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Chemical labelling of oyster shells used for time-calibrated high-resolution Mg/Ca ratios: a tool for estimation of past seasonal temperature variations

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Abstract:

The geochemical compositions of biogenic carbonates is increasingly used for paleoenvironmental reconstructions. The skeletal $\delta^{18}\text{O}$ temperature relationship is dependent on water salinity, so many recent studies have focused on the Mg/Ca and Sr/Ca ratios because those ratios in water do not change significantly on short time scales. Thus, those elemental ratios are considered to be good paleotemperature proxies in many biominerals, although their use remains ambiguous in bivalve shells. Here, we present the high-resolution Mg/Ca ratios of two modern species of juvenile and adult oyster shells, *Crassostrea gigas* and *Ostrea*

edulis. These specimens were grown in controlled conditions for over one year in two different locations. *In situ* monthly Mn-marking of the shells has been used for day calibration. The daily Mg/Ca ratios in the shell have been measured with an electron microprobe. The high frequency Mg/Ca variation of all specimens displays good synchronism with lunar cycles, suggesting that tides strongly influence the incorporation of Mg/Ca into the shells. Highly significant correlation coefficients ($0.70 < r < 0.83$, $p < 0.0001$) between the Mg/Ca ratios and the seawater temperature are obtained only for juvenile *C. gigas* samples, while metabolic control of Mg/Ca incorporation and lower shell growth rates preclude the use of the Mg/Ca ratio in adult shells as a paleothermometer. Data from three juvenile *C. gigas* shells from the two study sites are selected to establish a relationship : $T = 3.77Mg/Ca + 1.88$, where T is in °C and Mg/Ca in mmol/mol.

Key words: bivalve shells, seawater temperature, trace elements, growth rates, Mn-markings, cathodoluminescence.

1. Introduction

In paleoclimate research, sclerochronology allows the production of high-resolution environmental reconstructions. This type of study is based on the chemical analysis of bivalve mollusc shells, as these organisms build their mineralised carbonate parts from ions that they sample from their environment. Bivalves mineralise their shells by accretion, preventing the destruction of previously secreted parts of the shell. Therefore, this type of material allows the evaluation of water chemistry evolution and environmental variation throughout the life of the organism. Benthic inhabitants from coastal areas are particularly pertinent archives of the paleoclimatic conditions (i.e., seasonal range) because they are affected by temperatures close to those of the surface seawater.

The $\delta^{18}\text{O}$ in biogenic carbonates is widely used as a paleothermometer (Klein et al., 1996; Kirby et al., 1998; Andreasson and Schmitz, 2000). Unfortunately, it also depends on seawater isotopic composition (or salinity; Epstein and Mayeda, 1953), which is subject to frequent change. Consequently, this proxy is not always properly constrained (Rohling, 2000), particularly in coastal areas where freshwater runoff and/or evaporation occur, and several investigations have been made to estimate salinity, including stable isotopes (Gillikin et al., 2005) and trace elements (Dodd and Crisp, 1982).

To obtain more reliable paleotemperatures, other proxies were developed, such as trace elements ratios (Cronblad and Malmgren, 1981; Immenhauser et al., 2005). The Mg/Ca ratio in bivalve shells has been the subject of particularly intensive investigation (Dodd, 1965; Vander Putten et al., 2000; Freitas et al., 2005), but its utility as a reliable seawater thermometer is still unclear, producing positive or negative correlations according to seasonal environmental changes (Freitas et al., 2006). Despite the numerous pertinent studies that present geochemical analyses of recent mollusc shells to quantify the Mg/Ca and Sr/Ca molar ratios as environmental proxies (Boyden and Phillips, 1981; Lazareth et al., 2003; Takesue and van Geen, 2004; Wisshak et al., 2009), few of these works contain both a precise monitoring of the environmental parameters and a sclerochronological calendar of shell growth, both of which are essential to the establishment of a reliable model.

Chemical markings in bivalve shells, including fluorochromes and manganese, were developed for shell growth rate and growth pattern estimations (Hawkes et al., 1996; Kaehler and McQuaig, 1999; Lartaud et al., 2010a; Mahe et al., 2010). Several authors showed that manganese markings are particularly effective in the oyster hinge area (Langlet et al., 2006; Barbin et al., 2008), and they can be used to define high-resolution drilling samples of shell carbonate for geochemical analysis (Lartaud et al., 2010b). In this study, we use *Crassostrea gigas* and *Ostrea edulis* oyster shells cultured *in situ* coupled with monthly Mn-markings and

