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Lead accumulation in oyster shells, a potential tool for environmental monitoring

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ABSTRACT

Pb/Ca profiles were measured on ten live collected *Ostrea edulis* from three sites characterized by different levels of lead content. Intra-shell and inter-shell reproducibility were tested comparing several Pb/Ca profiles measured by LA-ICP-MS within a specimen, and within specimens from the same site. Results indicate that signals recorded are reproducible and mean shell Pb/Ca values are site-dependent. Second order variability is explained either by smoothing effects, biological effects or micro-environmental heterogeneities in lead distribution. Mean Pb contents measured in marine bivalve shells are reviewed here. Ranging from 0 to 50 ppm, they show a strong relationship with the environmental level of local lead contamination, and do not appear species-dependent. Our measurements show a linear relationship between mean shell Pb/Ca and surface sediment Pb concentrations, making marine bivalves and particularly *O. edulis* a potential accurate bio-monitoring tool able to monitor bioavailable lead along European coasts since Mesolithic, with an annual resolution.

1. Introduction

With mercury and cadmium, lead (Pb) is one of the most frequent and toxic contaminant in our environment. Lead is naturally introduced in marine environments by forest fires, volcanic activity and erosion and transport processes. It is supplied to water by gas exchanges, precipitations or fall-out of particles (Clark, 2001). Drained by rivers, it is transported dissolved as carbonate, hydroxide or chloride complexes (Libes, 1992). Tending to adsorb on organic matter particles, and being stocked in sediments under this particulate form, lead residence time is estimated to be less than five years in the surface waters (Veron et al., 1987; Wu and Boyle, 1997). However, in sediments and soils, its geochemical half-life is estimated about seven centuries (Semlali et al., 2004). Stocked in the soft surface sediments, lead can easily be remobilized and its remnant toxicity can poison the environment over millenniums.

Lead was extensively used during the Antiquity, in water supply systems or cooking utensils, having severe consequences on the health of the population (Harrison and Laxen, 1981). Smelters' activities locally contributed to lead supply in the sediments. Anthropogenic lead in the sediments of some antique harbors also reveals the impact of ore extraction and utilization for domestic purposes (Delile et al., 2014). High latitude ice-cores and high altitude varved lake deposits also show lead increases for this period, testifying the global environmental impact of lead use during Antiquity (Boutron et al., 2004; Guyard et al.,

2007). Later, the industrial revolution marks the first major global increase in lead fluxes to the ocean (Clark, 2001). A second increase coincides with the 1930–1970 period, and the atmospheric dispersal of residues from leaded gasoline (Boyle et al., 1986; Harrison and Laxen, 1981). Since this period, lead inputs into coastal environments have been decreasing, but local resuspension events are frequent, protracting the adverse environmental effects of these anthropogenic lead inputs (Lazareth et al., 2000; Gillikin et al., 2005). Locally, additional lead supplies can originate from dumping and opening-closing of ore mines in the watershed of coastal rivers (Richardson et al., 2001; Liehr et al., 2005).

Unlike copper or iron, lead is not a biologically essential metal. Its toxicity is conferred by its charge and ionic radius, which allow lead to imitate and substitute some major elements, and cross most biological barriers without being detected (Taylor and Maher, 2012). Though, it accumulates in most of organisms, causing severe neurological, cardiovascular or calcification troubles. Despite this bioaccumulation displays many negative effects on the health of organisms, it is also useful to detect lead environmental contaminations and assess temporal and spatial variations in its bioavailability (Schintu et al., 2010; Søndergaard et al., 2014). In coastal environments the lead content in the organs of shell mollusks like bivalves is extensively used to evaluate and monitor the environmental effects of local anthropogenic lead inputs (Søndergaard et al., 2014; Rainbow, 1995; Wang et al., 1996). A retrospective environmental monitoring is possible measuring lead

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content in the sedimentary deposits. However, such measurements are submitted to important biases (sediment remobilization, chemical diffusion, bioturbation...), and generally present a low time-resolution. A network of observations along the French coasts has been set up since 1974 and provides information about lead concentrations in surface sediments every 6 to 8 years. Such a spatial and time resolution is good enough to provide information about mid-term changes in lead concentrations but is insufficient to detect ancient lead contaminations as well as to trace the evolution of detailed (weekly to monthly) lead concentrations.

Bivalve shells are made of calcite or aragonite and are usually well preserved in the fossil record. Trace elements are incorporated into the successive layers of carbonate and can reflect the physico-chemical environment in which it was secreted (Elliot et al., 2009; Schöne and Gillikin, 2013). In bivalve shells, lead is substituted to calcium in the carbonate lattice (Bourgoin, 1987; Ramos et al., 2004). For a few filter-feeding species (*Mytilus edulis*, *Mya arenaria*, and *Crassostrea gigas*), relationships were observed between shell lead content and either suspended particulate lead concentration (Bourgoin, 1990) or dissolved lead concentration in the seawater (Pitts and Wallace, 1994; Almeida et al., 1998). However, Vander Putten et al. (2000) observed no direct correlation between the lead content of the calcite outer layer of *M. edulis* and either dissolved or particulate lead concentrations in the Netherlands estuaries, highlighting that the different layers of bivalve shells might respond differentially to environmental concentrations. Recently, two studies tracing the lead content along the growth increments of the long-lived bivalve *Arctica islandica* highlighted the possibility of building chronologies and visualizing ancient environmental lead concentrations (and thus anthropogenic lead inputs) over the last millennium through lead shells content (Krause-Nehring et al., 2012; Holland et al., 2014). These studies illustrate how marine bivalve shells can be precious environmental archives, and how identifying target promising species (widely distributed, well-known physiology...) and shell layers (with solid relationships with environmental concentrations) is required, before definitely endorsing bivalve shells as retrospective environmental bio-monitoring tools.

Ostrea edulis is an interesting species to target. This flat oyster is a filter-feeder bivalve that occupies subtidal and lower intertidal zones of rocky and silty shores. It displays a wide pan-European distribution (living from Mediterranean Sea to Norway, at temperatures between 5 °C and 25 °C), a longevity up to 20 years, and adult shells generally exceed 10 cm in diameter and 1 cm in thickness. Richardson et al. (1993) and Milner (2001) investigated the shell growth in live specimens from several locations around the British Isles. They showed that shell growth is mainly affected by temperature, salinity and food supply and slows down in colder months (February – March), forming annual growth lines and clefts at the boundary between the shell and the ligostracum, identifiable under the microscope.

This study aims to evaluate a potential tool for environmental monitoring, comparing variations in lead content recorded in ten flat oyster shells collected in three sites along the French Atlantic coasts. Each site was chosen as it is differentially affected by lead environmental inputs and monitored. One of them is supposed to be mainly impacted by atmospheric lead inputs, while the two others are differentially affected by riverine inputs of lead-rich sediments, from ancient mining activities. In order to evaluate the ability of *O. edulis* to monitor retrospectively the lead content of its environment, shell lead content was measured by LA-ICP-MS in the foliated calcite of the hinges of 3–4 specimens from each site. The mean lead content of shells was first compared between shells within the same site and then between different sites. The reproducibility of Pb/Ca signals recorded within a shell and between the different shells from a same site was initially investigated. Finally, confrontation of mean values measured in the surface sediments and mean values of Pb/Ca recorded in shells of each site allowed to propose and discuss the existence of a close relationship between shell lead content and environmental lead concentrations,

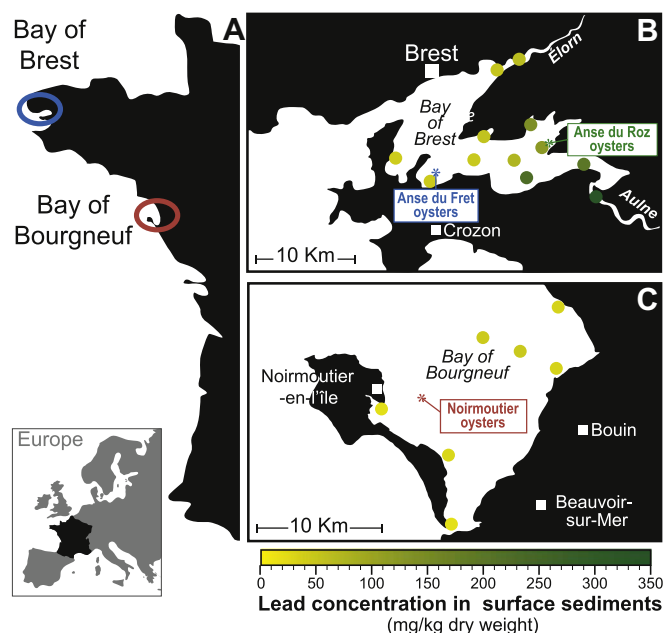


Fig. 1. A) Location of the Bay of Brest and the Bay of Bourgneuf along the French Atlantic coasts. B & C) Location of the sampled specimens and distribution of mean Pb concentration in the surface sediments between 2000 and 2015, according to the Quadrigé database (IFREMER).

making of *O. edulis* a key species for retrospective environmental bio-monitoring.

