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Sclerochronological and geochemical study of the carpet shell *Ruditapes decussatus* in archaeological contexts: a potential tool for season of collection and coastal paleo-temperature

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Keywords

Ruditapes decussatus, Bivalve Mollusk, Shell Midden, Sclerochronology, Sclerochemistry, Seasonality, Paleo-temperature, Mesolithic, European Atlantic coast

Abstract

Specimens of the carpet shell *Ruditapes decussatus* from the Mesolithic shell midden (6^{th} millennium BC) of Beg-an-Dorchenn (Brittany, France) were studied in order to assess their period of collection as well as provide some insight on Paleo-temperature reconstructions. Cross sectioned shells display very clear growth structures allowing an assessment of a growth rhythm of 2 increments per lunar day. From this temporal framework, daily growth increments and winter annual growth breaks were characterized. Our results show the *R. decussatus* shells were typically collected in the early spring period. Chemical analyses of shell carbonates were also performed to assess a preliminary range of potential sea temperature data from oxygen isotopes values ($\delta^{18}O_{shell}$). $\delta^{18}O_{shell}$ shows seasonal variations but do not record the whole seasonal temperature range as *R. decussatus* have a growth break during winter. However, results show predicted SSTs within the range of expected values. Thus, *R. decussatus* is particularly suited for paleo-environmental reconstructions at high temporal resolution such as seasonal timescales. Additional sclerochronological and chemical research on additional carpet shells and also other mollusk species from Beg-an-Dorchenn or other Mesolithic shell

middens along the European Atlantic coast should be considered. Such studies will contribute to the understanding of the seasonal occupation patterns of the last hunter-gatherers of the European Atlantic coast as well as the climate variability at the Mesolithic-Neolithic transition.

I. Introduction

The study of hunter-gatherer populations involves a deep understanding of their interactions with the local environment, especially concerning their spatial and temporal residential behaviors. The particular activity pattern of these communities (*i.e*. dependence on wild resources) implies a close interdependency with climatic variability and involves numerous environmental parameters at different timescales and periodicities (*e.g*. daily and monthly tidal cycles, seasonal faunal migrations, annual primary production) (Yesner 1980, Testart 1982, Zvelebil 1986, Moss 1993).

Archaeological and ethnographical studies show that coastal hunter-gatherer populations had an extensive knowledge of their terrestrial as well as maritime environments (Thomson 1939, Yesner 1980, Dupont and Marchand 2016). For the latter, the sea can bring a large diversity of wild resources such as mammals, fishes, crustaceans, mollusks, seaweed, and lithic materials. The remains of their activities (*i.e*. their waste products) create deposits rich in critical information that permits archaeologists to improve the understanding of these maritime populations (Milner et al. 2007, Claassen 1998). Shell deposits and shell middens are important faunal depositional environments within archaeological sites, especially during the Mesolithic along the European Atlantic coast (Gutiérrez-Zugasti et al. 2011).

Thus, *via* qualitative and quantitative diversity analyses, archaeomalacological studies give information about paleoenvironments, human diets or human behavior (Claassen 1998, Bar-Yosef Mayer 2005, Dupont 2006, Szabo et al. 2014). In this framework, sclerochronology, the study of accretional hard elements of biological organisms, provides useful insights on the species life traits (*e.g*. rhythm and variation of growth) as well as on the environment in which individuals grew (Buddemeier 1978, Jones 1983, Bessat and Tabeaud 1998, Schöne 2008, Schaffer et al. 2011, Wanamaker et al. 2011, Gosselin et al. 2013). Based on mollusk species, sclerochronological studies in archaeological shell middens offer the possibility to assess their season of collection and thus to deduce periods of coastal settlement residence, or fishing activity, by human communities. Moreover, complementary chemical analysis along the mollusk shells growth axis (sclerochemistry) gives additional clues on paleoclimatic and paleoenvironmental parameters such as sea surface temperature (SST), primary production or salinity (Andrus 2011). Along the European Atlantic coast more than 240 Mesolithic shell middens have been reported but evidence of clear residence pattern is still scarce (Dupont 2016). Among those settlements, the bivalve *Ruditapes decussatus* usually appears in the top three most abundant mollusk

species (Gutiérrez-Zugasti et al. 2011). This Veneridae is a widespread diachronic species with a large latitudinal modern distribution from Norway to Portugal (Quéro and Vayne 1998). Moreover, its shell thickness and size provide a substantial area for potential experiments making it particularly suitable to combine sclerochronological and geochemical analysis. Indeed, to date, studies focused on mollusks have typically employed only one method of investigation (sclerochronology or geochemical analysis) (Mannino et al. 2003, Deith 1983, Brock and Bouget 1989, Milner 2005). Here, we jointly apply sclerochronology and geochemistry to the archaeological *R. decussatus* shells from the Mesolithic settlement of Beg-an-Dorchenn (Brittany, France). Such studies have already been conducted on modern shells of the sympatric and currently commercialised *R*. *philippinarum* species (Poulain 2010, Poulain et al. 2010, Poulain et al. 2011). Sclerochronological studies showed that *R*. *philippinarum* daily growth follows a tidal rhythm (Poulain et al. 2011). Sclerochemical studies of *R*. *philippinarum* shells lead to an equation between the $\delta^{18}O$ values of sea water ($\delta^{18}O_W$) and salinity (Poulain 2010). However, no SST proxy derived from oxygen isotopes samples currently exists. To our knowledge there is no previous work on modern or archaeologic specimen of the native grooved carpet shell clam *Ruditapes decussatus* involving shell oxygen isotopes. Thus, there is no modern calibration study for palaeotemperature and one must keep in mind throughout the present study that physiological processes may be involved in the fractionation processes.

This paper aims to transfer knowledge gained from studying modern shells of *R. philippinarum* to archaeological shells of *R. decussatus*, enabling us to use the latter as a recorder of Mesolithic period human activities along the European Atlantic coasts. First, we conduct structural observations of the archaeological shells to 1) deal with potential diagenesis issues, 2) characterize increment readability and 3) confirm the temporal framework of shell growth patterns. From this basis, two shells (BAD-5 and BAD-18) were chosen to test jointly sclerochronological and biogeochemical analysis in order to identify the seasonality of shellfish collections and discuss the paleoenvironmental reconstruction process.

II. Materials and Methods

II.1. Material

II.1.1. Species biology

The native European carpet shell *Ruditapes decussatus* (Linneaus, 1758) is a mollusk from the Veneridae family living in the intertidal zone; from the mean high water level to the upper sublittoral level. This bivalve is a filter feeder and deposits feeder species which burrows in mud, sand or even muddy gravel at a maximum depth of 15 cm. Its growth, up to 8 cm (mean 4-5 cm), is mainly controlled

by environmental parameters such as sea temperature and food availability. *R. decussatus* is an euryhaline (20-50 ppt, optimum 30 ppt) (Le Treut 1986) and eurythermal (10° C-32° C, optimum 20-25° C) species (Sobral and Widdows 1997).

