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Nyctemeral variations of magnesium intake in the calcitic layer of a Chilean mollusk shell (*Concholepas concholepas*, Gastropoda)

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Abstract

Mollusk shells are increasingly used as records of past environmental conditions, particularly for sea-surface temperature (SST) reconstructions. Many recent studies tackled SST (and/or sea-surface salinity) tracers through variations in the elementary (Mg and Sr) or stable isotope ($\delta^{18}\text{O}$) composition within mollusk shells. But such attempts, which sometimes include calibration studies on modern specimens, are not always conclusive. We present here a series of Mg and Sr analyses in the calcitic layer of *Concholepas concholepas* (Muricidae, Gastropoda) with a very high time-resolution on a time window covering about 1 and a half month of shell formation, performed by Laser Ablation Inductively-Coupled Plasma Mass Spectrometry (LA-ICP-MS) and electron probe micro-analysis (EPMA). The selected specimen of this common Chilean gastropod was grown under controlled environmental conditions and precise weekly time-marks were imprinted in the shell with calcein staining. Strontium variations in the shell are too limited to be interpreted in terms of environmental parameter changes. In contrast, Mg incorporation into the shell and growth rate appear to change systematically between night and day. During the day, Mg is incorporated at a higher rate than at night and this intake seems positively correlated with water temperature. The nightly reduced Mg incorporation is seemingly related to metabolically controlled processes, formation of organic-rich shell increments and nocturnal feeding activity of the animals. The nyctemeral Mg changes in the *C. concholepas* shell revealed in this study might explain at least part of the discrepancies observed in previous studies on the use of Mg as a SST proxy in mollusk shells. In the case of *C. concholepas*, Mg cannot be used straightforwardly as a SST proxy.

1. INTRODUCTION

Reconstructions of past sea-surface temperatures (paleo-SST) is essential to understand past oceanographic and climatic changes. Part of the paleo-SST proxy data comes from elemental and isotopic geochemical variations in calcified structures of marine organisms (corals—e.g. Tudhope et al., 2001; Yu et al., 2005; Corrège, 2006; foraminifera—e.g. Elderfield and Ganssen, 2000; Barker et al., 2005; Schmidt et al., 2006; mollusk shells—e.g. Krantz et al., 1987; Richardson, 2001; Bojar et al., 2004). If data inferred

from foraminifera in marine sediment cores have provided century to millennia records of oceanographic conditions, mollusk shells may provide different paleoecological and paleoclimatic information at a much higher time-resolution, i.e. at daily to yearly scales (Jones, 1983; Weidman et al., 1994).

Since Epstein et al. (1953) pioneering work, d^{18}O has been the most commonly used proxy in attempts to infer SST from mollusk shell analyses (e.g. Andreasson and Schmitz, 1998; Schöne et al., 2004; Chauvaud et al., 2005). In biogenic carbonates, $\delta^{18}\text{O}$ is however dependent on both temperature and salinity variations (Epstein and Mayeda, 1953). Because it is difficult to obtain independent information on $\delta^{18}\text{O}_{\text{water}}$ or on paleosalinities,

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complementary geochemical proxies of SST have been actively sought in biogenic carbonates. Variations in Mg/Ca ratios might provide such independent information on paleo-SST (Klein et al., 1996; Lazareth et al., 2003; Takesue and van Geen, 2004). The Sr/Ca ratio has also been studied for paleo-SST reconstructions, but essentially in corals and sclerosponges aragonitic skeletons (e.g. McCulloch et al., 1994; Haase-Schramm et al., 2003; Rosenheim et al., 2004; Yu et al., 2005). In bivalve shells, it is still debated whether Sr/Ca ratios reflect SST or if kinetic effects are potentially involved in biomineral Sr intake (Gillikin et al., 2005; Lorrain et al., 2005; Carré et al., 2006).

The physical and physiological parameters (growth rate changes, pH variations of precipitating fluids, etc.), commonly grouped under the “vital effect” term, have also to be taken into consideration if it is shown that they affect the proxy record from bivalve shells (Owen et al., 2002; Lorrain et al., 2004; Geist et al., 2005; Lorrain et al., 2005). Also, growth rate changes or interruptions have to be considered to construct a time-series profile and to correct for time averaging problems (Goodwin et al., 2003). In an attempt to obtain an accurate paleo-SST proxy calibration, taking into account daily growth variations, we studied the geochemical variations of a fast growing gastropod, *Concholepas concholepas* on a short time window but at a daily to subdaily resolution. To our knowledge, such high time-resolution has never been reached for geochemical proxy calibration in mollusk shells.

For high temporal resolution records, high-resolution analytical techniques are necessary. With some techniques, a monthly to daily-scale resolution can be reached depending on the growth rate of the chosen species. Laser Ablation Inductively-Coupled Plasma Spectrometry (LA-ICP-MS) is becoming increasingly used to obtain geochemical profiles in biocarbonates (e.g. coral skeleton, bivalve shells) for environmental reconstructions. Since the pioneering works of Perkins et al. (1991) and Pearce et al. (1992), LA-ICP-MS trace element studies on bi carbonate concerned both calibration studies (van der Putten et al., 1999; Bellotto and Miekeley, 2000; Craig et al., 2000) and applications to environmental and paleoenvironmental reconstructions essentially based on corals (McCulloch et al., 1994; Sinclair et al., 1998; Quinn and Sampson, 2002; Fallon et al., 2003) and mollusk shells (Fuge et al., 1993; Raith et al., 1996; Stecher et al., 1996; van der Putten et al., 2000; Lazareth et al., 2003; Takesue and van Geen, 2004; Gillikin et al., 2005). These studies have shown that LA-ICP-MS allows accurate determination of trace element variations in the shells at a seasonal scale. Trace element profiles so produced can then reflect environmental changes during the shell formation. Some other studies were performed using Electron Probe Micro Analysis (EPMA) (Rosenberg and Hughes, 1991; Horng-Sheng and Grossman, 1994; Epplé, 2004) which in principle provides very high-resolution profiles. In addition, for elements with concentrations close to 1 wt%, EPMA gives quantitative results at a spatial resolution close to that of LA-ICP-MS. As LA-ICP-MS bi carbonate analysis still suffers from the lack of appropriate external standards, EPMA analyses can provide an assessment on LA-ICP-MS accuracy, as well as

an independent internal standardization for absolute concentration determinations (e.g. Poitrasson et al., 1996; Poitrasson, 2001).

We worked on the shell of *C. concholepas* [Bruguère, 1789], a large size subtidal endemic gastropod living in the nearshore area along the Peru–Chile coasts (between $\sim 10^{\circ}\text{S}$ and $\sim 55^{\circ}\text{S}$). This species has a similar economic importance than the Californian abalone (*Haliotis* spp.) and lives in comparable bathymetric ranges (0 to -50 m) but has quite different ecological niche, *C. concholepas* being a carnivorous predator, while, *Haliotis* is a grazer.

The northern half of the Peru–Chile area is under the influence of the El Niño phenomenon. Given the absence of other potential proxies like coral skeletons in this region, the *C. concholepas* species is potentially a promising paleo-environmental recorder because fossil shells are abundant in archeological sites and in well preserved Pleistocene marine terraces (Labonne and Hillaire-Marcel, 2000 ; Dauphin et al., 2003b ; Guzmán, 2004). This mollusk species also has the advantage of a high growth rate and particularly clear growth patterns, which make it well adapted for geochemical calibration purposes. Growth patterns were examined at the macro-, micro- and nanoscopic scales, with different techniques (Guzmán, 2004; Guzmán et al., 2007). These previous studies partly based on precise chronological control (at the hourly scale) involving fluorochrome staining indicate that *C. concholepas* shells are susceptible to record continuously temperature variations unlike most mollusk species (see review in Richardson, 2001). The biomineralisation process in the outer calcitic layer of *C. concholepas* is characterized by the formation at an hourly rhythm (between half-an-hour and 3 h) of growth units that measure about two micrometers in thickness (Guzmán, 2004; Guzmán et al., 2007). The pace at which these 21 μm -thick units are formed determines the growth rate, which varies throughout the day.

In this geochemical study, Mg and Sr determinations were obtained at a subdaily resolution for one and a half month on a laboratory-grown specimen. Precise temporal framework is provided by successive fluorescent markings of the shell. Reproducibility and accuracy of the geochemical obtained by LA-ICP-MS and EPMA is assessed. The Mg and Sr geochemical variations in the shell are then compared with water temperature fluctuations recorded during shell growth with the general aim to verify whether these elements can trace former thermal variations in shells of that species.

2. MATERIALS AND METHODS

2.1. Sample

The *C. concholepas* shell study in the present paper relies upon a population experimentally grown and submitted to staining experiments (Guzmán, 2004). The results are here-in used to allocate a date to each analysis point, thereby allowing an accurate comparison between geochemical variations and water temperature fluctuations. The detailed description of this experiment and of the growth patterns in the *C. concholepas* shell will be published elsewhere and only the main results are given here.

From August to December 2002, 85 specimens of *C. concholepas* were placed in an aquaculture tank at the Marine Resource Faculty of the Antofagasta University under natural light with almost the same length of time for day and night (23 °S). The specimens were fed ad libitum with mytilids (*Perumytilus purpuratus*). The experimental tank was supplied continuously with sea water and air. To produce temporal milestones in the shells, the organisms were stained through successive immersions in a fluorescent calcein solution (100 ppm) for 3 h on the 5, 8, 15, 22, 29 of November and 6 and 13 of December 2002 before their sacrifice on December 15. During the whole staining experiment, the water temperature was recorded every ten minutes by an automatic device with a precision of 0.2 °C ("tidbit" Onset[®]).