2. Material and methods

2.1. Sampling locations and environmental data

In January 2015, 20 samples of *Ostrea edulis* were collected alive in the Bay of Brest (Fig. 1), in the Anse du Fret (48°17'30"N; 4°28'39"O) and the Anse du Roz (48°19'27"N; 4°20'4"O), respectively. Specimens were collected by scuba diving, within a radius of 100 m. Oysters were 8–10 cm long and specialists of oyster fishing in the bay estimated their age around 8–10 years old, on the basis of size and growth rings visible on the shells. In November 2015, 5 live specimens were collected around the “La Chaussée” shoal (Fig. 1), near the harbour of Noirmoutier-en-l'île, in the Bay of Bourgneuf (46°59'32"N; 2°11'12"O), within a radius of 200 m. Ranging from 11 to 14 cm in length, those oysters were estimated to be around 10–12 years old by professionals of oyster fishing in the Bay of Bourgneuf. Soft tissues were removed immediately after collection for all oysters.

The Bay of Bourgneuf and the Bay of Brest were specifically chosen because flat oysters show similar growth rates. Water temperature and salinity are monitored sub-monthly by the REPHY network (Réseau de surveillance du Phytoplancton et des Phycotoxines) of the French Research Institute for Exploitation of the Sea (IFREMER). In the two areas, mean ranges of annual water temperatures are similar (13.5 °C ± 7 °C). In the Bay of Brest, salinity generally fluctuates between 35 and 33‰ (PSU). Low salinity values are more frequent in the Bay of Bourgneuf but rarely fall below 28‰ (PSU). In the two bays, metal concentrations in surface sediments (Fig. 1) are also monitored in several sites by IFREMER (Quadrigé database, Réseau d'Observation de la Contamination Chimique). Measurements were done in 2008 and 2014 in the Bay of Bourgneuf and in 2001, 2009 and 2015 in the Bay of Brest.

The Bay of Bourgneuf is largely opened to the Atlantic Ocean and partly protected by the peninsula of Noirmoutier. No major river drains into the bay but it is surrounded by large marshes, drained by dozens of creeks. The main known sources of Pb are atmospheric inputs and local draining (Amiard-Triquet, 1987). Pb concentrations measured in the

surface sediments by IFREMER, fluctuate between 48 and 15 ppm dry weight (d. w.) (Fig. 1). Measurements performed in 2008 in the vicinity of the sampling site indicated a value of 25 ppm d.w. of Pb in the surface sediments. In 2014, measurements indicated 16 ppm d.w., pointing to a mean value of 20.5 ppm d.w. over those last 8 years, and highlighting a slow decrease in the lead content of the surface sediments, also observed at the scale of the entire bay.

The Bay of Brest is a 180 km² shallow bay linked to the Atlantic Ocean by a 1.8 km wide strait, the Goulet of Brest. Three main rivers drain into the bay, the Aulne, the Elorn and the Penfeld. However, 2/3 of the bay water is renewed each tide, resulting in a rather homogeneous and normal marine salinity and temperature of the water body. Measurements performed over those last 15 years by IFREMER in the vicinity of the Roz and Fret sites revealed mean concentrations of respectively 115 ppm d.w. and 45 ppm d.w. in the surface sediments of those sites. Like in most locations across the bay, the successive measurements highlighted a slow decrease of Pb concentrations (≤ 2 ppm/yr) between 2001 and 2015. The distribution of Pb concentrations in the surface sediment of the bay shows a clear westward decrease in the mean Pb concentration, from up to 350 ppm in the Aulne estuary to less than 30 ppm near the Goulet (Fig. 1). This geographical distribution of the Pb is imputed to the draining of ancient silver mines in the vicinity of Huelgoat by small tributaries of the Aulne (Lemière et al., 2002). With diverse degrees of activity, those mines have probably been operational from the Bronze Age to 1934 (Monange, 1972). Analysis performed in the Aulne river (Lemière et al., 2002) showed that Pb is poorly transported under its dissolved form in the water ($< 2 \mu\text{g/L}$ – detection limit – of Pb dissolved in the surface waters in the Aulne estuary), and essentially remains in the sediment fraction ($< 80 \mu\text{m}$). According to French legislation, Pb concentrations in surface sediments up to 100 ppm d.w. (Level N1) are considered negligible. Over 200 ppm (N2), dredging or immersion operations are susceptible of being forbidden and a detailed study about the ecotoxicity of the sediment is required (Arrêté du 9 août 2006, see <https://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000000423497>). Thus, lead concentrations in the surface sediments of all sites investigated here were legally considered as negligible in 2015.

2.2. Shell selection and preparation

All collected shells were numbered in the field by the first letters of the collection site (NOI, FRET and ROZ respectively), the sample number, and 'l' or 'r' for left or right valve. In the lab, longitudinal sections were performed from the umbo to the ventral margin across the two valves of each specimen, using a low-speed precision saw equipped with a diamond-coated blade (125 mm in diameter). Sections showing the more continuous, numerous and parallel growth increments, as well as the less annelid burrows and conchiolin lines in the hinge area were selected for analysis, independently of the valve (Fig. 2). Finally three sections were selected for the Roz site (Roz1l, Roz7l and Roz9l), four sections for the Fret site (Fret5r, Fret7r, Fret7l and Fret9l) and five for the Noirmoutier site (Noi1r, Noi2r, Noi2l, Noi4r and Noi5r). Sections of the two valves of specimens Fret7 and Noi2 were specifically selected in order to test the reproducibility of signals recorded with the LA-ICP-MS within a shell. Then, slabs of approximately 1.5 mm were performed in the hinge areas, and mounted on glass slides with an Araldite® AY103* resin coupled to a HY956 hardener (*Huntsman), with a refractive index of RI = 1.538. Slabs were grounded to 1 mm thick with grinding disks from 220 to 1200 grit. 6 to 1 μm Al₂O₃ powders (AP-A suspensions STRUERS) were finally used on a polishing cloth (PAL F ESCIL) to obtain the final polishing. All samples were ultrasonically rinsed in milli-Q water before any sampling or analysis.

2.3. Trace elements analysis by LA-ICP-MS

Measurements were carried out with a quadrupole ICP-MS (inductively coupled plasma mass spectrometer) Varian 820-MS, coupled to a 193 nm eximer laser ablation system (Analyte G2, Photon Machine), at the Laboratory of Planetology and Geodynamics (LPG in Nantes University, France). The line-scan method was used to perform continuous tracks over the samples and standards. The isotopes ⁷Li, ²⁵Mg, ⁴³Ca, ⁵⁵Mn, ⁸⁸Sr, ¹³⁷Ba and ²⁰⁸Pb were monitored simultaneously.