daily *in situ* hydrological data (i.e., temperature, salinity), which allows a good comparison between the proxy and the environmental conditions. Oyster shells are appropriate for the establishment of a relevant model for seasonal paleo-contrast reconstructions, especially using geochemical investigations. Indeed, oyster shells are primarily composed of low-magnesium calcite, which is the most stable calcium carbonate with respect to diagenesis alteration. Furthermore, oysters are euryhaline molluscs and present large stratigraphic (slow evolution from 200 Ma; Stenzel, 1971), geographic (from intertidal to deep waters), and latitudinal distributions. The purpose of this paper is to investigate the potential of oysters to serve as high-resolution (seasonal) recorders of environmental variations. Using recent specimens from a breeding experiment of two oyster species, *Crassostrea gigas* and *Ostrea edulis*, the evolution of Mg incorporation is measured along the hinge. Geochemical markings, revealed by cathodoluminescence, allow time calibration throughout the growth direction, permitting an effective comparison between the analysed Mg/Ca ratios and the monitored environmental parameters.

2. Experimental details

2.1. Site description

The experiments were carried out in two IFREMER (Institut Français de Recherche pour l'Exploitation de la Mer) marine stations on the French west coast: Baie des Veys (Normandy) and Marennes-Oléron Bay (Charente-Maritime), which are among the largest oyster-farming areas in France (Fig. 1). During the experiment (from February 2005 to November 2006), the environmental parameters such as seawater temperature and salinity were monitored daily using a multi-parameter probe (YSI IFREMER) attached to the breeding tables right next to the packs. According to the measured seawater temperatures, the

breeding calendar was split into two main periods, from June to November for the summer period and from December to May for the winter period. Seawater samples for ICP-AES Mg/Ca analysis were collected during the experiment (10 samples at Baie des Veys and 6 at Marennes-Oléron).

Figure 1

2.2. *Specimens and rearing strategy*

Two different oyster species were selected: the cupped oyster, *Crassostrea gigas* (Thunberg, 1793), and the flat oyster, *Ostrea edulis* (Linné, 1758), both of which are mainly calcitic-mineralising molluscs that live in various environments close to the shore (mangrove, estuarine, lagoon, intertidal and subtidal habitats).

The details of the breeding conditions for the specimens used in this work are described in Lartaud et al. (2010b) (summarised here in Table 1). Juvenile *C. gigas* were sourced from wild broodstock collected at the Arcachon basin at the end of January 2005. Their date of birth was estimated based on the size of the individuals (< 10 mm from the umbo to the ventral margin) to have been during summer 2004. The spats were separated into several groups and transported in packs to be cultured on oyster tables at the different study locations from the beginning of February 2005 until November 2006 (Table 1). All of these individuals are considered to be juvenile oysters ('jn-gig' prefix on sample code) in contrast to those older than two years, which are considered to be adult specimens in this work.

Adult *C. gigas* ('ad-gig' prefix on sample code) were used to study the ontogenic influence on shell Mg/Ca records. These individuals were produced from the IFREMER hatchery at La Tremblade (Charente-Maritime) and transplanted into nursery tanks at Bouin when they remained until they were six months old. The spats were cultured for one year and a half on

oyster tables at Marennes-Oléron and then placed in Marennes marine ponds until they joined the marking protocol experiment at the Marennes-Oléron bay site, in September 2005. Adult *O. edulis* ('ad-edu' prefix) were also used to establish the inter-specific calibration of the Mg/Ca proxy based on the analysis of individuals bred under the same environmental conditions. These oysters were born in the summer of 2003 and placed on oyster tables in February 2004 at La Trinité (southern Brittany). At the end of February 2006, they were separated into two groups and transferred to the two study locations. On November 2006, all individuals were sacrificed for shell elemental analysis. In total, four juvenile *C. gigas* shells (one from Marennes-Oléron and three from Baie des Veys), two adult *C. gigas* shells (from Marennes-Oléron) and five adult *O. edulis* shells (three from Baie des Veys, two from Marennes-Oléron) were randomly selected for the high-resolution Mg/Ca ratio analyses.

Table 1

To compare the high-resolution geochemical data from the shells and the environmental parameters, a continuous and accurate age model of oyster shells is needed. All individuals were marked on site monthly by placing them in a tank filled with seawater doped with manganese (90 mg/l MnCl_2 for 4 hours). The artificial manganese incorporated into the shell calcite is revealed by a highly luminescent micro-growth band under cathodoluminescence (CL) microscopy (Langlet et al., 2006; Barbin et al., 2008). The identification of Mn markings in the shell sections under CL observation allows the precise estimation of growth rates at different time intervals (Lartaud et al., 2010b). The oysters were opened and emptied of their soft tissues immediately after collection. The shells were then placed in a 6% solution of hydrogen peroxide (H_2O_2) for 6 h, washed with a

diluted nitric acid (0.15 N for 20 min) and rinsed thoroughly with demineralised water. For each left valve, a 300 μ m-thick section was made along the maximum growth axis, through the middle of the hinge region to the ventral shell margin. The microscopic CL and geochemical analyses were performed on the foliated low-magnesium calcite of the hinge section because this area contains a good and well-preserved record of the life of the individual in its environment (Richardson et al., 1993; Kirby et al., 1998; Lartaud et al., 2006; 2010c).