After superficial cleaning, the shells were cut along the maximum growth axis (Fig. 1) and thick polished sections of the most recent part were prepared for microscopic observations and chemical analyses. The shell of one specimen, M77, was selected for chemical analysis investigations because of its clear growth pattern. Two samples (M77-1 and M77-2) taken on two successive ridges at the edge of the shell (Fig. 1) were prepared as described. The *C. concholepas* shell is made up of two layers. The internal layer is thin and aragonitic whereas the external is thick and calcitic (Dauphin et al., 2003b; Guzmán, 2004; Fig. 2 a). Under blue fluorescent light, shell sections revealed several very distinguished green lines corresponding to the successive calcein stains. Calcein baths for 3 h done in the morning do appear in the shell close to the middle of translucent increments (Guzmán, 2004) whereas calcein baths done around midnight appear in opaque increments. One day of growth thus results in the accretion of one translucent increment (day) and one opaque one (night) when viewed under diffuse natural light (Fig. 2 c). This gives the temporal framework required for high-spatial resolution geochemical analyses.

2.2. Analytical methods

Two high-resolution analytical techniques were used for this geochemical study, Laser Ablation Inductively-Coupled Plasma Spectrometry (LA-ICP-MS) and Electronic Probe Microanalysis

(EPMA). All analyses were done in the external calcitic layer of the *C. concholepas* specimen studied.

2.2.1. LASER ablation ICP-MS

Three LA-ICP-MS profiles along the maximum growth axis were done, one in M77-1 and two in M77-2. For the M77-1 section, analysis points were carefully located to get one analysis per day and another one per night for each 24-h day of growth. This resulted in a zigzag-like profile to avoid point overlapping (Fig. 2 a). Under the microscope coupled to the LASER device, growth layers do not appear with sufficient clarity to locate the analysis point exactly as wanted. Thus, some points overlapped day/night layers. Moreover, trying to locate the points one per day and one per night is too much time consuming in relation to the final precision of time allocation for each analysis point. Consequently, the two following LA-ICP-MS profiles in M77-2 were done perpendicular to the growth lines, every 65 µm (Fig. 2 a). A calendar date, considering the middle of the period of growth sampled, and time interval (in hours) was assigned to each analysis point through microscopic observation, each growth layer being previously dated using the calcein lines in the shell. Analyses along a single growth layer were done to give an indication on intrashell geochemical homogeneity.

The LA-ICP-MS equipment used was a Cetac LSX 200, a frequency quadrupled Nd-YAG operating at 266 nm, coupled with an Elan 6000 (Perkin-Elmer—SCIEX) ICP-MS from the "Laboratoire des Mécanismes de Transfert en Géologie" (LMTG, Toulouse, France). The LASER was operated in the Q-switched mode with a power of 5 mJ and a repetition rate of 10 Hz. Each analysis was done as a single point and lasted between 50 and 60 s with the first 6–8 s as background acquisition (gas blank). LASER operating conditions are summarized in Table 1. The resulting craters had a diameter of 60 µm (Fig. 2 b). The element and isotopes analyzed were ²⁴Mg, ⁴³Ca, ⁴⁴Ca, ⁵⁵Mn, ⁸⁸Sr, ¹³⁷Ba and ¹³⁸Ba. The signals were integrated on 10–15 s when stability has been reached (after the initial peak), with the first 5 s subtracted as blank.

To calculate trace element concentrations in the *C. concholepas* shell, several external standards were tested for calibration: (1) the NIST-610 silicate glass (US National Institute of Standard and Technology); a pressed pellet of (2) GSR-6 limestone (Institute of Geophysical and Geochemical Prospecting, China), (3) a calcite (C1) and (4) a fish otolith CRM-22 (National Institute for Environmental Studies, Japan). The GSR-6 pellet was prepared using 500 mg of powder pressed under a 10-ton/cm². For C1 we used a pressure of 8 ton/cm² on 350 mg and for CRM-22 10 ton/cm² on 500 mg. Element concentrations of the standards used are reported in Table 2. Five analyses in each standard were done at the beginning of each analytical session to assess reproducibility and

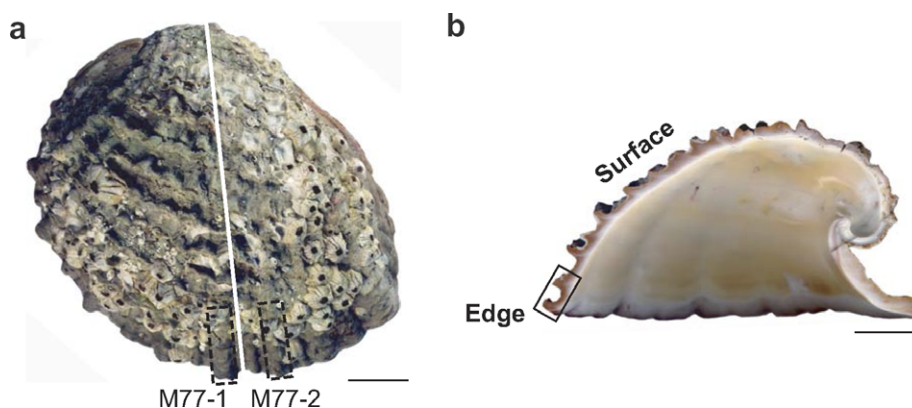


Fig. 1. The *Concholepas concholepas* specimen analyzed in this study (scale bars = 1 cm). (a) Dorsal view of the shell. White line: maximum growth axis, black broken boxes: samples studied. (b) Cross section of the shell. Black box: position of the M77-1 sample.

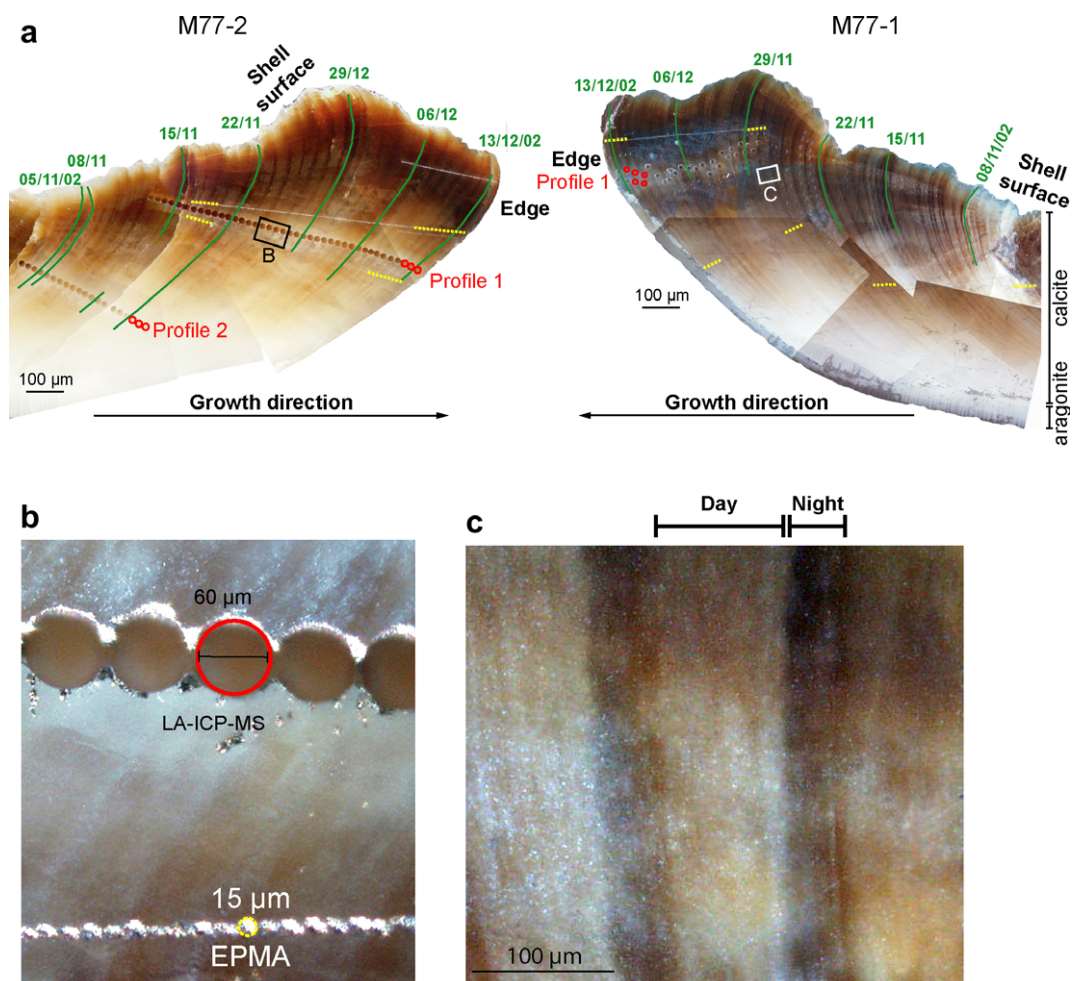


Fig. 2. Cross-section of the *Concholepas concholepas* shell studied (optical microscope, diffused natural light) after high-resolution analyses. Red circles: LA-ICP-MS, yellow dots: EPMA. (a) Global view of adjacent sections M77-1 and M77-2 showing the sub-daily growth pattern and the high-resolution profiles; green line: position and date of the weekly calcein markings. (b) Close-up of the high-resolution spots on the M77-2 section. (c) Close-up of the sub-daily growth pattern on the M77-1 section. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Table 1

LASER probe operating conditions

LASER mode	Q-switched
Laser power	5 mJ
Frequency	10 Hz
Preablation time (blank)	6–8 s
Acquisition time (laser firing)	50–60 s
Crater size	60 μm
Objective	5–10×
Depth profiling	1–5 μm s ⁻¹

accuracy, and to test standards for calibration. Then, each standard was analyzed every 10 samples for additional correction of possible instrumental drifts and calculation of element concentrations.