This paper mainly focuses on shell ²⁰⁸Pb and ⁴³Ca measurements. Two profiles of ⁵⁵Mn/⁴³Ca signals are also presented as a proxy for organic matter presence and supplementary evidence to strengthen proposed adjustments between profiles. Laser ablation tracks were performed across the oyster hinges, perpendicularly to the growth increments, from the most recently deposited calcite layers, toward the most juvenile parts of the shell (Fig. 2). The sampler was controlled by a software program (Chromium2). A colour CCD camera enabled to precisely visualize laser tracks on samples. Material was ablated in He. The ablated material from samples/standards was then transported through a Teflon coated tube in He with a flow rate of 1 L·min⁻¹ into the Ar plasma, for ionization and subsequent analysis in the mass spectrometer. Nickel-coated cones were used in the ICP-MS. Several tests were performed in order to optimize the measurements in biological calcite by finding the most appropriate repetition rate (10 Hz), laser beam energy (4.55 J/cm²), spot size (65 μm) and speed (10 $\mu\text{m/s}$). More details about operating conditions are given in supplementary material. Data were collected in time-resolved mode with one data point being recorded every 0.37 s, giving a spatial resolution of 3.7 μm /data point. The washout time of the ablation cell was approximately 3 s. General reproducibility of signals was controlled by measuring systematically at least two times along the same track or measuring two different tracks closely spaced ($\sim 100 \mu\text{m}$). The overall compositional homogeneity within hinges was also tested by performing successive tracks spaced from 1 to 4 mm in the hinge of the specimen Fret9l and across the left and right hinges of the specimens Fret7 and Noi2. All element data were background subtracted, and normalized to ⁴³Ca to correct for variations in ablation efficiency. As far as compared material is always *Ostrea edulis* shells in this study, and in order to avoid the approximation of a fixed Ca concentration in the shell, ratios in $\mu\text{mol/mol}$ were preferred to ppm concentrations. Nevertheless, when necessary and in order to facilitate comparison with other studies, Element/Ca ratios were also converted into ppm assuming a constant Ca concentration of 37 wt% in the shells (Sinclair et al., 2011). Such concentration is consistent with measurements performed in *Ostrea edulis* (Rodríguez and Lopez, 1985) and *Crassostrea gigas* (Almeida et al., 1998) exposed to low levels of contaminants. In the shells of *C. gigas* exposed to negligible to very high Pb water concentrations (790 $\mu\text{g Pb L}^{-1}$), Almeida et al. (1998) measured low variations in Ca content ($< 4\%$), and observed no shell shape anomaly. These results suggest that calcification rate in oysters is only marginally affected by the water Pb content. In *O. edulis* exposed to Zn and tributyltin (TBT), two components known for their influence on calcification processes and shell alteration of many mollusc species, Rodríguez and Lopez (1985) observed shell shape modifications but no Ca content variations, highlighting the high stability of Ca content in these oysters. Furthermore, Ca is at least 3000 times more abundant than Pb in shells. For these reasons, we consider that the assumption of a constant shell Ca content, and a low impact of potential minor Ca variations on element/Ca ratios, is appropriate here, and is independent of the Pb contamination level of the sites sampled in this study.

The synthetic glass NIST 612 was analysed continuously over a 30 s lasting track, before and after each track on an oyster sample. During the last three runs (Sept. 15, Dec. 15, Apr. 16), NIST 610 was also analysed this way. For those runs, trace element data were calibrated and drift corrected against both standards. No significant difference was

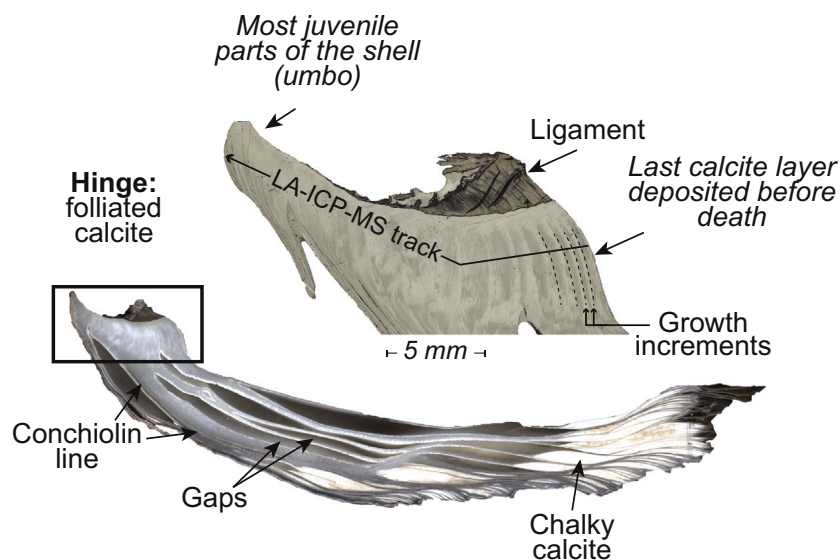


Fig. 2. Longitudinal section across the right valve of a specimen from the Bay of Brest (Noi2r), and the corresponding thick section in the hinge area, observed with a Keyence microscope.

noticed between both treatments for the considered elements. Thus, for homogeneity reasons we decided to keep calibrating and drift-correcting our shells against NIST 612 only, and to run and treat NIST 610 as samples for reproducibility/drift checks. The comparison of the mean Element/Ca ratios measured in standards for each run shows that a small instrumental drift can potentially occur within a run. For Mn and Pb, standards (NIST 612) are generally reproducible within 2–3%, but values up to 10% are occasionally observed for Pb. This variability is always related to a slow standard drift observed during the concerned run, which is taken in account in sample signal treatment (detrended). Thus, between the different runs, the NIST 610 treated as samples over 8 months are reproducible within 2.5% for both Mn and Pb showing a good day-to-day reproducibility, and the efficiency of the “standard drift correction” phase during sample data-processing. Finally, discrete Element/Ca peaks largely exceeding 3 standard deviations (SD) were considered as outliers and manually removed.

Detection limits (DL) were calculated as $DL = 3SD_{BG}$, where SD_{BG} is the standard deviation of the background. For both Pb and Mn, mean LA-ICP-MS signals measured in oysters, and background subtracted, are clearly above the DL, with a signal/background ratio of 157 and 13 respectively and a signal/DL of 20 and 30 respectively. For Pb, the DL of 107 cps/s calibrated against standards corresponds to 0.03 ppm.

2.4. Data cleaning, removing conchiolin layers and epoxy

Ostrea edulis hinges are often punctuated by layers of conchiolin (organic matter). Gaps (chambers) and burrows also occasionally disturb the calcite layers and are generally filled with epoxy during sample preparation (Fig. 2 and Fig. 3). We tried to avoid all these structures when measuring with the LA-ICP-MS but this was not always possible. A cleaning process of data was thus applied manually for each track, comparing photographs of the hinge, with ^{43}Ca counts recorded and Element/Ca relative concentrations along the track. Fig. 3 shows an example of scan crossing both a gap filled with epoxy and three conchiolin lines. The total absence of ^{43}Ca in epoxy or the strong reduction in ^{43}Ca in the conchiolin layers causes breaks in the ^{43}Ca profile, leading to abnormal Element/Ca values, which contrast with the shell signal. Both epoxy and conchiolin layers can easily be identified and unambiguously removed manually. The frequent occurrence of conchiolin lines in *O. edulis* hinges, and measurements of ^{43}Ca in these layers, underlines the necessity of being extremely careful in samplings for bulk analysis and in data cleaning for LA-ICP-MS analysis.

