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Uptake of Mg and Sr in the euryhaline ostracod *Cyprideis* determined from in vitro experiments

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Abstract

This paper describes a series of in vitro experiments during which juveniles of the euryhaline ostracod *Cyprideis australiensis* were grown to adulthood in waters of ranging salinities (from 10–70‰.) and Mg/Ca and Sr/Ca ratios, and under two different constant temperature regimes, 20° and 25°C. Results indicate the following thermodependence for Mg in the calcitic valves of adult *Cyprideis australiensis*: $T(\mathrm{Mg}) = 2.69 + 5230 \times [\mathrm{Mg/Ca}]_{\mathrm{ostracod}}/[\mathrm{Mg/Ca}]_{\mathrm{water}}$. A minor thermodependence was also found for this ostracod species with respect to the uptake of Sr. There is a very strong relationship between the Mg/Ca of the ostracods and the Mg/Ca of the waters in which they moulted. The same can be said between the Sr/Ca of the ostracods and the waters' Sr/Ca. There is no direct relationship between water salinity and either the Mg/Ca ratio nor the Sr/Ca ratio of the ostracod valves. The 20° and 25°C experiments established the following partition coefficients which we believe can be applied to any *Cyprideis* taxa grown in waters within the 5 to 20 Mg/Ca range: $K_{\mathrm{D}}[\mathrm{Mg}] = -0.000514 + 0.00019 \times \text{Temperature}\,^{\circ}\mathrm{C}$, and $K_{\mathrm{D}}[\mathrm{Sr}] = 0.223 + 0.0086 \times \text{Temperature}\,^{\circ}\mathrm{C}$. This relationship for Sr, which has been mentioned for the first time for *Cyprideis* is tentative. The Mg/Ca of ostracods is strongly dependant on temperature, but this effect can easily be masked by variations in the Mg/Ca of the host water commonly found in natural lakes. Ostracod physiology is adapted to constructing valves from low-Mg calcite, so when growing in waters with high (>30), or very low (<1) Mg/Ca, *Cyprideis* species will not take up Mg in accordance with the calculated K_{D}.

Keywords: ostracods; shell chemistry; trace elements; Mg; Sr; salinity; partition coefficient

1. Introduction

Since the publication of the experimental work of Chivas et al. (1983) on the uptake of trace elements, mainly Mg and Sr, in calcitic valves of the halobiont ostracod *Mytilocypris henricae* commonly found in Australian salt lakes, there have been a number of studies dealing with the reconstruction of trace-elemental composition of ostracods in non-marine environments, including some experimental work conducted in lakes as well as under laboratory conditions. Holmes (1992, 1996) provides a comprehensive review of those studies which deal with non-marine ostracods. Recently, the same approach has been applied by Dwyer et al. (1995)

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and Corrège and De Deckker (1997) who used the Mg content of deep-sea marine ostracod taxa for the reconstruction of past bottom-water temperatures. Nevertheless, some conflicting results appeared in the literature which seemed to contradict some of the earlier findings or interpretations on ostracod shell chemistry made by us (Chivas et al., 1985, 1986a,b; De Deckker et al., 1988).

For example, Teeter and Quick (1990) (p. 220) claimed that "the Mg concentration in the euryhaline *Cyprideis americana* is inversely related to salinity . . . ", and that " . . . temperature, over the range observed . . . " by these authors " . . . had little or no effect on the concentration of Mg in *Cyprideis*". Chivas et al. (1986b) and De Deckker et al. (1988), on the other hand, claimed that the Mg/Ca ratio in ostracod shells is positively related to the water temperature and the Mg/Ca ratio of the water. In Australian lakes, there is generally a positive correlation between the water's Mg content and salinity when the latter exceeds ~3‰. Therefore, this implies that the Mg/Ca ratio of ostracod shells from most Australian lakes can be positively correlated with salinity. For further details, refer to Chivas et al. (1986b).

Wansard's (1996) work on *Cyprideis* from north-eastern Spain provided a different partition coefficient K_D[Mg] for *Cyprideis torosa* to the value calculated by De Deckker et al. (1988) from different locations in Europe and Australia, as well as from experiments. Wansard's work implied that there are some conditions in lakes which induce departure from the expected partition coefficients. Chivas et al. (1986b), on the other hand, had identified that both the K_D[Mg][1] and the K_D[Sr] value is consistent for all the species grouped within the same genus, simply because congeneric taxa should possess fairly similar physiologies.

Xia et al. (1997) also contributed to the debate by adding that the "ostracode (*sic*) Sr/Ca, which should be temperature independent, shows strong positive covariance with shell Mg/Ca. These results imply that Sr uptake in ostracode calcite increases with the Mg content of the shell. The partitioning coefficient for Mg, on the other hand, appears to decrease at high values of Mg/Ca in the host water."

These uncertainties, in contrast to the original determinations made by Chivas et al. (1983) require verification and further comment, and therefore have engendered the preparation of the present paper. Results of in vitro experimental work done on the Australian taxon *Cyprideis australiensis* Hartmann, 1981 are reported here to provide a better link between ostracod valve chemistry with water conditions under which the valves calcified.

2. Material

2.1. Ostracods and experimental procedures

Live *Cyprideis australiensis* specimens were collected from Salt Dip Lake near Robe in South Australia. Samples were collected using a hand-held plankton net to scrape sediment containing ostracods at the sediment/water interface, and placed in 1-litre plastic jars. The following day, live and active juvenile (A-1)[2] specimens of *C. australiensis* were extracted from the sediment and placed in small glass scintillation vials in filtered lake water. Care was taken to prevent any sediment, or any other particulate matter, from being inserted into the vials. Up to 20 live specimens were therefore placed in each 10-ml vial filled with filtered Salt Dip Lake water, and placed in a refrigerator at approximately 4ºC. This low temperature induced the ostracods to go into torpor. When the vials were brought to room temperature (~20ºC), and particularly during examination under a binocular microscope when a heated lamp was used, the ostracods regained activity within minutes. Some specimens regained activity even af-

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[1] The annotation for partition coefficient is frequently referred to as K_D followed by the relevant chemical element, such as K_D[Mg]. Other authors use the alternative term distribution coefficient, abbreviated by the capital letter D and followed by the relevant chemical element, such as D(Mg). The term K_D is preferred in the present paper.

