New insights into oyster high-resolution hinge growth patterns

Huyghe Dimitri ^{1,2}, De Rafelis Marc ², Ropert Michel ³, Mouchi Vincent ^{1,4}, Emmanuel Laurent ⁴, Renard Maurice ⁴, Lartaud Franck ^{1,*}

¹ Sorbonne Université, CNRS, Laboratoire d'Ecogéochimie des Environnements Benthiques, LECOB, Observatoire Océanologique de Banyuls, 66650 Banyuls-sur-Mer, France

² Géosciences Environnement Toulouse, CNRS, IRD, Université Paul Sabatier Toulouse 3, 14 Avenue Edouard Belin, 31400 Toulouse, France

³ Ifremer, Laboratoire Environnement Ressource de Normandie, Avenue du General de Gaulle, BP 32, 14520 Port-en-Bessin, France

⁴ Sorbonne Université, CNRS-INSU, Institut des Sciences de la Terre Paris, IStEP, 75005 Paris, France

* Corresponding author : Franck Lartaud, email address : franck.lartaud@obs-banyuls.fr

Abstract :

While oyster shells are one of the most common mollusks used for the analysis of (paleo)environmental and (paleo)climatic records based on geochemical proxies, high-resolution growth rate changes still need to be determined. Promising previous works are restricted to small portions of shell sections due to difficulties in continuous growth increment revelation. Based on a mark and recapture experiment of *Magallana gigas* specimens reared in an intertidal area of Normandy (France) for 22 months, and a sclerochronological approach using cathodoluminescence microscopy, this study provides the longest high-resolution record of growth increments in oyster shells to date. Different growth patterns were identified likely related to the oyster age. After age 1 year, the formation of growth increments follows an expected tide-related model, leading to the mineralization of ~ 2 calcitic increments per day, together with growth rate changes at lunar and semi-lunar periodicities, and a seasonal trend with occasional growth breaks during winter when temperatures fall below ~ 6 °C. However, for oysters younger than 1 year, i.e., before reaching their sexual maturity, the growth increment analysis reveals unconventional patterns. In this case, oysters' growth is associated with either a large number (~ 5) or less than one increment per day depending on the period. This pattern is also associated with frequent growth cessations, although the growth rate of the shell is high at this period. Our results illustrate that the high-resolution sclerochronological approach is required for accurate paleoenvironmental reconstructions based on oyster shells.

Introduction

Bivalves inhabit a variety of different aquatic ecosystems, including freshwater, coastal and deep-sea environments. They produce shell carbonate that both support the general shape of the organisms as well as protect soft tissues against biotic or abiotic threats. However, the shell is not produced consistently over time (Goodwin et al. 2003). Periodic slowdowns of growth, controlled by environmental triggers and/or endogenous mechanisms, result in the formation of growth lines, which separate the growth into regular time intervals of (sub)equal duration, the growth increments (Schöne 2008). This typically results in the formation of annual, lunar, daily or tidal growth increments (Evans 1972; Schöne et al. 2003). The analysis of physical and chemical variations in the shell of organisms, and the temporal context in which they formed is called ‘sclerochronology’ (Hudson et al. 1976; Gröcke and Gillikin 2008). The sclerochronology of bivalve shells is widely used in ecological and (paleo)environmental studies. Growth increment counting provides a clue to estimating the life span of the organism, as for the ‘Methuselah’ bivalve *Arctica islandica* reaching 507 years (Butler et al. 2013), together with a potential archive of the organism’s environmental conditions (Schöne and Surge 2005). The latter raised a particular interest for shell material from inaccessible habitats (e.g., deep-sea hydrothermal vents, Schöne and Giere 2005; Nedoncelle et al. 2015) or for paleoclimatic reconstructions (Scourse et al. 2006; Butler et al. 2013; Schöne 2013). Additional geochemical proxies can also provide a complementary estimation of (past) water conditions, based on the analysis of stable isotopes and trace elements of the shell (Krantz et al. 1987; Dettman et al. 2004; Chauvaud et al. 2005; Ivany et al. 2008; Lartaud et al. 2010a). However, the accurate understanding of the growth pattern of calcifying mollusks and the accurate interpretation of geochemical studies require a robust

identification of growth rate variations and possible periods of non biomineralization of the shell.

Among the species used in sclerochronological studies, oysters have been given particular attention. Seasonal growth rate changes can be observed based on morphological growth anomalies in the ligament area (Kirby et al. 1998), winter growth line revelation using acetate peels (Richardson et al. 1993) or natural cathodoluminescence fluctuations in modern (Langlet et al. 2006; Lartaud et al. 2010b; Doldan et al. 2018) and fossil shells (Kirby et al. 1998; Lartaud et al. 2006). Numerous studies using oxygen stable isotopes (Surge et al. 2001; Lartaud et al. 2010c; Ullmann et al. 2010; Tynan et al. 2014) or magnesium-calcium ratios (Surge and Lohmann 2008; Mouchi et al. 2013; Tynan et al. 2017) highlight the ability of oyster shells to record properly the environmental conditions, even in the fossil realm (Harzhauser et al. 2011; Bougeois et al. 2014; Huyghe et al. 2015). However, this environmental archive was shown to be altered in the shell by local environmental settings (Lartaud et al. 2010c) or by an age effect (Mouchi et al. 2013). Thus, the representativeness of mean temperatures estimated from the analyses of a single oyster hinge could be questioned. This suggests that a high-resolution investigation of growth patterns is required to better define the timing and temperature restrictions in mineralization of oyster shells. Moreover, such high-resolution studies are of paramount importance, as recent geochemical analysis methods tend to favor high spatial resolution measurements in shell materials (Meibom et al. 2007; Mouchi et al. 2013, 2018; Füllenbach et al. 2017).

Due to their economic value, the growth of the modern oyster *Magallana gigas* (former *Crassostrea gigas*) (Salvi and Mariottini 2017), has been investigated for decades. It has been found that the seasonal growth fluctuations of this species are primarily controlled by temperature, salinity and food availability (e.g. Hall 1984; Brown 1988; Gangnery et al. 2003). However, few studies have focused on the characterization and significance of the

growth patterns over short periods. Based on the investigation of shell sections, Langlet et al. (2006) suggested that *M. gigas* form growth increments at a daily periodicity, but this study was carried out in a Mediterranean lagoon where tides are reduced and oysters are continuously immersed. Other studies observed increments formed at semidiurnal periods, likely produced by the tidal regime in intertidal areas of the Bay of Biscay, in Atlantic waters (Higuera-Ruiz and Elorza 2009). Both of these studies also revealed the existence of lower frequency growth changes: every seven days in shells from the Mediterranean (Langlet et al. 2006) and within fortnight cycles in shells from the Bay of Biscay (Higuera-Ruiz and Elorza 2009). In both cases, only a small portion of the shell, thus a small period of time, was analyzed. To the date, there is a lack of a continuous record of shell growth patterns in *M. gigas* to clearly define the growth dynamics at high-resolution.

To understand how the sclerocronological archive can be altered by environmental conditions or by the biologic activity of the organisms, we investigated the shell growth pattern of *M. gigas* at high-resolution over a long-term period (i.e., 22 months). To this aim, we developed a sclerochronological analysis based on cathodoluminescence increment counting. Oysters studied here come from the Baie des Veys (BDV), located in Normandy (France).