II.1.2. Archaeological site

The carpet shells studied in this paper came from one of the few French shell middens of the Mesolithic: Beg-an-Dorchenn (Brittany, France, Figure 1. A-B). This shell-midden is a key archaeological site to understand the Neolithization process along the French Atlantic coast (Figure 3.). Along the coast, only 6 sites are known for the Late Mesolithic. Beg-an-Dorchenn shows a clear dependence of the hunter-gatherers on the marine environments accessible in the vicinity of the settlement which was a maximum of one kilometre from the foreshore during occupation (Dupont et al. 2009). The wide diversity of remains of wild exploited resources demonstrates an expert use of the local biodiversity. Moreover, the temporal addition of the different seasonally available animal and vegetal exploited resources shows that this human population was able to survive throughout the year at the site (Dupont et al. 2009). So, the question of the permanent residence (nomadic VS sedentary) of this Mesolithic population can be asked.

Beg-an-Dorchenn shell-midden thickness decreased from more than one meter during the 19th century (Du Chatellier 1881) to 30 cm at the end of the 20th due to erosion (Figure 1. C). A supplementary sample excavation was performed in 2001 with an interdisciplinary focus in mind (Dupont et al. 2010) (see details in supplementary material 1). The aspect of the shell-midden is a black organic layer (Figure 2. A-B). It was composed of marine resources (shells, crabs, fishes, birds and mammals), terrestrial remains (birds, mammals, charcoals) and flints. A total of 26 kg of shells have been obtained from the sieved sample of 2001. The total minimum number of individuals (MNI) has been evaluated to 13,324 individuals of shells. 6.6% of them are *R. decussatus*. Radiocarbon dating attributes this Late Mesolithic occupation from the middle to the end of the sixth millennium BC (Marchand et al. 2009). Among the 50 fragments of the most complete carpet shells coming from Beg-an-Dorchenn, two very well-preserved valves of *Ruditapes decussatus* have been chosen for sclerochronolgical and biogeochemical analysis: a left one (BAD-5 from level 7) and a right one (BAD-18 from level 5)

II.2. Methods

II.2.1. Preparation of shell cross section

Incremental growth structures can be observed under a microscope from a shell cross section. One valve of each specimen of *R. decussatus* was cut along the axis of maximum growth from the umbo to the ventral margin. The valve was preliminarily embedded in epoxy resin (Araldite 2020©). Three cuts

are made perpendicular to the growth lines with a high concentration diamond-coated 0.43 mm metal blade (430CA Ø 152 mm) mounted on a low speed precision saw (Accutom 50, Struers©, 2700 rpm, feed speed 0.075 mm/s). Two consecutive embedded thin sections of 1.1 mm thickness are obtained and glued with Araldite on a sand blast glass slide. Next, the thickness and planeness of the thin section is adjusted to obtain a planar and smooth surface. This step enables growth structure observation of the shell by polishing the thin section with decreasing size grits of Al_2O_3 powders (1200, 2400, 4000 grain sizes) then with diamonded paste (Ø 3 and 1 µm) (Grinder-Polisher Tegra Force 5 Struers ©). Between each grinding/polishing step, thin sections were cleaned by ultrasonic bathing in MilliQ water (Bioblock Scientific ©). Thus, embedding, cutting and polishing steps leads to a thin section of the shell containing all its lifespan from birth to death.

Shell structure was observed by scanning electron microscope with chemical analysis by Raman spectrometry (see details in supplementary material 1).

II.2.2. Growth microstructure analysis

Growth microstructures were observed from the outer shell layer of the thin section under an optical microscope (Leica S8AP0) at 40-100x magnification with reflective light coupled with a camera (Leica EC3) to take digital photographs. Growth increment thicknesses were then measured from the ventral margin to the umbo direction using the image analysis software Visilog (V6.481) with an adapted sclerochronological macro (Noesis Co.) (Gosselin et al. 2007). One growth increment is defined as the couple of one thin dark growth line and one larger white growth line.

II.2.3. Shell geochemistry

Oxygen isotope analyses in mollusk shells ($\delta^{18}O_{Shell}$) reflect sea surface temperature (SST) and $\delta^{18}O$ seawater ($\delta^{18}O_W$) variations (Urey et al. 1951). $\delta^{18}O$ is a measure of the ratio of ^{18}O to ^{16}O relative to a standard material (VPDB). Carbonate oxygen stable isotopic ratios depend on two main factors: i) Water temperature during the calcification process ii) the oxygen isotope composition of the surrounding water. $\delta^{18}O_W$ variability encompass all the water cycle processes (*i.e*. evaporation, condensation, precipitation, surface run off) and can also often reflect salinity variations of the sea waters (Epstein and Mayeda 1953).

In this study, $\delta^{18}O_{Shell}$ analysis were performed in the outer shell layer of the thin section by drilling (Micromill NewWave ©) with a drill bit tip diameter of 200 µm. 83 powder samples from 33 to 94 µg were collected from the corresponding shell portion where sclerochronological analyses were performed. The drilled spots had a mean diameter of 380 ± 68 µm and a maximum depth of 400 µm while the distance between two consecutive holes was 68 ± 45 µm. Powder samples were then

processed in a Thermo Finnigan MAT 253 Isotope ratio mass spectrometer coupled to a Gas bench II. The calibration standard is NBS-19 ($\delta^{18}O$= -1.91 ‰) with an internal precision of 0.05 ‰ and an accuracy (1σ) of 0.07 ‰. $\delta^{18}O$ are expressed relative to the Vienna Pee Dee Belemnite international standard (VPDB) and given as per mil (‰). In the absence of a specific paleotemperature reconstruction equation for those two aragonitic species, we chose the corrected equation of Böhm et al. (2000) which is built from $\delta^{18}O$ datasets of different bioaragonitic seawater invertebrates. Moreover, in order to account for the seasonal variability of salinity on the $\delta^{18}O$ values of sea water we worked with the $\delta^{18}O_W$ / salinity equation of Poulain (2010).

II.2.4. Catch seasonality methodology

In order to assess the seasonality of shellfish collections and thus the presence of human population at the coastal site, we used two different methods. First, from the sclerochronological study we counted the number of increments after the last annual growth break. Second, from the sclerochemical study, we used the quartile method (Mannino et al. 2003, Prendergast et al. 2016). For the latter, the annual $\delta^{18}O$ variations were calculated from the third year of growth of BAD-5 shell and from the fourth year of growth of BAD-18 shell when the specimens displayed a maximum of growth days among a year (10 and 9 months respectively).

III. Results

III.1. Shell diagenesis and growth line readability

Structural and chemical analyses were performed on archeological shells. Raman analyses were carried out on a thin section on different spots of the shell of BAD-5. Raman spectra reveal that the calcium carbonate composition of the archaeological shells is aragonite (Wavelength 568 nm, wave number peaks at 155, 205, 271, 704 cm^{-1} and 1085 cm^{-1}). Those results are very similar to those found in modern shells of *Ruditapes decussatus* and *R. philippinarum* (Trinkler et al. 2011).

