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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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13

## 14 Keywords

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

## 17 Abstract

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

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

## 34 I. Introduction

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

41 Archaeological and ethnographical studies show that coastal hunter-gatherer populations had an  
42 extensive knowledge of their terrestrial as well as maritime environments (Thomson 1939, Yesner  
43 1980, Dupont and Marchand 2016). For the latter, the sea can bring a large diversity of wild resources  
44 such as mammals, fishes, crustaceans, mollusks, seaweed, and lithic materials. The remains of their  
45 activities (*i.e.* their waste products) create deposits rich in critical information that permits  
46 archaeologists to improve the understanding of these maritime populations (Milner et al. 2007,  
47 Claassen 1998). Shell deposits and shell middens are important faunal depositional environments  
48 within archaeological sites, especially during the Mesolithic along the European Atlantic coast  
49 (Gutiérrez-Zugasti et al. 2011).

50 Thus, *via* qualitative and quantitative diversity analyses, archaeomalacological studies give information  
51 about paleoenvironments, human diets or human behavior (Claassen 1998, Bar-Yosef Mayer 2005,  
52 Dupont 2006, Szabo et al. 2014). In this framework, sclerochronology, the study of accretional hard  
53 elements of biological organisms, provides useful insights on the species life traits (*e.g.* rhythm and  
54 variation of growth) as well as on the environment in which individuals grew (Buddemeier 1978, Jones  
55 1983, Bessat and Tabeaud 1998, Schöne 2008, Schaffer et al. 2011, Wanamaker et al. 2011, Gosselin  
56 et al. 2013). Based on mollusk species, sclerochronological studies in archaeological shell middens offer  
57 the possibility to assess their season of collection and thus to deduce periods of coastal settlement  
58 residence, or fishing activity, by human communities. Moreover, complementary chemical analysis  
59 along the mollusk shells growth axis (sclerochemistry) gives additional clues on paleoclimatic and  
60 paleoenvironmental parameters such as sea surface temperature (SST), primary production or salinity  
61 (Andrus 2011). Along the European Atlantic coast more than 240 Mesolithic shell middens have been  
62 reported but evidence of clear residence pattern is still scarce (Dupont 2016). Among those  
63 settlements, the bivalve *Ruditapes decussatus* usually appears in the top three most abundant mollusk

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

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

## 89 II. Materials and Methods

### 90 II.1. Material

#### 91 II.1.1. Species biology

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

96 by environmental parameters such as sea temperature and food availability. *R. decussatus* is an  
97 euryhaline (20-50 ppt, optimum 30 ppt) (Le Treut 1986) and eurythermal (10° C-32° C, optimum 20-  
98 25° C) species (Sobral and Widdows 1997).

## 99 II.1.2. Archaeological site

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

111 Beg-an-Dorchenn shell-midden thickness decreased from more than one meter during the 19<sup>th</sup> century  
112 (Du Chatellier 1881) to 30 cm at the end of the 20<sup>th</sup> due to erosion (Figure 1. C). A supplementary  
113 sample excavation was performed in 2001 with an interdisciplinary focus in mind (Dupont et al. 2010)  
114 (see details in supplementary material 1). The aspect of the shell-midden is a black organic layer (Figure  
115 2. A-B). It was composed of marine resources (shells, crabs, fishes, birds and mammals), terrestrial  
116 remains (birds, mammals, charcoals) and flints. A total of 26 kg of shells have been obtained from the  
117 sieved sample of 2001. The total minimum number of individuals (MNI) has been evaluated to 13,324  
118 individuals of shells. 6.6% of them are *R. decussatus*. Radiocarbon dating attributes this Late Mesolithic  
119 occupation from the middle to the end of the sixth millennium BC (Marchand et al. 2009). Among the  
120 50 fragments of the most complete carpet shells coming from Beg-an-Dorchenn, two very well-  
121 preserved valves of *Ruditapes decussatus* have been chosen for sclerochronological and biogeochemical  
122 analysis: a left one (BAD-5 from level 7) and a right one (BAD-18 from level 5)