[2] Adult ostracods are conventionally referred to by the capital letter A; juveniles by the same letter A followed by the negative sign which then precedes the juvenile moult stage from the adult stage, knowing that ostracods have to moult up to 8 times before reaching sexual maturity. Thus, 'A-1' refers to a juvenile ostracod at the growth stage just before reaching adulthood. For the experiment described herewith, only A-1 specimens were used.

ter having been kept under 'refrigeration' for up to 6 months.

Selected single specimens of A-1 ostracods were then placed alone in a small, capped glass vial containing approximately 10 ml of filtered (through 0.45-μm Millipore® filters) water (see below). No food was added to the vials so as to avoid any possible contamination of the waters or altering their chemical composition. The vials were immersed for up to 2/3 of their depth in water in a constant temperature bath covered with a removable acrylic lid.

Each vial was examined daily for any evidence of valve moulting and, as soon as each individual ostracod had reached adulthood, the 2 separated, juvenile valves which were clear of any tissue, were removed from the vials using a triple zero paint brush. After that operation, which took less than 5 minutes, the vial was returned to the bath for the experiment to continue. These moulted juvenile valves were discarded. Once an adult ostracod had died (probably through starvation), the specimen was removed from its vial, and the valves were then separated using standard entomological tungsten needles. Any soft tissue and appendages were carefully removed and also discarded. Upon separation, the cleaned valves were rinsed in MilliQ® water several times, further rinsed in pure ethanol, and finally stored in thoroughly-cleaned standard, plastic micropalaeontological slides for further examination. One valve of each adult specimen was later analysed using a built-in-house Inductively-Coupled Argon-Plasma Emission Spectrometer (ICPAES) at the Research School of Earth Sciences at ANU. Detection limits for Ca^{2+} are 0.02 ppb, for Mg^{2+} 0.04 ppb and for Sr^{2+} 0.03 ppb in solution (for further details on the ANU instrument, refer to our earlier paper: Chivas et al., 1983). Merck Suprapur HCl acid and MilliQ® water were used as well as SPEX Hipure standard solutions. Only *Cyprideis* valves which weighed more than 10 μg were used for trace-element analysis. The other lighter ($<$10 μg) valves were considered not to have reached sufficient calcification to warrant interpretation of the results, especially since the work of Chivas et al. (1983) demonstrated that poorly calcified ostracods yielded extremely high concentrations of Mg in comparison with Ca.

The experiments were run at two different temperatures, 20 and 25ºC. All the ostracods had died within 33 days, but most A-1 ostracods had moulted within a week of the cultures commencing. A total of 140 juvenile specimens were used for the different experiments, but only 47 reached adulthood and not all gained sufficient weight to allow Mg and Sr uptake interpretation. A total of 30 ostracods did reach a weight $>$ 10 μg and provide data for our analysis (see Table 2). In some cases, all the ostracods had died before reaching maturity, just a few days after the experiment had commenced, presumably because the waters (some of which were mixed — see below) were unsuitable for ostracod growth and calcification.

2.2. Waters

Waters were collected from a variety of lakes and from the ocean. All waters were filtered through a 0.45-μm filter and stored in clean plastic containers. Several waters were mixed together to give salinities that would be fairly similar, but with different ionic compositions or ratios, and in particular with respect to the three cations of interest to this study: Ca, Mg and Sr. Those waters used are listed in Table 1 and their relevant chemical characteristics are presented in Table 2.

3. Results

3.1. The uptake of Mg by *Cyprideis australiensis*

3.1.1. Temperature effect on Mg uptake

The growth experiment which involved using juveniles (A-1) ostracods to reach adulthood was conducted under 2 controlled temperatures, 20ºC and 25ºC, using water from Jervis Bay; this water type was chosen because it has comparable ionic ratios and chemistry to sea water. Only three ostracods reached adulthood and were sufficiently calcified to meet the analysis requirements at 20ºC, and four at 25ºC.

Examination of Fig. 1, which displays the individual results as well as the mean values for each of the two temperatures, shows that there is an increase in the uptake of Mg in the ostracod valves with temperature. The regression line passing through the two mean values gives the thermodependence of

Table 1
List of waters used in the experiments

Code name	Location
Natural waters	
Robe SW	Ocean water collected in Robe Harbour, South Australia
Jervis SW	Ocean water collected at Jervis Bay, Australian Capital Territory
SD	Salt Dip Lake, near Robe, South Australia
Fell	Lake Fellmongery, near Robe, South Australia
Chara	Chara Lake, near Robe, South Australia
SB	Shark Bay water collected in Hamelin Pool, Western Australia
Mixed waters	
FD + LG	Fresh Dip Lake (near Robe, South Australia) water mixed with water from Lake Gnotuk, Victoria
LG + FI	Lake Gnotuk water mixed with water from one lake on Fraser Island, Queensland
Fell + MQ	Lake Fellmongery (near Robe, South Australia) water mixed with milliQ® water

Cyprideis for Mg as follows:

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

Although this temperature relationship is based on only a few data points, it shows that a temperature increase of 1ºC represents about a 5% increase in the uptake of Mg in the calcite lattice of the ostracod valve. In order to apply our results to other *Cyprideis* ostracods grown in any water with a composition similar to that of sea water chemistry, and especially with a Mg/Ca ratio of less than 20 (see additional comments in Section 4.1), we have used in the vertical axis of Fig. 1 the $[\mathrm{Mg/Ca}]_{\mathrm{ostracod}}$ over the $[\mathrm{Mg/Ca}]_{\mathrm{water}}$. Thus, the following partition coefficient for Mg in *Cyprideis* was obtained:

$$K_{\mathrm{D}}[\mathrm{Mg}] = -0.000514 + 0.00019 \times \text{Temperature}\,^{\circ}\mathrm{C}$$

3.1.2. Mg/Ca in Cyprideis vs. salinity and the Mg/Ca of the host water

The 26 analyses of adult specimens considered to be suitable for interpretation show no connection between the water salinity and the Mg/Ca in *Cyprideis* (Fig. 2). In addition, data presented in Fig. 3 show that there is no correlation between water salinity and the K_{D}[Mg] values. It is worth pointing out also

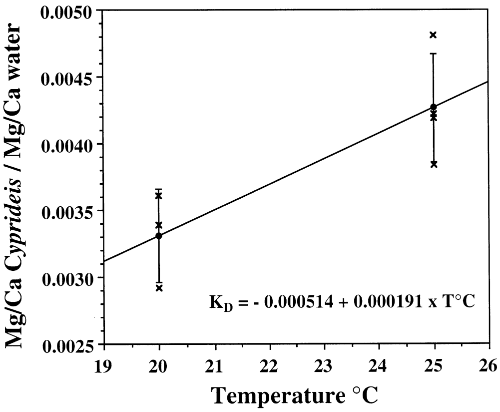

Fig. 1. Graphic representation of the data from the in vitro experiment using *Cyprideis australiensis* to show the relationship between the ratio of Mg/Ca of the ostracod and the Mg/Ca of the water in which it grew for two different temperatures 20º and 25ºC. Results for individual valves are indicated by ×, ● indicates the mean value for each temperature and the vertical bars delineate the standard deviation for all analyses at each of the two temperatures.

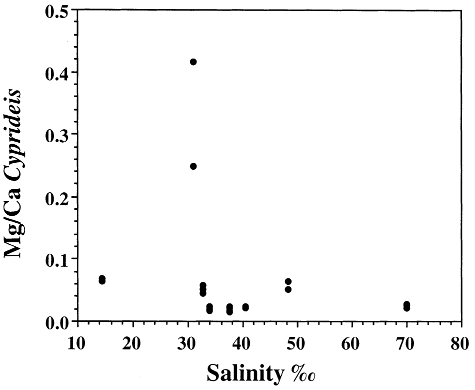

Fig. 2. Graphic representation of the data from the in vitro experiment using *Cyprideis australiensis* grown at 25ºC to show the results of the Mg/Ca analyses against the water salinity in which they grew.

Table 2
Chemical characteristics of the ostracod valves and the waters recovered from the experiments

Ostracod code no.	Water used	Temperature (°C)	Salinity	Mg/Ca water	Sr/Ca water	Mg/Ca ostracod	Sr/Ca ostracod	Mg/Ca ost:water	Sr/Ca ost:water
JB 20A	Jervis SW	20	34.0	4.71	0.00822	0.01595	0.00324	0.00339	0.39416
JB 20B	Jervis SW	20	34.0	4.71	0.00822	0.01701	0.00344	0.00361	0.41788
JB 20C	Jervis SW	20	34.0	4.71	0.00822	0.01375	0.00305	0.00292	0.37105
JB 25A	Jervis SW	25	34.0	4.71	0.00822	0.01987	0.00388	0.00422	0.47141
JB 25 B	Jervis SW	25	34.0	4.71	0.00822	0.01975	0.00327	0.00419	0.39793
JB 25C	Jervis SW	25	34.0	4.71	0.00822	0.02266	0.00376	0.00481	0.45754
JB 25D	Jervis SW	25	34.0	4.71	0.00822	0.01809	0.00347	0.00384	0.42202
SB 20A	SB	20	70.0	5.71	0.00815	0.02617	0.00333	0.00458	0.40798
SB 25A	SB	25	70.0	5.71	0.00815	0.02723	0.00326	0.00477	0.40000
SB 25B	SB	25	70.0	5.71	0.00815	0.02688	0.00340	0.00471	0.41718
SB 25C	SB	25	70.0	5.71	0.00815	0.02611	0.00373	0.00457	0.45718
SB 25D	SB	25	70.0	5.71	0.00815	0.02095	0.00353	0.00367	0.43301
CYP 1	SD	25	37.5	4.69	0.02260	0.01448	0.01079	0.00309	0.47730
CYP 3	SD	25	37.5	4.69	0.02260	0.02093	0.01039	0.00446	0.45978
CYP 7	SD	25	37.5	4.69	0.02260	0.02376	0.01050	0.00507	0.46451
CYP 10	SD	25	37.5	4.69	0.02260	0.01787	0.01184	0.00381	0.52398
CYP 21	Robe SW	25	40.6	5.45	0.00800	0.02145	0.00391	0.00394	0.48913
CYP 26	Robe SW	25	40.6	5.45	0.00800	0.02281	0.00396	0.00418	0.49463
CYP 32	Fell	25	32.8	11.82	0.05300	0.05082	0.02571	0.00430	0.48509
CYP 33	Fell	25	32.8	11.82	0.05300	0.04438	0.02734	0.00375	0.51583
CYP 38	Fell	25	32.8	11.82	0.05300	0.05877	0.02207	0.00497	0.41636
CYP 40	Fell	25	32.8	11.82	0.05300	0.04516	0.02355	0.00382	0.44436
CYP 41	Chara	25	48.2	10.33	0.04700	0.06497	0.02168	0.00629	0.46136
CYP 44	Chara	25	48.2	10.33	0.04700	0.05104	0.02213	0.00494	0.47074
CYP 54	FD + LG	25	–[a]	20.62	0.02400	0.09771	0.01194	0.00474	0.49763
CYP 55	FD + LG	25	–	20.62	0.02400	0.11435	0.01239	0.00555	0.51629
CYP 56	FD + LG	25	–	20.62	0.02400	0.10483	0.01123	0.00508	0.46771
CYP 58	FD + LG	25	–	20.62	0.02400	0.10568	0.01106	0.00513	0.46092
CYP 102	Fell + MQ	25	14.5	12.47	0.05140	0.06822	0.02916	0.00547	0.56722
CYP 109	Fell + MQ	25	14.5	12.47	0.05140	0.06538	0.02518	0.00524	0.48986
CYP 84[b]	LG + FI	25	31.0	31.51	0.02890	0.41696	0.01089	0.01323	0.37685
CYP 90[b]	LG + FI	25	31.0	31.51	0.02890	0.24981	0.01718	0.00793	0.59450