Materials and methods

The oyster Magallana gigas

Oysters of the species *Magallana gigas* live in shallow water and can tolerate large variations of water salinity, temperature and turbidity. They are suspension feeders and filter principally phytoplanktonic (diatoms) and zooplanktonic species. These oysters are

sequentially hermaphrodites and reach sexual maturity after 12 to 18 months of life in the studied area (Soletchnik et al. 1996). Reproduction occurs in the summer on the French Atlantic coast, but temperature generally remains too cold for reproduction in the BDV (Ropert et al. 2007). Thus, in the present study we will consider specimens of less than 12 months as juveniles (i.e. before summer 2005) and specimens older than 12 months as adults.

Field site and samples

In this work, we analyzed oyster shells bred on a farming site of the IFREMER (Institut Français de Recherche pour l'Exploitation de la Mer) located on the Normandy coast of the English Channel, at the Baie des Veys (BDV, 49°23.110'N 1°6.050'W; Fig. 1). The site is located in an open bay in a high intertidal setting with a semi-diurnal tidal regime (Fig. 1). Oyster spat of the species *Magallana gigas* came from wild broodstock from the Arcachon basin, located on the French Atlantic facade (Fig. 1A). They were recruited from the summer 2004 oyster farm ponds and were collected on 01/28/2005, and transplanted to the BDV on 01/29/2005 (see Lartaud et al. 2010b for further details). Then, they were allowed to breed in oyster pockets arranged on submerged tables until November 2006 (Fig. 1). The tables were located 50 cm above the sediment. Young oysters (< 1 year) were bred in 0.5 x 0.5 m pockets. Adult oysters were moved to larger pockets (1 x 0.5 m). Thus, the density of oysters remained low in the pockets throughout the experiment allowing the oysters to grow freely.

During the rearing experiment time at BDV, seawater temperature, salinity and water levels at the site were measured (data acquisition every 15 min) using a YSI multi-parameter probe attached to the oyster tables. Seawater samples for total chlorophyll *a* and pheopigment concentrations ($\mu\text{g.l}^{-1}$) were sampled fortnightly, filtered through Whatman GF/F filters to

estimate the trophic resources available for the oysters. Samplings were performed at low tide when tide coefficients were high, which guaranteed replicable conditions.

Fig. 1

Mn²⁺ labeling and cathodoluminescence analysis

The growth rate of *M. gigas* shells can be determined through cathodoluminescence (CL) observations of the hinge area, which gathers both the complete ontogenetic record of oysters and the environmental conditions experienced throughout their life (Barbin et al. 2008; Barbin 2013; Lartaud, et al. 2010b). The CL phenomenon results from the interactions between a light-emitting centre (chemical element or impurity) and the atomic environment inside the crystal lattice during excitation by an electron gun (Machel et al. 1991; Barbin and Schvoerer 1997). In calcite, CL emission (~620 nm) is principally induced by the presence of Mn²⁺ trapped into the lattice during mineral growth (El Ali et al. 1993; de Rafelis et al. 2000). Mn²⁺ concentrations have been monitored in the BDV and showed reduced seasonal fluctuations (Lartaud et al. 2010b). This work showed that in the BDV, natural CL fluctuation in the shells is not linked to seawater Mn concentration but rather to seawater temperature and shell growth rate.

For oysters, analysis of the hinge area is recommended instead of the whole shell section, as it gathers the growth history on a same shell portion. Moreover, the hinge is usually less impacted by shell boring species and algal deposits (Langlet et al. 2006; Lartaud et al. 2010b). Additionally, the hinge portion located under the ligamental area is composed of a single and more resistant microstructure (i.e., foliated calcite, Carter 1980). This is why

oyster hinges are more often the only shell region preserved in fossil remains and are more resistant to diagenetic alterations (Lartaud et al. 2006).

We stained the shells 15 times at regularly spaced intervals over the 22 months experiment at BDV using Mn^{2+} to obtain 15 temporal points of reference to measure shell growth increments, according to the approach described in Langlet et al. (2006) (Fig. 2). During low tide, the entire oyster pockets were immersed for 4 h in a tank filled with seawater sampled on site with the addition of 90 mg.l^{-1} of manganese chloride tetrahydrate ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$). Once marked, the pockets were immediately returned to the culture tables. Note that this protocol is not dangerous for oysters and does not affect their growth (Lartaud et al. 2010b).

Immediately after the final collection in November 2006, the specimens were sacrificed, and the shells were sectioned and mounted on slides for CL microscopy, as described in Lartaud et al. (2010b).

The slides were observed with a cold cathode (Cathodyne-OPEA, 15-20 kV and 200 to $400 \mu\text{A.mm}^{-2}$ under a pressure of 0.05 Torr) to reveal both the natural luminescence variability in the shells and the Mn labels (Fig. 3). A Nikon D5000 (1400 ASA) camera was used for luminescence image acquisition with a constant exposure time of 20 s. The analysis of growth intervals was conducted using the CL-mounted photographs with Adobe Photoshop CS6 image processing software. Mounted photographs, providing a complete and detailed panorama of the hinge area, were used to generate luminescence spectra by means of ImageJ software (Fig. 3B). Luminescence analyses are semi-quantitative because each shell has its own heterogeneity, which makes luminescence intensity normalization impossible (Langlet, et al. 2006; Lietard and Pierre 2008). Luminescence intensity is therefore expressed in arbitrary units (AU).

For each shell, the Mn²⁺ marking recognition allowed us to transform CL spectra along a growth profile into a calendar profile (Lartaud et al. 2010b; Mouchi et al. 2013). The least-squares method for non-linear regression analysis, following the Levenberg-Maquardt method (Statistica software), was performed to estimate parameters of the Von Bertalanffy relationship of oyster hinges growth. This equation enables the estimation of ontogenetic ages from shell hinge lengths:

$$L_t = L^\infty (1 - e^{-k(t-t_0)}) \quad (\text{equation 1})$$

where L_t is the hinge shell length (mm) at time t (in years), L^∞ is the maximum hinge shell length (mm), t_0 (in years) is the setting size and k is a time constant. It is then possible to determine the maximum size of the shell, as a linear relationship between hinge length and shell length size that was previously shown for oysters at the BDV (Lartaud et al. 2010b).

Analysis of the shell growth rate at higher resolution (i.e., at the scale of the calcite increment) corresponded to the study of micrometric alternations between highly luminescent and dull calcite CL increments (Fig. 3C). We defined a pair of bright-dull bands observed under CL as a CL increment. We counted the number of these pairs of bright-dull increments and measured the width between two successive bright increments with ImageJ to build sclerochronological profiles and determine high-resolution growth patterns of the shells throughout their lives (Nedoncelle et al. 2013).

Fig. 2 & 3

Principal component analysis

To examine which parameters controls the growth of oysters at high resolution, a Principal Component Analysis (PCA) was carried out using the software Matlab. The PCA was performed based on increment width fluctuation with respect to mean daily temperature, salinity, immersion duration, high tide level and low tide level.

Fast Fourier Transform analysis

The variability in growth increment width was analyzed via a spectral analysis, based on Fast Fourier Transform (FFT). In Matlab, we used the multi-taper method (Thomson 1982) and robust red noise modeling, as implemented in the singular spectrum analysis multi-taper method (SSA-MTM) toolkit (Ghil et al. 2002).

Mann-Whitney test

The Mann-Whitney test was used for comparison between mean growth rate between winter and summer and to determine if these values are statistically different or not. Before using this non-parametric test, the non-respect of the normality and homoscedasticity conditions were checked using the Shapiro and Bartlett tests respectively.

Results

Fluctuation Baie des Veys environmental parameters

We report the fluctuation of temperature, salinity, chlorophyll *a*, pheopigments concentration and tides measured in the BDV on Fig. 2. The temperature exhibited seasonal cyclic variations with minimum and maximum values of 5 °C between January and March and 20 °C from July to September, respectively. Salinity remained almost constant throughout the year at a value of ~ 33-34 psu with isolated transient decreases (~29 – 31 psu) due to freshwater input in response to elevated rainfall events.

