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

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17  
18       **Abstract:**

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

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

37

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

40

## 41 **1. Introduction**

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

51 The  $\delta^{18}\text{O}$  in biogenic carbonates is widely used as a paleothermometer (Klein et al., 1996;  
52 Kirby et al., 1998; Andreasson and Schmitz, 2000). Unfortunately, it also depends on  
53 seawater isotopic composition (or salinity; Epstein and Mayeda, 1953), which is subject to  
54 frequent change. Consequently, this proxy is not always properly constrained (Rohling,  
55 2000), particularly in coastal areas where freshwater runoff and/or evaporation occur, and  
56 several investigations have been made to estimate salinity, including stable isotopes (Gillikin  
57 et al., 2005) and trace elements (Dodd and Crisp, 1982).

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

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

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

91

## 92 **2. Experimental details**

### 93 *2.1. Site description*

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

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

105

106 Figure 1

107

## 108 2.2. *Specimens and rearing strategy*

109 Two different oyster species were selected: the cupped oyster, *Crassostrea gigas* (Thunberg,  
110 1793), and the flat oyster, *Ostrea edulis* (Linné, 1758), both of which are mainly calcitic-  
111 mineralising molluscs that live in various environments close to the shore (mangrove,  
112 estuarine, lagoon, intertidal and subtidal habitats).

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

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

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

138

139 Table 1

140

141 To compare the high-resolution geochemical data from the shells and the environmental  
142 parameters, a continuous and accurate age model of oyster shells is needed. All individuals  
143 were marked on site monthly by placing them in a tank filled with seawater doped with  
144 manganese (90 mg/l  $MnCl_2$  for 4 hours). The artificial manganese incorporated into the shell  
145 calcite is revealed by a highly luminescent micro-growth band under cathodoluminescence  
146 (CL) microscopy (Langlet et al., 2006; Barbin et al., 2008). The identification of Mn  
147 markings in the shell sections under CL observation allows the precise estimation of growth  
148 rates at different time intervals (Lartaud et al., 2010b).

149 The oysters were opened and emptied of their soft tissues immediately after collection. The  
150 shells were then placed in a 6% solution of hydrogen peroxide ( $H_2O_2$ ) for 6 h, washed with a

151 diluted nitric acid (0.15 N for 20 min) and rinsed thoroughly with demineralised water. For  
152 each left valve, a 300  $\mu\text{m}$ -thick section was made along the maximum growth axis, through  
153 the middle of the hinge region to the ventral shell margin. The microscopic CL and  
154 geochemical analyses were performed on the foliated low-magnesium calcite of the hinge  
155 section because this area contains a good and well-preserved record of the life of the  
156 individual in its environment (Richardson et al., 1993; Kirby et al., 1998; Lartaud et al., 2006;  
157 2010c).

158

### 159 *2.3. Geochemical analysis and data processing*

160 High-resolution analyses of the thin oyster shell sections were carried out with a CAMECA  
161 SX 50 electron microprobe (EPMA) at the CAMPARIS service of IStEP, UPMC, Paris. The  
162 cations analysed were Mg, Ca and Mn. The operating conditions employed a 25 kV potential  
163 with a 130 nA current and a 25  $\mu\text{m}$  defocused beam diameter. The detection limits were 5  
164 ppm for Ca, 160 ppm for Mg, and 10 ppm for Mn over a counting-time of 30 seconds. The  
165 instrument was calibrated with the following internal standards: a  $\text{MgCaSi}_2\text{O}_6$  (diopside)  
166 crystal for calcium and magnesium detection and a  $\text{MnTiO}_3$  crystal for Mn detection. The  
167 totals for analysis ranged between 92 and 100.6 wt% (average total of 96.5 wt%), which are  
168 within the acceptable error limits for low-magnesium calcite (England et al., 2007). For each  
169 shell, a 1 cm-long transect was chosen parallel to the growth axis, starting from the day of  
170 collection and providing 400 measurements per shell. To ensure that the analyses were always  
171 performed in a line perpendicular to the micro-increments, the precise location of the transect  
172 was made under CL observation prior to EPMA. As defined by Kirby et al. (1998) and  
173 Lartaud et al. (2010c), the best sampling zone is located just beneath the ligamental surface  
174 area, where the growth increments are all perpendicular to the external edge. The equally  
175 spaced analyses were then placed into a calendar scale using both the CL and Mn



176 concentrations (Fig. 2). Because the Mn concentration in oyster shells is naturally very low (at  
177 the approximate detection limit of the electron microprobe), the only recognisable spots on  
178 the Mn evolution curves of the shells are those that correspond to the Mn-markings. Thus, the  
179 geochemical Mn peaks were easily matched to the CL image. Using the AnalySeries software  
180 (Paillard et al., 1996) and making the assumption that the shell growth is constant between  
181 two consecutive markings, the spatial distribution of the geochemical data was transposed  
182 into a time scale with an accuracy that depended on the growth rate of each individual.