[a] – = no measurements taken.

[b] Measurements refer to poorly calcified ostracods grown in high-Mg waters.

All ratios presented in this table are molar ratios. Salinities were calculated as the total of dissolved solids through evaporation.

that, for the waters used in the culture experiments here, there is no obvious relationship between water salinity and the waters' Mg/Ca as shown in Fig. 4.

Some *Cyprideis* specimens grown at 25°C in Fresh Dip water with a salinity of less than 2‰ did moult but never reached a satisfactory weight (viz. $> 10\ \mu g$) (and therefore are not displayed in the figures nor Table 2), although their Mg/Ca values remained low — within the 0.025–0.067 range — and are consistent with the rest of the data.

For the 25°C experiment, and when ignoring the analyses of *Cyprideis* specimens grown in the water mixture of Fraser Island and Lake Gnotuk (because the moulted specimens did not reach a weight of 10 μg), the correlation between the Mg/Ca in *Cyprideis* and the Mg/Ca of the water ranging between 4.5 and 21 gives the following relationship:

$$(\mathrm{Mg/Ca})_{\mathrm{ostracod}}\ \text{at}\ 25°\mathrm{C} = -5.8 \times 10^{-3} + 5.4 \times 10^{-3} \times \mathrm{Mg/Ca}_{\mathrm{water}}$$

calculated from the data presented in Fig. 5. In this figure, we also show the results for the ostracods grown at 20°C, which were not used to calculate

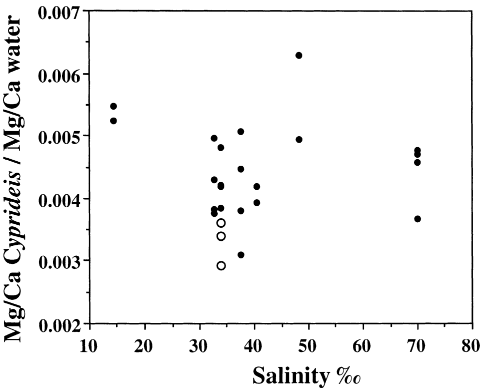

Fig. 3. Graphic representation of the data from the in vitro experiment using *Cyprideis australiensis* grown at 20° (○) and 25°C (●) to show the results of the Mg/Ca of the ostracods over that of the water against water salinity.

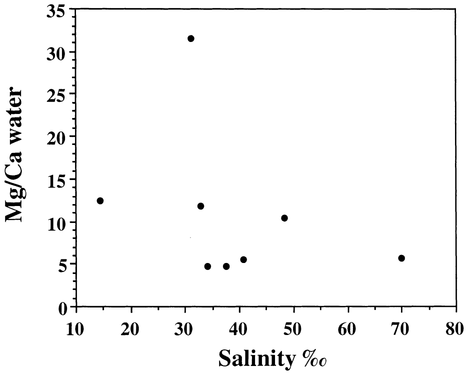

Fig. 4. Graphic representation of the poor relationship that exists between water salinity and the Mg/Ca ratios of the waters used for all experiments.

the above relationship. However, when the data from the mixed waters with a Mg/Ca of 31.51 are added to the rest of the data, no relationship is obtained. This is graphically represented in Fig. 6, for which the best regression fit produces a discontinuity. On this figure, we have also plotted the mean value for *Cyprideis torosa* measured by Wansard et al. (1998) from the freshwater Lake Banyoles in NE Spain which has a salinity of 0.8‰, and a very low water Mg/Ca: 0.65.

It is not surprising that the ostracods grown in the highly magnesian mixture of Lake Gnotuk + Fraser Island (LG + FI) water never reached complete calcification. The 2 points for LG + FI in Fig. 6 show a Mg/Ca of the ostracod valves typical of Mg-calcite

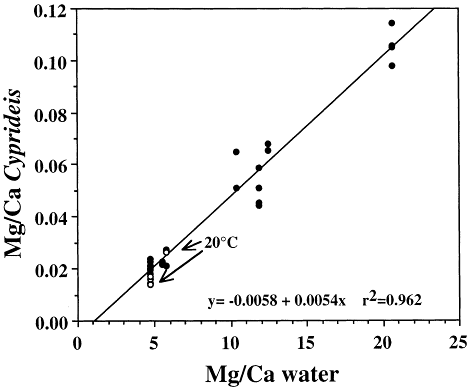

Fig. 5. Graphic representation of the data from the in vitro experiment using *Cyprideis australiensis* grown at 25°C to show the strong positive relationship between the Mg/Ca of the water against the Mg/Ca of the ostracod. Filled circles (●) represent ostracods grown at 25°C and for which the regression line was obtained. Open circles (○) represent values obtained for the ostracods grown at 20°C. Only those waters with a Mg/Ca less than 25 are used to calculate the regression line.