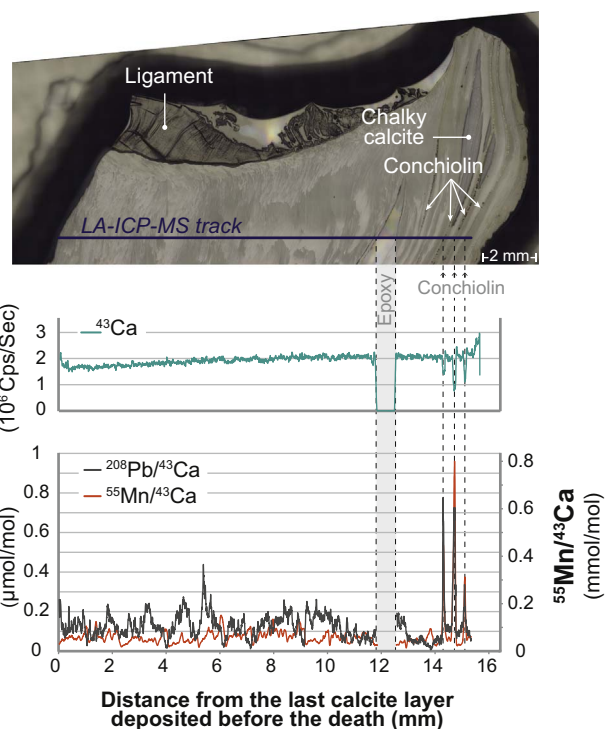


Fig. 3. Example of a LA-ICP-MS scan performed across the hinge of a specimen of the Bay of Bourgneuf (Noi1r), and associated records in ^{43}Ca , $^{55}\text{Mn}/^{43}\text{Ca}$ and $^{208}\text{Pb}/^{43}\text{Ca}$.

3. Results

3.1. Pb/Ca profiles

Fig. 4a shows the plot of one Pb/Ca profile obtained in each investigated specimen. Profiles show large variations in Pb/Ca content across the hinges, but no ontogenetic trend is observed in any profile. Mean Pb/Ca values and variability recorded are similar in specimens from the same site (Fig. 4b). On the contrary, they are significantly different between sites, forming three well identified clusters of measurements (Fig. 4b). Specimens of the Bay of Bourgneuf recorded relative Pb/Ca concentrations between 0 and 0.57 $\mu\text{mol/mol}$. The four specimens investigated respectively recorded mean values ranging from 0.08 $\mu\text{mol/mol}$ to 0.11 $\mu\text{mol/mol}$, which represent a range from 0.15 to

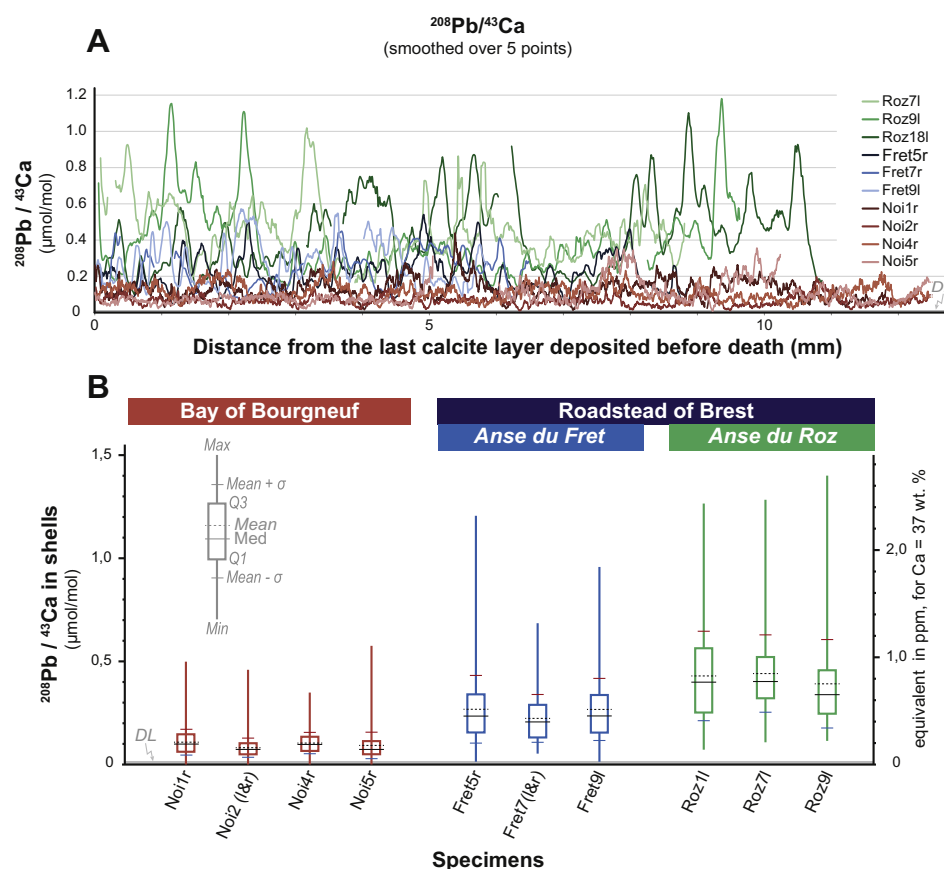


Fig. 4. A) Pb/Ca profiles recorded over the first 12 mm in the ten hinges investigated. B) Box plots summarizing information for each specimen, and highlighting the reproducibility of mean values and variation ranges observed for each site.

0.21 ppm of Pb. Oysters from the “Anse du Fret” site, in the Bay of Brest, recorded Pb/Ca values ranging from 0.01 $\mu\text{mol}/\text{mol}$ to 1.2 $\mu\text{mol}/\text{mol}$, but this last value is unusual and values generally fluctuate between 0.05 $\mu\text{mol}/\text{mol}$ and 0.7 $\mu\text{mol}/\text{mol}$, with mean values respectively of 0.22 $\mu\text{mol}/\text{mol}$, and 0.27 $\mu\text{mol}/\text{mol}$ (two specimens) for the three specimens investigated (~ 0.5 ppm of Pb). Oysters from the Anse du Roz recorded relative concentrations between 0.07 $\mu\text{mol}/\text{mol}$ and 1.4 $\mu\text{mol}/\text{mol}$. In the three specimens investigated, mean values reach 0.43 $\mu\text{mol}/\text{mol}$, 0.44 $\mu\text{mol}/\text{mol}$ and 0.39 $\mu\text{mol}/\text{mol}$ respectively, which corresponds to mean Pb values around 0.8 ppm (see Fig. 4).

3.2. Intra-shell reproducibility

Intra-shell reproducibility in the foliated calcite was tested by performing several scans, across the hinge area of a specimen in the Bay of Brest (Fret9l, Fig. 5a–b) and across the hinges of the left and right valves of a specimen in the Bay of Bourgneuf (Noi2l & r, Fig. 5c–d) and the Bay of Brest (Fret7l & r). In order to free from local growth rate differences, the AnalySeries 2.0.8 software was used to readjust the different scans (smoothed over five points) together. The longest track was taken as the reference one. The software calculated the linear correlation coefficients (r). Each adjustment was performed according with observations on photographs, and controlling the passive adjustment of other measured elements (Li/Ca, Mg/Ca, Mn/Ca, Sr/Ca, and Ba/Ca). In Fig. 5d the two profiles of Mn/Ca from the right and the left valves of the same shell are so reproducible ($r = 0.82$) that they are sometime difficult to observe once superimposed. We consider that this observation strongly justifies the matching exercise that was conducted, and strengthen the proposed correlations of the Pb/Ca profiles. In the specimen of the Bay of Brest, values generally oscillate between 0.05 and 0.7 $\mu\text{mol}/\text{mol}$, with two remarkable maxima around 1 mm and 2.3 mm along the reference scan (scan B in Fig. 5b). The general trend is similar for all scans, with linear correlation coefficients between 0.65

and 0.75. Minor and major peaks are observed simultaneously in all profiles. Except for scan A, in the vicinity of the two major peaks, each profile is always included within the 2σ envelope of the other scans. On major peaks, differences up to 0.3 $\mu\text{mol}/\text{mol}$ are observed in the maxima recorded in each profile. In a good agreement with mean data, values recorded are significantly lower in the specimen of the Bay of Bourgneuf (Fig. 5d). They rarely reach 0.2 $\mu\text{mol}/\text{mol}$ and frequently decrease to 0.02 $\mu\text{mol}/\text{mol}$. Like in the previous specimen, the two tracks fit very well ($r = 0.68$). Smoothed tracks are always included in the 2σ envelope of the other track. However, local differences up to 0.1 $\mu\text{mol}/\text{mol}$ are observed between the two Pb/Ca profiles.

