig. 5. Frequency distributions of Mg/Ca and Sr/Ca in single adult shells of *Cyprideis torosa* from Allhallows (see Fig. 3 for location). (a) smooth morphotypes. (b) noded morphotypes.

Table 1. Published and unpublished K_D [M] values for *Cyprideis*.

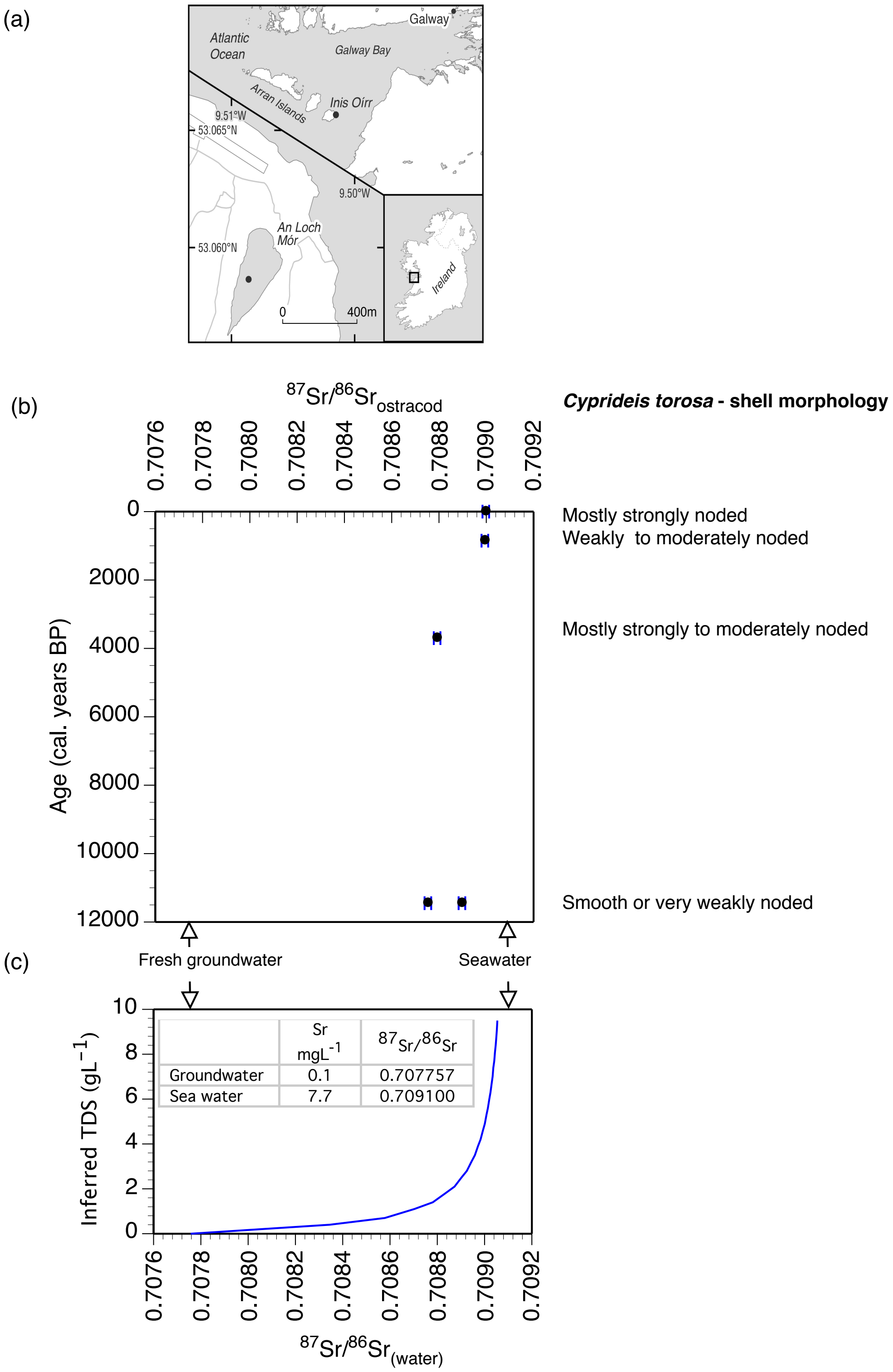
Supplementary material: Site information, analytical methods and data for previously-unpublished studies reported in the paper