183 Once the geochemical data were time calibrated, signal analysis (FFT) was performed to  
184 identify the different cyclicities and their meanings. The series were detrended according to  
185 the methodology described by Boulila et al. (2008) to remove the low-frequency components  
186 of the Mg/Ca curves. Then, the residuals were processed by the Multi-Tapper Method (MTM,  
187 Thomson, 1982) using the AnalySeries freeware (Paillard et al., 1996). This method is  
188 conventionally used for the analysis of high-noise signals; consequently, it is adapted for  
189 natural and metabolic records. To study the seasonal temperature variations, which are  
190 considered to be the low frequency signals in this study, the shell Mg/Ca curves were  
191 smoothed using a 4-point moving average. The covariations of the shell Mg/Ca ratios with the  
192 seawater temperature were studied on the smoothed curves (100 points). For each sample, the  
193 overall Mg/Ca temperature relationship was tested with the *Curve Fitting* tool from Matlab (v.  
194 7.5.0.342). To ensure the normal distribution of the Mg/Ca-temperature couple residuals, a  
195 Kolmogorov-Smirnov test was applied.

196

197 Figure 2

198

### 199 **3. Results**

200 *3.1. Seawater parameters*

201 At both sites, the environmental records showed small changes in the salinity, with mean  
202 values of  $33.4 \pm 0.7$  psu at Baie des Veys and  $34.4 \pm 2.8$  psu at Marennes-Oléron (Table 2,  
203 Fig. 3). The episodes of low salinity (down to 28 psu) were recorded in the two stations, but  
204 they were clearly linked to rainfall lasting for one or two days. The change in salinity was  
205 higher at Marennes-Oléron because of the higher frequency of heavy rain periods. No  
206 seasonal variation of the salinity was observed at any site. Conversely, the seawater  
207 temperature variations at both sites were related to strong seasonal fluctuations without any  
208 relevant correlations with salinity (Figure 3). Throughout the breeding period, the Baie des  
209 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  
210 23.1°C (mean:  $15.0 \pm 5.2^\circ\text{C}$ ) at Marennes-Oléron. The temperature records are typically  
211 sinusoidal, with lower values in the winter and higher values in the summer. The average  
212 seawater Mg/Ca ratios are  $2.57 \pm 0.15$  mmol/mol at Baie des Veys (n=10) and  $3.25 \pm 0.06$   
213 mmol/mol at Marennes-Oléron (n=6). At both sites, no relationship was observed between the  
214 salinity or the temperature and the Mg/Ca ratios of the seawater throughout the year (Table 2).

215

216 Table 2, Figure 3

217

### 218 3.2. Growth rates

219 Because the shell growth rate of oysters is not constant, even at a same site (Richardson et al.,  
220 1993; Lartaud et al., 2010b), the distribution of Mn-markings throughout the hinge is highly  
221 variable from one shell to another. Using the markings as time limits, the seasonal growth  
222 rates have been estimated for each shell assuming a constant growth rate between consecutive  
223 markings (Table 3). The seasonal growth rates of the juvenile *Crassostrea gigas* specimens  
224 range from 5.6 to 27.4  $\mu\text{m}/\text{day}$ , with the highest values obtained on the shells collected at the  
225 Baie des Veys station. The growth rates measured for adult *C. gigas* (only from the

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

230

231 Table 3

232

233 3.3. Mg/Ca evolution in oyster shells

234 3.3.1. At Baie des Veys (Table 4)

235 All of the spots of the analysed profile of the juvenile *C. gigas* shells are located in the part of  
236 the hinge corresponding to the experimental period. The profiles start between June 2005 and  
237 September 2005 (Fig. 4a) and end on the collection day, in November 2006. The Mg/Ca ratios  
238 in the juvenile *C. gigas* shells range from 0.87 to 9.92 mmol/mol and show similar trends. The  
239 highest Mg/Ca ratios correspond to the summer months (April to October), while lower  
240 Mg/Ca ratios are obtained during the winter months (November to March, Fig. 4a). Because  
241 of the short period of the Mn-marking experiment for the adult *O. edulis*, we have retained  
242 only the relevant geochemical data (i.e., those obtained between the reliable markings, Fig.  
243 4c). The Mg/Ca ratios recorded in adult *O. edulis* shells are slightly lower than those in the  
244 juvenile *C. gigas* (range between 0.84 and 8.07 mmol/mol, Table 4), and they increase  
245 slightly until the collection day.