3.3. Inter-shells reproducibility

Inter-shell reproducibility was tested at each locality. One track derived from each specimen was adjusted on a reference track, chosen to be long, continuous, and perpendicular to the growth lines across the whole hinge of the reference specimen. Fig. 6a shows the plot of the adjusted tracks of the three specimens sampled in the Anse du Fret. Similar trends are observed in the three specimens, and linear correlation coefficients fluctuate between 0.55 and 0.68, and most of the time, profiles are even included within the 1σ envelope of other scans. Differences up to 0.3 $\mu\text{mol}/\text{mol}$ are observed locally between profiles. Similar observations were performed in the other site of the Bay of Brest.

Specimens of the Bay of Bourgneuf also recorded simultaneously long-term and short-term variations in Pb/Ca (Fig. 6b). However, adjustments are characterized by significant but lower linear correlation coefficients, between 0.4 and 0.45. Differences rarely exceed 0.1 $\mu\text{mol}/\text{mol}$ between scans. Although long term variations are identified in each specimen, the specimen Noi5 recorded significantly lower values than the specimen Noi1 (reference) from 0 to 7 mm. To a lesser extent, this phenomenon is also observed in the specimen Noi2.

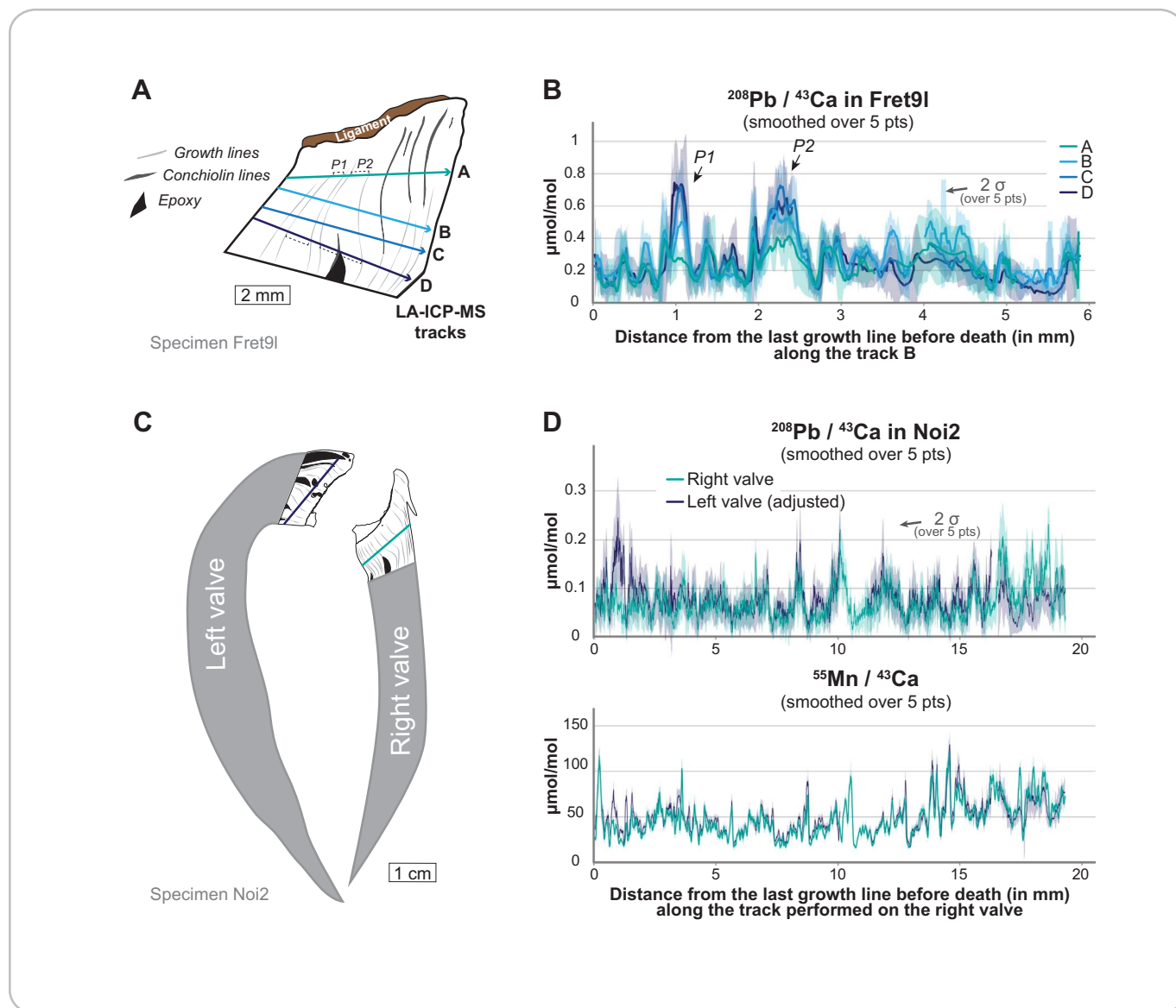


Fig. 5. Intra-shell reproducibility tests. A) Position of the four LA-ICP-MS tracks (from A to D) performed across the hinge of Fret91. B) Plot of the four Pb/Ca profiles recorded, smoothed over five points, and adjusted on the profile B with the software Anlyseries. The respective linear correlation coefficients are $r = 0.74$ for A, $r = 0.82$ for C and $r = 0.65$ for D. Translucent envelopes represent two times the standard deviation calculated over five points along each unsmoothed profile. In A, P1 and P2 indicate the position of the peaks P1 and P2 shown in B. Dashed lines highlight the same interval along tracks A and D. Along D, these intervals are twice longer than in A because of growth heterogeneities within the hinge. C) Pb/Ca and Mn/Ca profiles recorded across the left and right hinges of the specimen Noi2 from the Bay of Bourgneuf. D) Left valve profiles were adjusted on right valve profiles. For Pb/Ca profiles, the linear correlation coefficient is $r = 0.68$. For Mn/Ca, the linear correlation coefficient is $r = 0.82$ over the entire profiles.

4. Discussion

4.1. Testing reproducibility of Pb/Ca profiles

We first tested within-a-shell reproducibility (as done in Elliot et al., 2009) and the variability among individuals that grew within a same site, under the same environmental conditions (as done in Gillikin et al., 2005). These initial tests are necessary to evaluate and quantify shell Pb/Ca heterogeneity and inter-specimen variations.

We first compared parallel tracks within a shell (Fig. 5). Profiles have been 5 point smoothed, and 2σ envelopes are indicated. The data largely overlap and linear correlation coefficients systematically exceed $r = 0.65$. Our results illustrate that Pb/Ca signals are reproducible between different tracks performed across the same shell. Thus, signal measured along any track perpendicular to growth increments, in the foliated calcite of an *Ostrea edulis* can be considered as representative of the signal recorded by the animal. Occasional differences in Pb/Ca

between tracks never exceed $0.3 \mu\text{mol/mol}$ and $0.1 \mu\text{mol/mol}$ in specimens from the Bay of Brest and the Bay of Bourgneuf respectively (Fig. 5). We consider that some of the offsets observed are due to the fact that successive growth lines are not clearly parallel. As a result, the spot-size and the 5 point smooth we applied to all data sets will locally integrate different periods of time. For example in Fig. 5b, maxima recorded at 1 mm and 2.2 mm (identified as P1 and P2 on Fig. 5b), are lower in scan A than in scan B and scans C-D. On Fig. 5a, the corresponding intervals (dashed lines), are at least 50% longer along tracks B to D than along track A, and thus described by a significantly higher number of measurements. Combining to the “mean effect” of the laser spot, the 5 points smooth applied to the data can reduce the amplitude of peak values. In order to describe more precisely the concentrations recorded by the animal and to avoid “smoothing effects”, multiple scans crossing the maximum of large growth increments and combining these scans are recommended.