(a)
2.6°W
0
4km
50.65°N
The Fleet
Weymouth
50.6°N
Lyme Bay
2.5°W
(b)
Sr/Ca
0.005
0.004
0.003
0.002
0.001
0.000
0.000
0.005
0.010
Mg/Ca
(c)
T°C = 2.69 + 5230 (Mg/Ca_shell)/(Mg/Ca_water)
(De Deckker et al., 1999)
Mg/Ca_water
2
3
4
Mg/Ca-inferred temperature (°C)
30
25
20
15
10
5
0
Number of shells
8
6
4
2
0
0
0.004
0.008
0.012
Mg/Ca (Cyprideis)


(a)
5.75°W
5.7°W
57.29°N
57.27°N
Scottish mainland
Kyle of Lochalsh
Longay
Kyleakin
Loch Alsh
Isle of Skye
Scotland
0
2km
(b)
Sr/Ca
0.000
0.001
0.002
0.003
0.004
0.005
Mg/Ca
0.000
0.005
0.010
0.015
0.020
0.025
(c)
T°C = 2.69 + 5230(Mg/Ca$_{shell}$)/(Mg/Ca$_{water}$)
(De Deckker et al., 1999)
Mg/Ca$_{water}$
4.3
4.7
5.1
Mg/Ca-inferred temperature (°C)
0
5
10
15
20
25
30
Number of shells
0
2
4
6
8
10
12
14
Mg/Ca (Cyprideis)
0
0.01
0.02
0.03