Concentrations of chlorophyll *a* and pheopigments, assumed proxies of food available for oysters, are also reported in Fig. 2. Pheopigment concentrations remained low (< 2 mg/m³) during the whole interval compared to chlorophyll *a* concentrations, which fluctuated between 0.5 mg/m³ and 11.5 mg/m³. The higher concentrations were reached during phytoplanktonic blooms, which occurred mainly during spring. We also calculated the ratio between chlorophyll *a* and pheopigment concentration, which is related to the quality of food for oysters (Le Guitton et al. 2015; Belley et al. 2016). This ratio is mostly driven by the concentration in chlorophyll *a* and is thus high during spring and the end of summer and low during winter.

Variations in the high tide level ranged between 3 and 6 m (mean = 4 m) above the oyster tables, whereas the low tides ranged between -0.5 and 2 m (mean = 0.5 m) (Fig. 3). Thus, oysters remained completely immersed when tidal coefficients were low and could be exposed during 3 h when tidal coefficients were high.

General trends of hinge growth rates

The cumulative change of the hinge lengths measured between the successive markings on 11 shells is reported on Fig. 4. It highlights that the total hinge lengths ranged

between 13 and 21 mm after 22 months of growth. The growth of all oyster shells followed a Von Bertalanffy model ($r = +0.99$, $p = 0.01$ $n = 157$), with a fast growth rate during the beginning of their lives and a progressive slowing of growth as they mature, as observed for other oysters (Bayne and Newell 1983; Mitchell et al. 2000). The Von Bertalanffy growth function revealed an expected maximum hinge length of 27.6 mm for *M. gigas* shells at BDV. These measurements yield similar results to many other works on oysters including previous investigations in the BDV (Lartaud et al. 2010b) suggesting that the growth of the studied oysters was not disrupted by any unusual environmental or endogenous factors.

Fig. 4

Figure 5 presents the variation of the mean growth rate of the 11 oyster hinges between the successive Mn^{2+} markings and the mean daily seawater temperature. The general pattern of growth exhibited seasonal variations. The first part of the profile (i.e., February 2005) showed high variability in growth rates, leading to low values (mean of $25 \pm 15 \mu\text{m/day}$) immediately after transplantation. Very high values were observed during the rest of the month ($120 \pm 60 \mu\text{m/day}$). During the rest of 2005, mean growth rate fell to $\sim 20 \pm 7 \mu\text{m/day}$ from March to July and then increased during summer, with a maximum of $\sim 50 \pm 13 \mu\text{m/day}$ observed in September – October. Then, shell growth decreased during the winter to minimum values of $8 \pm 2 \mu\text{m/day}$ in February 2006, followed by a slightly but significant increase through the summer of 2006, reaching $24 \pm 8 \mu\text{m/day}$. Mann-Whitney U test ($n = 81$, $p < 0.001$) revealed that this increase between winter and summer values is statistically significant. The mean growth rate immediately before collection in November 2006 was $12 \pm 5 \mu\text{m/day}$.

Fig. 5

Figure 6 presents correlations between the mean growth rates and the mean temperature as well as the chlorophyll *a* concentration, calculated between the markings. Growth rate changes were consistent with the seasonal pattern of seawater temperatures, except for the youngest part (i.e., during the second half of February 2005), which showed high variability and high values in shell growth rates although temperatures remained low. Growth outlier of February 2005 was removed from comparison with environmental parameters. A low but positive correlation was observed between the mean shell growth rates and temperatures (Least mean squares regression, $r = +0.52$, $p = 0.05$; Fig. 6) and highlights that the extreme growth value of February 2005 is thus non-influenced by temperature variations (Fig. 6).

Unfortunately, chlorophyll *a* was not monitored during the high growth rate value of February 2005, but in the rest of the whole experiment, there was no relationship with shell growth rate (Least mean squares regression, $r = +0.001$, $p = 0.89$; Fig. 6), suggesting that this parameter has a lower influence on the shell growth rate compared to temperature.

Fig. 6

High-resolution growth patterns

Several specimens revealed a quasi-continuous record of CL increments during the two year monitoring and we counted the number of increments and measured the fluctuation of the increment widths for nine shells when CL observations were of quality (Table 1). The inferred sclerochronological profiles are presented in Fig. 7 for samples BDV3 and BDV5,

which present the most complete record (Table 1). Positions of Mn^{2+} chronological markings were reported on Fig. 7. The overall pattern of increment widths observed on Fig. 7 has five distinct periods. During the first period, in February 2005, the mean increment width was intermediate ($31 \pm 14 \mu m$ for BDV3 and $17 \pm 5 \mu m$ for BDV5) and increased during a second period from March to September ($45 \pm 24 \mu m$ for BDV3 and $50 \pm 19 \mu m$ for BDV5). Then, a third period showed a decrease in increment width from September 2005 to November 2005, reaching $32 \pm 20 \mu m$ for BDV3 and $32 \pm 10 \mu m$ for BDV5. Although the identification and measurement of increment widths were not possible for BDV3 from November 2005 to March 2006, due to very dull luminescence of the hinge, specimen BDV5 allowed increment counting to the end of January 2006. This fourth period corresponds to the beginning of winter and was marked by low mean increment width values ($10 \pm 6 \mu m$). No measurements were possible from February to May 2006 for specimen BDV5, because the width between two successive increments was too small to be clearly distinguishable from each other. For BDV3, values remained low from March to May 2006 (mean of $9 \pm 3 \mu m/inc.$), but displayed higher fluctuations (from 5 to $20 \mu m/inc.$) compared to the beginning of winter. During the fifth period (May to November 2006), the mean value of increment widths was high (mean of $20 \pm 9 \mu m$ for BDV3 and $18 \pm 8 \mu m$ for BDV5) and highly variable (between 3 and $58 \mu m$).

The number of CL increments secreted per day (inc./day) between two successive chemical markings was estimated from the analysis of nine shells (Table 1) and is reported for samples BDV3 and BDV5 on Fig. 7. The mean number of increments per day was variable according to the period considered. First, during February, a mean of $5.2 \pm 2.2 inc./day$ was calculated. The formation of infra-daily increments was highlighted by the duplication of the staining on 02/09/2005 (Fig. 8), corresponding to increment mineralization in less than four hours, the duration of the Mn^{2+} bath. From the end of February to September 2005, a mean of $0.8 \pm 0.1 inc./day$ was secreted. From September to November 2005, the number of

increments per day increased to 1.7 ± 0.1 . During the following winter and the beginning of spring, when increment counting was possible from November 2005 to January 2006 and from March to May 2006, the daily biomineralization rhythm fell to 1.6 ± 0.1 inc./day. Finally, from May to November 2006, 1.8 ± 0.2 inc./day were secreted. Except for February 2005, where numerous infra-daily increments occurred, the number of increment formed per day during the adult period (~ 2 increments per day) was significantly higher than in the oysters of < 1 year old (> 1 increment per day) (Student test, $n = 34$, $p < 0.001$).

Fig. 7, Fig. 8, Table 1

Discussion

Drivers of oyster shell growth

The analysis of growth increments formed during a 22 month *in situ* experiment revealed three distinct patterns. During February 2005, the oysters mineralized more than 5 increments per day. From the end of February to September 2005, corresponding to oysters aged 6 to 12 months, less than one increment per day was mineralized, with variability in the increment width. During the third period (September 2005 to November 2006), corresponding to oysters more than one year old, shells formed almost two increments per day, likely tide-related, as BDV has a semi-diurnal tide regime.