We then compared three specimens collected on the same day from

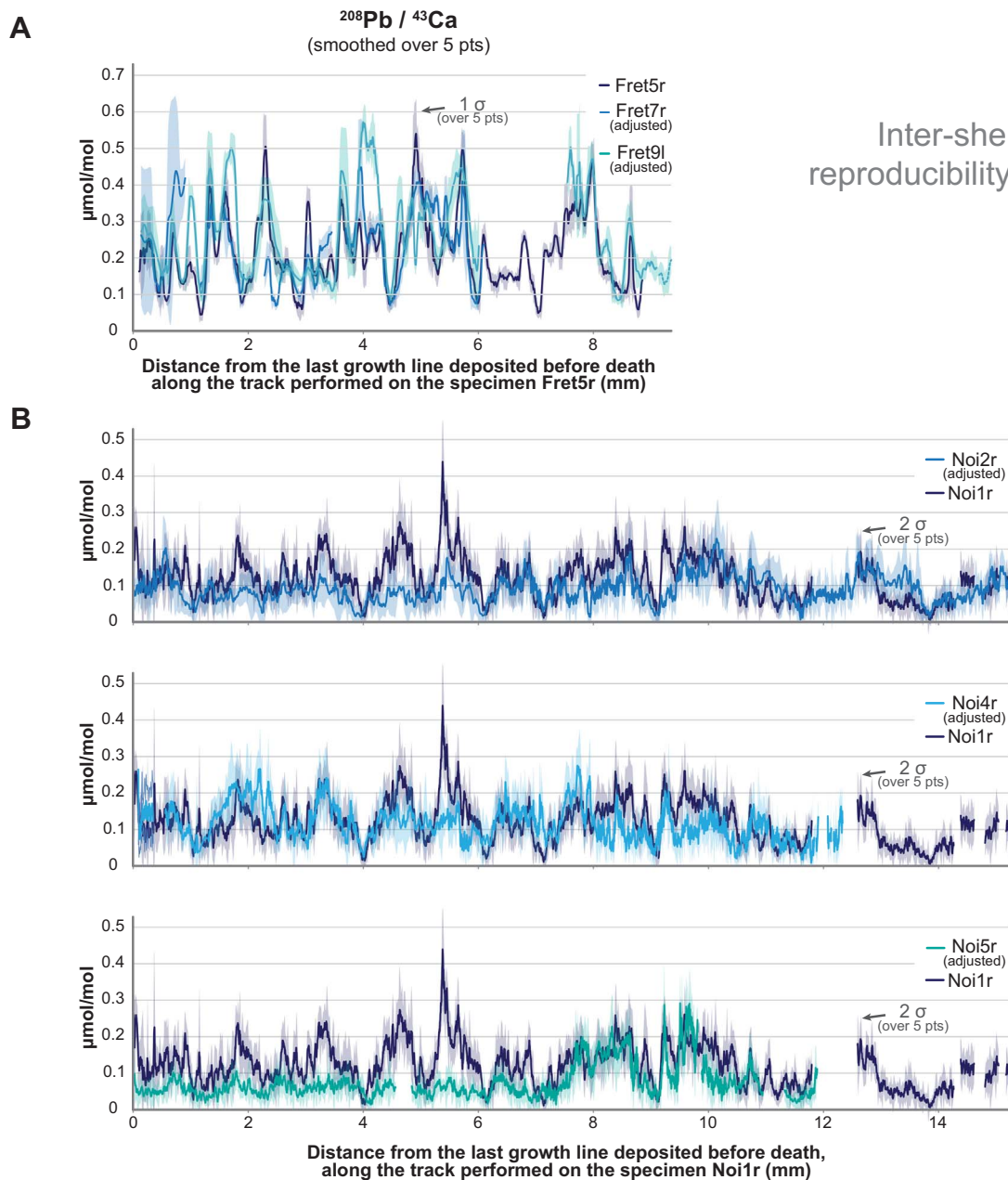
Inter-shell
reproducibility tests

Fig. 6. A) Pb/Ca profiles recorded across the hinges of three specimens of the Anse du Fret site, in the Bay of Brest. Profiles are adjusted on the profile Fret5r. Linear correlation coefficients are respectively $r = 0.55$ for Fret7r and $r = 0.68$ for Fret9l. B) Pb/Ca profiles recorded across the hinges of the four specimens of the Bay of Bourgneuf. Scans shown here are incomplete for Noi1r and Noi2r (arbitrarily cut at 15 mm). All scans are adjusted on the profile Noi1r. Linear correlation coefficients: $r = 0.45$ for Noi2r, $r = 0.40$ for Noi4r, $r = 0.41$ for Noi5r.

each site (Fig. 6). The adjustment of Pb/Ca signals between specimens of a site shows that the reproducibility of signals is comparable to the intra-shell reproducibility (Fig. 6): linear correlation coefficients are above 0.55 in the Bay of Brest and above 0.40 in the Bay of Bourgneuf, and differences between adjusted peaks never exceed $0.3 \mu\text{mol/mol}$ in the two bays. This comparison shows that tracks recorded in one specimen can be considered as representative of tracks recorded by each specimen of the site. This is particularly true in the Bay of Brest (Fig. 6a). A detailed comparison of the Bay of Bourgneuf specimens (Fig. 6b) shows more significant differences as illustrated by the lower linear coefficients (0.40–0.45). In Fig. 6b, Noi1 and Noi5 record similar values from 12 mm to 7 mm (early stages of growth). From 7 mm to 0 mm, Noi 1 records higher values than Noi5. However, it's only

between 4 and 6 mm that differences between Pb/Ca recorded in the two bivalve profiles fall outside the range of intra-shell differences ($0.1 \mu\text{mol/mol}$ in the Bay of Bourgneuf). Patchiness in environmental Pb distribution has been shown to potentially influence inter-shell reproducibility (Gillikin et al., 2005). The collection site in the Bay of Bourgneuf is submitted to a high hydrodynamics (waves and high tidal currents) and shells were collected within a couple of hundred meters. The extreme similarity of signals recorded by all specimens between 15 and 7 mm suggest that they were all very close to each other in their early stages. Noi5 were probably separated from Noi1 at 7 mm and started recording a different signal, with the same trends but significantly lower Pb/Ca values. If this scenario is correct, local patchiness of environmental Pb distribution within hundreds of meters seems

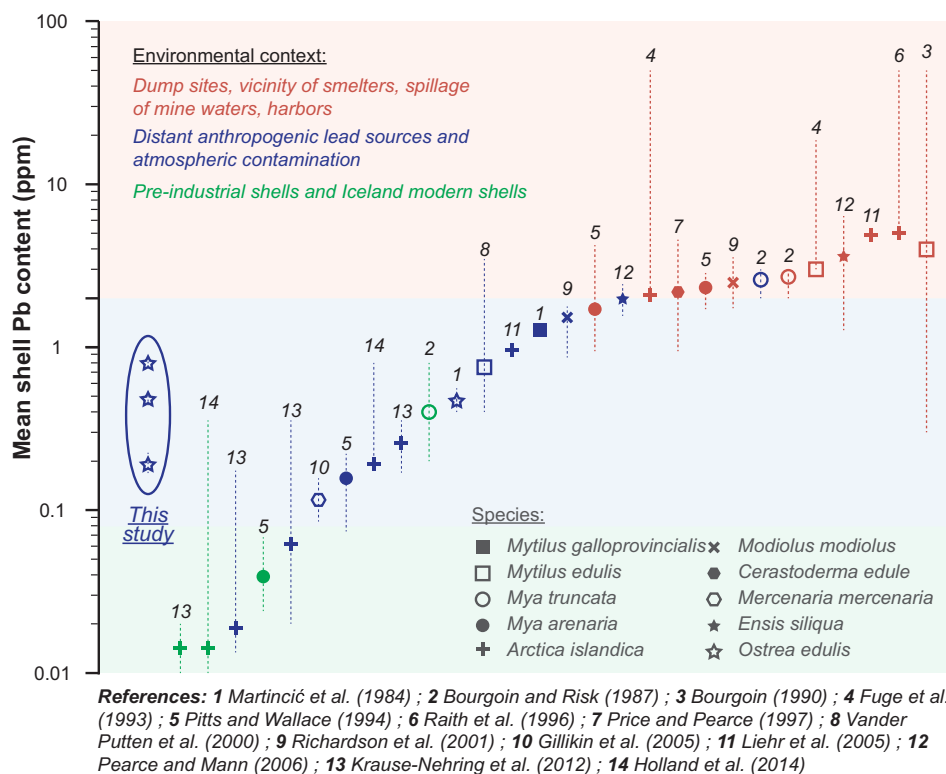


Fig. 7. Synthesis of mean shell Pb contents measured by authors in marine bivalves. Measurements initially given in Pb/Ca ratios (mmol/mol) were converted into Pb concentrations (ppm) assuming a Ca content of 37 wt% in shells. Symbols indicate the general mean value obtained within a site or during the study. Dashed lines indicate the range of values obtained within the different shells considered. In studies 4 and 6, highest values were obtained in the outer layer of *M. edulis* and the inner layer of *A. islandica*. Lower values were measured in the other layers of the same specimens. In study 3, numerous sites were tested, in the vicinity of a Pb-smelter and ore stockpiles. Lower values were measured in shells grown 1.5–5.7 km away from the stockpiles. Higher values were measured in shells grown nearby the Pb-smelter outfall.