2.2.2. Electron probe microanalysis (EPMA)

Trace elements were also determined by EPMA for comparison with the LA-ICP-MS analyses. The analyses were performed with a

Table 2

Element concentrations (ppm) of the external standards used for LA-ICP-MS analyses and of the *Concholepas concholepas* calcite layer (mean solution ICP-MS values)

	Mg	Ca	Sr
C1 (calcite)	2240	398,100	208
GSR-6 (limestone)	31,300	254,800	913
CRM-22 (otolith)	21	388,000	2360
NIST 610 (glass)	465.3	81,790	497.4
<i>C. concholepas</i> (calcite)	990	37,000	1200

C1, ICP-MS home-values (M. Valladon - LMTG, Toulouse, personal communication); GSR-6, recommended values from Govindaraju (1994); CRM-22, certified values from National Institute for Environmental Studies (Japan); NIST-610, values from Pearce et al. (1997); *C. concholepas* values from Guzmán (2004).

Cameca SX 100 at the "Centre Camparis" (IPG-Paris VI, France). The accelerating potential was set at 15 keV, the Faraday cup current at 40 nA for Ca and 400 nA for Mg, Mn, Sr and Ba. The

beam was defocused (10 μ m) and acquisition times were of 10 s for Ca, 20 s for Ba and 20–40 s for Mg, Mn and Sr. A diopside and a strontium silicate crystal of known composition were used as standards. The analytical precision expressed as 2 σ was calculated using the method developed by Ancey et al. (1977). Chemical profiles were conducted on the shell with an automated step of 10 μ m for one profile and 20 μ m for the others (Fig. 2 b). Six profiles were done, two in M77-1 and five in M77-2. To obtain trace element EPMA time-series, and because the points are small, numerous and regularly spaced, a date was allocated to points close to the calcein lines and the dates in between were interpolated.

With a mean daily growth rate of 95 μ m day⁻¹ for the considered period, the mean temporal resolution obtained is two analyses per day with LA-ICP-MS and ten analyses per day with EPMA.

3. RESULTS

Trace element (Mg and Sr) profiles were obtained in the *C. concholepas* shell by using two high-resolution techniques, LA-ICP-MS and EPMA. Barium and Mn were also analyzed using both techniques but remained below detection limits in the *C. concholepas* shell. Consequently, Ba and Mn will not be further discussed.

3.1. LA-ICP-MS: internal standardization and calibration

To correct for different ablation yields and loss of sensitivity through time, both ⁴³Ca (Longerich et al., 1996; Günther et al., 1999; Craig et al., 2000) and ⁴⁴Ca (Toland et al., 2000; Hendry et al., 2001) have been used as an internal standard in LA-ICP-MS analysis of carbonates. The best results are obtained for internal standardization when the analyte and internal standard have a similar behavior vs. time (Longerich et al., 1996). In our case, the ⁴⁴Ca signal yield was too high in comparison with those of the analytes of interest to provide a good internal standard and we thus choose to use ⁴³Ca as internal standard for all of our analyses.

To determine the best external standard for the calibration of the LA-ICP-MS data, the homogeneity of the different standards chosen has been evaluated in comparing the reproducibility of standard analyses. The reproducibility was checked on integrated stable signals after blank subtraction and internal normalization but without external calibration. Reproducibility is expressed as relative standard deviation expressed in percent (RSD).

For GSR-6 (n = 18), reproducibility is above 10% (Fig. 3). Fractionation effects related to variable particles sizes generated by the LASER ablation process have been reported, but these effects have been reported to be negligible for both Sr and Mg (Guillong and Günther, 2002; Kuhn and Günther, 2004). Even if such particle size fractionation effect cannot be excluded for the GSR-6 pressed pellet, optical observation of the pellet suggest that it remains heterogeneous because of a powder not fine enough. For C1 (n = 22), Mg and Sr reproducibility are around 8% and 6% respectively. For CRM-22 (n = 14), only Sr, the most abundant trace element in this substrate, gives good results with a RSD of 5.6%. For NIST-610, reproducibility is below 10% (n = 19). Hendry et al. (2001) obtained similar NIST-610 reproducibility. Better reproducibility, of less

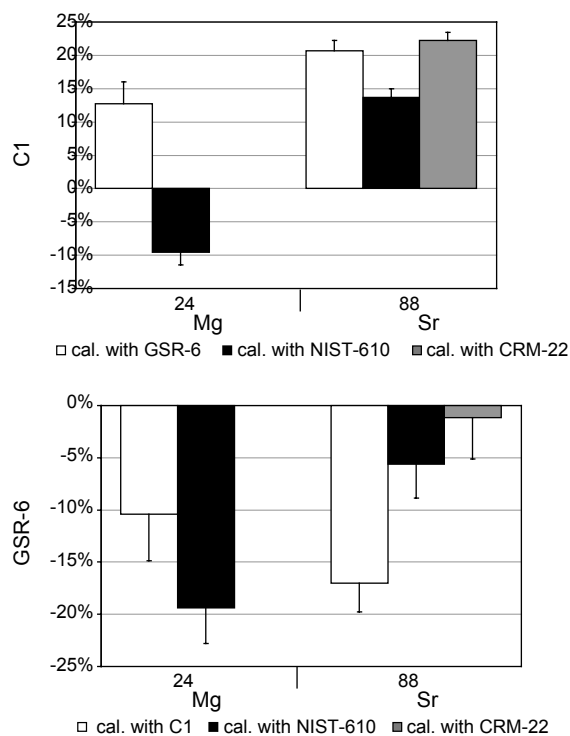


Fig. 3. Mean accuracies of LA-ICP-MS ²⁵Mg and ⁸⁸Sr data, expressed as the percentage of difference between calculated and recommended values, for C1 and GSR-6 calibrated with different external standards. Standard errors of the means are reported as error bars on the graphs. For C1 calibrated with GSR-6 and NIST-610, n = 22 (8 + 14 in two sessions), for GSR-6 calibrated with C1 and NIST-610, n = 18 (6 + 12 in two sessions) and for C1 and GSR-6 calibrated with CRM-22, n = 14 and 12, respectively.

than 5%, was obtained in other studies that used the same type of LASER but under different operating conditions, particularly using lower frequencies (6.5 Hz) and energy output (<1–3 mJ) (Norman et al., 1996; Epplé, 2004).

Carbonate reference materials are especially important for external calibration and to check the accuracy of biocarbonate LA-ICP-MS analysis given the difficulty that may be encountered during the LASER ablation of such material. However, use of ultraviolet LASER reduces matrix effects (Geertsen et al., 1994; Günther et al., 1995; Norman et al., 1996). Several types of potential external solid standards have been tested to calibrate analyses of biogenic CaCO₃ by LA-ICP-MS. These include silicate glass reference materials (e.g. Price and Pearce, 1997; Hendry et al., 2001; Fallon et al., 2002; Lee et al., 2004), “home-made” carbonate standards (Sinclair et al., 1998; Fallon et al., 1999; Bellotto and Miekeley, 2000; Runnalls and Coleman, 2003; Gillikin et al., 2005) and geological reference materials (Craig et al., 2000; Toland et al., 2000). Rosenheim et al. (2004) used the fish otolith reference material CRM-22 (Yoshinaga et al., 2000) for their sclerosponge LA-ICP-MS study. In the present work, we tested four reference materials, the NIST-610 glass, two geological materials (C1 and GSR-6) and the otolith CRM-22, the latter three as pressed pellets. The choice of an external

standard for the calibration of the *C. concholepas* analyses was determined on the basis of the reproducibility discussed above and accuracy of the calibrated analyses. The accuracy of the analyses of C1 and GSR-6 was evaluated when calibrated with the two other standards (Fig. 3). Accuracy is expressed as the difference between calibrated and known value divided by known value, and expressed in percent. Standard errors of the means of the calibrated data are reported in Fig. 3 and reflect the global reproducibility.

For Mg, accuracies are around 10%, except for GSR-6 when calibrated with NIST-610 (20% of difference with the recommended value). The accuracy obtained for GSR-6 when calibrated with NIST-610 is probably linked to the important difference in Mg content between GSR-6 and NIST-610 (cf. Table 2) but it remains reasonable for this substrate. Craig et al. (2000) obtained differences of -46% between measured and recommended contents of Mg in GSR-6 when their LA-ICP-MS analyses were calibrated using a calibration line obtained from different "home-made" synthetic pressed-powder pellet calibrations. When the LA-ICP-MS data were calibrated using reference material calibration line, Craig et al. (2000) obtained almost no difference between recommended and measured GSR-6 Mg content. But, in this last case, the reference material calibration line used to calibrate GSR-6 Mg data already includes the GSR-6 material (see Table 6 in Craig et al., 2000). The highly accurate result obtained in their study for Mg GSR-6 when using the reference material calibration line is probably related to the fact that GSR-6 was partly calibrated against itself.

For Sr, accuracies obtained for C1 and GSR-6 when calibrated with different external standards are variable; only the NIST-610 calibration gives values systematically within less than 15% of difference with recommended values (Fig. 3). The accuracies obtained on GSR-6 Sr data are better than those obtained by Craig et al. (2000) whatever the calibration line they used. The improvement is especially obvious for calibration with NIST-610 and CRM-22.