Circatidal growth patterns are frequently observed in bivalves from intertidal areas, such as the cockle *Cerastoderma edule* (Mahé et al. 2010), the mussel *Mytilus edulis* (Richardson 1989) and the clam *Chione cortezi* (Schöne et al. 2002a). Circatidal structures result from shell accretion during high tides, associated with periods of active pumping in

well-oxygenated waters, and growth cessation at low tides, during aerial exposure, when animals close their shells tightly leading to anaerobic conditions and internal pH modifications (Lutz and Rhoads 1977). Based on microscopic observations of small portions of shell sections deposited during spring, the increments of $\sim 10 \mu\text{m}$ found by Higuera-Ruiz and Elorza (2009) in the hinge area of *M. gigas* shells from intertidal zones of the Bay of Biscay were attributed to tidal periodicity. Our results thus support the growth model defined by these authors, adding an observation of growth increment over a long period (i.e., > 1 year). It is interesting to note that both bright and dull CL increments are formed during one tidal cycle. But at this stage it is unclear if a bright band corresponds to the flood or ebb current or, as oysters are not necessarily emerged at each tide in the studied area, to the maximum or minimum tide levels. Moreover, the process that drives the mineralization of bright and dull increments seems to be more difficult to explain during the youngest part of the life of the oysters, as they are able to mineralize two bright growth lines, i.e., two CL growth lines in a tank where tides are not recorded. It implies that processes other than environmental ones, probably related to biological factors, influence the mineralization of the CL increments. It also confirms that, at this scale, bright and dull intervals are not related to natural fluctuation of the Mn^{2+} concentration in seawater.

Our results contrast with the observations made by Langlet et al. (2006), which showed a single CL increment formed per day in *M. gigas* shells from Thau lagoon on the Mediterranean coast. In this area where tide fluctuations are only a few centimeters, oysters remain continuously submerged. Thus, a complex combination of other parameters, particularly evaporation and wind regimes, including the diurnal cycle of wind velocity (Bouin et al. 2012), together with the functioning of artificial channels connecting to Mediterranean seawaters, substantially modify the hydrology of the lagoon and can generate daily rather than tidal changes in the water masses (Audouin 1962).

Based on the observations described above, we can assume that oysters form two increments per day during the second year of life in our study. Thus, the decrease observed of this ratio to 1.5 to 1.6 inc/day during the winter to early spring period, a time when shell growth rate was reduced, suggests (1) that all CL growth increments may not be countable due to extremely reduced width and brightness and/or (2) not all increments are formed related to occasional growth breaks. CL increments were clearly distinguishable during the March to May 2006 period. Thus, the fact that oysters mineralize less than 2 inc./day cannot be explained by inaccurate observation of the shell. Thus, one can argue in favor of the second hypothesis. Occasional growth cessations are consistent with the model described by Schöne (2008) for shells from high-intertidal areas, leading to an incomplete record for several days. Observations done on the shell of the bivalve *C. cortezi* demonstrated that growth is slower during spring cool nights (Schöne et al. 2002a). The growth cessations observed during winter and spring for *M. gigas* could reflect these kinds of stressful conditions corresponding to occasional but significantly lower temperatures.

We analyzed the variability in growth increment width via FFT spectral analysis. Based on tide-related growth increments (i.e. 2 inc./day), this analysis revealed two periodicities of 64 and 28 to 32 increments corresponding to growth rhythms of 32 and 14 to 16 days. The ~15 days frequency relates to the fortnight cycle, while the 32 days frequency can be attributed to the synodic lunar cycle (29.5 days). Similar growth patterns have been observed in the Mg/Ca ratio of *M. gigas* oyster shells (Higuera-Ruiz and Elorza 2009; Mouchi et al. 2013) and are likely highlighted by the formation of dark/clear bands in the umbo (Higuera-Ruiz and Elorza 2009). However, to date, the description of such growth patterns in the shell mineralization has not been performed for such an extended period of time. Lunar cycles in the shell mineralization can be related to valve activity, with an increase in the duration of valve opening during highest tides and a decrease during the lowest ones (Tran et

al. 2011), and/or the role of current velocity changes, which modify food availability (Clark 2005; Lartaud et al. 2010a).

For BDV3 and BDV5, a comparison of the variability of CL increment width when ~2 increments were mineralized per day and the high tide sea level reveals that both for the autumn 2005 and spring to autumn 2006 periods, the larger increment width correlates most of the time with periods of lower high tide levels (Fig. 9; reversed scale), that occur a few days after neap tides in the BDV. In contrast, a narrow increment width coincides with higher high tide levels, which occur just after spring tides. However, we note two exceptions: the full moon of the 09/18/05 and the new moon of the 09/22/06, when large increment widths are observed. Contrary to the descriptions made by Schöne (2008) in high-intertidal areas, oyster shell production is favored at neap tides compared with spring tides. This is consistent with the pattern proposed by Ohno (1989) for oyster shells from mid- to low-intertidal settings, as also illustrated by observations on *C. cortezi* (Schöne et al. 2002a) and *Adamussium colbecki* (Lartaud et al. 2010a).

To test these assumptions and to better determine the causes of the increment widths fluctuation, we analyzed the relationship between this parameter and the mean daily temperature, salinity, immersion duration, high tide level and low tide level for samples BDV3 and BDV5 for periods when ~2 increments were mineralized per day using a Principal Component Analysis (PCA; Fig. 10). This analysis indicated that increment width is not correlated with salinity variations, immersion duration, high tide level or low tide level for both oysters. However, it highlights a correlation with temperature, which was greater during 2005 than 2006.

Thus, seawater temperature variation seems to be the most important factor controlling increment width. There is also evidence that the seasonal changes in growth rates are primarily driven by temperature (Fig. 5), and various studies report the role of temperature in

increment width variation, such as for the giant clam *Hippopus hippopus* (Schwartzmann et al. 2011) or the king scallop *Pecten maximus* (Chauvaud et al. 1998). At the CL increment width resolution, this relationship is obvious for 2005 but less for 2006, where increment width fluctuation seems to be correlated with high tide level (Fig. 9). Thus temperature changes in the BDV seawaters alone cannot explain small-scale growth variability in oyster shells. Cloern (1991) showed that during spring tides, phytoplankton concentration is dissipated by current flow velocity, which could lead to less available food and/or more energy consumption for shell growth during this period. Phytoplankton concentration can therefore appear as a secondary growth driver, when temperature is not a limiting factor.

Fig. 9, Fig. 10

Winter and early spring growth

The winter and spring periods of the second year are characterized by a slight decrease in the number of increments mineralized each day compared to the summer period (Fig. 7). We observe that the decrease in shell growth during the winter results from both a decrease in the number of increments and the deposition of narrower increments (Fig. 5 and 7). This decrease in growth rate, likely related to the relationship between oyster shell growth and temperature and, possibly, in a second order to food availability, has already been documented for oyster species and in particular for *M. gigas* (Brown and Hartwick 1988; Mitchell et al. 2000). The minimum temperature at which oysters stop mineralizing their shells is, however, subject to debate. Quayle (1988) and Kirby et al. (1998) set this temperature boundary near 10°C from the study of modern Eastern and Pacific oyster specimens. According to Ullmann et al. (2010), oysters should stop biomineralization when

seawater temperature is below 6°C. Here, we observed that oysters mineralize their shells even during winter (Fig. 4). In the BDV, the coldest interval occurred between the markings of the end of January and March, with a mean temperature of 5.9°C (Fig. 5). Between these two markings, temperatures ranged between 6.8 and 5°C. Thus, it is likely that oysters are able to mineralize their shells at least to a temperature of 6.8°C and even possibly to ~ 5°C. This observation is of crucial importance for paleoenvironmental reconstructions, because it shows that oysters are potentially good indicators for estimating cold seawater temperatures, as low as ~ 6°C.