(the Mg/Ca being >0.2, implying a calcite containing more than 20 mole% Mg), which is inconsistent with normal ostracod valve mineralogy; this is not surprising as the ostracods had not yet fully calcified. Such high Mg values are consistent with Chivas et al.'s (1983) observations which demonstrate that, at the early stages of calcification, Mg is taken in high concentrations in contrast to Ca.

3.2. The uptake of Sr by *Cyprideis australiensis*

3.2.1. Temperature effect on Sr uptake

Fig. 7 represents the plot of the ostracods Sr/Ca using Jervis Bay water with a Sr/Ca of 4.71 and grown at 20°C and 25°C. Again, only 3 specimens moulted at 20°C and 4 moulted at 25°C. The following regression line is obtained through the means:

$$K_D[\mathrm{Sr}] = 0.223 + 0.0086 \times T°(\mathrm{C})$$

Because the same water was used at both temperatures, we are able to calculate the thermodependence relation which is:

$$T°(\mathrm{C}) = -26.0 + 117.0 \times \frac{(\mathrm{Sr/Ca})_{\mathrm{ostracod}}}{(\mathrm{Sr/Ca})_{\mathrm{water}}}$$

Note that an increase of 1°C for the water is registered as a 2% increase in the Sr uptake in the

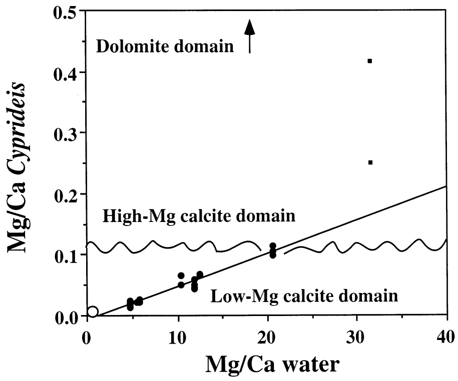

Fig. 6. Graphic representation of the data from the in vitro experiment using *Cyprideis australiensis* grown at 25°C as displayed in Fig. 4. The additional 2 measurements (■) for the poorly calcified ostracods grown in waters with a Mg/Ca > 30 show the apparent discontinuity that appears between the Mg/Ca of the ostracod and that of the water when large Mg/Ca of the waters are obtained. The open circle (○) shows the mean value obtained by Wansard et al. (1998) for modern *Cyprideis torosa* valves collected at Lake Banyoles in NE Spain. The area above the wavy line shows the realm of high-Mg calcite (note that the limit between low- and high-Mg calcite differs between authors) and eventually in the upper part of the diagram for dolomite which are inconsistent with ostracod valve mineralogy dictated by ostracod physiology.

calcite lattice of the ostracods. We are well aware that the data are too few to define a clear relationship between the Sr and temperature, but these preliminary data suggest the possibility of a small positive thermodependence for Sr uptake in *Cyprideis*.

3.2.2. Sr/Ca in Cyprideis australiensis vs. salinity

Only 26 specimens moulted from the A-1 stage to adult during the in vitro experiment at 25°C and reached a weight of >10 μg. Four specimens were cultured successfully at 20°C (see Table 2 for results which show quite convincingly that there is no relationship between the Sr/Ca of *Cyprideis australiensis* and water salinity). In Fig. 8 we demonstrate that there is no relationship between water salinity and the Sr/Ca of the ostracod "over that of" the water.

3.2.3. Sr/Ca in Cyprideis australiensis and the Sr/Ca of the water

Fig. 9 shows a very clear relationship between the Sr/Ca of the ostracods and the Sr/Ca of the water

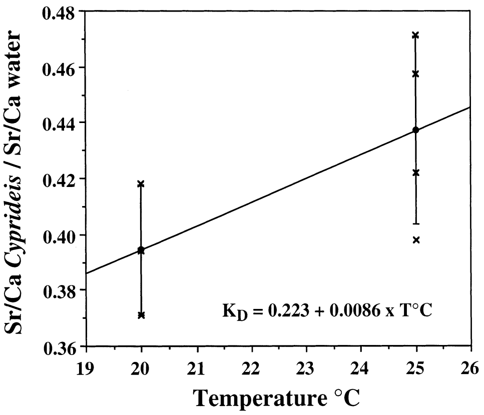

Fig. 7. Graphic representation of the data from the in vitro experiment using *Cyprideis australiensis* to show the relationship between the ratio of Sr/Ca of the ostracods and the Sr/Ca of the water in which they grew for two different temperatures 20° and 25°C. Results for individual valves are indicated by ×, ● indicates the mean value for each temperature and the vertical bars delineate the standard deviation for all analyses at each of the two temperatures.

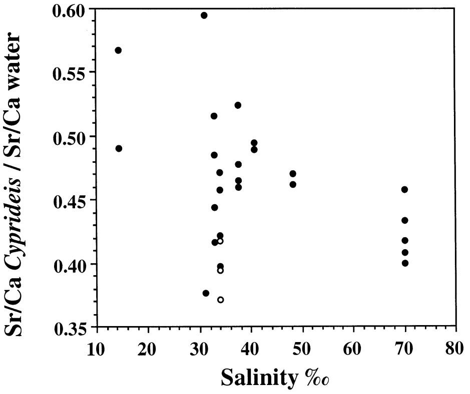

Fig. 8. Graphic representation of the data from the in vitro experiment using *Cyprideis australiensis* grown at 20° (○) and 25°C (●) to show the results of the Sr/Ca of the ostracods over that of the water against water salinity.

(all grown at 25°C), with the following partition coefficient:

$$\mathrm{Sr/Ca}_{\mathrm{Cyprideis}} = -4.08 \times 10^{-4} + 0.491 \times \mathrm{Sr/Ca}_{\mathrm{water}}$$

This figure also shows that ostracods grown in waters with a very high Sr/Ca ratio display a much greater variability in the Sr/Ca of their shells than do ostracods grown in waters with a seawater composition, ie. Sr/Ca ratio of 0.0082.