2.3. Geochemical analysis and data processing

High-resolution analyses of the thin oyster shell sections were carried out with a CAMECA SX 50 electron microprobe (EPMA) at the CAMPARIS service of IStEP, UPMC, Paris. The cations analysed were Mg, Ca and Mn. The operating conditions employed a 25 kV potential with a 130 nA current and a 25 μ m defocused beam diameter. The detection limits were 5 ppm for Ca, 160 ppm for Mg, and 10 ppm for Mn over a counting-time of 30 seconds. The instrument was calibrated with the following internal standards: a $\text{MgCaSi}_2\text{O}_6$ (diopside) crystal for calcium and magnesium detection and a MnTiO_3 crystal for Mn detection. The totals for analysis ranged between 92 and 100.6 wt% (average total of 96.5 wt%), which are within the acceptable error limits for low-magnesium calcite (England et al., 2007). For each shell, a 1 cm-long transect was chosen parallel to the growth axis, starting from the day of collection and providing 400 measurements per shell. To ensure that the analyses were always performed in a line perpendicular to the micro-increments, the precise location of the transect was made under CL observation prior to EPMA. As defined by Kirby et al. (1998) and Lartaud et al. (2010c), the best sampling zone is located just beneath the ligamental surface area, where the growth increments are all perpendicular to the external edge. The equally spaced analyses were then placed into a calendar scale using both the CL and Mn

concentrations (Fig. 2). Because the Mn concentration in oyster shells is naturally very low (at the approximate detection limit of the electron microprobe), the only recognisable spots on the Mn evolution curves of the shells are those that correspond to the Mn-markings. Thus, the geochemical Mn peaks were easily matched to the CL image. Using the AnalySeries software (Paillard et al., 1996) and making the assumption that the shell growth is constant between two consecutive markings, the spatial distribution of the geochemical data was transposed into a time scale with an accuracy that depended on the growth rate of each individual. Once the geochemical data were time calibrated, signal analysis (FFT) was performed to identify the different cyclicities and their meanings. The series were detrended according to the methodology described by Boulila et al. (2008) to remove the low-frequency components of the Mg/Ca curves. Then, the residuals were processed by the Multi-Tapper Method (MTM, Thomson, 1982) using the AnalySeries freeware (Paillard et al., 1996). This method is conventionally used for the analysis of high-noise signals; consequently, it is adapted for natural and metabolic records. To study the seasonal temperature variations, which are considered to be the low frequency signals in this study, the shell Mg/Ca curves were smoothed using a 4-point moving average. The covariations of the shell Mg/Ca ratios with the seawater temperature were studied on the smoothed curves (100 points). For each sample, the overall Mg/Ca temperature relationship was tested with the *Curve Fitting* tool from Matlab (v. 7.5.0.342). To ensure the normal distribution of the Mg/Ca-temperature couple residuals, a Kolmogorov-Smirnov test was applied.

Figure 2

3. Results

3.1. Seawater parameters

At both sites, the environmental records showed small changes in the salinity, with mean values of 33.4 ± 0.7 psu at Baie des Veys and 34.4 ± 2.8 psu at Marennes-Oléron (Table 2, Fig. 3). The episodes of low salinity (down to 28 psu) were recorded in the two stations, but they were clearly linked to rainfall lasting for one or two days. The change in salinity was higher at Marennes-Oléron because of the higher frequency of heavy rain periods. No seasonal variation of the salinity was observed at any site. Conversely, the seawater temperature variations at both sites were related to strong seasonal fluctuations without any relevant correlations with salinity (Figure 3). Throughout the breeding period, the Baie des Veys seawater temperatures ranged from 4.8 to 20.0°C (mean: $12.9 \pm 4.7^\circ\text{C}$), and from 4.8 to 23.1°C (mean: $15.0 \pm 5.2^\circ\text{C}$) at Marennes-Oléron. The temperature records are typically sinusoidal, with lower values in the winter and higher values in the summer. The average seawater Mg/Ca ratios are 2.57 ± 0.15 mmol/mol at Baie des Veys (n=10) and 3.25 ± 0.06 mmol/mol at Marennes-Oléron (n=6). At both sites, no relationship was observed between the salinity or the temperature and the Mg/Ca ratios of the seawater throughout the year (Table 2).