## 123 II.2. Methods

### 124 II.2.1. Preparation of shell cross section

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

128 are made perpendicular to the growth lines with a high concentration diamond-coated 0.43 mm metal  
129 blade (430CA Ø 152 mm) mounted on a low speed precision saw (Accutom 50, Struers®, 2700 rpm,  
130 feed speed 0.075 mm/s). Two consecutive embedded thin sections of 1.1 mm thickness are obtained  
131 and glued with Araldite on a sand blast glass slide. Next, the thickness and planeness of the thin section  
132 is adjusted to obtain a planar and smooth surface. This step enables growth structure observation of  
133 the shell by polishing the thin section with decreasing size grits of Al<sub>2</sub>O<sub>3</sub> powders (1200, 2400, 4000  
134 grain sizes) then with diamonded paste (Ø 3 and 1 µm) (Grinder-Polisher Tegra Force 5 Struers ©).  
135 Between each grinding/polishing step, thin sections were cleaned by ultrasonic bathing in MilliQ water  
136 (Bioblock Scientific ©). Thus, embedding, cutting and polishing steps leads to a thin section of the shell  
137 containing all its lifespan from birth to death.

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

#### 140 II.2.2. Growth microstructure analysis

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

#### 147 II.2.3. Shell geochemistry

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

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

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

#### 168 II.2.4. Catch seasonality methodology

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

### 176 III. Results

#### 177 III.1. Shell diagenesis and growth line readability

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

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

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

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

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

### 193 III.2.1. From annual to tidal growth marks

194 Depending on the degree of growth line readability, the sclerochronological work took place along  
195 almost all the thin section of the BAD-5 valve (3.3 cm for 1388 cumulated growth increments) while  
196 the BAD-18 valve was studied over the last 1 cm of cumulated growth (657 increments) (Figure 5 B.).  
197 The annual growth marks of each shell were investigated by observing their external surface as well as  
198 the cross sections (Figure 5 A-B). They underline a break, or a drastic slowing, of growth; probably  
199 attributable to the low sea temperature and/or low food availability occurring during winter. Those  
200 annual growth marks are characterized by a V shape notch at the outer shell layer in cross section and  
201 as a groove at the external shell surface. They usually appear during a period of low mean increment  
202 thickness with a slow growth decrease before the notch and a fast increase after the notch (Sato,  
203 1999). Each shell displays a total of 4 breaks following the annual growth marks criteria (N1 to N4 in  
204 Table 2. and Figure 5.). Thus BAD-5 and BAD-18 are around 4 years old with different growth duration  
205 depending on the year.

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

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

### 221 III.3. Oxygen isotopic analysis

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

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

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

249 With  $\delta^{18}\text{O}_{\text{w}} = 0$  ‰ (standard mean ocean water), the reconstructed SST were  $20.80 \pm 1.87$  ° C (min  
250  $16.37$  ° C; max  $24.35$  ° C) and  $21.29$  ° C  $\pm 1.82$  ° C (min  $18.47$  ° C; max  $23.81$  ° C) for BAD-5 and BAD-18  
251 respectively. The reconstructed SST from BAD-5  $\delta^{18}\text{O}_{\text{Shell}}$  were also calculated with  $\delta^{18}\text{O}_{\text{w}}$  “seasonal”  
252 values for warm/high salinity (mean  $20.50$  ° C  $\pm 1.87$  ° C; min  $16.07$  ° C; max  $24.05$  ° C), cold/low salinity  
253 period ( $15.73$  ° C  $\pm 1.87$  ° C; min  $11.30$  ° C; max  $19.28$  ° C) and cold/isolated lowest salinity events ( $10.64$  °  
254 C  $\pm 1.87$  ° C; min  $6.21$  ° C; max  $14.19$  ° C). Thus, from the two archaeological shells, the reconstructed

255 SST for Beg-an-Dorchenn may have roughly been around a mean  $21 \pm 2$  ° C with extrema between 6 °  
256 C (cold/punctual lowest salinity events) and 24° C (warm/high salinity event). All these values are  
257 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  
258 to 23.6° C (Mean 18.4° C) currently recorded at Loch River, 100 km East of Beg-an-Dorchenn, (Poulain  
259 2010, Poulain et al. 2011).