Consequently, given the reproducibility and accuracy obtained, the NIST-610 standard was chosen for calibration in this study. This calibration procedure has been verified by standard liquid ICP-MS analyses on micromilled powders sampled on another section of the same shell (Electronic annex, EA-1). Absolute trace element contents in the *C. concholepas* shell were calculated using the mean of NIST-610 analyses bracketing the different profiles. When a signal bias of more than 5% was observed on NIST-610 data, calibration calculations were performed on sub-sections of the profiles. The mean Ca content of the shell part analyzed with EPMA ($Ca = 38.2 \pm 0.3\%$) was used in the internal standardization for calculation of Mg and Sr concentrations.

3.2. Growth and temperature variations

For convenience and consistency in the following sections, we will use the term "day" for the period with natural sunlight as opposed to the term "night". The adjective "daily" will be used when both "day" and "night" are taken into account, from one morning to the next one (24-h).

To evaluate the possible implications of growth rate variations on geochemical changes in the shell, daily growth was measured under the microscope as the thickness (in μm) of one couplet of translucent and opaque increment. In addition, day vs. night growth was measured as the thickness of translucent and opaque increments respectively (two growth values per day, Gr_{day} and Gr_{night}). Daily growth varied between $19\mu\text{m}$ to $150\mu\text{m}$. A Mann-Whitney test ($p < 0.05$) shows that growth during day (mean $Gr_{\text{day}} = 50 \pm 9\mu\text{m}$, 1 standard deviation, SD) is significantly faster than growth during the night (mean $Gr_{\text{night}} = 34 \pm 8\mu\text{m}$, 1SD).

The recorded water temperature increased regularly from 17 to $22\text{ }^\circ\text{C}$ during the month and a half experiment with several peaks ($1\text{ }^\circ\text{C}$ in range) in between, the broadest one occurring between the 5th and 10th of December 2002 (see Fig. 7 a). To compare the recorded water temperature with shell growth, we compute mean daily water temperature (from 6:00 to 6:00 next day) as well as mean day and night water temperature ($T_{\text{day}} =$ from 6:00 to 18:00, $T_{\text{night}} =$ from 18:00 to 6:00) for each day of growth.

No relation was found between daily growth and daily water temperature. When the data are grouped in terms of day and night, $T_{\text{day}}/Gr_{\text{day}}$ and $T_{\text{night}}/Gr_{\text{night}}$, these two groups are significantly different (discriminant analysis, $p < 0.05$). No relation was found between Gr_{day} and T_{day} (Fig. 4 a) whereas 12% of the variability of Gr_{night} can be explained by T_{night} changes with a weak, but significant ($p < 0.05$) positive linear relationship ($r^2 = 0.12$) (Fig. 4 b).

3.3. Trace element profiles

3.3.1. Strontium

The mean Sr concentration of the *C. concholepas* shell measured by LA-ICP-MS is of $1045 \pm 83\text{ ppm}$ (1 standard deviation, SD). The RSD of the LA-ICP-MS analyses of Sr content along a single growth layer is of 4% ($n = 16$) and of 8% when crossing the growth lines. Furthermore, the analytical error on NIST-610 LA-ICP-MS Sr measurements is of 6%. Consequently, Sr content can be considered as homogeneous and variations observed using LA-ICP-MS can hardly be considered as significant. The mean Sr content in the *C. concholepas* shell as measured by EPMA is of $1272 \pm 210\text{ ppm}$ (1SD). Three profiles were not considered because Sr concentrations measured by EPMA were below detection limits (840 ppm). The mean standard deviation for EPMA is 125 ppm. Electron microprobe Sr variations are thus not significant (Fig. 5). The mean Sr abundance measured by EPMA is higher by 18% than LA-ICP-MS determinations (Wilcoxon-Mann-Whitney test, $p < 0.05$). Strontium contents in the *C. concholepas* shell studied were also measured by standard liquid ICP-MS technique and the results confirm the values obtained by LA-ICP-MS (EA-1). On the other hand, the measurements of Sr content on the *C. concholepas* shell using EPMA appears too high. This is probably because the Sr contents of the shell are too close to EPMA detection limits. This is confirmed by the lack of agreement between the Sr variations found along the profiles with the two analytical technique used.

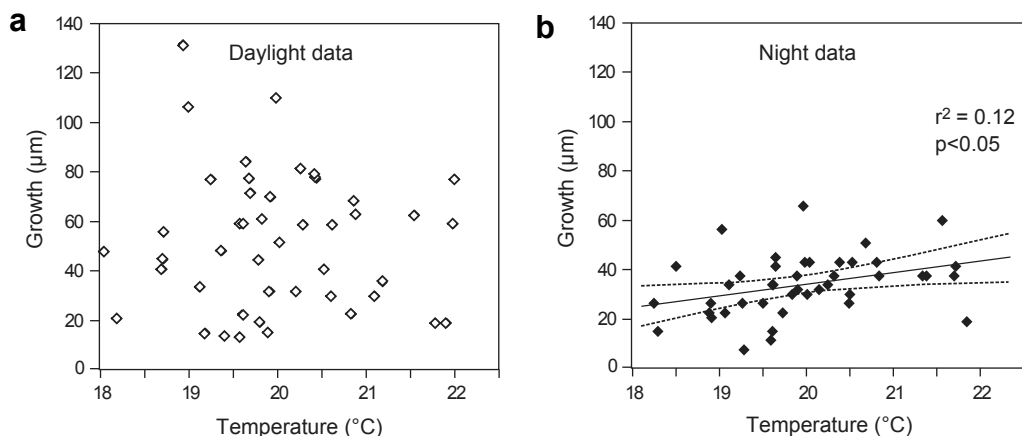


Fig. 4. Relation between water temperature and *Concholepas concholepas* shell growth. (a) Daylight data (water temperature mean from 6:00 to 18:00 h; growth: thickness of translucent increments). (b) Night data (water temperature mean from 18:00 to 6:00 h; growth: thickness of opaque increments) with least square correlation line and 95% confidence level (dotted lines).

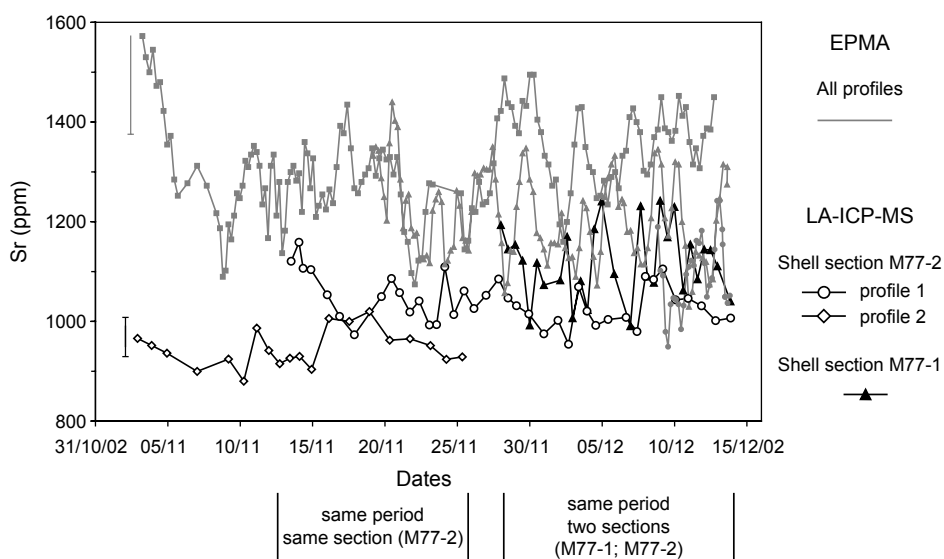


Fig. 5. Strontium profiles in the *Concholepas concholepas* shell obtained by LA-ICP-MS and EPMA (data smoothed using a four points running average; distinct profiles are represented by different symbols; see Figs. 1 and 2 for location). LA-ICP-MS error bar = Sr RSD along a single growth line (4%); EPMA error bar: mean analytical standard deviation.

3.3.2. Magnesium

The mean abundance of Mg in the *C. concholepas* shell, as measured by using LA-ICP-MS, is 487 ± 201 ppm (1SD; 41% of RSD). Magnesium variations observed by LA-ICP-MS (Fig. 6) are highly significant as the RSDs of the LA-ICP-MS analyses of Mg content along a single growth layer and the analytical error on NIST-610 LA-ICP-MS are only of 8%. Further, two parallel LA-ICP-MS profiles in the same shell section M77-2 show that these Mg variations reproduce well within the same growth period (Fig. 6). On the other hand, results for the same growth period but in two different sections, M77-1 and M77-2, show slight differences between 28/11 to 08/12/02 (Fig. 6). This is probably because of the larger distance between the two profiles (Fig. 1). Magnesium profiles measured by EPMA are highly noisy and are presented as a 4 point running average in the

Fig. 6. The mean analytical standard deviation for EPMA Mg analyses is 20 ppm. The general pattern shown by EPMA and LA-ICP-MS for the month-and-a-half period is roughly similar (Fig. 6). Magnesium variations across the analyzed part of the shell display four maxima (excluding the margin of the shell) more clearly shown by LA-ICP-MS, with an amplitude decreasing through time of shell formation (i.e., from left to right on Fig. 6).