246

247 Table 4

248 Figure 4

249

250 3.3.2. At Marennes-Oléron (Table 4)

251 As shown before, the growth rates of the oysters bred at Marennes-Oléron are half of those  
252 bred at Baie des Veys. Moreover, when the oysters reached an age of two years, their growth  
253 rates decreased consistently. For these two reasons, the sampling resolution after time  
254 calibration was much lower, implying that the geochemical interpretations at Baie des Veys  
255 became more uncertain, especially with regards to adult specimens. The Mg/Ca ratios of the  
256 juvenile *C. gigas* shells exhibit values ranging from 1.27 to 10.51 mmol/mol (mean  $4.14 \pm$   
257  $1.54$  mmol/mol), and the highest Mg/Ca ratios were recorded during the summer period. The  
258 Adult *C. gigas* shells (Fig. 4b, grey lines) yielded higher mean values ( $4.82 \pm 2.06$  mmol/mol  
259 and  $7.10 \pm 1.68$  mmol/mol) and wider amplitudes (from 1.25 to 16.60 mmol/mol, Table 4).  
260 Similar to the Baie des Veys shells, the very low growth rate of the adult *O. edulis* shells at  
261 Marennes-Oléron bias the high-resolution investigation of the Mg/Ca ratios, with one point  
262 corresponding to 15 days at best (Fig. 4d). The Mg/Ca ratios in the adult *O. edulis* shells show  
263 the highest values of all of the samples, with a range between 3.25 and 17.57 mmol/mol  
264 (Table 4). However, the mean values (mean  $5.13 \pm 1.87$  mmol/mol and  $6.84 \pm 3.02$   
265 mmol/mol) are similar to those from the adult *C. gigas*. Finally, no clear trend is conspicuous  
266 during the time interval analysed.

267

## 268 **4. Discussion**

### 269 *4.1. Shell growth analysis*

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

276 annual growth break can be revealed as this can be done on other bivalve shells (Schöne et al.,  
277 2004). These results differ from those of the eastern oyster *Crassostrea virginica*, which  
278 undergoes growth breaks when temperatures fall below 10°C (Kirby et al., 1998). An  
279 ontogenetic trend is observed that leads to lower growth performance in adult shells. Because  
280 *Ostrea edulis* grew only from February 2006 to November 2006 in Marennes-Oléron and  
281 from March 2006 to November 2006 in the Baie des Veys, the winter estimates of the growth  
282 rate are not available for this study. However, the two adult species cultured at Marennes-  
283 Oléron show that the *C. gigas* shells grow almost twice as fast as *O. edulis* (7.7 vs. 3.5  
284  $\mu\text{m/day}$ ). Additionally, regardless of their species, the oysters at Baie des Veys grow faster  
285 (mean 12.5  $\mu\text{m/day}$ ) than those from Marennes-Oléron (mean 5.6  $\mu\text{m/day}$ ).

286

#### 287 4.2. Shell Mg/Ca ratios and ontogeny in short-lived oysters

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

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

307

#### 308 *4.3. High frequency Mg/Ca variation in oyster shells*

309 The Mg/Ca evolution in shells shows different cyclicities. Because this work focuses on the  
310 use of the calcite Mg/Ca molar ratio as a paleothermometer for seasonal contrast estimations,  
311 it is imperative to determine the origin of these cyclicities (i.e., environmental control or  
312 analytical bias). The FFT performed on the Mg/Ca record of all the shells reveals two main  
313 cyclicities. Two main ranges of periodicity (13.7 to 15,4 and 25.6 to 31.3 solar days) are  
314 identified in both sites for all juvenile oysters, while nothing can be seen on the adult  
315 specimens because of the weakness of the sampling resolution for the studied time span  
316 (except for ad-edu-BV-1, Table 5). Based on the sclerochronological approaches, these types  
317 of periodicities have been already identified on mollusc bivalve shells and related to tidal  
318 cycles (Evans, 1972; Pannella, 1976; Higuera-Ruiz and Elorza, 2009; Lartaud et al., 2010a).  
319 The reported lunar calendar shows that the Mg/Ca ratio is higher during full-moon spring  
320 tides and lower during new-moon spring tides (Fig. 5). Surprisingly, Higuera-Ruiz and Elorza  
321 (2009) show the opposite result, with a lower Mg/Ca ratio during a single spring tide (without  
322 any distinction between the new or full-moon spring tide) in an oyster shell from the Bay of  
323 Biscay (Spain). Based on the relationship between the shell Mg/Ca ratio and the temperature  
324 (Lerman, 1965; Vander Putten et al., 2000; Freitas et al., 2009), the authors attributed this