#### 260 III.4. Catch seasonality

261 BAD-5 shows its 4<sup>th</sup> and last annual notch very close to the ventral margin (11 days). On the other hand,  
262 the BAD-18 shell shows 6 weeks of growth from the 4<sup>th</sup> annual break to the ventral margin. Thus BAD-  
263 5 and BAD-18 shells were thus probably collected at the early spring period during the restart of growth  
264 after winter growth break/reduction. We also estimated the season of collection with the quartile  
265 method by comparing the  $\delta^{18}\text{O}$  variation over the course of a year of growth and the  $\delta^{18}\text{O}$  at the  
266 outermost growing edge of the shells (Table 3). The upper quartile (>75%) of  $\delta^{18}\text{O}$  values reflects the  
267 lowest SST during winter ( $> -0.015$  ‰; max 0.680 ‰; (N=30) and  $> 0.239$  ‰; max 0.547 ‰; (N=18) for  
268 BAD-5 and BAD-18 respectively). The last weeks of growth of BAD-5 and BAD-18 shells displayed  $\delta^{18}\text{O}$   
269 values close to the lower limit of the upper quartile (0.189 ‰ (N=1) and 0.303 ‰ (N=2) respectively).  
270 Those stable isotope samples encompass a period of several weeks because of low growth rates. Thus,  
271 although those isotope data show less temporal precision than the increment data, the sclerochemical  
272 study also suggests a catch during early spring. This period of human occupation and mollusks feeding  
273 are in accordance with seasonality from other zooarcheological remains of Beg-an-Dorchenn site such  
274 as fishes and crabs (Dupont et al. 2010).

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

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

## 291 IV. Discussion

### 292 IV.1. Shell diagenesis and increment readability

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

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

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

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

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

317

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 ( $\pm 1$  month) is similar to that of modern *Ruditapes philippinarum*  
321 specimens of the same region (Loch River distant of  $\pm 100$  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 4<sup>th</sup> 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 3<sup>rd</sup> and 4<sup>th</sup> 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

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

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

## 360 V. Conclusion

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

364 This paper was firstly an attempt to adapt sclerochronological methods to archeological materials and  
365 eventually use it routinely in further studies. Cross sectioned shells display very clear growth structures  
366 allowing us to assess a growth rhythm of 2 increments per lunar day and to characterize daily growth  
367 increments as well as winter annual growth breaks. The daily growth thickness and the annual growth  
368 duration, are both ontogenetically and environmentally dependent. Sclerochronological and  
369 sclerochemical results show that the *R. decussatus* studied shells were collected at early spring. Intra  
370 and inter species analyses will help to strengthen the present results and to facilitate future  
371 comparisons with other shell middens studied along European Atlantic coasts. We recommend to  
372 preferentially select *R. decussatus* specimens of 3-4 years old when the number of growing days per  
373 year is the largest. Moreover, additional chemical analysis (*e.g.* mapping) of growth dependent  
374 elements such as Manganese, Magnesium, Strontium or Barium could help in the interpretation of  
375 growth marks and temporal frameworks (Langlet et al. 2006, Poulain et al. 2015, Hausmann et al.  
376 2019.).

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

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

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

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

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## 409 **VII. Author contributions**