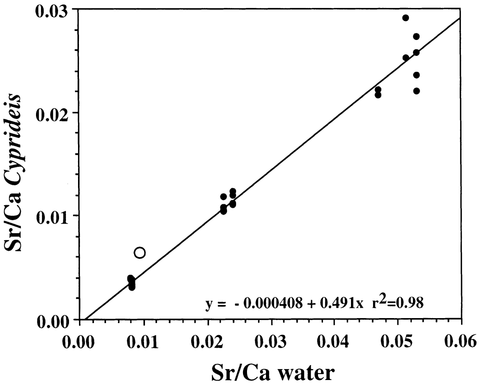

Fig. 9. Graphic representation of the data from the in vitro experiment using *Cyprideis australiensis* grown at 25°C to show the strong positive relationship between the Sr/Ca of the water against the Sr/Ca of the ostracod. Filled circles (●) represent ostracods grown at 25°C and for which the regression line was obtained. The open circle (○) shows the mean value obtained by Wansard et al. (1998) for modern *Cyprideis torosa* valves collected at Lake Banyoles in NE Spain; note that the water temperature range estimated for La Draga by Wansard et al. (1998) is quite broad.

4. Discussion

4.1. Mg uptake by Cyprideis australiensis

The composition of the water with respect to its Mg/Ca ratio plays a particularly significant role towards the uptake of Mg. If the ostracods are to follow their prescribed K_D[Mg], it is obvious that for waters with substantially elevated Mg/Ca, a disequilibrium will occur. Since all ostracods should always have a carapace consisting of low-Mg calcite, at substantially elevated Mg/Ca the ostracod valve's chemistry could not comply with the thermodynamic equilibrium under which the valves have to form. If we were to extend both axes in Fig. 5, the resulting regression line extrapolated for waters with higher Mg/Ca (e.g. for waters with a Mg/Ca > 25), would show that the ostracods would have to 'make' high-Mg calcite and, at even higher values, they would have to construct their valves of dolomite! This is physiologically impossible since the crystallographic characteristics of these minerals are very different, and the organism cannot change mineralogies. Consequently, ostracods would have to calcify their valves 'out of equilibrium'. In fact, this phenomenon has been indirectly observed by researchers who analysed ostracod valves grown in waters with extremely high Mg/Ca ratios. This is the case for Xia et al. (1997) who examined the ostracod *Candona rawsoni* grown in waters with a Mg/Ca > 30. A simple projection of the relationship line between the Mg/Ca of the waters and that of *C. rawsoni* for 25°C in fig. 8 of Xia et al.'s (1997) publication would show that, under normal circumstances, the partition coefficient of that species would require the ostracod to have a valve with a Mg/Ca of $>$ 0.1 (= high-Mg calcite) for those ostracods grown in Coldwater Lake. Instead, the ostracod Mg/Ca valves show a broad scatter with values remaining within the range of low-Mg calcite.

Xia et al. (1997) stated that "the partition coefficient for Mg ... appears to decrease at high values of Mg/Ca of the host water". Similarly, Wansard (1996) indicated in his chemical investigations of *Cyprideis torosa* from Lake Banyoles that this ostracod did not follow the partition coefficient obtained for that species by De Deckker et al. (1988) from European collections and which were also found for the Australian species *C. australiensis*. Similarly, in their work on the Australian Lake Keilambete, Chivas et al. (1985) determined a K_D[Sr] for the large congeneric ostracods *Mytilocypris* and *Australocypris*, quite different from the K_D[Sr] for collections of the same ostracod taxa from other Australian lakes. The reason is that Lake Keilambete has a very unusual water chemistry, with a water Mg/Ca ranging from 79 to 112. In fact, the fossil ostracods analysed by Chivas et al. (1985) must have followed a 'modified' K_D[Sr] for the entire Holocene sequence, since these authors established a strong relationship between water salinity changes through time and the ostracod Sr/Ca. This was possible simply because this crater lake is an almost completely closed system, with high alkalinity, and is strongly buffered with respect to maintaining fairly 'constant' ionic ratios through time. The relationship between salinity and ostracod Sr/Ca established by Chivas et al. (1985), and clearly explained in their paper with respect to the uniqueness of lake Keilambete, has caused some confusion in the literature and led some researchers to believe that it is true for other lacustrine situations. The same confusion has arisen among workers dealing with the Mg/Ca in fossil ostracod sequences because, once again, Chivas et al. (1986b) had demonstrated for

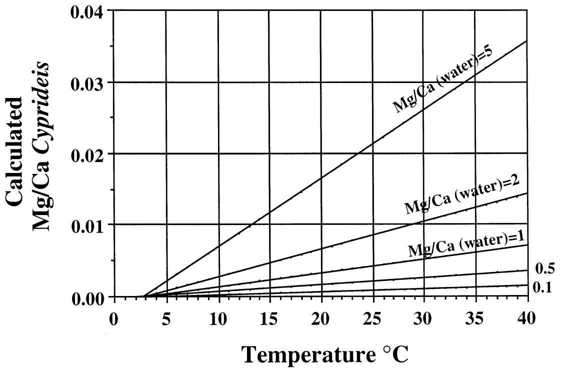

Fig. 10. Diagram to illustrate the relationship between the water temperature and the Mg/Ca of *Cyprideis australiensis* using the K_D[Mg] $= 0.0229 + 0.00857 \times T^o$. This graph is plotted for waters with a Mg/Ca having the following values: 0.1, 0.5, 1, 2 and 5. Note that for temperatures lower than 3ºC, the K_D could not ‘operate’ properly. It is fair not to envisage such situation as we have demonstrated with our experiments, which consisted in keeping juveniles in a refrigerator for several months, that this species goes into torpor at low temperatures.