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

337

338 Figure 5, Table 5

339

#### 340 4.4. Mg/Ca vs seawater temperature (SST)

341 The seasonal variation of the Mg/Ca ratios in bivalve shells is not linked to changes in the  
342 seawater Mg/Ca ratio for salinities greater than 10 psu (Dodd and Crisp, 1982). However, in  
343 estuarine environments, the seawater Mg/Ca ratio can vary significantly over a salinity range  
344 from 10 to 37 psu (Surge and Lohmann, 2008). In this study, because salinity remains fairly  
345 constant at approximately 33 psu in both locations (see § 3.1), changes in the seawater Mg/Ca  
346 ratio should be low throughout the year. Highly significant good correlations ( $r = 0.55$ ,  $p <$   
347  $0.0001$ ) to high correlations ( $0.70 < r < 0.83$ ,  $p < 0.0001$ ) between the SST and the Mg/Ca  
348 ratio have been observed for juvenile *C. gigas* shells from both Baie des Veys and Marennes-  
349 Oléron (Fig. 6). The Mg/Ca ratios of the two adult specimens of *C. gigas* from Marennes-

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

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

368

369 Figure 6

370

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



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

$$380 \quad T = 3.77Mg/Ca + 1.88 \quad (1)$$

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

383

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

$$385 \quad T = 1.39Mg/Ca + 0.23 \quad (2).$$

386 Beyond the fact that the studied species is different, the main  
387 differences between their work and the present study concern the environment of the living

388 oysters. During their experiments (conducted in the Gulf of Mexico), Surge and Lohmann

389 recorded seawater temperatures ranging from 19.6 to 31.6 °C and salinity ranging from 7.9 to

390 38.5 psu. Furthermore, these authors described a poor correlation between the temperature

391 and the shell Mg/Ca because of an ontogenetic effect that influenced the incorporation of Mg

392 into the *C. virginica* shell during the first years of growth. They also observed a decrease of

393 the accuracy of date assignments further back in the geochemical record. The most accurate

394 equation was finally obtained using the geochemical data corresponding to the most recent

395 part of the adult shells. Klein et al. (1996) demonstrated that the Mg/Ca ratio from the calcitic

396 part of *M. edulis* is well correlated with the SSTs, and they proposed the following overall

397 equation:  $T = 3.33Mg/Ca - 7.5$  (3). During their experiment, the mussels were grown at

398 Squirrel Cove (British Columbia, Canada), where the SSTs were between 6.1 and 22.7°C,

399 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

400 temperatures. The geochemical data used for the temperature calculation are those of the three  
401 selected juvenile *C. gigas* shells from both sites. The estimated SSTs obtained using the  
402 Equation 2 and the Equation 3 underestimate the seawater temperatures by approximately ten  
403 degrees (with some negative temperatures during the winter months). In detail, the calculated  
404 temperatures determined from the equation of Surge and Lohmann (2008) smooth the  
405 sinusoidal shape of the temperature curve, with a seasonal variation of less than 6-7°C. The  
406 comparison of our temperature estimates with the one calculated by the method of Klein et al.  
407 (1996) suggests that the Mg incorporation differs between the *M. edulis* and *C. gigas* shells,  
408 and thus that an inter-specific calibration is necessary for paleoclimatic investigations. In  
409 contrast, *C. virginica* and *C. gigas* are closely related species, but the experiments were  
410 carried out in significantly different environments. The Surge and Lohmann equation is  
411 suitable for high temperatures and variable salinities; therefore, it is most likely not valid for  
412 the French temperate environments.

413

414 Figure 7

415

## 416 **6. Conclusion**

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

425 Mg/Ca incorporation into the adult shells, preventing any available correlation with the  
426 seawater temperature. On the seasonal scale, a highly significant correlation is observed  
427 between the Mg/Ca ratios and the seawater temperatures, especially in regard to the *C. gigas*  
428 juvenile specimens. Using data from the two study sites, an overall relationship between the  
429 Mg/Ca shell ratios and the seawater temperatures is given by the following equation:  
430  $T = 3.77Mg/Ca + 1.88$  (T in °C and Mg/Ca in mmol/mol). The comparison with previously  
431 published equations highlights the potential of *Crassostrea gigas* shell Mg/Ca variations for  
432 seasonal temperature estimations in temperate environments.

433

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444

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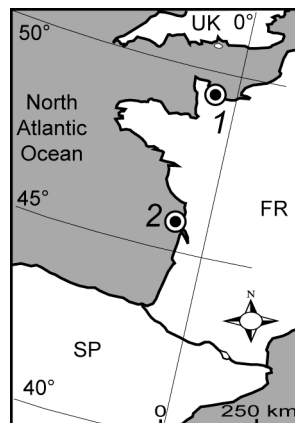