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

414 **VIII. Declaration of interest**

415 Declarations of interest: none.

416 **VIII. References**

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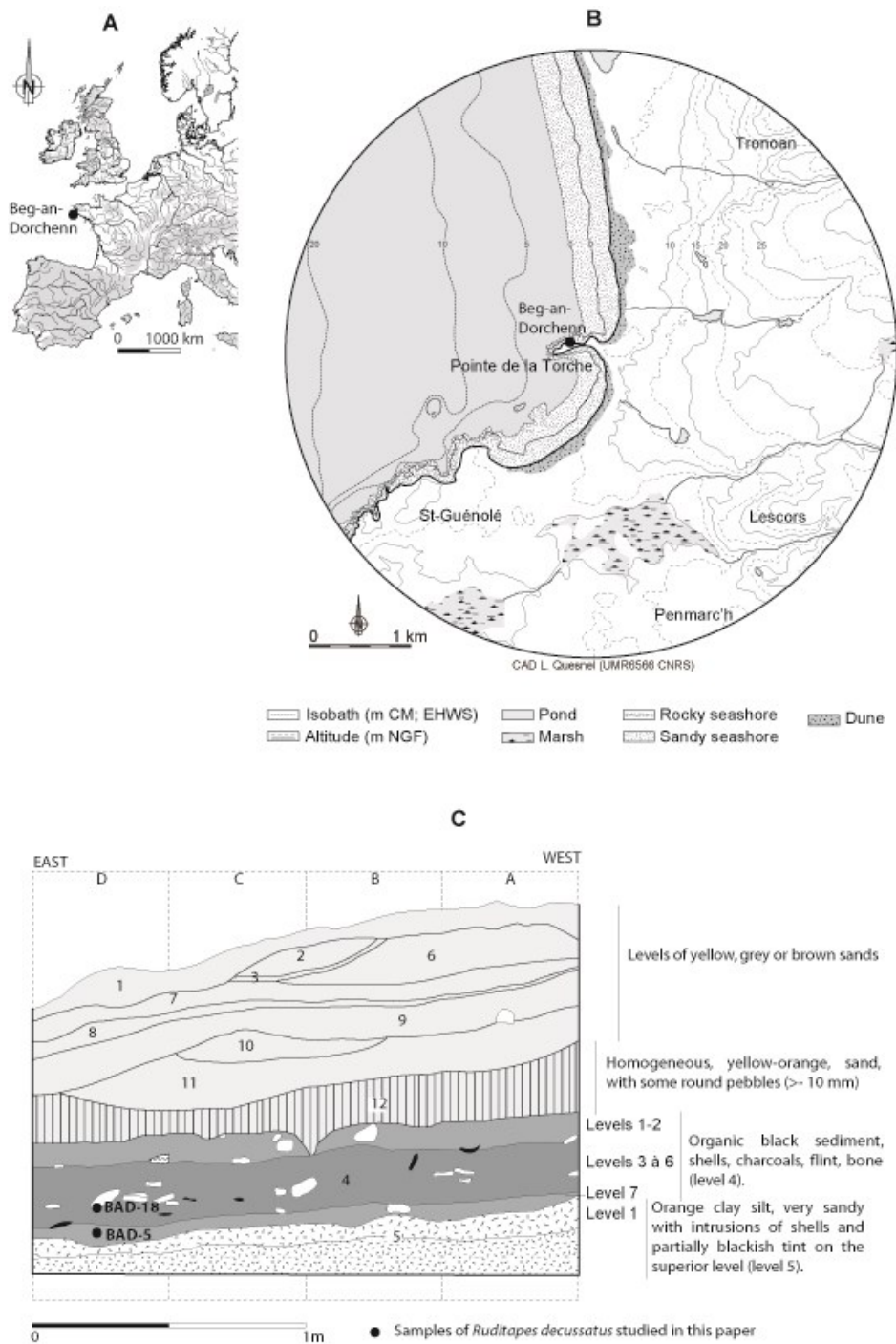
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556