Fragments of *R. decussatus* broken by hand were observed on SEM. Three archaeological shells from Beg-an-Dorchenn have been observed including shell BAD-18. They display two layers of aragonite: a homogeneous inner layer and a prismatic outer layer (Figure 4) Growth increments exhibit an exceptional readability for these 6^{th} millennium BC specimens.

III.2. Growth analysis: Time frameworks, periodicities, aging

Growth marks of the two shells of *Ruditapes decussatus* were counted and inter-mark distances measured in order to build their respective growth trajectory curves from the high frequency analysis of the growth increments of the thin sections to the external notches of the valves (Figure 5 and Table

1). The two shells of *Ruditapes decussatus* were nearly of the same size (*i.e*. 30.6 mm and 27.7 mm along the maximum growth axis for BAD-5 and BAD-18 respectively).

III.2.1. From annual to tidal growth marks

Depending on the degree of growth line readability, the sclerochronological work took place along almost all the thin section of the BAD-5 valve (3.3 cm for 1388 cumulated growth increments) while the BAD-18 valve was studied over the last 1 cm of cumulated growth (657 increments) (Figure 5 B.). The annual growth marks of each shell were investigated by observing their external surface as well as the cross sections (Figure 5 A-B). They underline a break, or a drastic slowing, of growth; probably attributable to the low sea temperature and/or low food availability occurring during winter. Those annual growth marks are characterized by a V shape notch at the outer shell layer in cross section and as a groove at the external shell surface. They usually appear during a period of low mean increment thickness with a slow growth decrease before the notch and a fast increase after the notch (Sato, 1999). Each shell displays a total of 4 breaks following the annual growth marks criteria (N1 to N4 in Table 2. and Figure 5.). Thus BAD-5 and BAD-18 are around 4 years old with different growth duration depending on the year.

Indeed, while BAD-5 and BAD-18 exhibit a common increment thickness range between 5 and 80 µm, their distributions (24.0 ± 13.4 µm and 15.1 ± 7.0 µm respectively) are significantly different (Wilcoxon-Mann-Withney test $U = 2.673 \times 10^5$; $P < 0.0001$) (Figure 5 C.). In the same line, BAD-5 shows larger increments than BAD-18 in their last 1 cm of growth (19.3 ± 8.9 µm and 15.1 ± 7.0 µm respectively; Wilcoxon-Mann-Withney test $U = 1.184 \times 10^5$; $P < 0.0001$). The ontogeny of the bivalve has an effect on its growth: as the shells grow older, their mean growth increment thickness as well as their length of the growth season decrease (Gosling 2003).

Between the third and fourth annual mark of BAD-18 a total of 510 increments were counted suggesting a growth rhythm of two daily increments that may be influenced by tidal periodicity. Indeed, modern tidal cycle in Brittany is semi-diurnal with two daily low/high tides. Thus, tidal growth increments analysis of BAD-5 suggests a growth duration of 7, 10 and 5 months during 2^{nd}, 3^{rd} and 4^{th} year respectively while BAD-18 shows 9 months of incremental growth in its 4^{th} year. Other tidal cycles such as the neap/spring tide succession with a fortnightly periodicity are not observed from the high-resolution growth patterns of the two *R. decussatus* archaeological shells (REDFIT spectral analysis on tidal daily increments with software PAST V3.22).

III.3. Oxygen isotopic analysis

$\delta^{18}O_{Shell}$ mean sample values are statistically similar in BAD-5 and BAD-18 (Wilcoxon-Mann-Withney test U = 538; *P*= 0.33) even if BAD-5 $\delta^{18}O_{Shell}$ data show a larger range of values (Table 2 and Figure 5. D). They follow sinusoidal shapes with a range between -0.78 and 1.02 ‰. In BAD-5, the 2nd and 3rd years of incremental growth are negatively correlated with the related $\delta^{18}O_{Shell}$ values while those parameters are positively correlated during the 4th year. In fact, a small but significant negative correlation is observed between $\delta^{18}O_{Shell}$ values and growth increment thickness (Spearman correlation test, *P*< 0.001, R^2_{BAD-5} = 0.115, R^2_{BAD-18} = 0.144). The positive correlation between the third and the fourth growth break in BAD-5 may be the results of fresh water impact. Indeed, due to physicochemical processes, the $\delta^{18}O_w$ is correlated to the salinity (Epstein and Mayeda 1953, Chauvaud et al. 2005).

The annual growth marks of each shell are interpretated as negative environmental impacts occurring during winter. During those breaks/slowing of growth, the corresponding $\delta^{18}O_{Shell}$ values are high, implying low temperature periods which are in accordance with wintry conditions (Figure 5. C, D). An estimate of paleo-SST was made using the corrected equation of Böhm et al. (2000) which was built from $\delta^{18}O$ datasets of different bioaragonitic marine invertebrates [SST = 20.0 - 4.42 x ($\delta^{18}O_{shell}$ – ($\delta^{18}O_W$ – 0.2 ‰)] with the sea surface temperature expressed in ° C (SST), the $\delta^{18}O$ values in ‰ for the aragonite shell ($\delta^{18}O_{shell}$) and the sea water ($\delta^{18}O_W$). $\delta^{18}O_W$ is corrected by -0.2 ‰ to reflect unit change from SMOW to PDB. For a $\delta^{18}O_W$ parameter set as a constant, a $\delta^{18}O_{Shell}$ variation of 1 ‰ shift is equivalent to a 4.42° C variation in SST and the spectrometer accuracy of 0.07 ‰ is equivalent to 0.31° C.

In order to account for the seasonal variability of salinity on the $\delta^{18}O$ values of sea water ($\delta^{18}O_W$), we used the following equation described by Poulain (2010): $\delta^{18}O_W$ = 0.144 x Salinity – 4.707 (N=35, R^2=0.97, P_V < 0.001) with the salinity values of Locmariaquer (distant of ± 100 km East from BAD site). The maximum salinity of 35 ppt and minimal winter values of 27.5 ppt gave respectively a “seasonal” $\delta^{18}O_W$ value of 0.333 ‰ and -0.747 ‰. During the desalinated winter period some isolated low values of 19,5 may occur with a corresponding calculated value of -1.899 ‰. This latter salinity value is close to the minimal salinity that *R*. *decussatus* can physiologically managed (Le Treut 1986).