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576

577 **Figure**

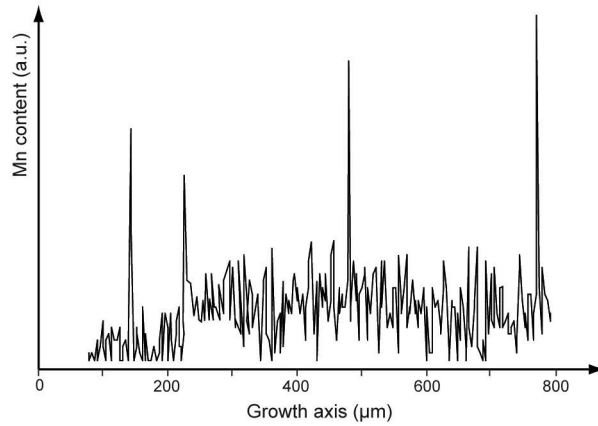
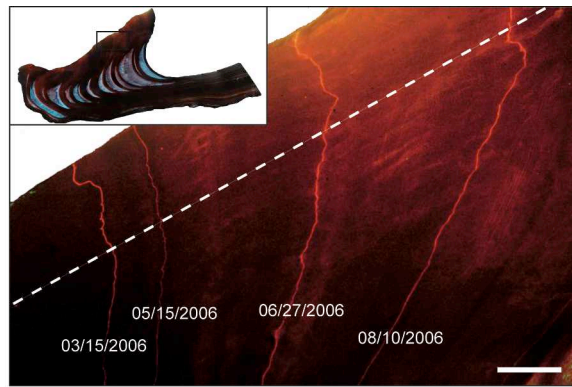
578 Figure 1: Location map of the studied breeding sites: French oyster farming of (1) Baie des  
579 Veys (Normandy) and (2) Marennes-Oléron (Charente-Maritime).



580

581

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

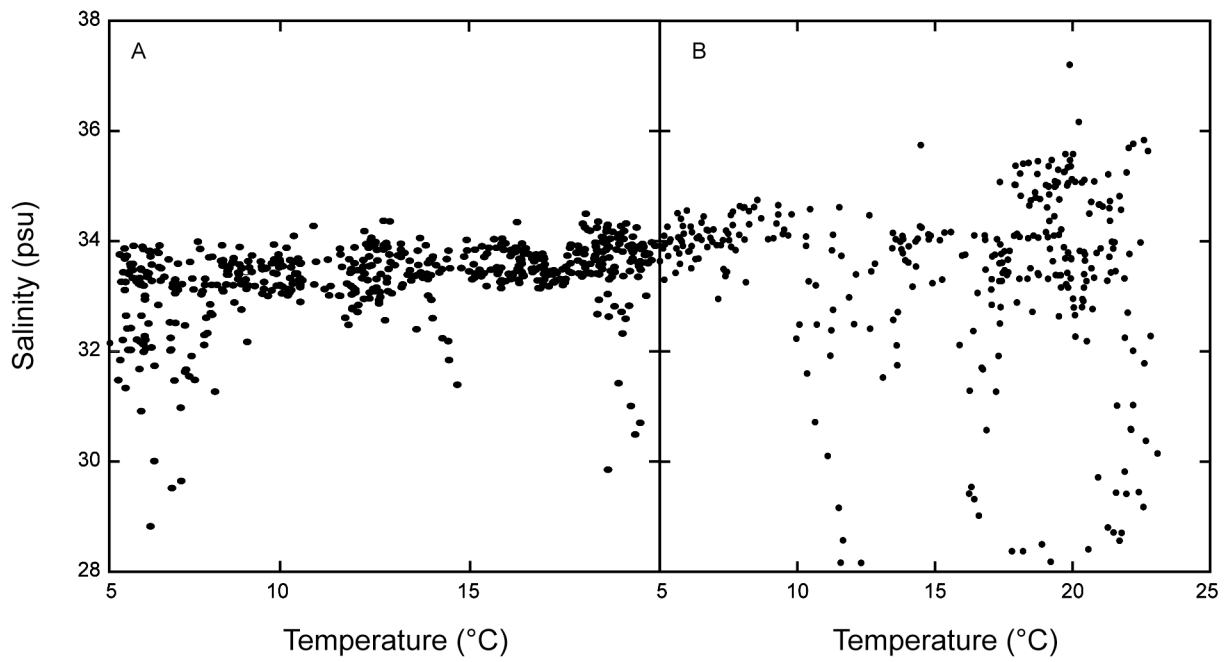


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590 Figure 3 : Measured seawater salinity and temperature in both locations : A- Baie des Veys,