Australian lakes — which, in most cases, follow the sea-water chemical pathway of Na-Cl dominated waters — that the Mg content in saline waters follows an almost direct correlation with salinity.

Figs. 10 and 11 are calculated using the K_D[Mg] described earlier in this paper, to show graphically the expected relationship between water temperature and the ostracod Mg/Ca. These two figures illustrate the conditions under which the ostracod valves would have to *calcify out of equilibrium for low temperatures*, as well as for waters with either extremely low or very high Mg/Ca.

It is clear that the effect of temperature on the uptake of Mg by ostracods is much more substantial than a change in the Mg/Ca of the host water which would be unusual in lakes during a single season. Fig. 12 illustrates this phenomenon. Using our calculated K_D[Mg], we can show that for a temperature change of 5ºC and a water Mg/Ca of 5, the ostracod would register a Mg/Ca increase of approximately 0.004 in its valves. The same order of magnitude increase in the shell Mg/Ca would be registered if the water Mg/Ca was to increase from 1 to 2. At those low water Mg/Ca values, the temperature effect is not as consequential as a change in water ionic ratio. Nevertheless, at more elevated water Mg/Ca, the temperature effect becomes greater.

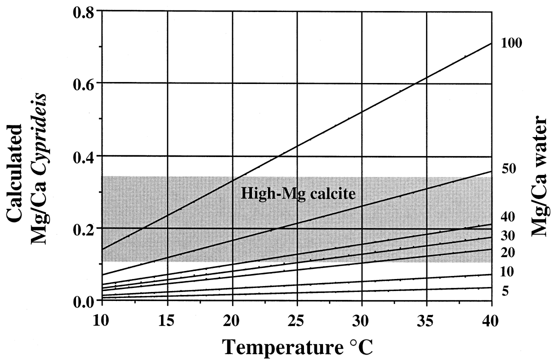

Fig. 11. The same computation applied in Fig. 10 has been used in here to show the calculated relationship with higher water Mg/Ca values. This graphic representation demonstrates that at high water Mg/Ca, the ostracod — if following the K_D calculated using in vitro experiments — would have to produce valves that are made of high-Mg calcite (shaded area) or dolomite for higher Mg/Ca values.

The only study that has contradicted our preliminary investigations (Chivas et al., 1986a,b), and is in conflict with our results presented here is the work of Teeter and Quick (1990) which bears the following conclusion: “magnesium concentration in the carapace … of *Cyprideis americana* … is inversely related to salinity”. We have examined the data presented in this study, and also through the courtesy of those authors who made their raw data available, we have been able to reproduce one of their figures.

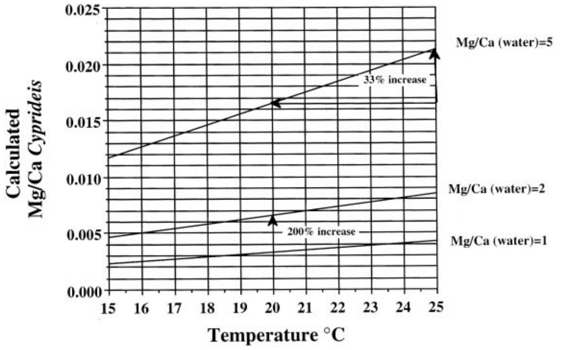

Fig. 12. Graphic representation using the K_D[Mg] $= 0.0229 + 0.00857 \times T^o$ for 3 waters with different Mg/Ca to help visualise (1) the effect water temperature on the uptake of Mg in the ostracod valve, and for comparison (2) against a change in the Mg/Ca of the water on the same uptake. For example, a change of 5ºC temperature for *Cyprideis* grown in a water with Mg/Ca of 5 causes an increase in ~0.004 which is approximately the same when the water Mg/Ca is increased from 1 to 2.

However, we believe that there are several flaws in their study carried out on ostracod samples from the Bahamas, and which were analysed by electron microprobe.