To compare geochemical variations with water temperature fluctuations, we calculated a mean recorded temperature for the time interval sampled for each LA-ICP-MS analysis point. No correlation is found between Mg content and water temperature (Fig. 7 a). The Mg variations of the *C. concholepas* shell analyzed do not follow the water temperature increase during the month-and-a-half recorded and do not display significant variations during the water

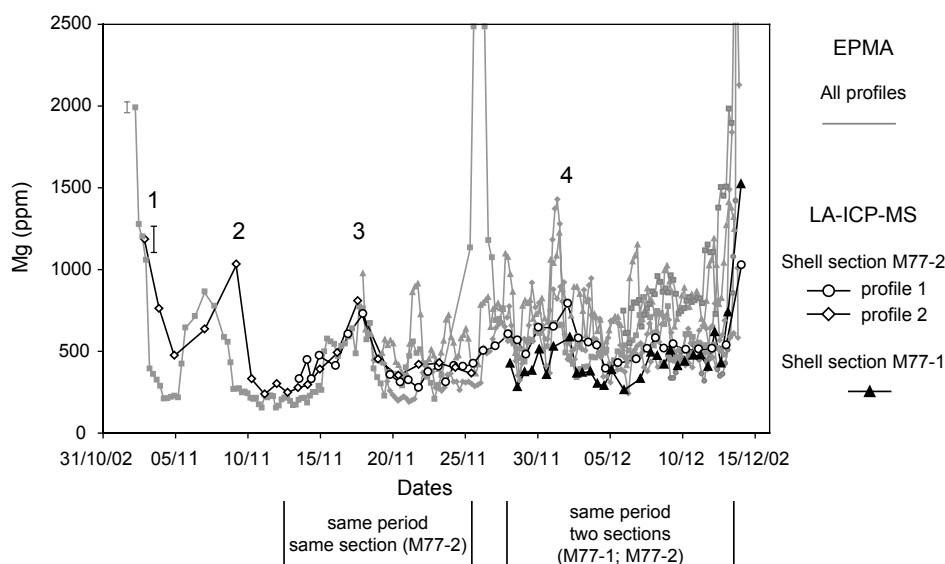


Fig. 6. Magnesium variations in the *Concholepas concholepa* shell obtained by LA-ICP-MS and EPMA (data smoothed using a 4 points running average, different profiles are represented by different symbols; see Figs. 1 and 2 for location) measurements. LA-ICP-MS error bar = NIST-610 Mg RSD (8%); EPMA error bar: mean analytical standard deviation; numbers 1–4 correspond to the maxima described in the Section 3.

temperature peaks observed. Furthermore, no clear relation is found between Mg variations and daily growth changes either (Fig. 7 b).

To better understand the Mg incorporation processes into the shell, we separated, through optical-microscope observation of the M77-1 and M77-2 profiles, Mg analyses that covered mainly day shell formation ($[Mg]_{\text{day}}$) from those that correspond mainly to night increments ($[Mg]_{\text{night}}$). It appears that the mean $[Mg]_{\text{day}}$ content ($[Mg]_{\text{day}} = 396 \pm 21$ ppm, 1 SE) is significantly lower than the mean $[Mg]_{\text{night}}$ content ($[Mg]_{\text{night}} = 521 \pm 30$ ppm, 1 SE; t-test, $p < 0.05$). When the water temperature and Mg day vs. night are considered, no relation between $[Mg]_{\text{night}}$ and T_{night} is observed. In contrast, a weak but significant positive relationship between $[Mg]_{\text{day}}$ and T_{day} is observed ($r^2 = 0.30$; $p < 0.05$) (Fig. 8). No relation between Mg content and growth rate was found for both day and night.

4. DISCUSSION

4.1. Analytical and intra-shell reproducibility

In this study, the mean Sr abundance measured by EPMA is 18% higher than that determined by LA-ICP-MS. No such large differences between the two techniques and for the different LA-ICP-MS profiles are observed for Mg. Furthermore, standard liquid ICP-MS analyses on the same sample do validate the Sr content as determined by LA-ICP-MS. On *Arctica islandica* shells, Eplé (2004) also obtained EPMA analyses of Sr content that appeared to be 14% higher than measurements made by LA-ICP-MS on the same samples. The agreement between LA-ICP-MS/ICP-MS suggests that the observed inconsistencies between analytical techniques on Sr abundance in *C. concholepa* shell are probably linked to calibration problems of the

EPMA technique for Sr in biocarbonates. In our experiment with *C. concholepa*, we found no significant changes in Sr concentrations in the shell. Consequently, either Sr incorporation is not influenced by water temperature or shell growth variations, or environmental/physiological changes were too low to impact Sr incorporation into the shell.

The results of EPMA Mg measurements in the *C. concholepa* shell are particularly noisy. These variations show that the incorporation of Mg into the shell does occur at the same timescale as the biomineralisation process, which is hourly in the case of *C. concholepa*. With the EPMA setting used, the rough correspondence observed between EPMA and LA-ICP-MS Mg variations suggests that EPMA might be used to obtain Mg profiles in biocarbonates with a very-high spatial resolution provided analytical improvements. For the same period of growth (13/11 to 25/11/02) of the section M77-2, Mg variations obtained for two overlapping LA-ICP-MS profiles are identical (Fig. 6). In distinct sections close to the maximum growth axis (M77-1 and M77-2), similar Mg results are obtained. The Mg variations in the *C. concholepa* shell are therefore spatially reproducible. This demonstrates that, in our case, interpretation of shell Mg variations as an environmental proxy is reliable whatever the considered section of the shell, at least for transects close to the maximum growth axis.

4.2. The *C. concholepa* external shell layer: a very-low Mg calcite

In this study, the mean Mg content of the calcite layer of the *C. concholepa* shell (~ 500 ppm with both techniques used) compares well with values previously obtained in other *C. concholepa* shells (~ 700 ppm by using Energy Dis-

Shell Mg nyctemeral variations in *C. concholepas*

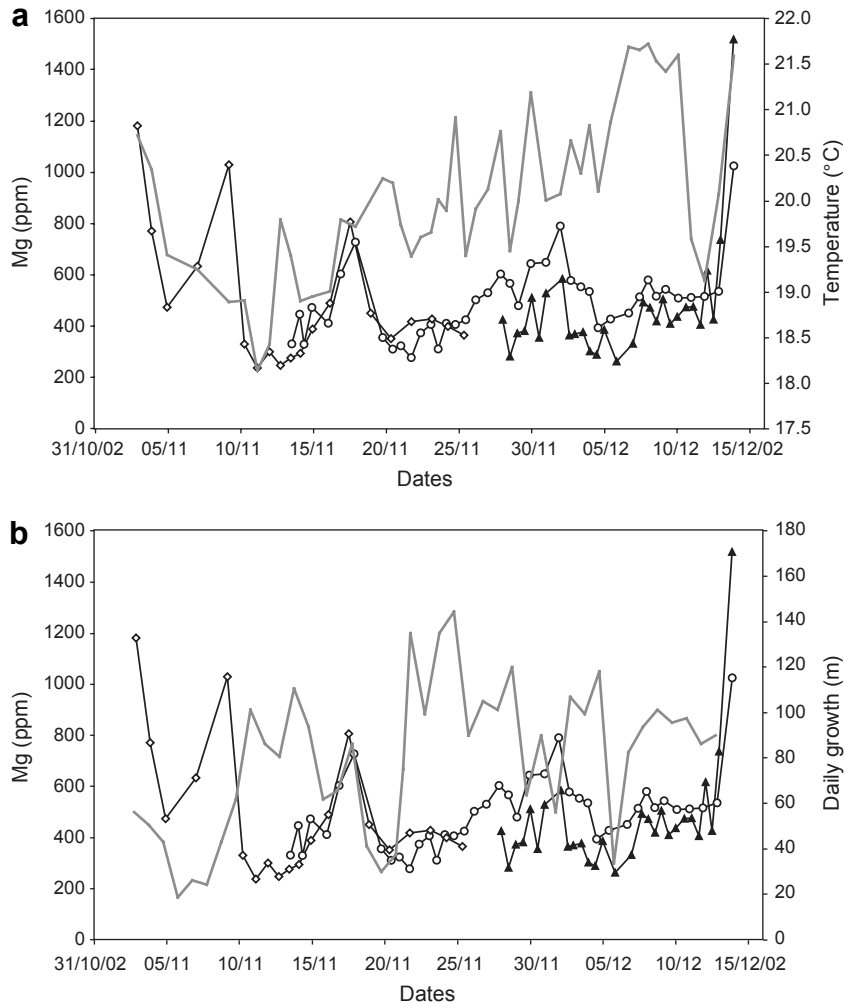


Fig. 7. Comparison between high-resolution measurements of Mg variations determined by LA-ICP-MS in the *Concholepas concholepas* shell (lines with symbols, same as Fig. 6) with thermal and growth rate data. (a) Plot of Mg and water temperature (gray line) vs. time. (b) Plot of Mg and daily growth (black line) vs. time.