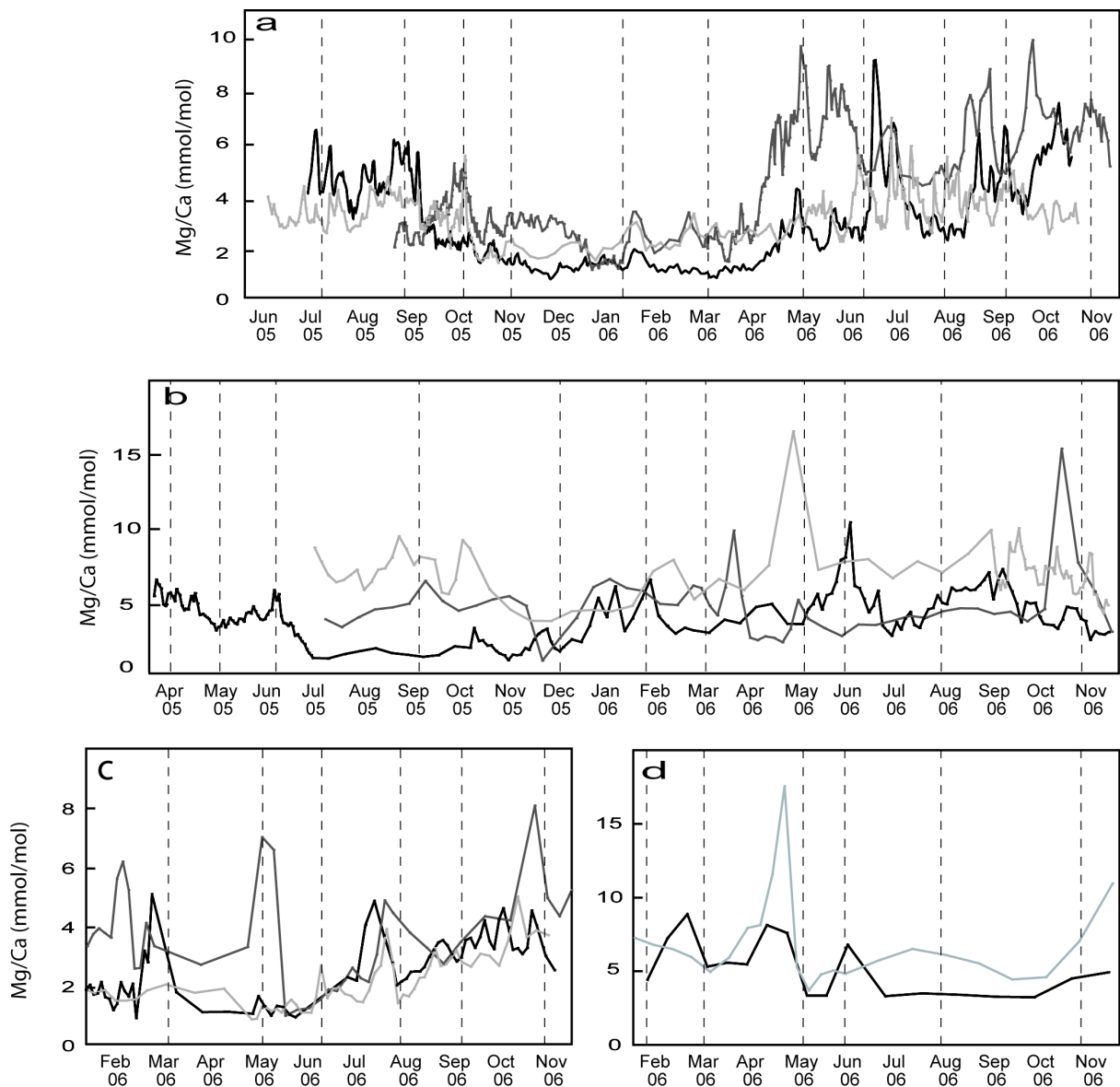
591 B- Marennes-Oléron.