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

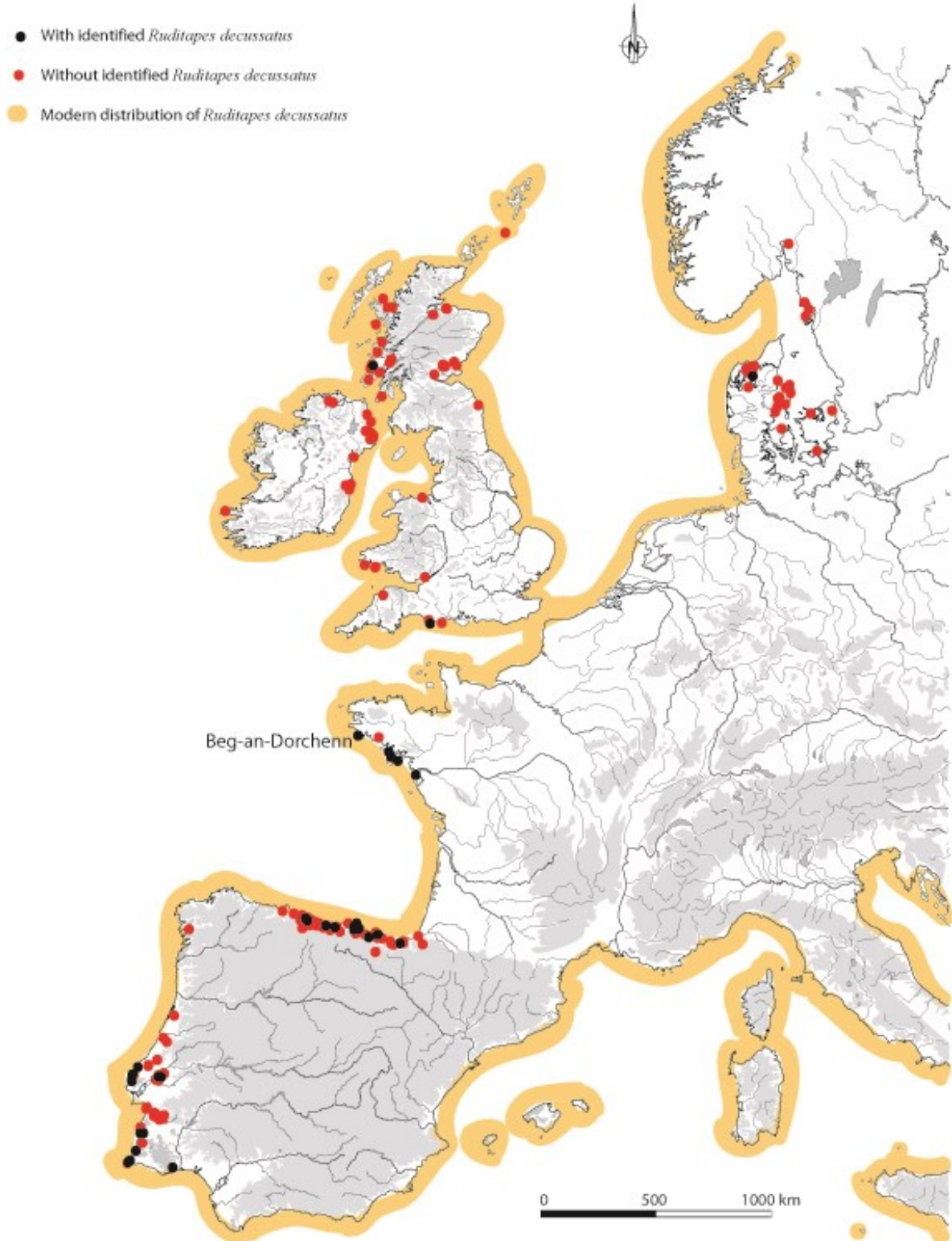


560

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

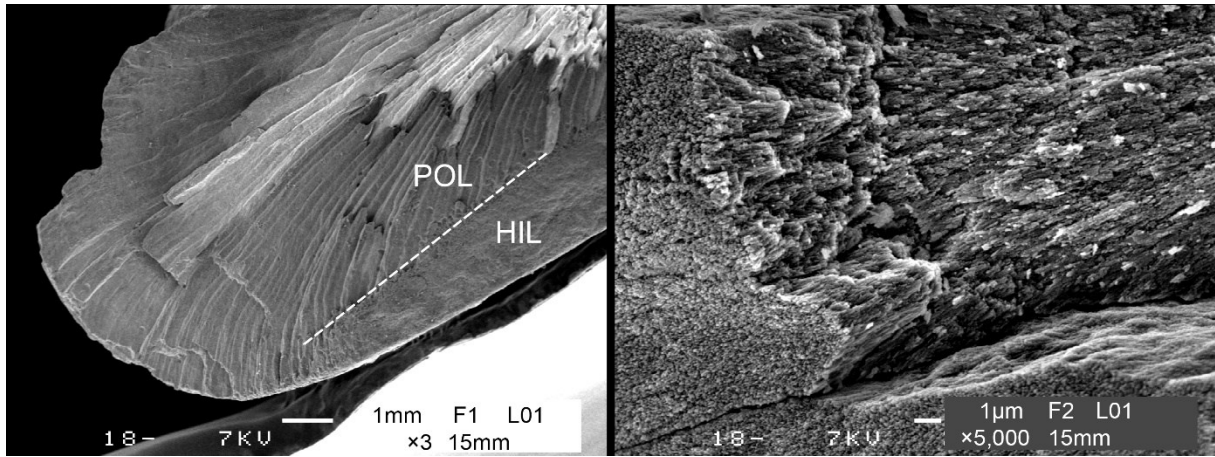
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### Mesolithic shell-middens of the European Atlantic façade