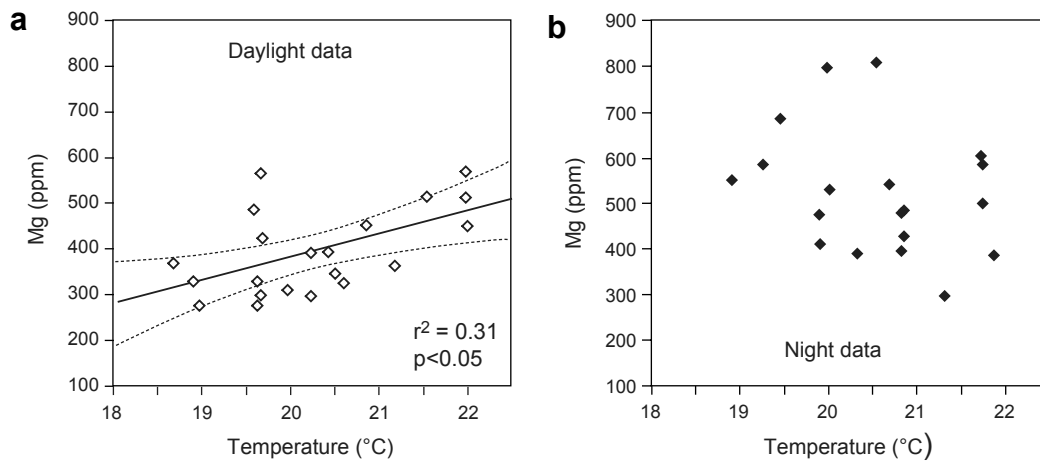


Fig. 8. Relationship between water temperature and Mg abundance in the *Concholepas concholepas* shell, measured by LA-ICP-MS, at the nyctemeral rhythm. (a) Daylight data with least-square correlation line and 95% confidence level (dotted lines). (b) Night data.

persive Spectroscopy in Dauphin et al., 2003b ; between 500 and 1000 ppm by EDS and solution ICP-MS in Guzmán, 2004). This Mg content corresponds to a mean Mg/Ca ratio around 2 mmol/mol, a low value when compared with other calcitic mollusks (e.g. Masuda and Hirano, 1980; Klein et al., 1996; van der Putten et al., 2000; Freitas et al., 2005; Lorrain et al., 2005 ; Fig. 9), or even those referred to as low-Mg calcite species (e.g. rudists, Immenhauser et al., 2005; Steuber and Rauch, 2005), and to foraminifera (Nürnberg et al., 1996; Lea et al., 1999; Lear et al., 2002). This very low Mg content in the *C. concholepas* calcitic layer is actually comparable to those reported for aragonite biocarbonates, which is around 2 mmol/mol for aragonitic bivalves (e.g. Foster and Cravo, 2003; Takesue and van Geen, 2004; Gillikin et al., 2005), around 4–5 mmol/mol in corals (e.g. Quinn and Sampson, 2002; Kilbourne et al., 2004) and around 1 mmol/mol for sclerosponges (e.g. Rosenheim et al., 2005) (Fig. 9). Lorens and Bender (1977, 1980) postulated that *Mytilus edulis* physiologically exclude Mg from their extrapalleal fluid to precipitate their calcite shell. The Mg partition coefficient, $D_{Mg} = (Mg/Ca)_{calcite}/(Mg/Ca)_{seawater}$ for the *C. concholepas* calcite layer is of $0.04 \cdot 10^{-2}$ (using a $Mg/Ca_{seawater}$ value of 5.15 mmol/mol; Broecker and Peng, 1982), lower than that observed for *M. edulis* ($0.12\text{--}0.13 \cdot 10^{-2}$; Lorens and Bender, 1980; Gillikin, 2005) and far lower than that of inorganic precipitated calcite ($1.7 \cdot 10^{-2}$; Mucci, 1987). This might

indicate a strong physiological control of Mg incorporation into the *C. concholepas* shell.

4.3. Magnesium incorporation in *C. concholepas* a complex processes at a nyctemeral rhythm

Microgrowth increment studies on molluskan shells have led to conclude that growth lineation is a reflection of the alternation of “calcium-carbonate-rich” and “organic-rich” regions (Lutz and Rhoads, 1980). This succession of more or less organic-rich increments has been observed using different methods (optical and electron microscope) and shell preparations (thin sections, acetate peels, etc.). For *C. concholepas* shells, daily growth results in the accretion of one translucent and one dark increment when viewed with an optical microscope under diffused light. Staining experiments showed that translucent increments correspond to shell growth during the day and that opaque increments correspond to shell growth during the night. Darker increments are usually interpreted as organic rich laminae in mollusks shell (e.g. Pirker and Schiel, 1993; Shepherd et al., 1995) and otoliths (e.g. Panella, 1980; Morales-Nin, 1987; Wright et al., 2002). It has been established that sulfur is associated with the organic phase in biocarbonates, both corals and mollusks (Lorens and Bender, 1980; Rosenberg et al., 2001; Dauphin et al., 2003a; Cuif and Dauphin, 2005; Dauphin et al., 2005). The S is present

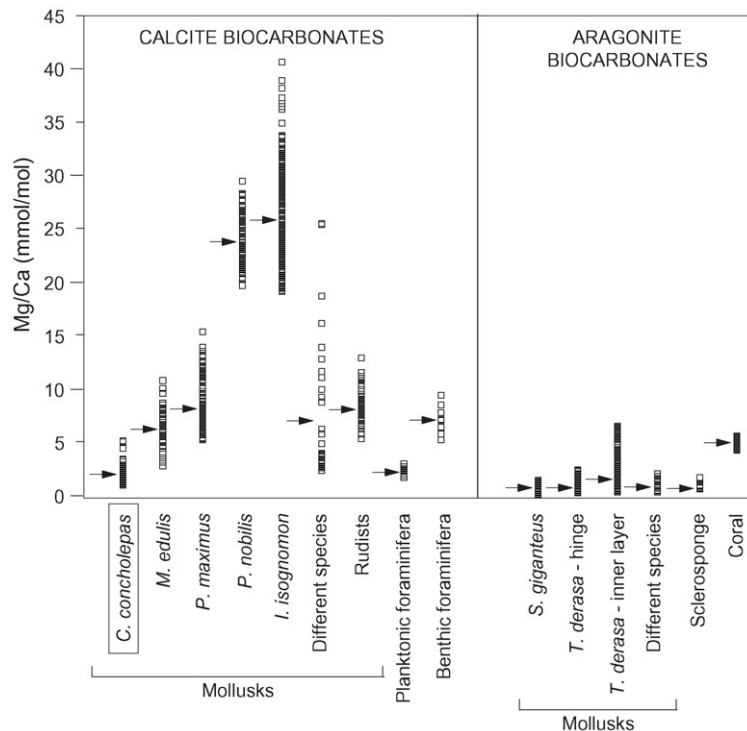


Fig. 9. Comparison of Mg/Ca ratios from the calcitic layer of *Concholepas concholepas* (this study; LA-ICP-MS data) with Mg/Ca from other biominerals. Squares: Mg/Ca values; arrows: point average values. Other calcite data from: bivalves (*Mytilus edulis*, Klein et al. (1996) ; *Pecten maximus*, Lorrain et al. (2005)); *Pinna nobilis*, Freitas et al. (2005)); *Isognomon ephippium* EA-2 ; different species, Masuda and Hirano (1980) ; rudists, Immenhauser et al. (2005)) and foraminifera (Lear et al., 2002; Anand et al., 2003 ; calculated for temperatures between 17 and 23°C). Aragonite data from: bivalves (*Saxidomus giganteus* Gillikin (2005)); *Tridacna derasa*, Elliot, personal communication), gastropod species (Foster and Cravo, 2003), *Ceratoporella nicholsoni* sclerosponge (Rosenheim et al., 2005) and *Porites lutea* coral (Quinn and Sampson, 2003). Arrow: mean value.

as sulfated polysaccharides which repartition is directly related to growth layers (Cuif et al., 2003; Guzmán et al., 2007). Guzmán et al. (2007) evidenced the sulfated polysaccharide nature of S in *C. concholepas* and its link with the shell banding pattern (see Fig. 9 in Guzmán et al., 2007). An EPMA map of S distribution, that will be published elsewhere, shows that dark increments in *C. concholepas* shell have a higher S content than translucent ones. The fact that S is well linked to growth layers and microstructural organization, together with Guzmán et al. (2007) results, strongly suggests that it is linked to biological growth process and in this way it can not be considered as “pure mineral” nature. At last, several *C. concholepas* shell samples were etched using the “Mutvei’s solution” that includes glutaraldehyde, a fixative molecule that reduces the solubility of organic compounds (Schöne et al., 2005). The etched samples observed by scanning electron microscopy reveal that translucent growth layers were more sensible to etching, meanwhile dark growth layers were less attacked and their homogeneity was preserved. Thus, *C. concholepas* dark increments that correspond to night shell growth are richer in organic matrix than the translucent (day shell growth) increments.

In the *C. concholepas* shell studied, distinct processes are at work at a nyctemeral rhythm, in terms of global Mg content and growth. The *C. concholepas* shell exhibits a faster growth during the day ($Gr_{\text{day}} = 50 \pm 9 \mu\text{m}$; 1SD) than during the night ($Gr_{\text{night}} = 34 \pm 8 \mu\text{m}$; 1SD), when opaque, organic-rich increments are observed. From their study on *M. edulis* shell metabolism, Rosenberg and Hughes (1991), conclude that “matrix-rich shell is metabolically more expensive to produce than mineral-rich shell”, an affirmation supported by Palmer (1983, 1992). While Palmer (1983, 1992) works rely on populations and whole shells studies, Rosenberg and Hughes (1991) made their observation on different parts, close to the umbo/slow growth vs. edge/fast growth, of *M. edulis* shells. Rosenberg and Hughes (1991) found high Mg and S content close to the slow growing umbo, where they previously revealed a high mantle metabolism activity (Rosenberg et al., 1989). In the case of *C. concholepas*, this is somehow different because various Mg content, organic-phase richness and shell growth are observed in the shell at the nyctemeral scale. Nevertheless, if we transpose to this study the Rosenberg and Hughes (1991) metabolic model, we would then interpret that the metabolic activity of the mantle of *C. concholepas* changes at a subdaily rhythm, with a high metabolic activity at night (high Mg, low growth rate, high organic content) and a low metabolic activity during daytime (low Mg, high growth rate, low organic content).