The authors did not measure the Mg/Ca of the waters in which they collected the ostracods. Our experiments show this to be extremely important since there is a direct relationship between the Mg/Ca of the water and that of the ostracod valves. Teeter and Quick (1990) were already aware of this relationship which is discussed in the paper by De Deckker et al. (1988). In addition, although ostracods had been collected live by Teeter and Quick (1990) who chose to analyse ostracod valves from animals which had soft parts at the time of collection, we cannot ascertain when the ostracod had moulted into the adult stage. The *C. americana* specimens could have moulted at a very different time of the year, and perhaps been transported by tidal currents to the sites of collection. Teeter and Quick (1990) mentioned strong tides having affected some sites between the different collecting times. Thirdly, and perhaps more importantly, our concern relates to the quality of the material collected by Teeter and Quick (1990). These authors stated that, upon collection, the ostracods were preserved in buffered formalin. This preservative is commonly avoided by researchers who study ostracods, especially if they want to study and photograph ostracod valves under the scanning electron microscope. It is well known that formalin, even if buffered, causes some decalcification of thin ostracod valves; formalin bleaches the ostracod pigments and tissues, and often renders ostracod valves to become ‘soft’, even after just a few days of immersion! We have established that slight dissolution of ostracod valves can cause the ‘leaching out’ of Mg from ostracod calcitic valves. This was achieved by us when dissolving valves of *Cyprideis* specimens in 30% H_2O_2 in less than 1 hour (one ostracod valve, belonging to a single specimen was analysed for its Mg/Ca content, and the opposite valve placed in H_2O_2 prior to the same analysis). This analysis showed an almost complete absence of Mg in the ostracod valve which had been immersed in H_2O_2. We also analysed fossil valves of *Cyprideis* from core GC2 from the Gulf of Carpentaria (see De Deckker et al., 1988). Specimens from several horizons showed evidence of post mortem partial dissolution as a result of oxidation of pyrite crystals which had adhered to the ostracod valves after thanatocoenosis (see De Deckker (1988) for illustration of such specimens). Ostracods which had been partly dissolved contained little Mg in their valves compared to the other, well preserved specimens for the same horizons in the core. In all cases, for the specimens partly dissolved in H_2O_2 and those affected by dissolution from the Gulf of Carpentaria core, the Sr/Ca of the ostracod valves gave similar values to those valves which had not undergone any dissolution. These observations indicate that Sr atoms are less likely to be ‘extracted’ under dissolution conditions and that this element can still be utilised for study even on specimens that have undergone some minor level of diagenesis. The final point to make here is that it is likely that the ostracods collected by Teeter and Quick (1990) from the high salinity (within the 60–90‰ range) localities, were less well calcified in comparison with those from the lower salinities (~10–40‰). This is a well established phenomenon as ostracods are stressed while attempting to calcify at extremely high salinities when, often calcite saturation levels of the host waters are low especially when dealing with marine-derived waters. Rosenfeld and Vesper (1977) have documented that the sensory pores that abound on the carapace of *Cyprideis torosa* are irregular when the ostracods are collected from high salinity waters (although these authors have not established this relationship in in vitro experiments). The irregularity of the pores is indicative of calcification under ‘abnormal’ conditions. The thin, poorly calcified ostracods grown at high salinities could more easily suffer partial dissolution, and therefore be preferentially leached of Mg if preserved in formalin. We believe that Teeter and Quick’s (1990) results can be discounted until chemical analyses of the waters in their Bahamas sites are obtained and compared with new collections believed to contain ostracods grown in those waters.

Another important concern we have relates to the choice of lake when considering analysing the trace-element content of fossil ostracods from a core so as to reconstruct past water chemistries, and possibly palaeotemperatures for the lake. It is necessary to eliminate sites which have waters with elevated Mg/Ca, and possibly also with Mg/Ca below unity as well, because the ostracod valves are unlikely to follow the 'dictates' of their partition coefficient. Xia et al. (1997) indicated that at high water Mg/Ca, the Sr content in ostracods increases with the Mg content of the valves. Perhaps under those circumstances, Sr may take the role of Mg in adopting a strong thermodependence. In the deep sea, where analyses have been done by Cadot (1974) and Cadot and Kaesler (1977) on several ostracod taxa (analysed under a microprobe), Corrège (1993) and De Deckker (unpubl.) found that at a very low temperature threshold (~1–3ºC) or above a certain temperature threshold (values differ between species), the Sr/Ca of the ostracods appears to show a linear relationship with water temperature, whereas the Mg/Ca of the ostracod does not. This phenomenon requires further investigation. Fig. 13 represents an attempt to explain empirically the changes that have been observed for several ostracods collected in the deep sea.

Note that this particular phenomenon has been documented using benthic foraminifera collected at low temperatures (−2 to 6ºC) by Rathburn and De Deckker (1997). In this particular study, these authors demonstrated a positive relationship between the Sr/Ca of those organisms, which from previous studies (Bender et al., 1975), had shown no temperature dependence. Obviously, additional experimentation and observations are required before this phenomenon can be fully quantified and understood.

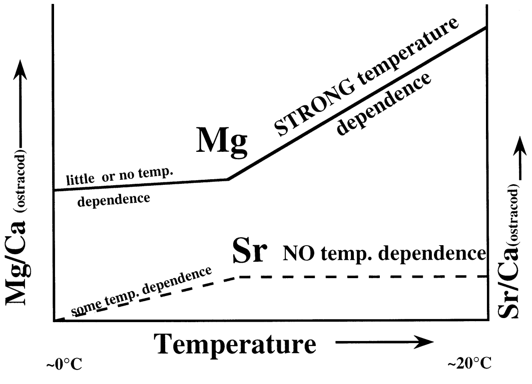

Fig. 13. Schematic diagram to show the possible relationship that may exist between the uptake of Mg and Sr in ostracod valves under 'stressed' conditions caused by either elevated or very low Mg/Ca of the host water, or low temperatures. For example, at low temperatures bordering zero such as in the left-hand part of the diagram, the Mg uptake in the ostracod does not follow the linear relationship with respect to temperature; Sr shows some thermodependence instead. Once temperature is more suitable for growth of the ostracod, then Mg shows thermodependence, but not Sr.

5. Conclusion

We have established the following relationships with respect to the trace-elemental composition of *Cyprideis australiensis*:

(1) The Mg/Ca of this ostracod is strongly correlated with the Mg/Ca of the water, as well as water temperature. However, depending on the partition coefficient of individual ostracods, when calcifying in water with high, and perhaps also low (<1) Mg/Ca, the ostracods are unlikely follow their 'normal' partition coefficient 'guidelines' to avoid having to make valves which would otherwise be different from low-Mg calcite. We anticipate this would apply to all ostracod taxa.

(2) There is no relationship between the Mg/Ca and the Sr/Ca of the ostracod and water salinity. Exceptional cases exist for lakes in which increasing water salinity is accompanied by increasing Mg/Ca and/or Sr/Ca of their waters.

(3) Although ostracods are strongly thermodependent with respect to the Mg/Ca of their valves, any shift in the Mg/Ca of the host water can have a significant effect on the Mg/Ca of the ostracod valves. Thus, it is important to study sites where the Mg/Ca of the water changes little, such as lakes in limestone terrains (e.g. karstic lakes) that may be buffered and display little ionic change such as in the Mg/Ca ratio during evaporation or dilution. Also, the choice of a site for chemical analyses of ostracod valves is paramount for ending up with sound and clear palaeoenvironmental interpretations.

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