With $\delta^{18}O_W$ = 0 ‰ (standard mean ocean water), the reconstructed SST were 20.80 ± 1. 87° C (min 16.37° C; max 24.35° C) and 21.29° C ± 1.82° C (min 18.47° C; max 23.81° C) for BAD-5 and BAD-18 respectively. The reconstructed SST from BAD-5 $\delta^{18}O_{Shell}$ were also calculated with $\delta^{18}O_W$ “seasonal” values for warm/high salinity (mean 20.50° C ± 1.87° C; min 16.07° C; max 24.05° C), cold/low salinity period (15.73° C ± 1.87° C; min 11.30° C; max 19.28° C) and cold/isolated lowest salinity events (10.64° C ± 1.87° C; min 6.21° C; max 14.19° C). Thus, from the two archaeological shells, the reconstructed

SST for Beg-an-Dorchenn may have roughly been around a mean 21 ± 2 ° C with extrema between 6 ° C (cold/punctual lowest salinity events) and 24° C (warm/high salinity event). All these values are within the limitation range of 3 to 28° C to use the Böhm equation as well as in the SST range of 3.2° C to 23.6° C (Mean 18.4° C) currently recorded at Loch River, 100 km East of Beg-an-Dorchenn, (Poulain 2010, Poulain et al. 2011).

III.4. Catch seasonality

BAD-5 shows its 4th and last annual notch very close to the ventral margin (11 days). On the other hand, the BAD-18 shell shows 6 weeks of growth from the 4th annual break to the ventral margin. Thus BAD-5 and BAD-18 shells were thus probably collected at the early spring period during the restart of growth after winter growth break/reduction. We also estimated the season of collection with the quartile method by comparing the $\delta^{18}O$ variation over the course of a year of growth and the $\delta^{18}O$ at the outermost growing edge of the shells (Table 3). The upper quartile (>75%) of $\delta^{18}O$ values reflects the lowest SST during winter (> -0.015 ‰; max 0.680 ‰; (N=30) and > 0.239 ‰; max 0.547 ‰; (N=18) for BAD-5 and BAD-18 respectively). The last weeks of growth of BAD-5 and BAD-18 shells displayed $\delta^{18}O$ values close to the lower limit of the upper quartile (0.189 ‰ (N=1) and 0.303 ‰ (N=2) respectively). Those stable isotope samples encompass a period of several weeks because of low growth rates. Thus, although those isotope data show less temporal precision than the increment data, the sclerochemical study also suggests a catch during early spring. This period of human occupation and mollusks feeding are in accordance with seasonality from other zooarcheological remains of Beg-an-Dorchenn site such as fishes and crabs (Dupont et al. 2010).

An intent to specify the growing months for *Ruditapes decussatus* archaeological shell was made using both increment and isotope data. A year of growth was characterized as the number of growth increments between two consecutive (winter) growth breaks. For each $\delta^{18}O$ shell value, the number of days encompassing the isotope samples and the corresponding mean daily growth were calculated. Then the calendar day of 15th of August (current highest SST) was attributed to the lowest $\delta^{18}O$ shell value (highest SST). From this day, a calendar date for each couple increment/isotope sample was generated (forwards and backwards). This work has been applied to the 3rd and 4th year of growth of BAD-5 and to the 4th year of growth of BAD-18. During the 3rd year, BAD-5 grew between the beginning of May and mid-November; the following year (4th) the growth starts at mid-march and stops at the end of December. During its 4th year of growth BAD-18 grew from mid-April to early December. By centering the growing days at mid-August, the shells of *Ruditapes decussatus* display a mean growing season between early April and early December with a standard deviation is 3 to 4 weeks which is very similar to the growing season of *Ruditapes philippinarum* from March to November (Poulain, 2010). It

has to be noted that the growth break between the 3rd and 4th year of growth of BAD-5 can be estimated to 4 months which, with the duration of growth of the 3rd year of BAD-5 (7 months), allow one to assess the complete growth activity pattern of BAD-5 during that year.

IV. Discussion

IV.1. Shell diagenesis and increment readability

The two shells of *Ruditapes decussatus* show a very high state of preservation at structural as well as chemical and microstructure levels. This absence of apparent diagenesis allowed development of sclerochronological and sclerochemical analyses on specimens dated between 5700 and 5500 Cal. BC.

IV.2. Growth parameters and life traits of Beg-an-Dorchenn *R. decussatus* specimens

Shell growth rhythm (number of daily increment) are usually species-specific and related to the astronomical cycle (Sun-Moon-Earth tryptic) while shell growth amplitude (increment thickness variation) is environmentally dependent (Gosselin et al. 2013). Detailed analysis of growth marks from daily to annual periodicities allow one to determine a growth rhythm of two increments per day for *Ruditapes decussatus*, similar to its close taxonomic relative *Ruditapes philippinarum* which follows a tidal rhythm (Poulain et al. 2011). Following this hypothesis, the sections of *R. decussatus* shells in this study grew 3.3 cm in 23 months (BAD-5) and 1 cm in 11 months (BAD-18). Thus, the great readability of the growth increments gave access to up to 3 partial years of daily growth.

Ruditapes decussatus archaeological shells show a winter growth break from 2 to 7 months depending on the year of growth. With annual recording/growing window periods between 5 and 10 months, Beg-an-Dorchenn bivalves were probably growing from spring up to early winter when temperatures were close to their physiological optimum and thus were recording maximal annual SST. However, they did not record the minimum annual SST (winter growth break) leading to an overestimation of the mean reconstructed temperature. Thus, the real SST occurring at Beg-an-Dorchenn and the potential shift with current SST is difficult to currently assess.

We recommend to preferentially select *R. decussatus* specimens of 3-4 years old when the number of growing days per year is the largest. Tidal growth bands analyses from annual growth breaks allow a smaller uncertainty than isotope analyses (infra-daily VS 10 days) but sclerochemical analyses, by revealing the lowest $\delta^{18}O$ shell value (highest SST), offer the possibility compare the growing period at intra and inter specimen level as well as between different human occupation duration.

318 The duration of the annual break is inter and intra-species dependent (ontogeny) as well as
319 environmentally impacted (*e.g*. SST variations). However, this mean duration of growth breaks
320 between April and December (± 1 month) is similar to that of modern *Ruditapes philippinarum*
321 specimens of the same region (Loch River distant of ± 100 km from BAD site) which show a mean winter
322 growth break of 5 months between November and March (Poulain 2010). Sea and sediment
323 temperature along Atlantic French coasts usually range from minimum temperatures in February to
324 maximum temperature in August which respectively match the period of lowest and highest growth in
325 the Beg-an-Dorchenn shells confirming that the growth breaks occur in winter (Gómez-Gesteira et al.
326 2008).

327 However, restart of growth after the winter break is still not easy to determine in archaeological shells
328 as the duration of growth break is age-dependent and can also vary from year to year due to energy
329 reserves kept from the previous year (Chauvaud et al. 2012). That could be particularly the case during
330 the third year of growth of BAD-5 with a growing season of ten months suggesting that some extra
331 energy from the second year may have been used at the beginning of the third years and that a higher
332 part of the third year energy reserved may have been redirected for shell growth activity leading to a
333 longer growing season.