Table 2, Figure 3

3.2. Growth rates

Because the shell growth rate of oysters is not constant, even at a same site (Richardson et al., 1993; Lartaud et al., 2010b), the distribution of Mn-markings throughout the hinge is highly variable from one shell to another. Using the markings as time limits, the seasonal growth rates have been estimated for each shell assuming a constant growth rate between consecutive markings (Table 3). The seasonal growth rates of the juvenile *Crassostrea gigas* specimens range from 5.6 to 27.4 $\mu\text{m/day}$, with the highest values obtained on the shells collected at the Baie des Veys station. The growth rates measured for adult *C. gigas* (only from the

Marennes-Oléron location) range from 2.0 to 7.4 $\mu\text{m/day}$. The adult shells of *Ostrea edulis* show growth rates ranging from 2.0 to 10.7 $\mu\text{m/day}$, depending on the season. As for juvenile *C. gigas*, higher growth rates of *O. edulis* are obtained for specimens bred at the Baie des Veys station.

Table 3

3.3. Mg/Ca evolution in oyster shells

3.3.1. At Baie des Veys (Table 4)

All of the spots of the analysed profile of the juvenile *C. gigas* shells are located in the part of the hinge corresponding to the experimental period. The profiles start between June 2005 and September 2005 (Fig. 4a) and end on the collection day, in November 2006. The Mg/Ca ratios in the juvenile *C. gigas* shells range from 0.87 to 9.92 mmol/mol and show similar trends. The highest Mg/Ca ratios correspond to the summer months (April to October), while lower Mg/Ca ratios are obtained during the winter months (November to March, Fig. 4a). Because of the short period of the Mn-marking experiment for the adult *O. edulis*, we have retained only the relevant geochemical data (i.e., those obtained between the reliable markings, Fig. 4c). The Mg/Ca ratios recorded in adult *O. edulis* shells are slightly lower than those in the juvenile *C. gigas* (range between 0.84 and 8.07 mmol/mol, Table 4), and they increase slightly until the collection day.

Table 4

Figure 4

3.3.2. At Marennes-Oléron (Table 4)

As shown before, the growth rates of the oysters bred at Marennes-Oléron are half of those bred at Baie des Veys. Moreover, when the oysters reached an age of two years, their growth rates decreased consistently. For these two reasons, the sampling resolution after time calibration was much lower, implying that the geochemical interpretations at Baie des Veys became more uncertain, especially with regards to adult specimens. The Mg/Ca ratios of the juvenile *C. gigas* shells exhibit values ranging from 1.27 to 10.51 mmol/mol (mean 4.14 ± 1.54 mmol/mol), and the highest Mg/Ca ratios were recorded during the summer period. The Adult *C. gigas* shells (Fig. 4b, grey lines) yielded higher mean values (4.82 ± 2.06 mmol/mol and 7.10 ± 1.68 mmol/mol) and wider amplitudes (from 1.25 to 16.60 mmol/mol, Table 4). Similar to the Baie des Veys shells, the very low growth rate of the adult *O. edulis* shells at Marennes-Oléron bias the high-resolution investigation of the Mg/Ca ratios, with one point corresponding to 15 days at best (Fig. 4d). The Mg/Ca ratios in the adult *O. edulis* shells show the highest values of all of the samples, with a range between 3.25 and 17.57 mmol/mol (Table 4). However, the mean values (mean 5.13 ± 1.87 mmol/mol and 6.84 ± 3.02 mmol/mol) are similar to those from the adult *C. gigas*. Finally, no clear trend is conspicuous during the time interval analysed.

4. Discussion

4.1. Shell growth analysis

Taking into account all samples, oyster shell growth rates vary seasonally. Higher growth rates are observed during summer (with a mean value of $16.1 \mu\text{m/day}$) than winter (with a mean value of $11.5 \mu\text{m/day}$, Table 3) except for the specimen jn-gigBV-1. The fact that shell deposition is clearly visible between all winter markings confirms that, as previously reported by Lartaud et al. (2010b), *Crassostrea gigas* shell mineralisation can occur at both sites during cold months. During this period, no evidence of growth line (so called winter line) or

annual growth break can be revealed as this can be done on other bivalve shells (Schöne et al., 2004). These results differ from those of the eastern oyster *Crassostrea virginica*, which undergoes growth breaks when temperatures fall below 10°C (Kirby et al., 1998). An ontogenetic trend is observed that leads to lower growth performance in adult shells. Because *Ostrea edulis* grew only from February 2006 to November 2006 in Marennes-Oléron and from March 2006 to November 2006 in the Baie des Veys, the winter estimates of the growth rate are not available for this study. However, the two adult species cultured at Marennes-Oléron show that the *C. gigas* shells grow almost twice as fast as *O. edulis* (7.7 vs. 3.5 $\mu\text{m/day}$). Additionally, regardless of their species, the oysters at Baie des Veys grow faster (mean 12.5 $\mu\text{m/day}$) than those from Marennes-Oléron (mean 5.6 $\mu\text{m/day}$).