	Sr mgL^{-1}	Sr/Ca
Groundwater	0.35	0.0012
Sea water	7.7	0.0088



	Sr mgL^{-1}	$^{87}Sr/^{86}Sr$
Groundwater	0.1	0.707757
Sea water	7.7	0.709100

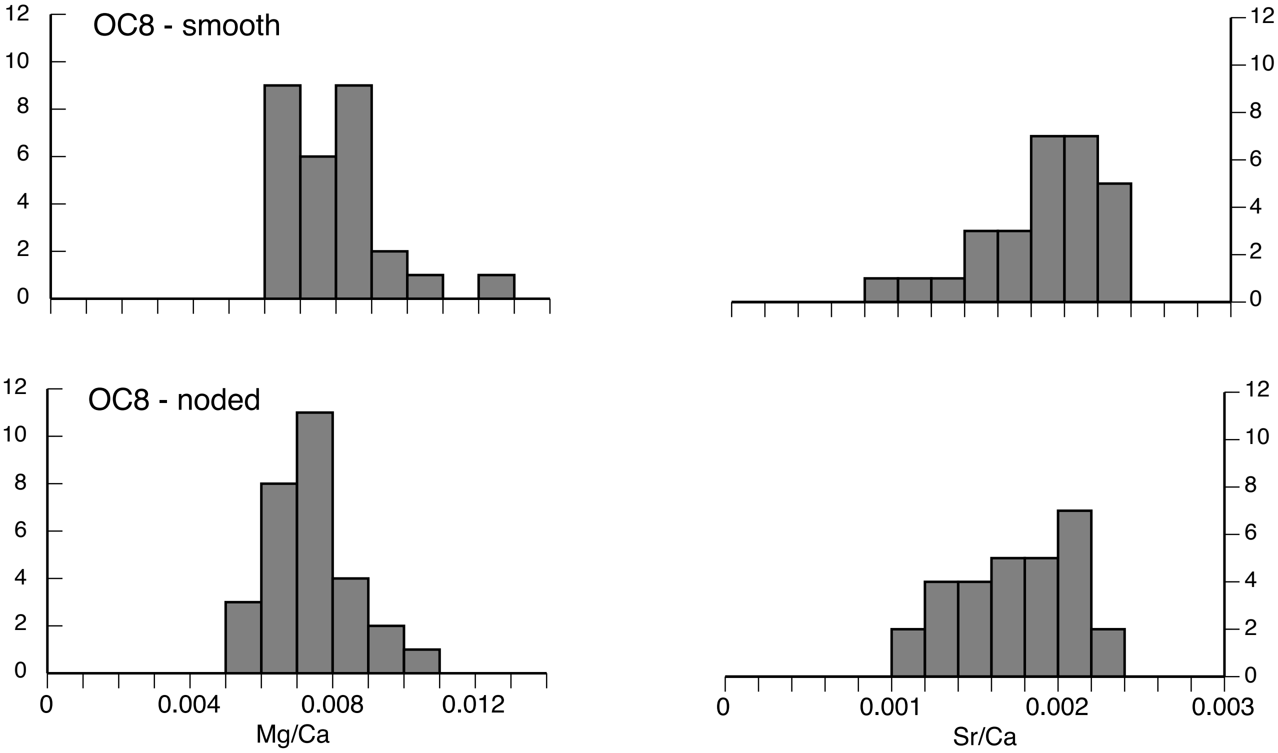

OC8 - smooth
OC8 - noded
0
2
4
6
8
10
12
0
0.004
0.008
0.012
Mg/Ca
0
0.001
0.002
0.003
Sr/Ca

Table 1. Published and unpublished K_D [M] values for *Cyprideis*. T = temperature (˚C)

KD[Sr]	KD[Mg]	Species	Site details	Reference
0.471 ± 0.061		*C. australiensis*	Diluted or evaporated seawater. Natural waters, Australia.	Chivas et al. (1986)
0.475 ± 0.057	0.0046 ± 0.001	*C. australiensis*	Diluted or evaporated seawater. Laboratory cultures. T = 25˚C	Chivas et al. (1986)
0.223+0.0086T	-0.000514 + 0.00019T	*C. australiensis*	Marine-type water. Laboratory cultures	De Deckker et al. (1999)
0.470 ± 0.053	0.0030 ± 0.0012	*C. torosa*	Lake Qarun, Egypt.T = ~19˚C	Keatings et al. (2007)
0.602-0.830	0.0169	*C. torosa*	Lake Banyoles, NE Spain. K_D[Mg] quoted for 25˚C	Wansard et al. (1998)
0.67 ± 0.09	0.00214 ± 0.0037	*C. torosa*	The Fleet Lagoon, S. England. T = 19-16˚C	G. Eisenhauer, unpublished data

Supplementary material to ‘Trace-element and stable-isotope composition of the *Cyprideis torosa* (Crustacea, Ostracoda) shell’ Journal of Micropalaeontology, XX: xxx-xxx.

Jonathan A. Holmes & Patrick De Deckker

Site information, analytical methods and data for previously-unpublished studies reported in the paper

1. Kyleakin, Isle of Skye, Scotland

Living *Cyprideis torosa* specimens were collected from An-t-Ob, a small tidal inlet behind the village of Kyleakin, Isle of Skye by D. J. Horne (Queen Mary, University of London) on 7 and 9 June 2004 (see main paper for location map). At the time of sampling, water temperature was 14 – 18 ˚C and optical salinity 27 - 32 S. Specimens of *Cyprideis torosa* were picked from the sediment residues, preserved in ethanol and dissected. Forty single valves were used for analysis, brush cleaned under a low power binocular microscope using a fine brush and deionised water. Clean valve material was placed into acid-washed 5 mL polypropylene tubes and dissolved in 1 mL 0.6M Merck Aristar™ HCl. The Ca, Mg, and Sr content of the ostracod valves were determined using a JY Ultima 2C ICP-AES calibrated using multi-element standards prepared with Spectrosol™ standard solutions for ICP. The results were corrected for blank contamination in the solvent acid and for instrumental drift using an external drift monitor. Results are given in Supplementary Table 1. Based on multiple analyses of a calcite standard (PE3), precision was ±0.63% RSD (relative standard deviation) for Mg/Ca and ±0.51% RSD for Sr.

2. The Fleet Lagoon, Dorset, UK

Living *Cyprideis torosa* specimens were collected from the low-salinity (TDS ~6 gL^{-1}, temperature = 10.5 ˚C at the time of sample collection in October 1994) end of The Fleet lagoon in Dorset, southern England (see main paper for a location map). Twenty one individual valves were used for analysis, brush cleaned under a low power binocular microscope using a fine brush and deionised water. Clean valve material was placed into acid-washed 25ml sterilin tubes and dissolved in 2 mL 0.6M Merck Aristar™ HCl. The Ca, Mg, and Sr content of the ostracod valves were determined using a JY-70 Plus inductively coupled plasma-atomic emission spectrometer (ICP-AES) calibrated using multi-element standards prepared with Spectrosol™ standard solutions for ICP. The results were corrected for blank contamination in the solvent acid and for instrumental drift using an external drift monitor. Results are given in Supplementary Table 2. Based on multiple analyses of the 'in-house' aragonite standard, precision was ±2.1% RSD (relative standard deviation) for Mg/Ca and ±1.4% RSD for Sr/Ca.