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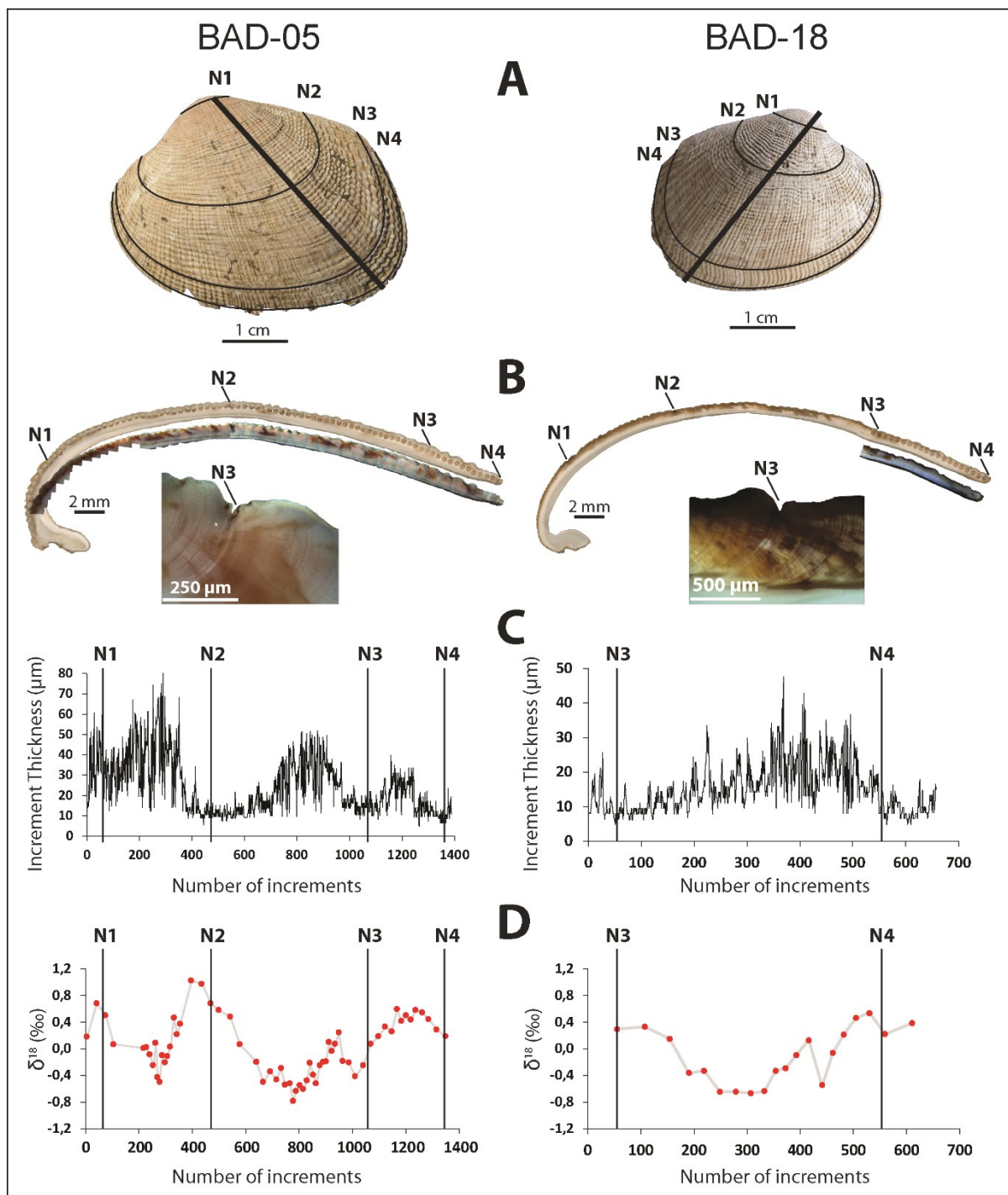
565 Figure 3: Map of the Mesolithic shell-middens along the European Atlantic coast with the modern and  
566 archaeological presence of *Ruditapes decussatus* (Data 2017, unpublished C. Dupont).