4.2. Shell Mg/Ca ratios and ontogeny in short-lived oysters

The use of metal-to-calcium ratios in bivalve shells as paleothermometers, unlike its use in other taxa such as foraminifera or corals, is still poorly documented. Stecher et al. (1996) and Carré et al. (2006) described a positive ontogenetic trend of Mg incorporation in the shells of various clam species. These observations contrast with the results from Strasser et al. (2008) concerning *Mya arenaria* and Higuera-Ruiz and Elorza (2009) for *C. gigas*, who noted a decrease in the Mg incorporation with size. Other studies have emphasised the correlations between the shell Mg record and its growth rate (Takesue and van Geen, 2004) or age (Freitas et al., 2005). Although the investigation of an ontogenetic trend is difficult because of the short life span of the specimens used in the present study, juvenile *C. gigas* from both sites exhibit similar Mg/Ca ratios between six months and two years-old, whereas an increase in the Mg/Ca ratio is recorded for adult *C. gigas* shells (i.e., ages between two and a half and three years, Table 4).

The Mg/Ca records of the two species between the two breeding sites are slightly different, with chemical ratios higher in the shells from Marennes-Oléron. These differences are closely linked to the seawater Mg/Ca ratios of the sites (Table 2). Furthermore, adult *C. gigas* and *O. edulis* shells cultured at the same site exhibit similar Mg/Ca ratios, suggesting that the species-related ‘vital effect’ is weak and that it does not significantly influence the Mg incorporation into the carbonate lattice of those oysters. A similar result was reported for $\delta^{18}\text{O}$ by Kirby et al. (1998).

4.3. High frequency Mg/Ca variation in oyster shells

The Mg/Ca evolution in shells shows different cyclicities. Because this work focuses on the use of the calcite Mg/Ca molar ratio as a paleothermometer for seasonal contrast estimations, it is imperative to determine the origin of these cyclicities (i.e., environmental control or analytical bias). The FFT performed on the Mg/Ca record of all the shells reveals two main cyclicities. Two main ranges of periodicity (13.7 to 15.4 and 25.6 to 31.3 solar days) are identified in both sites for all juvenile oysters, while nothing can be seen on the adult specimens because of the weakness of the sampling resolution for the studied time span (except for ad-edu-BV-1, Table 5). Based on the sclerochronological approaches, these types of periodicities have been already identified on mollusc bivalve shells and related to tidal cycles (Evans, 1972; Pannella, 1976; Higuera-Ruiz and Elorza, 2009; Lartaud et al., 2010a). The reported lunar calendar shows that the Mg/Ca ratio is higher during full-moon spring tides and lower during new-moon spring tides (Fig. 5). Surprisingly, Higuera-Ruiz and Elorza (2009) show the opposite result, with a lower Mg/Ca ratio during a single spring tide (without any distinction between the new or full-moon spring tide) in an oyster shell from the Bay of Biscay (Spain). Based on the relationship between the shell Mg/Ca ratio and the temperature (Lerman, 1965; Vander Putten et al., 2000; Freitas et al., 2009), the authors attributed this

fortnightly pattern to the input of cold water during the spring tides. However, evidence of such a temperature changes with the tide is not observed in either Baie des Veys or Marennes-Oléron. Parameters other than the temperature have been reported to modify the Mg concentration in the shells, such as metabolically controlled processes that lead to variations in Mg in the organic matrix (Vander Putten et al., 2000; Takesue et al., 2008). The primary energy provider in all organisms is adenosine triphosphate (ATP). The formation of this molecule mainly derives from the catabolism of carbohydrates. The reactions that use ATP require Mg^{2+} as a cofactor. This requirement leads, for example, to nycthemeral changes in the uptake of Mg^{2+} (Lazareth et al., 2007). Tides are recognised as environmental pacemakers for endogenous timekeeping mechanisms, the so-called biological clocks (Richardson, 1996; Schöne, 2008), and metabolic control of the Mg concentration in the organic matrix that depends on spring (new-moon or full-moon) and neap tides cannot be precluded.

Figure 5, Table 5

4.4. Mg/Ca vs seawater temperature (SST)

The seasonal variation of the Mg/Ca ratios in bivalve shells is not linked to changes in the seawater Mg/Ca ratio for salinities greater than 10 psu (Dodd and Crisp, 1982). However, in estuarine environments, the seawater Mg/Ca ratio can vary significantly over a salinity range from 10 to 37 psu (Surge and Lohmann, 2008). In this study, because salinity remains fairly constant at approximately 33 psu in both locations (see § 3.1), changes in the seawater Mg/Ca ratio should be low throughout the year. Highly significant good correlations ($r = 0.55$, $p < 0.0001$) to high correlations ($0.70 < r < 0.83$, $p < 0.0001$) between the SST and the Mg/Ca ratio have been observed for juvenile *C. gigas* shells from both Baie des Veys and Marennes-Oléron (Fig. 6). The Mg/Ca ratios of the two adult specimens of *C. gigas* from Marennes-