able to influence Pb/Ca records in shells within the range of 0.3 $\mu\text{mol/mol}$ (maximum difference of values between Noi1 and Noi5, recorded at 5.5 mm). Alternatively, biological factors cannot be excluded. This inter-shell comparison illustrates the need to analyse several specimens within one locality to capture high-resolution (weekly to monthly) Pb/Ca variations.

The intra-shell variability of *O. edulis* Pb/Ca shows that profiles from within a same shell can be reproduced in detail, with correlation coefficients above 0.65 and adjusted peaks within the 2σ envelope (over 5 points) of other tracks. Local differences never exceed 50% of the local mean signal. Smoothing the Pb/Ca profiles seems to have an effect on the reproducibility test. The intra-site test shows that the major trends are reproducible in samples collected within the same site. Again smoothing the Pb/Ca profiles between specimens can affect the reproducibility tests and patchiness in Pb distribution within sediments of a given site may also be a source of variability between shell Pb/Ca profiles.

4.2. Temporal variability of Pb in *O. edulis* shells

At the scale of the animal life-span, Pb/Ca scans reveal a heterogeneous distribution of Pb content across shells, but no ontogenetic trend. This suggests that growth rate is not directly associated with Pb incorporation in the shells (Vander Putten et al., 2000; Strasser et al., 2008). In more details, each profile of Pb/Ca reveals first order cycles approximately every $2 \text{ mm} \pm 1 \text{ mm}$. Shells collected in the Bay of Brest show between 8 and 10 Pb/Ca cycles, while shells from the Bay of Bourgneuf record 10–12 cycles (Fig. 6). The number of cycles observed in each site is thus close to the estimated ages, suggesting these Pb/Ca cycles could be annual, and reflect an intra-annual variability in Pb inputs in the shells. Several studies observed similar cyclic variability in shell Pb concentration (Price and Pearce, 1997; Vander Putten et al., 2000; Richardson et al., 2001; Gillikin et al., 2005). Biological effects such as reproduction cycles (Jørgensen, 1976; Amiard-Triquet, 1987; Lares and Orians, 1997), environmental factors such as variable Pb runoff (Pitts and Wallace, 1994) or sediment resuspension (Price and

Pearce, 1997), and crystallography and organic matrix content (Vander Putten et al., 2000), were invoked to explain such cyclic signals. Furthermore, in hazardous chemicals contaminated areas, morphological and chemical composition changes in shells were observed in many molluscs (Alzieu, 2000; Márquez et al., 2011; Begliomini et al., 2017). Important Ca content anomalies seem particularly frequent, and could also be involved in the Pb/Ca fluctuations observed here. In tributyltin (TBT) and zinc contaminated environments, oysters of different species showed abnormally concave shells, related to an increased development of gaps between shell layers, (Rodríguez and Lopez, 1985; Alzieu et al., 1986). In *O. edulis*, Rodríguez and Lopez (1985) showed that gaps were associated with the development of a thin calcite layer with disorganized crystals. This layer showed the same Ca content than other layers but a rather different chemical composition (higher Cl, Na, Fe). Not involved as a biologically important metal in calcification processes (enzymes), Pb is not mentioned as a factor favouring gap creation, or as an abnormally abundant element in layers surrounding gaps.

In this study, recorded ^{43}Ca signals (see Fig. 3 for an example) do not show any cycle, suggesting fluctuations in the organic matter content between crystals are not responsible of Pb/Ca decreases and falls. Furthermore, neither Ca, nor Pb drastic fluctuations were observed in layers surrounding gaps (Fig. 5). Based on these observations we hypothesize that the cyclic changes observed in shell Pb/Ca are seasonal and reflect seasonal changes in environmental Pb content, in flesh Pb content, or in the way oysters incorporate Pb in their shell. The precise timing of the Pb/Ca peaks and the discrimination of those hypotheses would require more detailed sclerochronological studies and an accurate confrontation with metabolic cycles of *O. edulis*.

4.3. Mean shells Pb content

The published mean Pb concentrations measured in shells of marine bivalve are sorted in increasing order (Fig. 7), and distinguished as a function of the different environmental contexts and species. General trends observed in Fig. 7 show that shell Pb content range from around 0.01 ppm in sub-fossil shells of *Arctica islandica* from Northeast Iceland

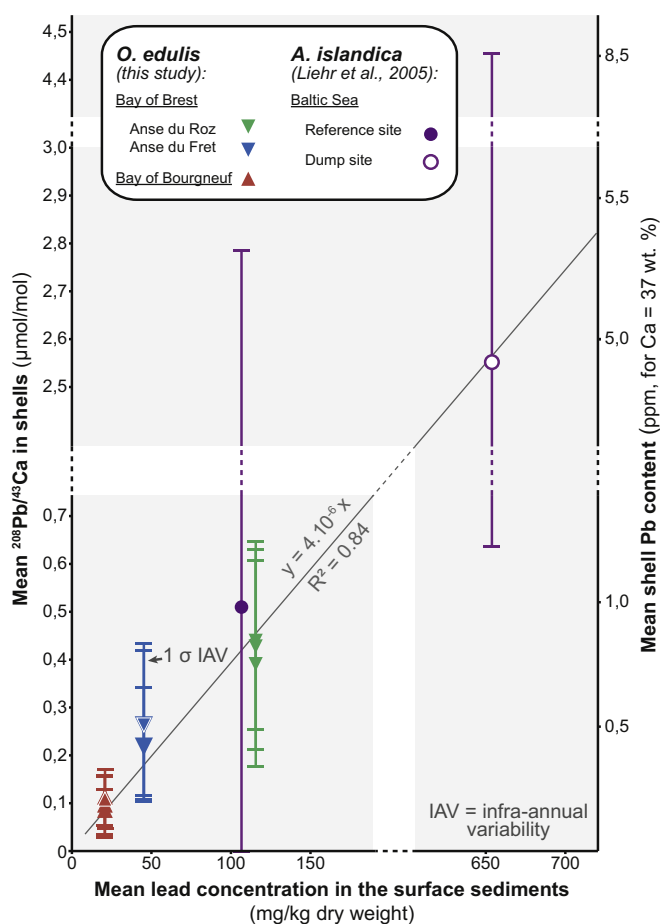


Fig. 8. Relationship between Pb concentrations in the surface sediments (mg/kg d.w.) and within the foliated calcite of *Ostrea edulis* hinges (this study) and the aragonite of the cardinal teeth of *Arctica islandica* (Liehr et al., 2005).