For a more accurate interpretation in terms of different metabolic activity between night and day, we consider the Mg flux into the shell. The experiment took place under natural light at 23 °S at a time when daylight and night were almost equal. Because the *C. concholepas* shell formation is an hourly process (Guzmán et al., 2007), we made the assumption that translucent (day) and dark (night) increments represent the same amount of time (12 h). Consequently, a LASER ablation crater of 60 μm of diameter centered on an increment formed during the day generally

encompasses less than 12 h of shell formation. We thus estimate the amount of Mg incorporated into the shell per hour (Mg flux), for day vs. night analyses, as the content of Mg measured by LA-ICP-MS divided by an estimation of the mean number of hours of shell formation sampled (mean of 12 h per night analysis vs. mean of 9 h per day analysis for a 60 μm analysis point). It appears that this proxy of the Mg flux into the shell during night growth ($\sim 35 \text{ ppm/h}$) is lower than that of the day Mg flux into the shell ($\sim 45 \text{ ppm/h}$).

What could explain such a difference between day and night Mg intake in the shell carbonate? As stated before, a matrix-rich shell portion is more metabolically expensive to produce than a shell portion with less matrix content. The production of the nocturnal organic rich increments of the *C. concholepas* shell thus requires a larger amount of energy. In addition, during several years of growth experiment on many individuals of *C. concholepas* at the University of Antofagasta facilities, we observed that the animals ate almost exclusively at night. Clearly, *C. concholepas* spends much more energy at night, moving and eating, while the individuals keep still during the day. The primary energy provider in all organisms is the adenosine triphosphate (ATP) molecule. The formation of this molecule mainly derives from the catabolism of carbohydrates. The reactions that imply ATP need Mg^{2+} as a cofactor. We can assume that during the night the high amount of energy needed by the *C. concholepas* for locomotion, nutrition and organic-matrix rich shell formation involves more ATP molecules than during less active daylight periods. Concerning the matrix-rich shell formation, this hypothesis is supported by the work of Wilbur and Saleuddin (1983) that showed that more ATP molecules are needed to produce protein than to produce calcium carbonate. As Mg^{2+} ions are required for the use of ATP, we suggest that Mg^{2+} ions are more specifically allocated for the production of energy during the night, thereby explaining the less important flux of Mg into the shell grown during the same period. This hypothesis can explain the different relationships between Mg and water temperature during the day and at night. If shell Mg incorporation process is preferentially controlled by metabolism during the night, we can assume a minor control of the organism metabolism on Mg during the day, when *C. concholepas* is motionless and when less organic-rich shell is formed. As we observe a positive relation between water temperature and Mg for day data only, it might be interpreted that water temperature influence on shell Mg incorporation is more important during the day. Finally, we surmise that the weak but significant positive relationship between water temperature and growth during the night probably reflects an increase of metabolic efficiency with water temperature.

4.4. Implications for paleo-SST reconstructions

The complexity of the nyctemeral interrelation between water temperature, growth and shell Mg incorporation revealed in this study can explain the difficulties encountered in the use of mollusk shell Mg content as a proxy for SST reconstructions. For calcite mollusks, the use of shell

Mg/Ca ratio as SST proxy is still debated. Klein et al. (1996) found a significant positive relation between Mg/Ca and SST in *Mytilus trossulus* and then used the equation established to conduct paleo-SST and paleo-salinity reconstructions by combining Mg/Ca and $\delta^{18}\text{O}$ profiles from fossil *M. trossulus* (Klein et al., 1997). In *Isognomon ehippium* shells from Kenya, Mg variations were shown to closely follow those of SST (Lazareth et al., 2003). However, other studies point to the non-systematic relationship between Mg/Ca ratio and SST, for single individuals as well as between individuals. From a one year study of growth of *M. edulis*, van der Putten et al. (2000) observed a positive correlation between Mg/Ca and SST only during spring. Similarly, Freitas et al. (2005) showed that in several *Pinna nobilis* shells, a reliable SST can be calculated from Mg/Ca ratios only for the first 4.5 years of growth. An increasing ontogenetic effect was evidenced after this early growth phase of the *P. nobilis* shells. In an earlier work, Richardson et al. (2004) also evidenced a positive relation between Mg/Ca and SST for the first, or two first year(s) of growth of some *P. nobilis* shells, but the different Mg/Ca ratios observed between different specimens growing at the same time led the authors to conclude that it was not possible to deduce an accurate SST directly from Mg/Ca records. It is thus clear that the Mg (or Mg/Ca) relationship with SST is species-dependent and that Mg incorporation in calcite shells is also physiologically controlled. To our knowledge, no other studies investigated the Mg variations in calcite shells at the subdaily resolution as done in the present study, but the nyctemeral difference observed in the *C. concholepas* shell may exist in other species. This can explain at least some of the discrepancies observed between shell Mg (or Mg/Ca) contents and paleo-SST reconstructions. Our results show that for species like *C. concholepas* it is important to take into account the period of the day corresponding to the area analyzed (i.e. translucent vs. opaque bands) to produce meaningful paleo-SST reconstructions. Similar studies are needed on other *C. concholepas* specimens but also for other species of mollusks before a generalization of the processes and mechanisms proposed here can be extrapolated. In the same way, other hypothesis, as changes in saturation state of the calcifying fluid, could explain Mg/Ca differences within the growth layers, but research needs to be extended in this sense.

5. CONCLUSIONS

In this study, geochemical analyses were performed on a *C. concholepas* shell with a subdaily time-resolution. Concurrently, high-time resolution information on growth conditions were acquired during the experimental growth of the animals using a highly precise timescale provided by repeated calcein staining. In situ geochemical measurements show that Sr variations in the shell are not sufficiently important to be interpreted in terms of environmental parameter changes. The very-low Mg content found in the *C. concholepas* calcite layer, measured by LA-ICP-MS, most probably indicates a strong physiological control on Mg incorporation into the shell. In detail, this control appears partly influenced by water temperature when there

is daylight. No relation between Mg concentration, growth rate and water temperature is observed at the daily (24 h) scale. Instead, distinct processes, both in terms of Mg incorporation into the shell and shell formation, are at work at a nyctemeral rhythm. During the night, thin matrix-rich increments are formed and Mg intake into the shell carbonate is less important than during the day. This suggests a strong metabolic control that might be related to the increased demand of energy of the *C. concholepas* during the night, at a time when these animals are the most active. This metabolic influence is probably reduced during the day and leaves the temperature impact on Mg incorporation into the shell more apparent. Clearly, this study shows that Mg variations in the calcite layer of *C. concholepas* shells cannot be directly used as a proxy of SST changes without a strong optical control and characterization of the growth increments in which analyses are made.

This is the first time that such differences between day and night shell formation and shell composition are found. The complexity of these metabolic processes (yet to be fully understood) should not be forgotten when Mg variations within mollusk shells are investigated with the aim to reconstruct paleo-SST. These processes might account for the inconsistencies reported in the literature regarding the use of Mg as a paleo-SST proxy in other mollusk species.

Further work is needed to better understand the nyctemeral differences observed in the *C. concholepas* shell and to study in more detail the possible metabolic changes observed between day and night periods. To validate the assumption of a 12-h period of formation for both day and night increments, new staining experiments are already planned. In addition, more individuals must be analyzed and *C. concholepas* grown in natural conditions should be investigated. The nyctemeral metabolic differences could be studied in more detail, for example by measuring respiratory activity changes and/or $\delta^{13}\text{C}$ ratios in the shell.

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APPENDIX A. SUPPLEMENTARY DATA