Oléron show conflicting results, with negative and positive correlations ($r = -0.24$ for ad-gig-MO-2 and $r = 0.73$ for ad-gig-MO-3). It is remarkable that the positive correlation corresponds to the shell with the higher growth rate, especially in summer (Table 3). For *O. edulis* shells, the correlations are weak to absent ($-0.1 < r < 0.47$) at Baie des Veys, according to the shell growth performance (Table 3), and negative correlations are observed at Marennes-Oléron ($r = -0.80$ and $r = -0.36$, Fig. 5), where the shell growth rate is low (Table 3). These results suggest that the Mg/Ca ratio in the shells is correlated with the seawater temperature only when growth rate is high. At low growth rates, metabolic/kinetic effects appear to control metal incorporation into the shells. This control is linked to calcium selectivity against magnesium, which complicates the environmental Mg/Ca relationship (Vander Putten et al., 2000; Schöne et al., 2011).

When the SST-Mg/Ca relationship is significant (e.g., for juvenile shells), Mg and Ca exhibit different pathways in different seasons. In the same temperature range, the shell Mg/Ca ratios are lower when the seasonal SST trend decreases (autumn) and higher when it increases (spring). Considered separately, the Mg/Ca-temperature relationships can be described by two slightly different equations, which have higher correlation coefficients than the global set of data. Because the temperature range is the same for both seasonal data sets, these results may reflect the impact of metabolic effects on the incorporation of Mg/Ca in the shell.

Figure 6

A Mg/Ca-temperature equation has been calculated from the Mg/Ca ratios of juvenile oyster shells from both sites, with one exception: shell jn-gig-BV-1. The Mg/Ca evolution curve from this individual does not follow the same pattern as that of the other specimens, with depleted values between July and August 2006 and anomalously increasing ratios during the

last autumn. Nevertheless, the CL age model remains in correlation with the effective seasonal periods. This result implies that Mg/Ca evolution in the jn-gig-BV-1 shell is not based on temperature variations but rather to stress or illness during experiment. Excluding this irrelevant datum from the complete juvenile Mg/Ca data set, the calculated Mg/Ca-temperature linear relationship is given by the following expression:

$$T = 3.77Mg/Ca + 1.88 \quad (1)$$

where T is the temperature in °C and Mg/Ca is the elemental ratio of the juvenile *C. gigas* shells (in mmol/mol).

This equation differs from the one obtained for *C. virginica* by Surge and Lohmann (2008):

$$T = 1.39Mg/Ca + 0.23 \quad (2).$$

Beyond the fact that the studied species is different, the main differences between their work and the present study concern the environment of the living oysters. During their experiments (conducted in the Gulf of Mexico), Surge and Lohmann recorded seawater temperatures ranging from 19.6 to 31.6 °C and salinity ranging from 7.9 to 38.5 psu. Furthermore, these authors described a poor correlation between the temperature and the shell Mg/Ca because of an ontogenetic effect that influenced the incorporation of Mg into the *C. virginica* shell during the first years of growth. They also observed a decrease of the accuracy of date assignments further back in the geochemical record. The most accurate equation was finally obtained using the geochemical data corresponding to the most recent part of the adult shells. Klein et al. (1996) demonstrated that the Mg/Ca ratio from the calcitic part of *M. edulis* is well correlated with the SSTs, and they proposed the following overall equation: $T = 3.33Mg/Ca - 7.5$ (3). During their experiment, the mussels were grown at Squirrel Cove (British Columbia, Canada), where the SSTs were between 6.1 and 22.7°C, which is very similar to the temperature range of the present study. Figure 7 depicts the comparison of the calculated SSTs using the different models with the measured seawater

temperatures. The geochemical data used for the temperature calculation are those of the three selected juvenile *C. gigas* shells from both sites. The estimated SSTs obtained using the Equation 2 and the Equation 3 underestimate the seawater temperatures by approximately ten degrees (with some negative temperatures during the winter months). In detail, the calculated temperatures determined from the equation of Surge and Lohmann (2008) smooth the sinusoidal shape of the temperature curve, with a seasonal variation of less than 6-7°C. The comparison of our temperature estimates with the one calculated by the method of Klein et al. (1996) suggests that the Mg incorporation differs between the *M. edulis* and *C. gigas* shells, and thus that an inter-specific calibration is necessary for paleoclimatic investigations. In contrast, *C. virginica* and *C. gigas* are closely related species, but the experiments were carried out in significantly different environments. The Surge and Lohmann equation is suitable for high temperatures and variable salinities; therefore, it is most likely not valid for the French temperate environments.