(Holland et al., 2014) to 50 ppm in many specimens of *Mytilus edulis* and *A. islandica* collected in the vicinity of a smelter or submitted to the spillage of mine waters (Bourgoin, 1990; Fuge et al., 1993; Raith et al., 1996). Data shown in this figure suggest that lower values (below 0.08 ppm) are representative of sub-fossil shells and shells from poorly contaminated sites (relatively isolated from atmospheric contamination). Values ranging from 0.08 ppm to 2 ppm appear associated to moderately contaminated environments, mainly influenced by distant anthropogenic sources and atmospheric inputs. Values ranging from 2 to 5 ppm were essentially measured in shells grown in environments submitted to the spillage of mine waters or in the vicinity of dump sites (Bourgoin and Risk, 1987; Fuge et al., 1993; Raith et al., 1996; Price and Pearce, 1997; Richardson et al., 2001; Liehr et al., 2005; Pearce and Mann, 2006). Finally, mean values higher than 5 ppm were only measured in shells from highly contaminated sites in the immediate vicinity of a Pb smelter effluent (Bourgoin, 1990) or in specimens submitted to artificially highly contaminated waters (Almeida et al., 1998). These general trends appear independent of the considered species. However, in some species, Pb values can sometimes be significantly higher in different areas of the shell, illustrating potential shell heterogeneities and the necessity to avoid bulk sampling. For example, in specimens submitted to the spillage of mine waters, values up to 50 ppm were observed by Fuge et al. (1993) and Raith et al. (1996) in the inner layer of *A. islandica* and the outer prismatic layer of *M. edulis*, while other layers in the same specimens recorded values from 2 to 5 ppm. A similar Pb partitioning was also observed in *Crassostrea gigas*, areas formed by prismatic calcite being enriched in Pb by comparison with areas in foliated calcite (Almeida et al., 1998). In our study, *O. edulis* specimens

have mean Pb/Ca values between 0.08 μmol/mol (~0.15 ppm) and 44 μmol/mol (~0.84 ppm) (Fig. 4 and Fig. 7). Such values generally coincide with the range of mean values measured in shells of marine bivalves from sites influenced by distant Pb sources or atmospheric inputs. Thus, concentrations measured here are in a good agreement with the environmental context of the two bays and the typical values usually measured in such contexts. Overall, Fig. 7 shows that Pb concentrations measured in shells of marine bivalves seem closely related to the degree of Pb available in the environment. Although methodological bias exist, this relationship is strengthened by the fact that it seems both independent of the considered species and of the method used to quantify the mean Pb values in the shells.

4.4. Site effect: relationship with surface sediment concentrations

Oysters are filter-feeding bivalves and Pb concentration in their flesh is thought to depend on both Pb dissolved in the water and their ingestion of particulate Pb-species (insoluble PbSO₄ or PbS) in suspension in the water (Fuge et al., 1993; Søndergaard et al., 2014). In good agreement with those assumptions, Pitts and Wallace (1994) established a linear relationship between Pb content in *Mya arenaria*, shells and dissolved Pb concentration in the seawater. Bourgoin (1990) also observed a linear correlation between, tissue-Pb concentrations and suspended particulate matter (SPM) Pb concentrations in the surrounding water, in *Mytilus edulis* grown near a smelter. Most of all, Bourgoin (1990) highlighted a linear relationship between those two parameters and the nacre-Pb levels in shells, and revealed that the relationship between SPM-Pb level and nacre-Pb level has a particularly low variability and provides a good spatial resolution.

Pb concentrations in the water are difficult to obtain, especially because of very low concentrations and potential interference of the salt matrix (Søndergaard et al., 2014). In our sites, no such data were available, but studies performed in the fresh waters of the Aulne river suggest that dissolved Pb input in the Bay of Brest is low (2 μg/L), stable and essentially reflects the geological background. Remnant industrial Pb is essentially transported downstream in the bay, incorporated in the < 80 μm fraction of the sediments (Lemière et al., 2002).

In our dataset, shell Pb/Ca values from the Bay of Brest samples are significantly higher than in the Bay of Bourgneuf (Fig. 4). We hypothesize that this most probably reflects the historical anthropogenic Pb inputs into the bay of Brest, mainly via the Aulne River (Fig. 1). This is further supported by the differences in mean shell Pb/Ca values between the Fret and the Roz sites: higher mean values are recorded in shells of the Anse du Roz site, which is the closest site to the Aulne mouth and the Pb source. In order to test the existence of a relationship between Pb mean concentration in the shells of *Ostrea edulis*, and in Pb content in its immediate environment, our specimens were adequately sampled in sites where Pb concentration in the surface sediments was monitored over the lifespan of the animals (mean values measured over those 15 last years in the Roz site, the Fret site and the Bourgneuf site of 115 ppm, 45 ppm and 20 ppm respectively). These values were used to plot the mean Pb/Ca ratio obtained in shells of each site, as a function of the Pb content in the surface sediments measured in the vicinity of sample locations (Fig. 8). This plot reveals a strong linear relationship ($R^2 = 0.84$) between shell Pb content in the shells of our specimens and in the surface sediments surrounding them. The three sites from this study (Fig. 1) can all be considered as poorly Pb-contaminated, yet, the shell Pb/Ca from specimens at each site are significantly different. We consider that this result illustrates the high sensitivity of *O. edulis* to Pb content in the surface sediments, and its capacity to highlight small or remnant Pb inputs and to help localize the source.

At higher environmental concentrations, Liehr et al. (2005) analysed the shell-Pb content in the hinges of some *Arctica islandica* specimens from two localities in the vicinity of a dump site in the North Sea. Although the species are different, mean values obtained in shells of *A. islandica* appear to distribute along the linear regression settled in

our *O. edulis* specimens. Additional tests are obviously required, but this suggests that the relationship postulated here between shell-Pb content and surface sediments-Pb content could be independent of both the species and the mineralogy (calcite vs. aragonite).

5. Conclusions

The Pb content was measured in ten hinges of modern *Ostrea edulis*, collected in three sites along the French Atlantic coast, which are characterized by different levels of Pb content in the surface sediments. LA-ICP-MS tracks perpendicular to growth increments enabled to monitor intra-annual Pb/Ca ratio fluctuations recorded by oysters over several years. No ontogenetic trend was observed. Within-a-shell reproducibility tests performed in the foliated calcite of hinges show reproducible Pb/Ca profiles in each *O. edulis*. This result illustrates a homogeneous distribution of Pb/Ca within each growth line. Secondly, inter-shell reproducibility was tested by comparing adjusted Pb/Ca profiles recorded in the specimens of each site. Results show that all oysters within a given site record the same Pb/Ca variations within error of the method. Second order differences in peaks intensity up to 50% can be observed between specimens, which we consider can be explained either by the smoothing effect, or averaging method we used, which can become important when growth lines are narrow. A second source of variability comes from potential micro-environmental heterogeneities in Pb distribution at the water-sediment interface. A synthesis of Pb mean values measured in marine bivalves sampled in multiple environmental contexts shows a range of values from 0.01 ppm up to 50 ppm, and a general distribution of values independent of the considered species, but highly dependent from the environmental context (pre-industrial shells and modern shells from Iceland < 0.08 ppm < shells from environments influenced by distant anthropogenic sources and atmospheric inputs < 2 ppm < shells from Pb-contaminated sites – vicinity of smelters, spillage of mine waters – < 5 ppm < shells from exceptionally contaminated sites or specific layers < 50 ppm). Mean values measured in *O. edulis* shells in this study range from 0.15 ppm to 0.84 ppm, and are in a good agreement with the environmental context of the sampling sites. Furthermore, the confrontation of mean values measured in the surface sediments of each site and mean values of Pb/Ca recorded in shells show a linear relationship. *O. edulis* presents a wide geographical distribution along European coasts and is abundant in shell middens since the Mesolithic. Thus, the existence of such a relationship suggests that *O. edulis* can potentially be an accurate bio-monitoring tool of both modern and ancient environmental bioavailable Pb.

Future laboratory experiments should be helpful to quantify more accurately the factors involved in infra-annual Pb-fluctuations, and go further in the development of this bio-monitoring tool. Accurate interspecific comparisons are now also required to test our hypothesis of a non-specific relationship between sediment Pb-content and shell Pb-content. If this hypothesis revealed true, data from archaeological *O. edulis* could be combined to data obtained in other species with different environmental requirement (like *A. islandica*). Then, accurate maps, showing the evolution of bio-available Pb along European coasts since the Mesolithic, could be reconstructed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.marpolbul.2017.07.075>.

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