Juvenile growth patterns

In our study, during most of the first year of their life i.e. before reaching their sexual maturity (January to September 2005), oysters exhibited completely different shell growth patterns compared with the rest of the studied life history. Increment counting pointed out the formation of several increments each day (mean of $\sim 5 \pm 2$ inc./day) in February 2005. Then from March to September 2005, less than one increment mineralized per day instead of two in the adult period (see above). Although we have only one cohort - which limits extrapolations to a general feature at the species level - this different pattern during the juvenile stage was observed for all shells analyzed. CL increments were easily identifiable and bias in increment counting cannot explain this observation. Measurements of the environmental parameters (temperature, salinity and food availability) do not indicate different specific conditions compared to the second year of the experiment (Fig. 2) and no elevated mortality (> 10 %) was reported in the BDV breeding station during the experiment (Mary et al. 2006; Ropert et al. 2007).

Nearly one growth increment mineralized per day has also been reported in the shell of the bivalve *C. cortezi* (Goodwin et al., 2001; Schöne et al., 2002b), but to date the drivers remain unknown. Interestingly, the formation of numerous growth increments per day during the February 2005 period coincides with high shell growth rates (Fig. 4). This pattern could be the consequence of unusual environmental conditions in the BDV during February 2005, but the parameters monitored do not exhibit any such anomalies. Additionally, a similar high shell growth rate has been identified in other oysters bred in different sites on the French Atlantic coast and coming from the same spat as the ones analyzed in this work (Lartaud et al., 2010b). However, in the other sites, the growth peak occurred when the oysters were three to four months older (i.e. during the spring). Moreover, the hinge length mineralized between 02/09/05 and 02/24/05, which varies significantly according to the individuals, is a function of the number of increments produced (Fig. 11). It is thus difficult to directly relate the shell growth of oysters to environmental parameters during this period, and should rather imply internal biological processes such as physiological factors and/or genetic features, as suggested by Rodland et al. (2006) based on the analysis of behavior patterns of bivalves at ultradian time-scales.

From the end of February to September 2005, the absence of a bi-daily rhythm in increment formation corresponds to periodic growth cessations. However, during the first year of life, increment widths are larger compared to the second year of life (Fig. 7), which is consistent with a Von Bertalanffy growth model (Berthome et al. 1986; Brown and Hartwick 1988; Mitchell et al. 2000). This suggests that shells grew more rapidly, but less frequently during the first months of life, corresponding to a fast growth but not at each tide (i.e., less than half of the tidal increments are recorded).

It is still unclear why young oysters exhibit such growth patterns, with a growth history more discontinuous than during the adult phase. The impact of energy budget

allocation to sexual reproduction and gamete production instead of shell mineralization cannot be invoked as a possible cause, as oysters had not yet reached sexual maturity in the spring of 2005. This metabolic trait occurs after 12 to 18 months of life (Soletchnik et al. 1996). However, this emphasizes the importance of input provided by a sclerochronological approach, compared to measurements resulting from mark and recapture techniques only, because the former provides a better description of growth evolution at fine scale. Thus, oysters have a more complete record of their environmental conditions after the first year of life and more precisely during summer and autumn periods, with less growth breaks and shell biomineralization during each tide.

Fig. 11

Conclusions

Based on a cathodoluminescence approach, this study provides the longest high-resolution record of growth in oyster shells, showing two distinct patterns. The general feature is the formation of circatidal CL growth increments, composed of a pair of bright/dull luminescent bands likely tide-related, and associated with semi-lunar and lunar growth rate changes. A seasonal trend shows growth rate decrease, with growth cessations in winter, even if oysters are able to mineralize at temperatures of $\sim 5^{\circ}\text{C}$. In addition, our *in situ* experiment suggests variable growth patterns during the first months of life, characterized by either (1) numerous infra-daily growth increments or (2) frequent growth breaks during the younger period (below 1 year old), despite high annual shell growth rates. These unconventional growth patterns require further investigations.

Considering the contribution of CL analysis in growth increment revelation, these results are of particular interest for ecological studies of bivalves in general and oysters in particular, but also for the use of the shells as (paleo)environmental proxies. Indeed, shell growth rate changes and growth cessations at high-resolution, should be taken into account for paleoclimatic reconstructions based on growth indices or geochemical proxies. The numerous growth cessations observed during the first months of life can lead to misinterpretations in the reconstruction of environmental conditions. Therefore, we recommend to avoid sampling this period in further paleoenvironmental and paleoclimatic studies.

Acknowledgments

This work was financially supported by the ANR Amor ‘Data Model Reconstruction of the Cenozoic Climate’ and the BQR project from Sorbonne Université, ‘High frequency to very high frequency recordings of environmental changes to climate by biomineralization.’ Special thanks may be due to Brian Mitchell for improving the English of the manuscript. Thoughtful comments by Editor A. Checa and two anonymous reviewers helped to improve the original version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

- Audouin J (1962) Hydrologie de l'étang de Thau. Rev Trav Inst Pech Marit 26(2): 5-104
- Barbin V (2013) Application of cathodoluminescence microscopy to recent and past biological materials: a decade of progress. Mineral Petrol 107(3): 353-362
- Barbin V, Schvoerer M (1997) Cathodoluminescence and geosciences. C R Acad Sci Paris 325: 157–169
- Barbin V, Ramseyer K, Elfman M (2008) Biological record of added manganese in seawater: a new efficient tool to mark in vivo growth lines in the oyster species *Crassostrea gigas*. Int J Earth Sci 97(1): 193-199
- Bayne BL, Newell RIE (1983) Physiological energetics of marine molluscs. In The Mollusca: Physiology, Part 1. (ed. A.S.M. Saleuddin and K.M. Wilbur), Academic Press, pp 407-415
- Belley R, Snelgrove PV, Archambault P, Juniper SK (2016) Environmental drivers of benthic flux variation and ecosystem functioning in Salish Sea and Northeast Pacific sediments. PloS 11(3): e0151110
- Berthome JP, Prou J, Bodoy A (1986) Performances de croissance de l'huître creuse, *Crassostrea gigas* (Thunberg) dans le bassin d'élevage de Marennes-Oléron entre 1979 & 1982. Haliotis 15: 183-192
- Bougeois L, de Rafelis M, Reichart GJ, de Nooijer LJ, Nicollin F, Dupont-Nivet G (2014) A high resolution study of trace elements and stable isotopes in oyster shells to estimate Central Asian Middle Eocene seasonality. Chem Geol 363: 200–212
- Bouin MN, Caniaux G, Traulle O, Legain D, Le Moigne P (2012) Long-term heat exchanges over a Mediterranean lagoon. J Geophys Res: Atmospheres 117(D23)

589 Brown JR (1988) Multivariate analyses of the role of environmental factors in seasonal and
590 site-related growth variation in the Pacific oyster *Crassostrea gigas*. Mar Ecol Prog Ser
591 45(3): 225-236

592 Brown JR, Hartwick EB (1988) Influences of temperature, salinity and available food upon
593 suspended culture of the Pacific oyster, *Crassostrea gigas*: I. Absolute and allometric
594 growth. Aquaculture 70(3): 231-251