334 To gain insight of the particular annual growth of the archaeological specimens among the species
335 growth range, growth curves deducted from the four winter breaks of the studied shells were
336 calculated and then compared with length-at-age data of southern modern *Ruditapes decussatus*
337 populations (Figure 6.). Archaeological shells are smaller during their first and second years than the
338 populations growing in Atlantic southern France (Arcachon and Arguin Bays) or North-Eastern Adriatic
339 (Pag Bay) before reaching similar sizes during their 4th year of growth. The modern shells of *R.*
340 *decussatus* grew mainly during their first two years while the archaeological shells show a higher
341 growth rate later catching up with the modern ones during the 3rd and 4th years.

342 The growth discrepancy between modern and archaeological specimens could be explained by a
343 difference in life history-traits, timing events and energetic management (*e.g*. winter growth break,
344 size at first reproduction) driven directly or indirectly by climatic and environmental variations (*e.g*.
345 temperature variability, food availability VS population density, river fresh water VS oceanic marine
346 water or opened bay VS semi-closed embayment). Genetic variability could underlie such growth
347 variability patterns with appearance of different populations from successive glacial/interglacial
348 periods. Testing this hypothesis is beyond the scope of the present study.

349 The latitudinal factor acts as a driver of growth heterogeneity in bivalves (Sato 1999). Indeed Chauvaud
350 et al. (2012) show that Atlantic *Pecten maximus* population exhibit a lower growth rate and a higher

potential maximum size in higher latitude. This is also the case with the Northern Atlantic Mesolithic shells of *R. decussatus* compared to the Southern Atlantic and Mediterranean modern populations. Thermal gradient is closely related to the latitudinal gradient and acts on growth parameters such as the mean daily growth increment thickness as well as the period of growing season. Disentangling the latitudinal effect (space) from the temperature variations between the Mesolithic and current era stills a challenge.

Finally, the growth patterns displayed by the Mesolithic specimens such as a low growth rate, a high maximum size and growth break in winter tend to situated those individuals close to the Northern limit of *R. decussatus* range.

V. Conclusion

Two specimens of the carpet shell *Ruditapes decussatus* from the Mesolithic shell midden of Beg-an-Dorchenn were studied in order to assess their period of collection as well some insight into paleo-temperature reconstruction.

This paper was firstly an attempt to adapt sclerochronological methods to archeological materials and eventually use it routinely in further studies. Cross sectioned shells display very clear growth structures allowing us to assess a growth rhythm of 2 increments per lunar day and to characterize daily growth increments as well as winter annual growth breaks. The daily growth thickness and the annual growth duration, are both ontogenetically and environmentally dependent. Sclerochronological and sclerochemical results show that the *R. decussatus* studied shells were collected at early spring. Intra and inter species analyses will help to strengthen the present results and to facilitate future comparisons with other shell middens studied along European Atlantic coasts. We recommend to preferentially select *R. decussatus* specimens of 3-4 years old when the number of growing days per year is the largest. Moreover, additional chemical analysis (*e.g*. mapping) of growth dependent elements such as Manganese, Magnesium, Strontium or Barium could help in the interpretation of growth marks and temporal frameworks (Langlet et al. 2006, Poulain et al. 2015, Hausmann et al. 2019.).

A second approach of this paper was a climatic concern about the thermal variability of coastal oceanic waters in the west part of France 7700 to 7500 years ago reconstructed from oxygen stable isotope ratios. Sclerochemical analyses of shell carbonates were conducted to test the feasibility of such analyses and display seasonal variability. Moreover, the range of reconstructed SST did not show nonsensical values. However further interpretations are difficult to deduce from the present data. Indeed, we must be careful to the uncertainties introduced into our results: the Böhm equation used

for SST reconstruction was of broad-spectrum including a large variety of marine invertebrates and the $\delta^{18}O_W$ data could have been more geographically closer, restricting the understanding of the impact of the salinity. Thus, specific SST- $\delta^{18}O_{shell}$ and Salinity- $\delta^{18}O_W$ equations could be developed for *Ruditapes decussatus* from the Beg-an-Dorchenn shell midden and its surrounding waters in order to reduce some uncertainties in future $\delta^{18}O$ analyses.

Recorders of different environmental and ecological parameters will lead to a better understanding of past climate variations and could specify how far the Mesolithic sea temperatures were different from the current ones. *R. decussatus* is particularly suited to address paleo-environmental issues at high resolutions such as the seasonal periodicity.

To conclude, this paper is a first study on the carpet shell *Ruditapes decussatus* from an archaeological context. These promising results are an incentive to extend these investigations in the others Beg-an-Dorchenn archaeological levels and on the whole excavated area. Such a high spatio-temporal level of analyses is fundamental to better understand the rhythm of residence of these late hunter-gatherer-fishers on the French Atlantic coast. Additional sclerochronological and chemical research on Mesolithic and modern mollusk species along the European Atlantic coast will also contribute to the problematic seasonal occupation as well as climate variability at the Mesolithic-Neolithic transition.

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VII. Author contributions

Conceptualization CD, MG, YMP; Methodology CD, CeP, FP, GM, MG, XLC, YG; Formal analysis MG; Investigation CD, CeP, ChP, FP, GM, MG, XLC, YG; Resources CD, CeP, FP, GM, MG, XLC, YG; Writing - Original Draft CD, MG; Writing - Review & Editing CD, ChP, GM, MG, YMP; Visualization CD, MG; Supervision CD; Project administration CD; Funding acquisition CD, MG.

VIII. Declaration of interest

Declarations of interest: none.