Figure 7

6. Conclusion

In this study, an electron microprobe Mg/Ca analysis was performed on *Crassostrea gigas* and *Ostrea edulis* shells. The animals were bred over two years in two different locations on the French Atlantic coast, where the seawater salinities and temperatures were recorded daily. The shells were marked monthly using Mn-doped seawater. After collection, chemical markings were used to time-calibrate the Mg/Ca shell profiles with daily precision. Frequency analysis of the chemical records shows that the high frequency variation is well synchronised with the spring tides, suggesting that Mg incorporation into the shell is partly influenced by physiological processes. Furthermore, ontogenetic processes strongly affect

Mg/Ca incorporation into the adult shells, preventing any available correlation with the seawater temperature. On the seasonal scale, a highly significant correlation is observed between the Mg/Ca ratios and the seawater temperatures, especially in regard to the *C. gigas* juvenile specimens. Using data from the two study sites, an overall relationship between the Mg/Ca shell ratios and the seawater temperatures is given by the following equation:

$$T = 3.77 \text{Mg/Ca} + 1.88$$

(T in °C and Mg/Ca in mmol/mol). The comparison with previously published equations highlights the potential of *Crassostrea gigas* shell Mg/Ca variations for seasonal temperature estimations in temperate environments.

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Figure

Figure 1: Location map of the studied breeding sites: French oyster farming of (1) Baie des
Veys (Normandy) and (2) Marennes-Oléron (Charente-Maritime).

Figure 2: Cathodoluminescence microphotograph of oyster shell section showing both natural
luminescence and bright luminescent bands corresponding to the Mn-marking days. White
dashed line represents the electron microprobe transect along which the measurements were
taken along the hinge region (scale bar is 200 μ m). The graph beneath shows the relative Mn
profile along the transect (a.u. = arbitrary unit) where each Mn Marking (bright luminescent
increment) corresponds with high Mn content.

Figure 3 : Measured seawater salinity and temperature in both locations : A- Baie des Veys,
B- Marennes-Oléron.

Figure 4: Electron microprobe Mg/Ca evolution curve of the studied osyster shells (hinge region). All curves are time calibrated using monthly Mn-markings. a- juvenile *C. gigas* shells from Baie des Veys: jn-gig-BV-1 (dark grey line, n= 394), jn-gig-BV-2 (black line, n=394), jn-gig-BV-3 (light grey line, n=398); b- juvenile and adult *C. gigas* shells from Marennes-Oléron: jn-gig-MO-1 (black line, n=190), ad-gig-MO-2 (dark grey line, n=54), ad-gig-MO-3 (light grey line, n= 96); c- adult *O. edulis* shells from Baie des Veys: ad-edu-BV-1 (black line, n=67), ad-edu-BV-2 (dark grey line, n=39), ad-edu-BV-3 (light grey line, n=59); d- adult *O. edulis* shells from Marennes-Oléron: ad-edu-MO-1 (black line, n=18), ad-edu-MO-2 (light grey line, n=23). The vertical dashed lines correspond to Mn-marking days.

Figure 5: Shell Mg/Ca evolution curve (dashed black line), detrended Mg/Ca curve (red line) compared with a 28-days cycles (green line) and with a 14-days cycles (blue line). New moon spring tides (grey circles) and full moon spring tides (white circles) are indicated.

Figure 6: Smoothed Mg/Ca ratios plotted versus seawater temperature. Correlation coefficients R are obtained using a simple linear regression. Square symbols are Mg/Ca ratios corresponding to the increasing temperatures (spring to summer) and cross symbols the Mg/Ca ratios during the decreasing temperatures (autumn to winter).

Figure 7: Seawater temperatures calculated from Mg/Ca ratios in juvenile *C. gigas* shells using this study equation (black dotted curves) and the equation from Klein et al. (1996; grey dotted curves). a- Baie des Veys measured seawater temperatures (solid curve), calculated SST using Mg/Ca of shells jn-gig-BV-2 (dashed curve) and jn-gig-BV-3 (dotted curve). b- Marennes-Oléron measured seawater temperatures (solid curve), calculated SST using Mg/Ca of shell jn-gig-MO-1 (dashed line).

Table

Table 1: Simplified schedule of the oyster breeding program.

Location	Shells	Birth	Hatchery Nursery	Oyster tables (before marking phase)	Marine ponds	Oyster tables (during marking phase)	Collection of oysters
Baie des Veys	C. gigas juv.	Summer 2004				February 2005	November 06
	O. edulis ad.	Summer 2003		February 2004		February 2006	November 06
Marennes Oléron	C. gigas juv.	Summer 2004				February 2005	November 06
	C. gigas ad.	March 2003	March 2003	February 2004	June 05	September 2005	November 06
	O. edulis ad.	Summer 2003		February 2004		February 2006	November 06

Table 2: Measured seawater Mg/Ca ratio, temperature and salinity at the two breeding locations.