595 Butler PG, Wanamaker Jr, AD, Scourse JD, Richardson CA, Reynolds DJ (2013) Variability
596 of marine climate on the North Icelandic Shelf in a 1357-year proxy archive based on
597 growth increments in bivalve *Arctica islandica*. Palaeogeogr Palaeoclimatol Palaeoecol
598 373: 141–151

599 Carter, JG, 1980. Guide to bivalve shell microstructures. In: Rhoads, D.C. and Lutz, R.A.
600 (Eds), Skeletal growth of aquatic organisms. Plenum Press, New York: 645-673

601 Chauvaud L, Thouzeau G, Paulet YM (1998) Effects of environmental factors on the daily
602 growth rate of *Pecten maximus* juveniles in the Bay of Brest (France). J Exp Mar Biol
603 Ecol 227(1): 83-111

604 Chauvaud L, Lorrain A, Dunbar RB, Paulet YM, Thouzeau G, Jean F, Guarini JM,
605 Mucciarone D (2005) Shell of the Great Scallop *Pecten maximus* as a high frequency
606 archive of paleoenvironmental change. Geochem Geophys Geosyst 6: 1–34

607 Clark GR (2005) Daily growth lines in some living Pectens (Mollusca: Bivalvia), and some
608 applications in a fossil relative: time and tide will tell. Palaeogeogr Palaeoclimatol
609 Palaeoecol 228(1): 26-42

610 Cloern JE (1991) Tidal stirring and phytoplankton bloom dynamics in an estuary. J Mar
611 Res 49(1): 203-221

612 de Rafelis M, Renard M, Emmanuel L, Durllet C (2000) Apport de la cathodoluminescence à
 613 la connaissance de la spéciation du manganèse dans les carbonates pélagiques. C R
 614 Acad Sci Paris 330: 391–398

615 Dettman DL, Flessa KW, Roopnarine PD, Schöne BR, Goodwin DH (2004) The use of
 616 oxygen isotope variation in shells of estuarine mollusks as a quantitative record of
 617 seasonal and annual Colorado River discharge. Geochim Cosmochim Acta 68:1253–
 618 1263

619 Doldan MS, de Rafelis M, Kroeck MA, Pascual MS, Morsan EM (2018) Age estimation of
 620 the oyster *Ostrea puelchana* determined from the hinge internal growth pattern. Mar Biol
 621 165: 119

622 El Ali A, Barbin V, Calas G, Cervelle B, Ramseyer K, Bouroulec J (1993) Mn²⁺ activated
 623 luminescence in dolomite, calcite and magnesite: quantitative determination of
 624 manganese site distribution by EPR and CL spectroscopy. Chem Geol 104 : 189–202

625 Evans JW (1972) Tidal growth increments in the cockle *Clinocardium nuttalli*. Science 176:
 626 416–417

627 Füllenbach CS, Schöne BR, Shirai K, Takahata N, Ishida A, Sano Y (2017) Minute co-
 628 variations of Sr/Ca ratios and microstructures in the aragonitic shell of *Cerastoderma*
 629 *edule* (Bivalvia) - Are geochemical variations at the ultra-scale masking potential
 630 environmental signals? Geochim Cosmochim Acta 205: 256-271

631 Gangnery A, Chabirand J-M, Lagarde F, Le Gall P, Oheix J, Bacher C, Buestel D (2003)
 632 Growth model of the Pacific oyster, *Crassostrea gigas*, cultured in Thau Lagoon
 633 (Méditerranée, France). Aquaculture 2015: 267-290

634 Ghil M, Allen RM, Dettinger MD, Ide K, Kondrashov D, Mann ME, Robertson A, Saunders
 635 A, Tian Y, Varadi F, Yiou P (2002) Advanced spectral methods for climatic time series.
 636 Rev Geophys 40: 31-341

637 Goodwin DH, Flessa KW, Schöne BR, Dettman DL (2001) Cross-calibration of daily growth
638 increments, stable isotope variation, and temperature in the gulf of California bivalve
639 mollusk *Chione cortezi*: implications for paleoenvironmental analysis. *Palaios* 16: 387–
640 398.

641 Goodwin DH, Schöne BR, Dettman DL (2003) Resolution and fidelity of oxygen isotopes as
642 paleotemperature proxies in bivalves mollusk shells: models and observations. *Palaios*
643 18: 110-125.

644 Gröcke DR, Gillikin DP (2008) Advances in mollusc sclerochronology and sclerochemistry:
645 tools for understanding climate and environment. *Geo-Marine Lett* 28: 265–268

646 Hall S (1984) A multiple regression model of oyster growth. *Fish Res* 2: 167-175

647 Harzhauser M, Piller WE, Müllegger S, Grunert P, Micheels A (2011) Changing seasonality
648 patterns in Central Europe from Miocene Climate Optimum to Miocene Climate
649 Transition deduced from the *Crassostrea* isotope archive. *Global Planet Change* 76: 77-
650 84, doi: 10.1016/j.gloplacha.2010.12.003

651 Higuera-Ruiz R, Elorza J (2009) Biometric, microstructural, and high-resolution trace
652 element studies in *Crassostrea gigas* of Cantabria (Bay of Biscay, Spain):
653 Anthropogenic and seasonal influences. *Estuar Coast Shelf Sci* 82(2): 201-213

654 Hudson JH, Shinn EA, Halley RB, Lidz B (1976) Sclerochronology: a tool for interpreting
655 past environments. *Geology* 4: 361–364

656 Huyghe D, Lartaud F, Emmanuel L, Merle D, Renard M (2015) Palaeogene climate evolution
657 in the Paris Basin from oxygen stable isotope ($\delta^{18}\text{O}$) compositions of marine molluscs. *J*
658 *Geol Soc* 172(5): 576-587

659 Ivany LC, Lohmann KC, Hasiuk F, Blacke DB, Glass A, Aronson RB, Moody RM (2008)
660 Eocene climate record of a high southern latitude continental shelf: Seymour Island,
661 Antarctica. *Geol Soc Am Bull* 120: 659-678

662 Kirby MX, Soniat TM, Spero HJ (1998) Stable isotope sclerochronology of Pleistocene and
663 Recent oyster shells (*Crassostrea virginica*). *Palaios* 13: 560–569

664 Krantz DE, Williams DF, Jones DS (1987) Ecological and paleoenvironmental information
665 using stable isotope profiles from living and fossil molluscs. *Palaeogeogr*
666 *Palaeoclimatol Palaeoecol* 58(3-4): 249-266

667 Langlet D, Alunno-Bruscia M, Rafélis M, Renard M, Roux M, Schein E, Buestel D (2006)
668 Experimental and natural cathodoluminescence in the shell of *Crassostrea gigas* from
669 Thau lagoon (France): ecological and environmental implications. *Mar Ecol Prog*
670 *Ser* 317: 143-156

671 Lartaud F, Langlet D, De Rafelis M, Emmanuel L, Renard M (2006) Description of seasonal
672 rythmicity in fossil oyster shells *Crassostrea aginensis* Tournouer, 1914 (Aquitanian)
673 and *Ostrea bellovacina* Lamarck, 1806 (Thanetian). Cathodoluminescence and
674 sclerochronological approaches. *Geobios* 39(6): 845-852

675 Lartaud F, Chauvaud L, Richard J, Toulot A, Bollinger C, Testut L, Paulet YM (2010a)
676 Experimental growth pattern calibration of Antarctic scallop shells (*Adamussium*
677 *colbecki*, Smith 1902) to provide a biogenic archive of high-resolution records of
678 environmental and climatic changes. *J Exp Mar Bio Ecol* 393(1): 158-167