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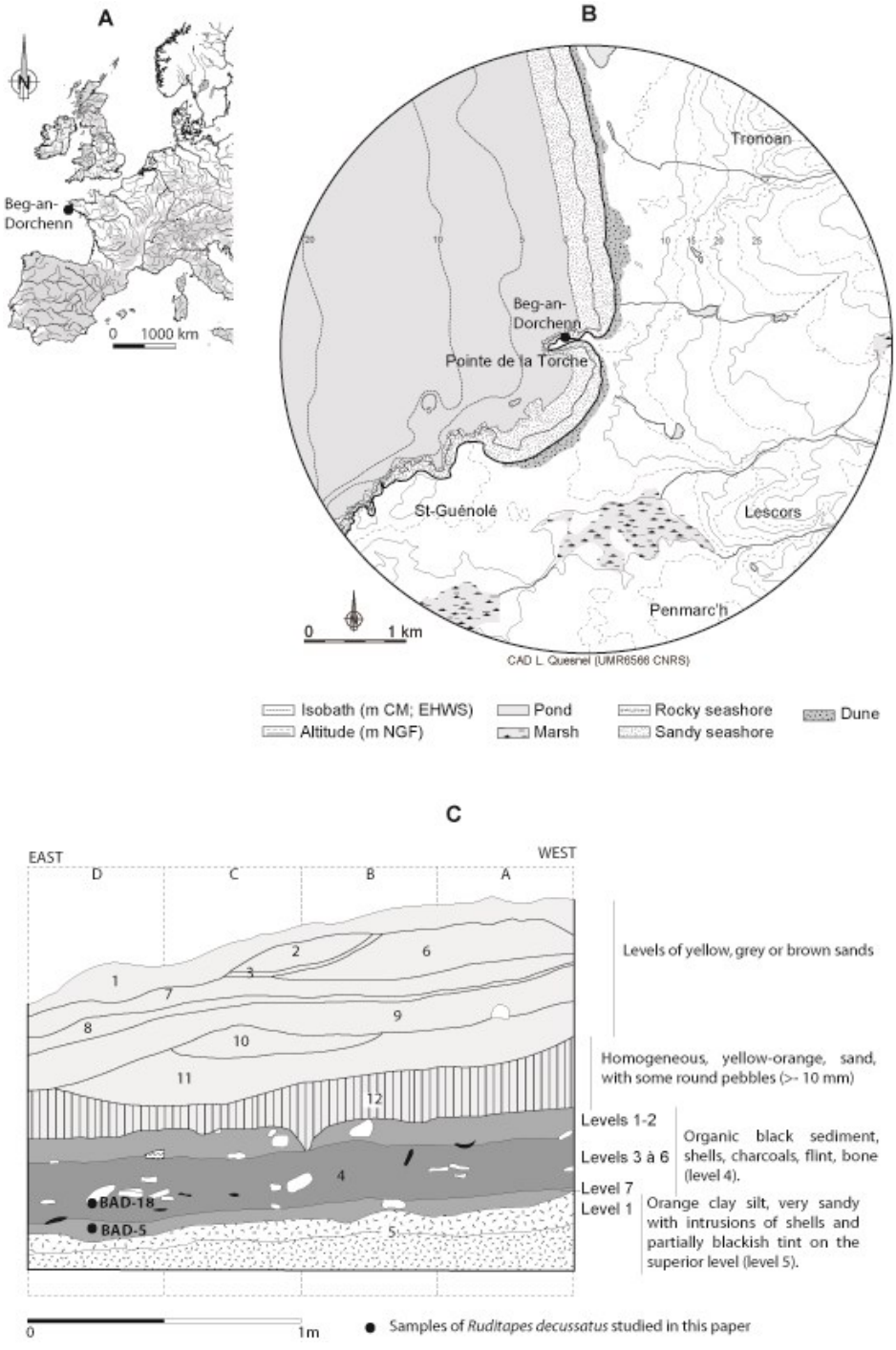
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556

557 Figure 1: A. Location map of the shell midden of Beg-an-Dorchenn B. in the present marine
558 environment (CAD L. Quesnel); C. Stratigraphy of the 2001 survey (CAD G. Marchand).

559

A



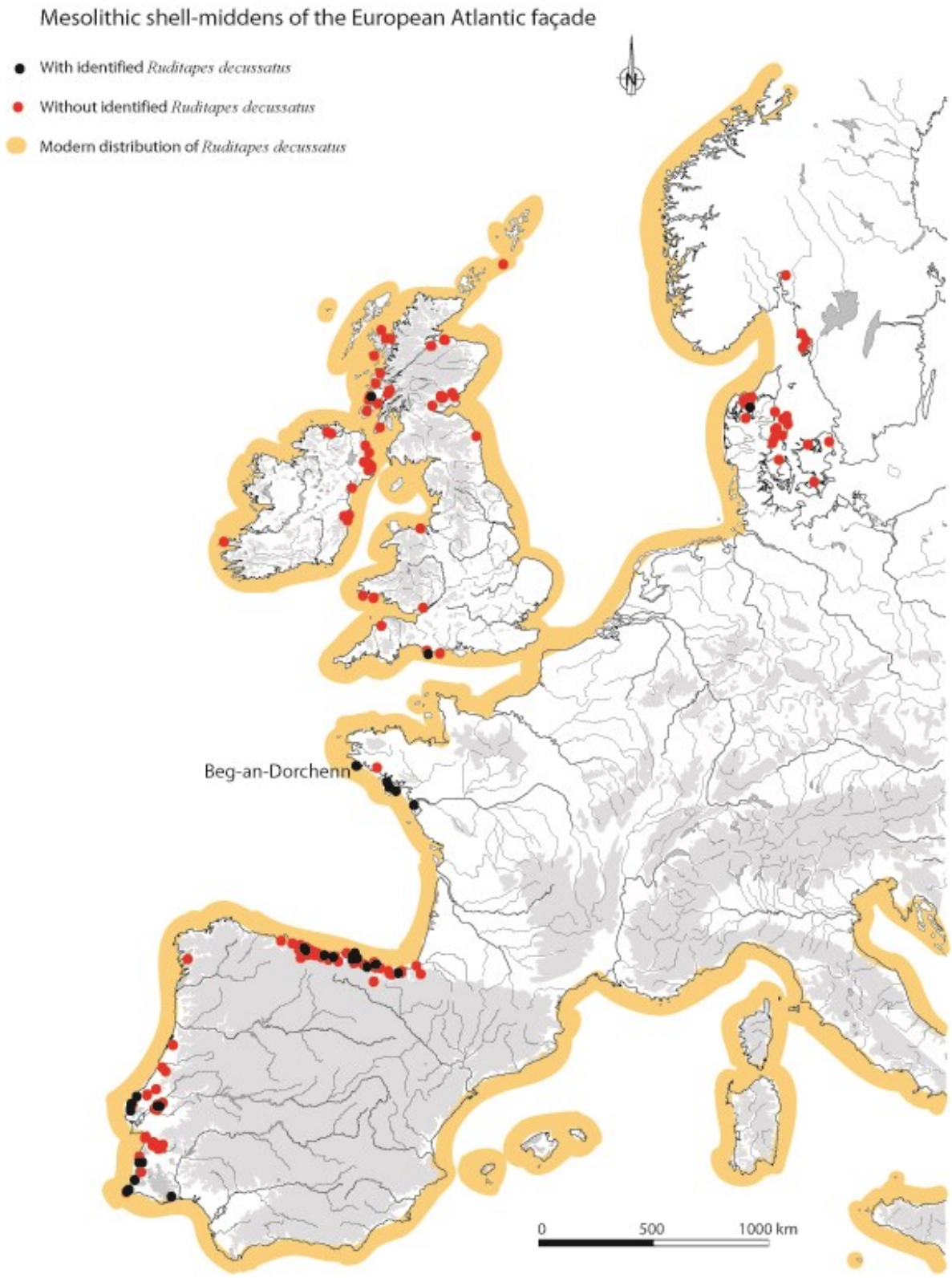
B



C



Figure 2: Detail of the shell midden of Beg-an-Dorchenn; A. survey in 2001; B. detail of the shell midden in May 2001; C. Coarse mesh of sieving process (Credit C. Dupont).



564

565 Figure 3: Map of the Mesolithic shell-middens along the European Atlantic coast with the modern and
566 archaeological presence of *Ruditapes decussatus* (Data 2017, unpublished C. Dupont).