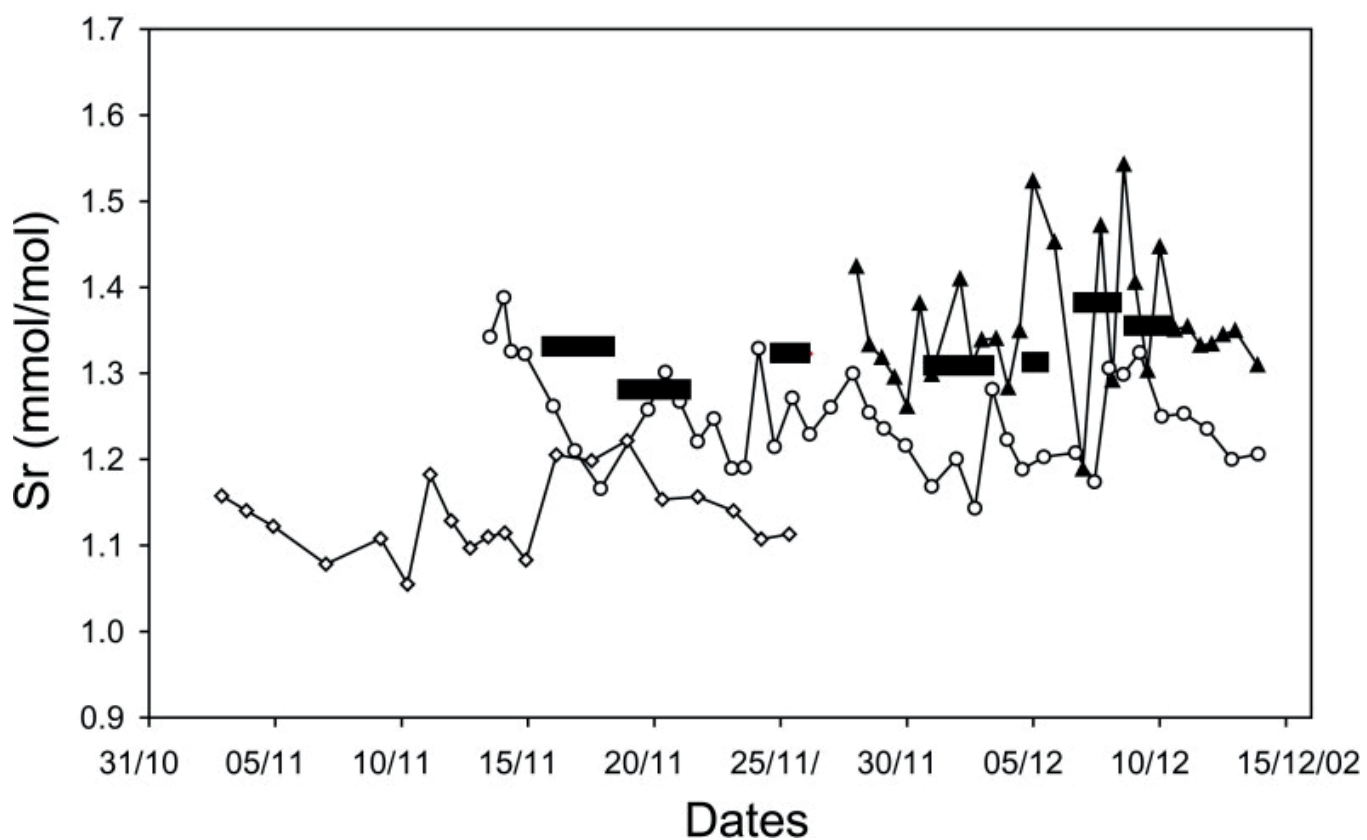
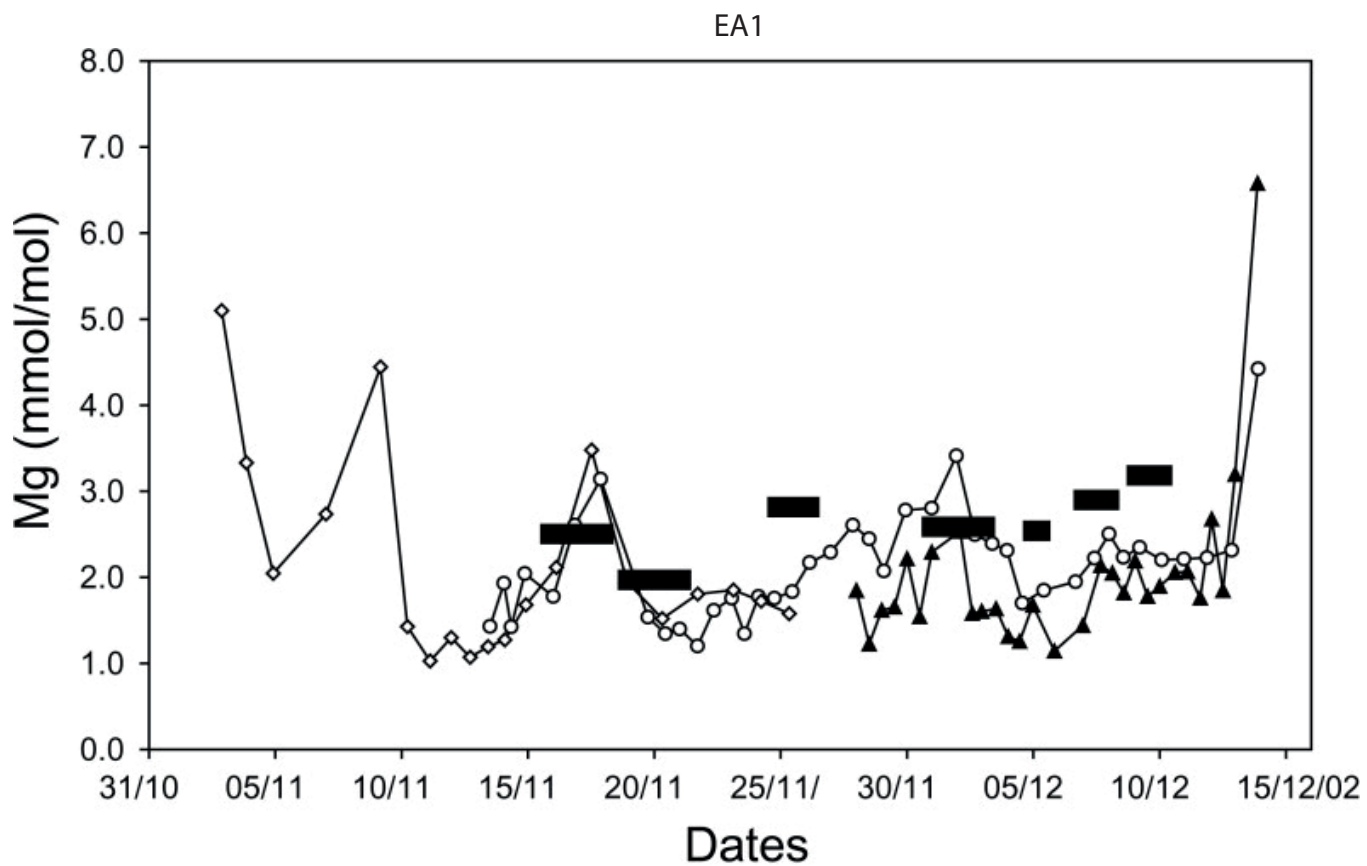
Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.gca.2007.07.031](https://doi.org/10.1016/j.gca.2007.07.031).

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Supplementary Figure 1. Electronic annex 1: Comparison of LASER ICP-MS profiles and standard liquid ICP-MS analyses done on micromilled samples from a third section of the *C. concholepas* shell studied. Standard liquid ICP-MS are represented as a bar which represents the amount of time of shell growth averaged.

Mg/Ca (mmol/mol)	Mg/Ca (mmol/mol) suite	Mg/Ca (mmol/mol) suite	Mg/Ca (mmol/mol) suite
23.15	23.19	31.52	23.79
24.86	22.08	30.37	24.42
24.27	22.30	31.95	24.21
23.90	23.35	31.82	24.37
24.08	23.62	31.92	23.70
23.76	24.11	31.73	24.77
23.39	23.41	32.00	25.01
23.03	25.03	31.68	24.83
23.86	24.83	31.96	26.33
23.15	24.35	33.75	24.69
21.92	23.58	32.84	26.10
21.70	22.78	33.68	27.41
21.99	21.89	33.77	29.84
22.35	23.02	30.59	29.49
21.97	23.77	29.08	30.30
22.22	22.98	29.33	30.64
22.61	23.28	28.98	31.18
21.12	23.95	29.20	29.25
20.59	23.35	29.56	29.61
20.42	23.71	29.19	29.75
20.55	22.99	29.55	28.46
22.45	24.00	30.45	29.73
22.62	22.90	31.17	29.36
22.31	24.39	29.14	28.73
23.26	24.18	27.93	28.52
22.93	23.35	25.90	28.24
22.26	23.77	25.92	27.92
23.00	24.90	25.76	26.89
21.59	25.21	25.30	26.51
22.04	23.36	25.87	27.06
22.15	23.45	24.75	26.52
20.90	24.79	24.49	24.86
20.95	25.67	24.77	24.12
19.89	23.21	24.80	23.56
19.44	23.32	24.56	23.78
19.72	22.62	24.37	24.33
20.17	22.21	24.12	24.76
19.70	23.09	25.01	25.40
19.55	20.98	25.97	25.29
19.11	20.20	26.36	24.85
19.11	22.25	26.75	26.72
19.92	21.58	27.10	27.37
19.60	21.99	26.79	31.93
19.56	20.18	27.47	28.63
21.11	20.48	27.69	28.81
20.44	20.04	27.60	29.58
19.98	19.25	28.02	30.35
21.02	20.50	28.82	31.31
20.67	20.56	28.31	33.11
21.50	20.95	28.85	33.01
21.85	20.96	29.18	31.34
20.57	21.45	29.40	28.91
20.23	20.86	30.50	24.39
20.89	20.54	30.59	23.68
21.70	21.76	29.97	24.93
21.16	21.09	29.09	32.92
21.54	21.73	28.17	29.43
21.83	22.16	29.21	30.24
21.55	21.79	29.42	31.72
21.87	21.75	29.10	30.74
22.16	23.47	28.49	32.02
21.49	23.83	28.69	31.32
21.71	23.35	29.92	32.48
21.80	23.11	29.98	32.01
23.32	23.60	29.80	32.09
22.57	24.17	31.42	31.50
23.63	24.50	30.78	31.72
23.76	25.09	29.67	32.04
24.25	24.72	27.75	33.55
23.18	26.47	27.87	34.89
22.76	23.81	28.37	36.21
22.27	26.31	27.84	36.49
22.25	27.97	26.33	37.29
	28.64	26.16	38.17
	29.72	23.94	36.75
			38.92
			40.68

Supplementary data. Electronic annex 2: Isognomon ephippium Mg/Ca shell ratios. Data obtained by LA-ICP-MS at the Royal Museum for Central Africa, Tervuren, Belgium. This work was supported by a Post Doctoral Grant to C.E. Lazareth under the European Union Training and Mobility of Researchers (TMR) Program, Contract FMBICT983440 and by EC-INCO research project IC18-CT96-0065 and FWO Flanders Contract.