679 Lartaud F, de Rafélis M, Ropert M, Emmanuel L, Geairon P, Renard M (2010b) Mn labelling
680 of living oysters: artificial and natural cathodoluminescence analyses as a tool for age
681 and growth rate determination of *C. gigas* (Thunberg, 1793) shells. *Aquaculture* 300(1):
682 206-217

683 Lartaud F, Emmanuel L, de Rafelis M, Ropert M, Labourdette N, Richardson CA, Renard M
684 (2010c) A latitudinal gradient of seasonal temperature variation recorded in oyster
685 shells from the coastal waters of France and The Netherlands. *Facies* 56: 13–25

686 Le Guitton M, Soetaert K, Damsté JS, Middelburg JJ (2015) Biogeochemical consequences of
687 vertical and lateral transport of particulate organic matter in the southern North Sea: a
688 multiproxy approach. *Estuar Coast Shelf Sci* 165: 117-127

689 Lietard C, Pierre C (2008) High-resolution isotopic records ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) and
690 cathodoluminescence study of lucinid shells from methane seeps of the Eastern
691 Mediterranean. *Geo-Mar Lett* 28(4): 195-203

692 Lutz RA, Rhoads DC (1977) Anaerobiosis and a theory of growth line formation. *Science*
693 198(4323): 1222–1227

694 Machel HG, Mason RA, Mariano AN, Mucci A (1991) Causes and emission of luminescence
695 in calcite and dolomite, and their applications for studies of carbonates diagenesis. In:
696 Barker, C.E., Kopp, O.C. (Eds.), *Luminescence Microscopy: Quantitative and*
697 *Qualitative Aspects*. *SEPM* 25: 9–25

698 Mahé K, Bellamy E, Lartaud F, de Rafélis M (2010) Calcein and manganese experiments for
699 marking the shell of the common cockle (*Cerastoderma edule*): tidal rhythm validation
700 of increments formation. *Aquat Living Resour* 23(3): 239-245

701 Mary C, Pien S, Ropert M, Blin J-L (2006) Rapport REMONOR, Résultats 2005, Ifremer, 67
702 pp.

703 Meibom A, Mostefaoui S, Cuif JP, Dauphin Y, Houlbreque F, Dunbar R, Constantz B (2007)
704 Biological forcing controls the chemistry of reef-building coral skeleton. *Geophys Res*
705 *Lett* 34(2): 1-5

706 Mitchell IM, Crawford CM, Rushton MJ (2000) Flat oyster (*Ostrea angasi*) growth and
707 survival rates at Georges Bay, Tasmania (Australia). *Aquaculture* 191(4): 309-321

708 Mouchi V, de Rafélis M, Lartaud F, Fialin M, Verrecchia E (2013) Chemical labelling of
709 oyster shells used for time-calibrated high-resolution Mg/Ca ratios: a tool for estimation

710 of past seasonal temperature variations. *Palaeogeogr Palaeoclimatol Palaeoecol* 373:
711 66-74

712 Mouchi V, Briard J, Gaillot S, Argant T, Forest V, Emmanuel L (2018) Reconstructing
713 environments of collection site from archaeological bivalve shells: case study from
714 oysters (Lyon, France). *J Archaeol Sci: Reports* 21: 1225-1235, doi:
715 10.1016/j.jasrep.2017.10.025

716 Nedoncelle K, Lartaud F, de Rafélis M, Boulila S, Le Bris N (2013) A new method for high-
717 resolution bivalve growth rate studies in hydrothermal environments. *Mar Biol* 160(6):
718 1427-1439

719 Nedoncelle K, Lartaud F, Contreira Pereira L, Yücel M, Thurnherr AM, Mullineaux L, Le
720 Bris N (2015) *Bathymodiolus* growth dynamics in relation to environmental fluctuations
721 in vent habitats. *Deep Sea Res I Oceanogr Res Pap* 106: 183–193

722 Ohno T (1989) Palaeotidal characteristics determined by micro-growth patterns in
723 bivalves. *Palaeontology* 32(2): 237-263

724 Quayle DB (1988) Pacific oyster culture in British Columbia. *Can Bull Fish Aquat Sci* 218:
725 241

726 Richardson CA (1989) An analysis of the microgrowth bands in the shell of the common
727 mussel *Mytilus edulis*. *J Mar Biol Assoc U K* 69(2): 477-491

728 Richardson CA, Collis SA, Ekaratne K, Dare P, Key D (1993) The age determination and
729 growth rate of the European flat oyster, *Ostrea edulis*, in British waters determined
730 from acetate peels of umbo growth lines. *ICES J Mar Sci* 50(4): 493-500

731 Rodland DL, Schöne BR, Helema S, Nielsen JK, Baier S (2006) A clockwork mollusc:
732 ultradian rhythms in bivalve activity revealed by digital photography. *J Exp Mar Bio*
733 *Ecol* 334: 316–323

734 Ropert M, Pien S, Mary C, Bouchaud B (2007) Rapport REMONOR, Résultats 2006, Ifremer,
735 70 pp

736 Salvi D, Mariottini P (2017) Molecular taxonomy in 2D: a novel ITS2 rRNA sequence-
737 structure approach guides the description of the oysters' subfamily Saccostreinae and
738 the genus *Magallana* (Bivalvia: Ostreidae). Zool J Linnean Soc 179: 263–276

739 Schöne BR (2008) The curse of physiology—challenges and opportunities in the
740 interpretation of geochemical data from mollusk shells. Geo-Mar Lett 28(5): 269-285

741 Schöne B (2013) *Arctica islandica* (Bivalvia): a unique paleoenvironmental archive of the
742 northern North Atlantic Ocean. Global Planet Change 111: 199–225

743 Schöne BR, Surge, D (eds) (2005) Looking back over skeletal diaries -high-resolution
744 environmental reconstructions from accretionary hard parts of aquatic organisms.
745 Palaeogeogr Palaeoclimatol Palaeoecol 228:1–191

746 Schöne BR, Giere O (2005) Growth increments and stable isotope variation in shells of the
747 deep-sea hydrothermal vent bivalve mollusk *Bathymodiolus brevior* from the North Fiji
748 Basin. Pacific Deep Sea Res Part 1 Oceanogr Res Pap 52(10): 1896-1910

749 Schöne BR, Lega J, Flessa KW, Goodwin DH, Dettman DL (2002a) Reconstructing daily
750 temperatures from growth rates of the intertidal bivalve mollusk *Chione cortezi*
751 (northern Gulf of California, Mexico). Palaeogeogr Palaeoclimatol Palaeoecol 184(1):
752 131-146

753 Schöne BR, Goodwin DH, Flessa KW, Dettman DL, Roopnarine PD (2002b)
754 Sclerochronology and growth of the bivalve molluscs *Chione* (*Chionista*) *fluctifraga*
755 and *Chione* (*Chionista*) *cortezi* in the northern Gulf of California, Mexico. The Veliger
756 45: 45–54

757 Schöne BR, Oschmann W, Rössler J, Freyre Castro AD, Houk SD, Kröncke I, Dreyer W,
758 Janssen R, Rumohr H, Dunca E (2003) North Atlantic oscillation dynamics recorded in

759 shells of a long-lived bivalve mollusk. *Geology* 31: 1237–1240

760 Scourse J, Richardson C, Forsythe G, Harris I, Heinemeier J, Fraser N, Briffa K, Jones P
761 (2006) First cross-matched floating chronology from the marine fossil record: data from
762 growth lines of the long-lived bivalve mollusc *Arctica islandica*. *Holocene* 16: 967–974