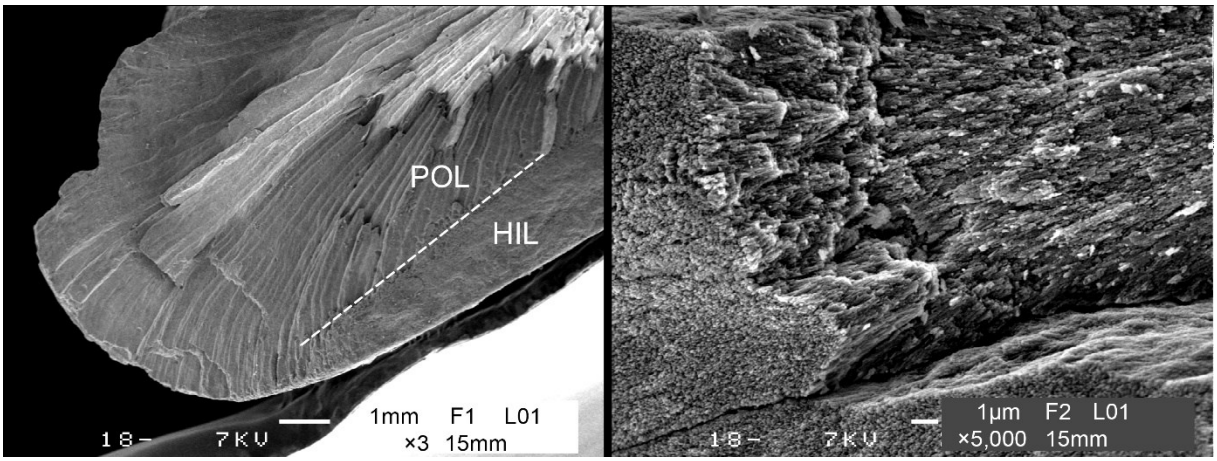


Figure 4: SEM images of shell samples (BAD-18) manually broken in order to observe aragonitic structures along the longitudinal and transversal growth axis. Left image: Calcium carbonate crystals show a homogeneous inner layer (HIL) and a prismatic outer layer (POL). Right image: detail of the prismatic layer. Except slightly less sharpened crystal side and apex than modern shells, no sign of diagenesis has been detected. White scale bar: 1 mm (left image) and 1µm (right image) (Credit: J. Le Lannic).

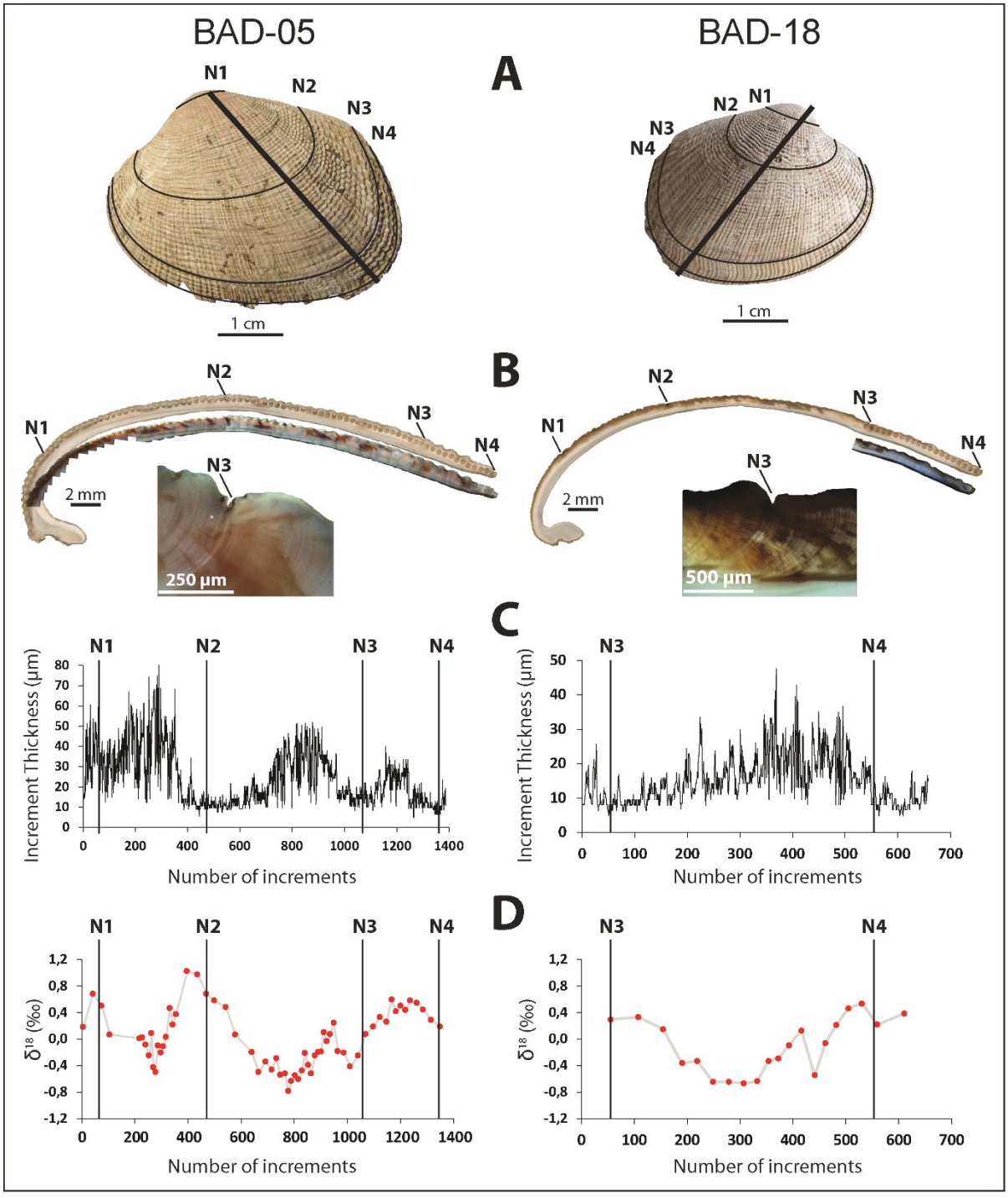
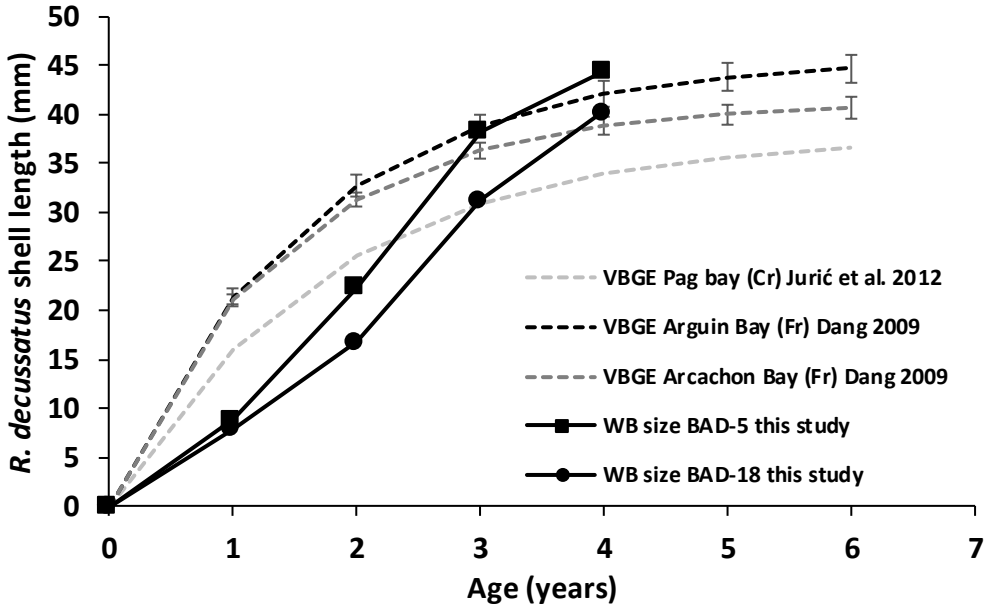