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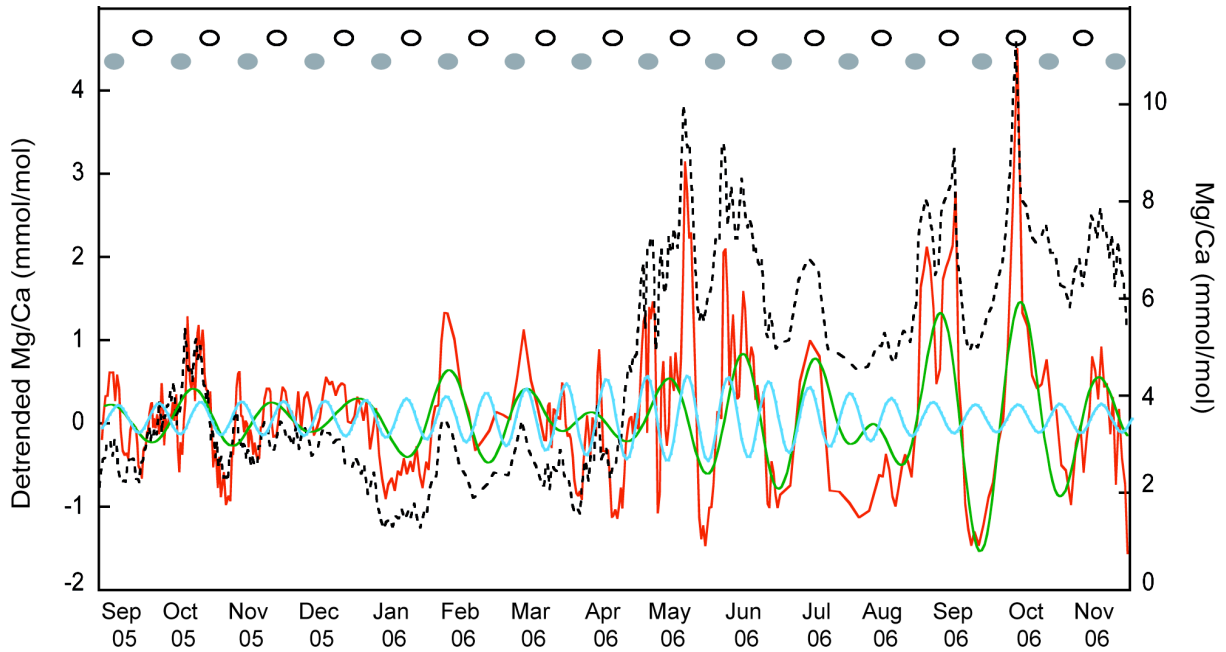
594 Figure 4: Electron microprobe Mg/Ca evolution curve of the studied osyter shells (hinge  
 595 region). All curves are time calibrated using monthly Mn-markings. a- juvenile *C. gigas* shells  
 596 from Baie des Veys: jn-gig-BV-1 (dark grey line, n= 394), jn-gig-BV-2 (black line, n=394),  
 597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



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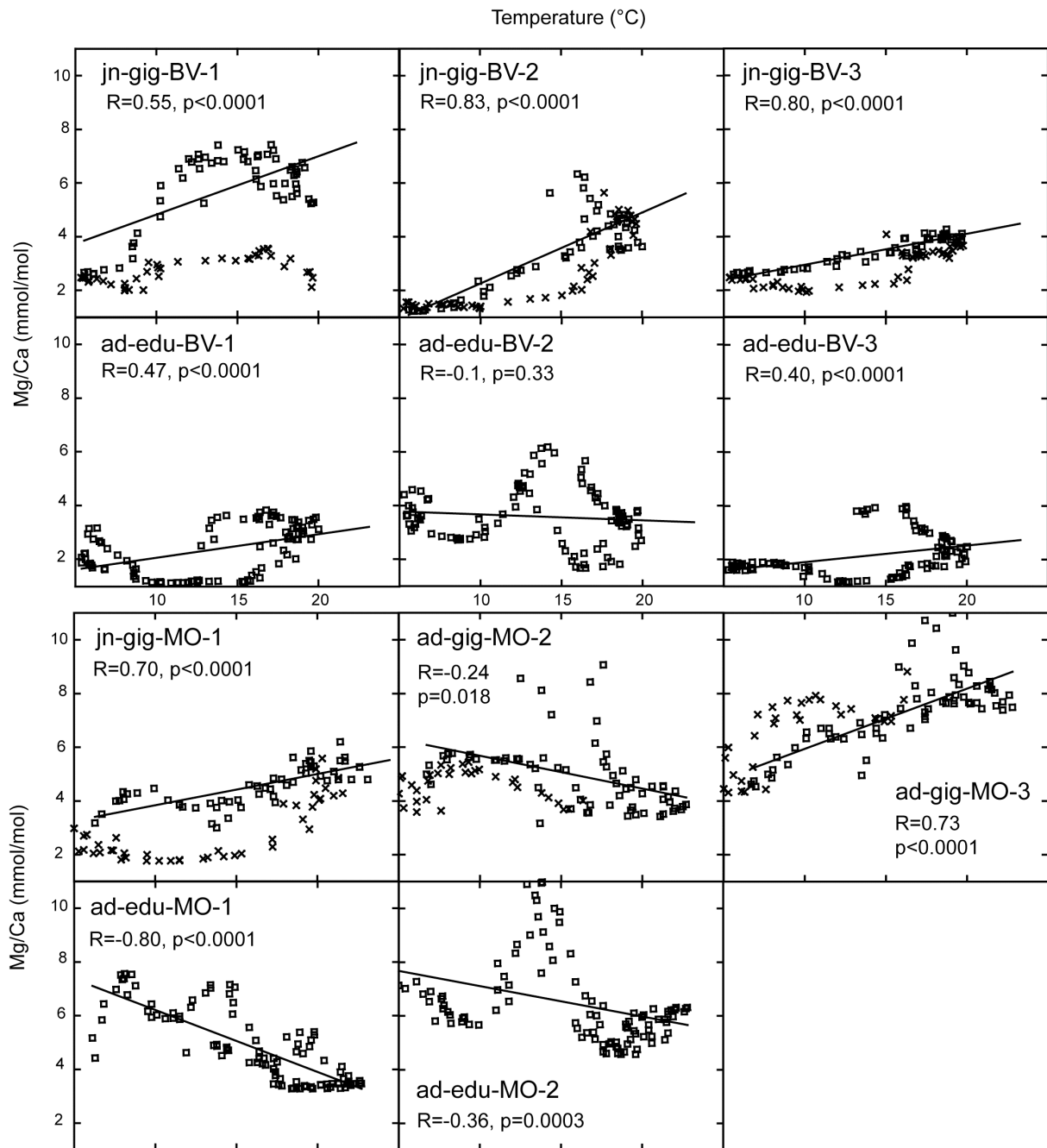
605 Figure 5: Shell Mg/Ca evolution curve (dashed black line), detrended Mg/Ca curve (red line)  
606 compared with a 28-days cycles (green line) and with a 14-days cycles (blue line). New moon  
607 spring tides (grey circles) and full moon spring tides (white circles) are indicated.