3. Allhallows Proto-Thames-Medway site, Eastern England

Samples were collected from a section exposed at Allhallows, Kent, Eastern England (see main paper for a location map and Bates et al. (2002) for details of the stratigraphical levels sampled). Level OC8 from Allhallows contains a mixture of smooth and noded ecophenotypes of *Cyprideis torosa*, whereas OC30 contains only noded forms. In this study, noded and smooth forms of *C. torosa* were selected from levels OC8 and OC30 (28 smooth specimens and 29 noded specimens, respectively, from OC8 and 30 noded specimens from OC30) and their trace-element content analysed. Single shells were selected and then brush cleaned with deionised water under a low-power binocular microscope. Clean valves were placed into acid-washed 5ml polypropylene tubes and dissolved in 2 mL 0.6M Merck Aristar™ HCl. The Ca, Mg, and Sr contents of the ostracod valves were determined using a JY-70 Plus inductively coupled plasma-atomic emission spectrometer (ICP-AES) calibrated using multi-element standards prepared with Spectrosol™ standard solutions for ICP. The results were corrected for blank contamination in the solvent acid and for instrumental drift using an external drift monitor. Results are given in Supplementary Table 3. Based on multiple analyses of an 'in-house' calcite standard, precision was ±1.8% RSD (relative standard deviation) for Mg/Ca and ±0.3% RSD for Sr.

4. An Loch Mór, Iniss Oírr, Ireland

One recent sample of *Cyprideis torosa* was collected from the northern end of An Loch Mór, Inis Oírr, Ireland, in 0.8 m of water, with TDS of 5 gL^{-1}. (see main paper for a location map). Four additional samples were selected from different levels of Holocene sediments from a core recovered from the lake. Further details of the sediment record from An Loch Mór are in Holmes et al. (2007). Individual samples composed of up to 27 valves of *Cyprideis torosa* were analysed. Further analytical details are in Holmes et al. (2007). Data are presented in Holmes et al. (2007) and in Supplementary Table 4

Supplementary references

Bates, M.R., Keen, D.H., Whittaker, J.E., Merry, J.S. & Wenban-Smith, F.F. 2002. Middle Pleistocene molluscan and ostracod faunas from Allhallows, Kent, UK. *Proceedings of the Geologists Association*, **113**: 223-236.

Holmes, J.A., Jones, R.L., Haas, J.N., McDermott, F., Molloy, K., & O'Connell, M. 2007. Multi-proxy evidence for Holocene lake-level and salinity changes at An Loch Mór, a coastal lake on the Aran Islands, western Ireland. *Quaternary Science Reviews,* **26:** 2438-2462.

Supplementary Table 1. Trace-element ratios of single adult shells of *Cyprideis torosa* from Kyleakin

	Sr/Ca (molar)	Mg/Ca (molar)
	0.00412	0.01768
	0.00420	0.01705
	0.00387	0.02013
	0.00445	0.01355
	0.00383	0.01587
	0.00395	0.01912
	0.00420	0.01622
	0.00455	0.01316
	0.00414	0.01820
	0.00384	0.01477
	0.00393	0.02147
	0.00400	0.00896
	0.00398	0.01721
	0.00368	0.01648
	0.00377	0.00819
	0.00373	0.01376
	0.00375	0.00790
	0.00368	0.01132
	0.00399	0.00863
	0.00397	0.01741
	0.00388	0.01212
	0.00355	0.01715
	0.00340	0.01382
	0.00330	0.01763
	0.00302	0.01660
	0.00322	0.01677
	0.00363	0.01698
	0.00395	0.01175
	0.00320	0.01661
	0.00348	0.01458
	0.00331	0.01169
	0.00360	0.01210
	0.00335	0.01449
	0.00363	0.01216
	0.00370	0.01604
	0.00363	0.01528
	0.00356	0.01320
	0.00333	0.01284
	0.00353	0.01264
	0.00333	0.01400
Mean	0.00373	0.01464
1s	0.00034	0.00320
RSD%	9	22

Supplementary Table 2. Trace-element ratios of single adult shells of *Cyprideis torosa* from The Fleet