Figure 5: Sclerochronological and geochemical analysis of the *Ruditapes decussatus* shells BAD-5 (left) and BAD-18 (right). A. The well-preserved archaeological valves with annual marks (*i.e*. winter breaks) and fortnightly concentric ridges at the shell surface. B. Cross sections with isotopic samples (upper) and growth lines (lower). A snapshot of one characteristic winter break is provided for each valve (white bar scale of 250 µm). C. Growth increment thickness variation. D. $\delta^{18}O_{shell}$ isotopic values (Red spots). Annual notches (N1-N4 and vertical solid lines).

583



584

585 Figure 6: Length-at-age data of the bivalve *Ruditapes decussatus*. Comparison between winter break
586 (WB) sizes of Beg-an-Dorchenn archaeological specimens BAD-5 and BAD-18 (Table 1. N1-N4) and Von
587 Bertalanffy growth equation (VBGE) of modern European population. East Atlantic South France
588 population (Arguin and Arcachon) from Dang 2009 and North East Adriatic population from Jurić et al.
589 2012. The Height-at-age to Length-at-age transformation of archaeological shell data was estimated
590 from the relationship described in Jurić et al (2012). Standard deviation in vertical bars.

591

592 Table 1: Growth parameters (µm) for the two studied valves of *Ruditapes decussatus*. Increment
593 parameters; SD standard deviation. External annual growth marks parameters along the maximum
594 growth axe; N1-N4: Number 1 to 4 notches (Cf. Figure 5-A); VM: ventral margin.

	N increment	Mean	SD	Min	Max	N1	N2	N3	N4	VM
BAD-5	1388	23.96	13.4	4.68	83.09	6.61	15.63	26.25	30.26	30.56
BAD-18	657	15.07	7.03	4.8	47.5	5.98	11.9	21.55	27.51	27.72

595

596

597

598 Table 2: $\delta^{18}O_{Shell}$ parameters (‰) for the two studied valves of *Ruditapes decussatus*. SD: standard
599 deviation.

	N $\delta^{18}O_{Shell}$	Mean	SD	Min	Max	Median
BAD-5	63	0.02	0.42	-0.78	1.02	0.02
BAD-18	20	-0.09	0.41	-0.66	0.55	-0.08

600

601

602

603 Table 3: Quartile method to assess the season of shellfish collection through the study of the annual
604 range of the $\delta^{18}O_{Shell}$ (‰). BAD-5 data (N=30) come from the 3rd year of growth over 10 months. BAD-
605 18 data (N=18) come from the 4th year of growth over 9 months.

	Quartile ranges	BAD-5 $\delta^{18}O$ (‰)	BAD-18 $\delta^{18}O$ (‰)	Inferred season
Quartile Model	Maximun value	0.68	0.547	winter
	75th percentile	-0.015	0.238	winter
	Median	-0.25	-0.19	Autumn/Spring
	25th percentile	-0.505	-0.573	Summer
	Minimun value	-0.78	-0.663	Summer
Time of collection	Last weeks of growth	0.189	0.303	Late Winter / Early Spring

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Supplementary material 1 : Detailed methodology

SP1.1. Beg-an-Dorchenn excavation protocol in 2001.

The excavation realised in 2001 at Beg-an-Dorchenn consisted in sampling the whole sediment of $1m^3$ of the shell-midden by horizontal levels (thickness: 5 cm) and per quarter of square meter (Dupont et al. 2010). In a second step, the sediment was sieved (5 and 1.6 mm) with fresh water in a laboratory. The whole fraction of the taller mesh and only a small fraction of the fine mesh (50 ml of a quarter of square meter) have been sorted. A first evaluation of the season of gathering of the carpet shell has been made at Beg-an-Dorchenn after an external observation of the growth rate of 299 fragments from the squares A and C of the 2001 excavation (Dupont 2006). This observation led to the conclusion of a capture window between the winter and end of spring. (Dupont 2006). Unfortunately, this method remains rough and new developments in biology can be adapted to archaeological samples to have a better precision of the date of collection of the shell linked to climatic data. this observation lead to the sclerochronological and sclerochemical approach developed in this paper.

SP1.2. Shell structural analysis SEM and Raman

Prior to any growth structure studies (*e.g*. growth increment), microstructural and chemical analyses need to be carried out on the calcium carbonated shells in order to deal with diagenesis potential issues (Nouet et al. 2015). Indeed, structural and chemical changes in the bivalve shell specimen may appear after its death and lead to a lower increment readability and/or to a misinterpretation of subsequent geochemical analysis (Nouet et al. 2015). Mainly, diagenesis affects the crystal structure of the calcium carbonate and modifies its form from aragonite into calcite.

Shell structural concerns have been observed by Scanning electron microscope and chemical analysis by Raman spectrometry. (JSM-6310F Scanning microscope JEOL) from a shell sample metallized with a 20/80 Palladium/Gold coat. Chemical analysis of the calcium carbonate form was provided by Raman spectroscopy (T64000 Jobin-Yvon-Horiba) following the same analysis performed in manila clams, *Ruditapes philippinarum* (Trinkler et al. 2011). It is a non-destructive technic of investigation using an Argon-Krypton laser at a micrometer level and based on the shift of its energy when interacting with molecular vibrations of the studied material (hereby the mollusk shell).

Additional references from supplementary material

Nouet J., Chevallard C., Farre B., Nehrke G., Campmas E., Stoetzel E., El Hajraoui M.A. , Nespoulet R., 2015. Limpet Shells from the Aterian Level 8 of El Harhoura 2 Cave (Temara, Morocco): Preservation State of Crossed-Foliated Layers. PLoS ONE, Public Library of Science. pp.0137162.