Location	Date	Mg/Ca (mmol/mol)	Temperature (°C)	Salinity (psu)
Baie des Veys	13/03/05	2.50	5.9	32.3
	27/04/05	2.66	10.2	33.7
	26/05/05	2.50	13.0	33.2
	22/06/05	2.67	16.6	33.7
	18/08/05	2.67	19.8	33.8
	07/12/05	2.66	9.9	33.1
	22/12/05	2.22	8.2	33.2
	03/02/06	2.66	5.6	33.6
	16/05/06	2.45	12.0	33.0
	09/08/06	2.68	19.7	33.9
Marennes Oléron	13/06/05	3.19	17.8	34.1
	21/06/05	3.28	18.7	34.1
	01/07/05	3.27	19.5	33.4
	20/09/05	3.27	18.0	35.2
	22/03/06	3.17	8.3	29.3
	22/05/06	3.31	12.9	29.6

Table 3: Seasonal growth rate measurements of the hinge area of *C. gigas* and *O. edulis* marked shells.

Location	Group	Sample	Season	Growth rate
Baie des Veys	<i>O. edulis</i> (adult)	ad-edu-BV-1	summer	7.8 $\mu\text{m/day}$
		ad-edu-BV-2	summer	2.9 $\mu\text{m/day}$
		ad-edu-BV-3	summer	10.7 $\mu\text{m/day}$
	<i>C. gigas</i> (juvenile)	jn-gig-BV-1	summer	13.7 $\mu\text{m/day}$
			winter	19.9 $\mu\text{m/day}$
			summer	16.8 $\mu\text{m/day}$
		jn-gig-BV-2	summer	14.2 $\mu\text{m/day}$
			winter	13.2 $\mu\text{m/day}$
			summer	27.4 $\mu\text{m/day}$
		jn-gig-BV-3	summer	23.9 $\mu\text{m/day}$
			winter	15.9 $\mu\text{m/day}$
			summer	20.0 $\mu\text{m/day}$
Marennes- Oléron	<i>O. edulis</i> (adult)	ad-edu-MO-1	summer	2.0 $\mu\text{m/day}$
		ad-edu-MO-2	summer	3.4 $\mu\text{m/day}$
	<i>C. gigas</i> (juvenile)	jn-gig-MO-1	summer	9.8 $\mu\text{m/day}$
			winter	5.6 $\mu\text{m/day}$
			summer	12.9 $\mu\text{m/day}$
	<i>C. gigas</i> (adult)	ad-gig-MO-2	summer	2.0 $\mu\text{m/day}$
			winter	2.5 $\mu\text{m/day}$
		ad-gig-MO-3	summer	7.4 $\mu\text{m/day}$
			winter	2.8 $\mu\text{m/day}$

Table 4: Mean shell Mg/Ca ratios of *C. gigas* and *O. edulis* used in this study.

Location	Sample code	Number of analyses	Mg/Ca (mmol/mol) min/max	Mean $\pm$ standard deviation (mmol/mol)
Baie des Veys	jn-gig-BV-1	394	1.25/9.92	4.37 $\pm$ 2.05
	jn-gig-BV-2	394	0.87/9.26	3.16 $\pm$ 1.64
	jn-gig-BV-3	398	1.52/7.13	3.42 $\pm$ 0.85
	ad-edu-BV-1	67	0.89/5.08	2.59 $\pm$ 1.08
	ad-edu-BV-2	39	0.96/8.07	3.64 $\pm$ 1.65
	ad-edu-BV-3	59	0.84/5.00	2.10 $\pm$ 0.89
Marennes-Oléron	jn-gig-MO-1	190	1.27/10.51	4.14 $\pm$ 1.54
	ad-gig-MO-2	54	1.25/15.43	4.82 $\pm$ 2.06
	ad-gig-MO-3	96	3.89/16.60	7.10 $\pm$ 1.68
	ad-edu-MO-1	18	3.25/8.88	5.13 $\pm$ 1.87
	ad-edu-MO-2	23	3.71/17.57	6.84 $\pm$ 3.02

Table 5: Main periodicities deduced from FFT analysis of the time calibrated Mg/Ca

evolution curves of *C. gigas* and *O. edulis* shells (days are solar days).

	25.6-31.3	13.7-15.4
ad-edu-BV-1	-	X
ad-edu-BV-2	-	-
ad-edu-BV-3	-	-
jn-gig-BV-1	X	X
jn-gig-BV-2	X	X
jn-gig-BV-3	X	X
ad-gig-MO-1	-	-
ad-gig-MO-2	-	-
ad-edu-MO-1	-	-
ad-edu-MO-2	-	X
jn-gig-MO-1	X	X