608

609

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



614

615

616 Figure 7: Seawater temperatures calculated from Mg/Ca ratios in juvenile *C. gigas* shells

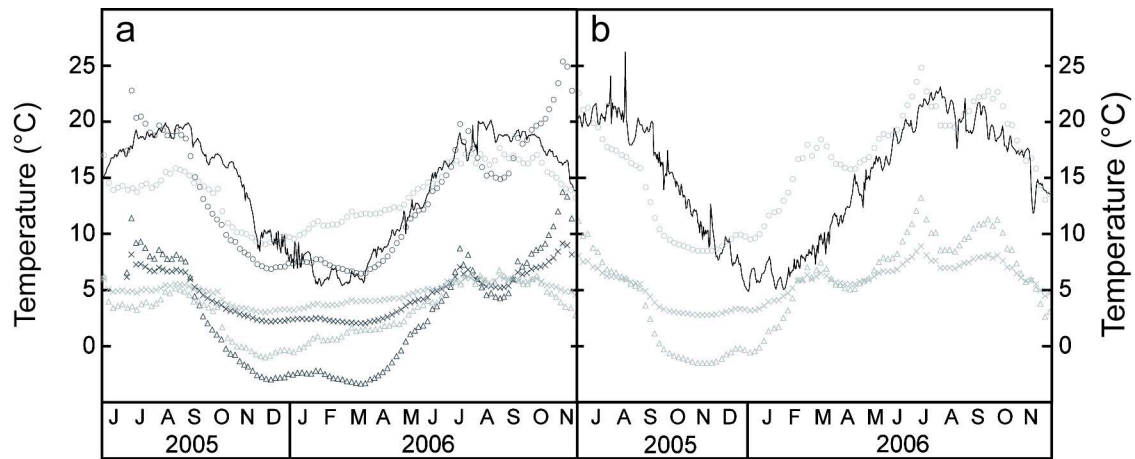
617 using this study equation (black dotted curves) and the equation from Klein et al. (1996; grey

618 dotted curves). a- Baie des Veys measured seawater temperatures (solid curve), calculated

619 SST using Mg/Ca of shells jn-gig-BV-2 (dashed curve) and jn-gig-BV-3 (dotted curve). b-

620 Marennes-Oléron measured seawater temperatures (solid curve), calculated SST using Mg/Ca

621 of shell jn-gig-MO-1 (dashed line).



622

623

624 **Table**

625 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

626

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

628 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
Marennes Oléron	09/08/06	2.68	19.7	33.9
	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

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630 Table 3: Seasonal growth rate measurements of the hinge area of *C. gigas* and *O. edulis*  
631 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 $\mu\text{m/day}$
		jn-gig-BV-2	summer	16.8 $\mu\text{m/day}$
			winter	14.2 $\mu\text{m/day}$
			summer	13.2 $\mu\text{m/day}$
		jn-gig-BV-3	summer	27.4 $\mu\text{m/day}$
			winter	23.9 $\mu\text{m/day}$
			summer	15.9 $\mu\text{m/day}$
		Marennes- Oléron	<i>O. edulis</i> (adult)	ad-edu-MO-1
ad-edu-MO-2	summer			2.0 $\mu\text{m/day}$
<i>C. gigas</i> (juvenile)	jn-gig-MO-1		summer	3.4 $\mu\text{m/day}$
			winter	9.8 $\mu\text{m/day}$
	summer		5.6 $\mu\text{m/day}$	
<i>C. gigas</i> (adult)	ad-gig-MO-2		summer	12.9 $\mu\text{m/day}$
			winter	2.0 $\mu\text{m/day}$
	ad-gig-MO-3		summer	2.5 $\mu\text{m/day}$
			winter	7.4 $\mu\text{m/day}$
		2.8 $\mu\text{m/day}$		

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633 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

634

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

636 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

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