567

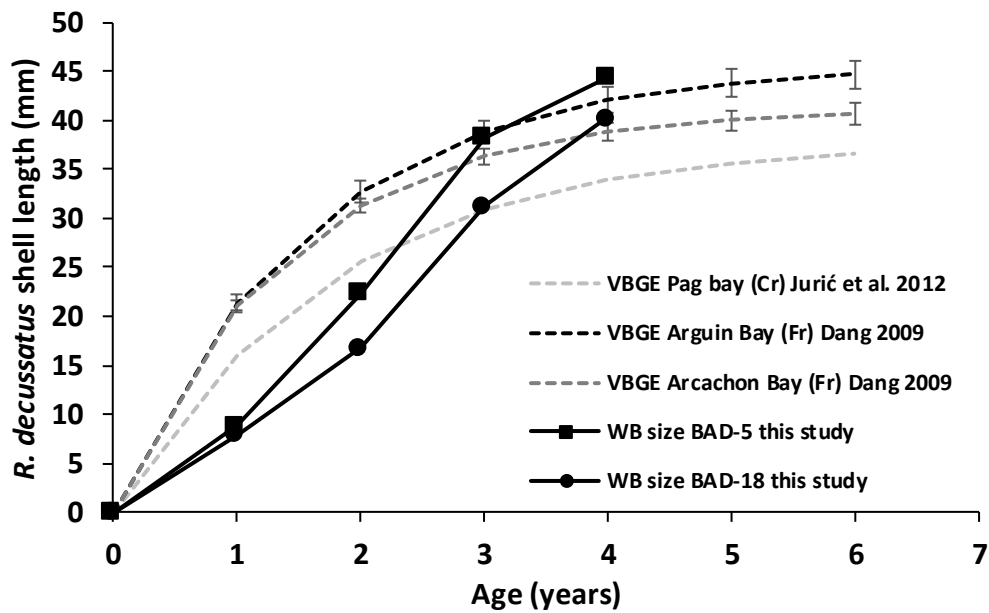
568 Figure 4: SEM images of shell samples (BAD-18) manually broken in order to observe aragonitic  
569 structures along the longitudinal and transversal growth axis. Left image: Calcium carbonate crystals  
570 show a homogeneous inner layer (HIL) and a prismatic outer layer (POL). Right image: detail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

574



576

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



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 ( $\mu\text{m}$ ) 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

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

	N $\delta^{18}\text{O}_{\text{Shell}}$	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

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603 Table 3: Quartile method to assess the season of shellfish collection through the study of the annual  
 604 range of the  $\delta^{18}\text{O}_{\text{Shell}}$  (‰). 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.

	<b>Quartile ranges</b>	<b>BAD-5 <math>\delta^{18}\text{O}</math> (‰)</b>	<b>BAD-18 <math>\delta^{18}\text{O}</math> (‰)</b>	<b>Inferred season</b>
<b>Quartile Model</b>	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
<b>Time of collection</b>	Last weeks of growth	0.189	0.303	Late Winter / Early Spring

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1 Supplementary material 1 : Detailed methodology

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

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

14 SP1.2. Shell structural analysis SEM and Raman

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

21 Shell structural concerns have been observed by Scanning electron microscope and chemical analysis  
22 by Raman spectrometry. (JSM-6310F Scanning microscope JEOL) from a shell sample metallized with a  
23 20/80 Palladium/Gold coat. Chemical analysis of the calcium carbonate form was provided by Raman  
24 spectroscopy (T64000 Jobin-Yvon-Horiba) following the same analysis performed in manila clams,  
25 *Ruditapes philippinarum* (Trinkler et al. 2011). It is a non-destructive technic of investigation using an  
26 Argon-Krypton laser at a micrometer level and based on the shift of its energy when interacting with  
27 molecular vibrations of the studied material (hereby the mollusk shell).

28 Additional references from supplementary material

29 Nouet J., Chevillard C., Farre B., Nehrke G., Campmas E., Stoetzel E., El Hajraoui M.A. , Nespoulet R.,  
30 2015. Limpet Shells from the Aterian Level 8 of El Harhoura 2 Cave (Temara, Morocco): Preservation  
31 State of Crossed-Foliated Layers. PLoS ONE, Public Library of Science. pp.0137162.