763 Schwartzmann C, Durrieu G, Sow M, Ciret P, Lazareth CE, Massabuau JC (2011) In situ
764 clam growth rate behavior in relation to temperature: a one-year coupled study of high-
765 frequency noninvasive valvometry and sclerochronology. *Limnol Oceanogr* 56: 1940–
766 1951

767 Soletchnik P, Geairon P, Razet D, Goulletquer P (1996) Physiologie de la maturation et de la
768 ponte chez l'huître creuse *Crassostrea gigas*. Rapport Ifremer, pp. 2-34

769 Surge D, Lohmann KC (2008) Evaluating Mg/Ca ratios as a temperature proxy in the
770 estuarine oyster, *Crassostrea virginica*. *J Geophys Res Biogeosci* 113 (G2)

771 Surge D, Lohmann KC, Dettman DL (2001) Controls on isotopic chemistry of the American
772 oyster, *Crassostrea virginica*: implications for growth patterns. *Palaeogeogr*
773 *Palaeoclimatol Palaeoecol* 172: 283–296

774 Thomson DJ (1982) Spectrum estimation and harmonic analysis. *Proc IEEE* 70(9): 1055-
775 1096

776 Tran D, Nadau A, Durrieu G, Ciret P, Parisot JP, Massabuau JC (2011) Field chronobiology
777 of a molluscan bivalve: how the moon and sun cycles interact to drive oyster activity
778 rhythms. *Chronobiol int* 28(4): 307-317

779 Tynan S, Dutton A, Eggins S, Opdyke B (2014) Oxygen isotope records of the Australian flat
780 oyster (*Ostrea angasi*) as a potential temperature archive. *Mar Geol* 357 195–209

781 Tynan S, Opdyke BN, Walczak M, Eggins S, Dutton A (2017) Assessment of Mg/Ca in
782 *Saccostrea glomerata* (the Sydney rock oyster) shell as a potential temperature record.
783 *Palaeogeogr Palaeoclimatol Palaeoecol* 484 : 79–88

784 Ullmann CV, Wiechert U, Korte C (2010) Oxygen isotope fluctuations in a modern North Sea
785 oyster (*Crassostrea gigas*) compared with annual variations in seawater temperature:
786 Implications for palaeoclimate studies. Chem Geol 277(1): 160-166
787

Figure Captions

Figure 1: A: Location of the Arcachon basin where the juvenile oysters were recruited and of the breeding site (red rectangle) in Normandy, northwestern France. B: Aerial photo of the Baie des Veys (BDV) and geographic location of the breeding site. C: Illustration of the disposition of the tables and pockets where oysters were bred. D: Detail of figure 1C.

Figure 2: Variations of the environmental parameters during 2005 and 2006, directly measured above the oyster tables. Temperature and salinity were measured every 15 mn. Chlorophyll *a* and pheopigment concentrations were measured fortnightly. The elevation of the water level above or below the oyster tables is reported for each high and low tide, as well as the daily emersion duration. The dotted line represents the top of the oyster tables.

Figure 3: Observation of the section of an oyster hinge (sample BDV5) under cathodoluminescence (CL). A: Thick section of the whole hinge of the oyster. B: Detail of part of the hinge observed under CL and the associated luminescence profile. Seventy nine increments were counted in 44 days and 77 in the following 43 days. The dotted white line shows the analyzed profile. C: Example of revelation of the chemical markings (high luminescence highlighted by the date of staining). Detail of the CL image illustrating the alternating bright and dull bands. The white arrows show one CL increment as the distance between two successive bright intervals.

Figure 4: Size-at-age and Von Bertalanffy growth model estimated for *Magallana gigas* at the Baie des Veys (Normandy, France) based on 11 oyster hinge analyzes. Growth is fast during the first year of life and then decreases through time. A significant variability is

observed between the 11 shells and could be related to inter-specific variability. Black dots show the date of the 15 chemical markings and the date of death are reported. The hinge length refers to the length measured in the Baie des Veys, after transplantation from the Arcachon Basin.

Figure 5: Variations of the mean growth rate and mean daily temperature calculated for the intervals between successive Mn^{2+} markings in the Baie des Veys during 2005 and 2006. The growth rate of the oysters was determined from measurements on cathodoluminescence images of the hinge between two consecutive chemical markings. The vertical bars correspond to the standard deviation of the growth rate determined from the measurements on 11 individuals.

Figure 6: Determination of the relationships between temperature and growth rate and chlorophyll *a* and growth rate of oysters. The black dot represents the growth peak of February 2005 and is not considered in the correlation. Correlations were tested using Spearman coefficients.

Figure 7: CL increment width changes in the hinge of two *M. gigas* shells for samples BDV3 and BDV5. The mean number of increments formed per day between two successive markings was calculated.

Figure 8: The staining of 02/09/05 appears twice, which corresponds to increment formation in less than 4 hours.

Figure 9: Correlation between increment widths and sea surface temperature for periods when ~2 increments per day are mineralized in 2005 and in 2006 for A: sample BDV 3 and B: sample BDV5. Note that the scale of the high tide level is inverted for a better visualization of the correlation with increment width fluctuations.

Figure 10: Principal component analysis for samples BDV3 and BDV5 during the intervals when ~2 increments are mineralized per day. We tested the relationship between the increment width and mean daily temperature, salinity, immersion duration, high tide level and low tide level.

Figure 11: Correlation between the number of increments and the length of carbonate mineralized in the hinge area between the markings of 02/09/05 and 02/24/05.

Table 1: Number of CL increments counted between consecutive chemical markings and determination of the mean number of increments mineralized per day for each interval.

Figure 1

853
854
855

Figure 2

856
857

Figure 3

861
862
863

Figure 4

864
865

866
867
868
869

Figure 5

870
871
872
873
874
875

875

Figure 6

876
877

877

Figure 7

878
879
880
881
882

882

Figure 8

883
884
885
886
887

887

Figure 9

888
889
890
891

891
892

Figure 10

893
894
895
896
897

897

Figure 11

898
899
900
901
902

902
903
904
905

	02/09/05	02/24/05	05/09/05	07/21/05	09/15/05	10/17/05	11/15/05	03/15/06	05/15/06	06/27/06	08/10/06	09/22/06
	02/24/05	05/09/05	07/21/05	09/15/05	10/17/05	11/15/05	01/31/06	05/15/06	06/27/06	08/10/06	09/22/06	11/07/06
BDV2	-	-	56	51	53	-	-	-	-	-	68	85
BDV3	89	56	53	46	57	52	-	94	77	79	-	-
BDV4	116	54	60	43	64	49	-	-	-	-	-	-
BDV5	68	54	54	52	61	54	123	-	77	79	80	92
BDV6	38	41	43	52	55	47	-	83	70	-	67	70
BDV-FL1	-	-	-	-	-	-	-	-	-	-	81	-
BDV-FL2	-	-	-	-	-	-	-	-	61	82	76	-
BDV-FL3	-	-	-	-	-	-	-	-	75	76	-	-
BDV-FL5	-	-	-	-	-	-	-	-	-	73	-	-
increment mean	77.75	51.25	53.2	48.8	58.0	50.5	123.0	88.5	72.0	77.8	74.4	82.33
standard deviation	32.9	6.8	6.3	4.1	4.5	3.1	-	7.8	6.8	3.4	6.6	11.2
number of days	15	74	73	56	32	29	77	61	43	44	43	46
increments / day	5.2	0.7	0.7	0.9	1.8	1.7	1.6	1.4	1.7	1.8	1.7	1.8
standard deviation	2.2	0.1	0.1	0.1	0.1	0.1	-	0.1	0.2	0.1	0.2	0.2

906
907
908