Specimen	Sr/Ca	Mg/Ca
BPC1	0.003612	0.009176
BPC2	0.003596	0.003510
BPC3	0.002493	0.005626
BPC4	0.003512	0.007679
BPC5	0.002604	0.006703
BPC6	0.003497	0.007073
BPC10	0.002673	0.008246
BPC11	0.002468	0.006985
BPC12	0.003264	0.004884
BPC13	0.003106	0.004260
BPC14	0.002758	0.007400
BPC15	0.002773	0.008150
BPH2	0.003390	0.005774
BPH3	0.003261	0.003682
BPH4	0.004080	0.007392
BPH5	0.003256	0.007923
BPH6	0.003025	0.006649
BPH7	0.003024	0.007103
BPH8	0.002869	0.006161
BK2	0.003233	0.007129
BK3	0.003308	0.007566
Mean	0.003133	0.006622
1s	0.000417	0.001518
RSD %	13	23

Supplementary Table 3. Trace-element ratios of single adult shells of *Cyprideis torosa* from Allhallows.

	Sr/Ca (molar)	Sr/Ca (molar)	Sr/Ca (molar)	Mg/Ca (molar)	Mg/Ca (molar)	Mg/Ca (molar)
	OC8 smooth	OC8 noded	OC30 noded	OC8 smooth	OC8 noded	OC30 noded
	0.00225	0.00165	0.00210	0.00825	0.00713	0.00866
	0.00224	0.00183	0.00230	0.00754	0.00788	0.00917
	0.00110	0.00178	0.00236	0.00675	0.00626	0.00787
	0.00201	0.00172	0.00139	0.00841	0.00953	0.00752
	0.00090	0.00234	0.00209	0.00837	0.00806	0.00635
	0.00195	0.00126	0.00200	0.00824	0.00780	0.00707
	0.00184	0.00214	0.00178	0.00683	0.00760	0.00800
	0.00155	0.00198	0.00182	0.01249	0.00653	0.01238
	0.00205	0.00192	0.00159	0.00771	0.01047	0.00593
	0.00204	0.00203	0.00231	0.00849	0.00817	0.00879
	0.00182	0.00222	0.00244	0.00942	0.00590	0.00867
	0.00170	0.00183	0.00203	0.00985	0.00677	0.00776
	0.00123	0.00130	0.00134	0.00829	0.00642	0.00670
	0.00148	0.00171	0.00239	0.00688	0.00756	0.00791
	0.00198	0.00144	0.00231	0.00826	0.00757	0.00885
	0.00238	0.00214	0.00209	0.00739	0.00577	0.00716
	0.00189	0.00216	0.00189	0.00717	0.00824	0.00833
	0.00180	0.00184	0.00188	0.00643	0.00671	0.00807
	0.00214	0.00161	0.00216	0.01014	0.00610	0.01112
	0.00204	0.00156	0.00171	0.00680	0.00529	0.00594
	0.00155	0.00121	0.00206	0.00664	0.00745	0.00706
	0.00171	0.00211	0.00190	0.00793	0.00998	0.00909
	0.00226	0.00119	0.00238	0.00875	0.00777	0.00661
	0.00201	0.00110	0.00226	0.00858	0.00705	0.00757
	0.00224	0.00216	0.00236	0.00752	0.00815	0.00850
	0.00189	0.00144	0.00138	0.00669	0.00608	0.00859
	0.00164	0.00132	0.00139	0.00678	0.00665	0.00928
	0.00206	0.00156	0.00202	0.00686	0.00746	0.00704
		0.00218	0.00239		0.00765	0.00830
			0.00242			0.00864
Mean	0.00185	0.00175	0.00202	0.00798	0.00738	0.00810
1s	0.00036	0.00036	0.00034	0.00133	0.00121	0.00138
RSD%	19	21	17	17	16	17

Supplementary Table 4. Strontium-isotope ratios of mutliple shell samples of *Cyprideis torosa* from An Loch Mór, Ireland.

Sample no.	Age (cal. a BP)	$^{87}Sr/^{86}Sr$	2s error
TIMECHS 1 An Loch Mor, N. end	modern	0.708998	0.000014
TIMECHS 6 MOR 1 IIA 25.84	850	0.708994	0.000014
TIMECHS 4 MOR 1 V1B 30.34	3700	0.708792	0.000014
TIMECHS 2 MOR 1 V1B 34.67	11450	0.708897	0.000014
TIMECHS 3 MOR 1 V1B 34.69	11450	0.708753	0.000014