

# 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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- 1 Sclerochronological and geochemical study of the carpet shell *Ruditapes decussatus* in archaeological
- 2 contexts: a potential tool for season of collection and coastal paleo-temperature
- 3
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#### 14 Keywords

15 Ruditapes decussatus, Bivalve Mollusk, Shell Midden, Sclerochronology, Sclerochemistry, Seasonality,

16 Paleo-temperature, Mesolithic, European Atlantic coast

#### 17 Abstract

Specimens of the carpet shell Ruditapes decussatus from the Mesolithic shell midden (6<sup>th</sup> millennium 18 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 potential sea temperature data from oxygen isotopes values ( $\delta^{18}O_{shell}$ ).  $\delta^{18}O_{shell}$  shows seasonal 25 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

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).

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 along the mollusk shells growth axis (sclerochemistry) gives additional clues on paleoclimatic and 59 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 have typically employed only one method of investigation (sclerochronology or geochemical analysis) 68 (Mannino et al. 2003, Deith 1983, Brock and Bouget 1989, Milner 2005). Here, we jointly apply 69 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  $\delta^{18}$ O values of sea water ( $\delta^{18}$ O<sub>w</sub>) 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 archaeologic 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.

This paper aims to transfer knowledge gained from studying modern shells of R. philippinarum to 81 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 archaeological shells to 1) deal with potential diagenesis issues, 2) characterize increment readability 84 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 identify the seasonality of shellfish collections and discuss the paleoenvironmental reconstruction 87 88 process.

#### 89 II. Materials and Methods

90 II.1. Material

91 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 2025° 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.

Beg-an-Dorchenn shell-midden thickness decreased from more than one meter during the 19<sup>th</sup> century 111 112 (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 sclerochronolgical 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

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

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 is adjusted to obtain a planar and smooth surface. This step enables growth structure observation of 132 the shell by polishing the thin section with decreasing size grits of Al<sub>2</sub>O<sub>3</sub> powders (1200, 2400, 4000 133 134 grain sizes) then with diamonded paste ( $\emptyset$  3 and 1  $\mu$ m) (Grinder-Polisher Tegra Force 5 Struers  $\mathbb{C}$ ). 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.

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

140 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.

147 II.2.3. Shell geochemistry

Oxygen isotope analyses in mollusk shells ( $\delta^{18}O_{Shell}$ ) reflect sea surface temperature (SST) and  $\delta^{18}O_{Shell}$ seawater ( $\delta^{18}O_w$ ) variations (Urey et al. 1951).  $\delta^{18}O$  is a measure of the ratio of <sup>18</sup>O to <sup>16</sup>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 160 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 161 accuracy (1 $\sigma$ ) of 0.07 ‰.  $\delta^{18}$ O are expressed relative to the Vienna Pee Dee Belemnite international 162 standard (VPDB) and given as per mil (‰). In the absence of a specific paleotemperature 163 164 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. 165 166 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). 167

168 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).

#### 176 III. Results

#### 177 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<sup>-1</sup> and 1085 cm<sup>-1</sup>). 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<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 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).

#### 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.

Indeed, while BAD-5 and BAD-18 exhibit a common increment thickness range between 5 and 80  $\mu$ m, their distributions (24.0 ± 13.4  $\mu$ m and 15.1 ± 7.0  $\mu$ m respectively) are significantly different (Wilcoxon-Mann-Withney test U = 2.673x10<sup>5</sup>; *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  $\mu$ m and 15.1 ± 7.0  $\mu$ m respectively; Wilcoxon-Mann-Withney test U = 1.184x10<sup>5</sup>; *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).

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 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> 216 217 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  $\delta^{18}O_{\text{Shell}}$  mean sample values are statistically similar in BAD-5 and BAD-18 (Wilcoxon-Mann-Withney 223 test U = 538; P= 0.33) even if BAD-5  $\delta^{18}O_{\text{shell}}$  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 2<sup>nd</sup> and 3<sup>rd</sup> 224 years of incremental growth are negatively correlated with the related  $\delta^{18}O_{Shell}$  values while those 225 226 parameters are positively correlated during the 4<sup>th</sup> year. In fact, a small but significant negative correlation is observed between  $\delta^{18}O_{Shell}$  values and growth increment thickness (Spearman 227 228 correlation test, P < 0.001,  $R^2_{BAD-5} = 0.115$ ,  $R^2_{BAD-18} = 0.144$ ). 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  $\delta^{18}$ O<sub>w</sub> is correlated to the salinity (Epstein and Mayeda 1953, Chauvaud 231 et al. 2005).

232 The annual growth marks of each shell are interpretated as negative environmental impacts occurring 233 during winter. During those breaks/slowing of growth, the corresponding  $\delta^{18}O_{\text{shell}}$  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  $\delta^{18}$ O datasets of different bioaragonitic marine invertebrates [SST = 20.0 - 4.42 x ( $\delta^{18}$ O<sub>shell</sub> – ( $\delta^{18}$ O<sub>w</sub> – 0.2 ‰)] with the sea surface temperature expressed in ° C (SST), the  $\delta^{18}$ O values in ‰ for the 237 238 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 239 240 equivalent to a 4.42° C variation in SST and the spectrometer accuracy of 0.07 ‰ is equivalent to 0.31° 241 C.

In order to account for the seasonal variability of salinity on the  $\delta^{18}$ O values of sea water ( $\delta^{18}$ O<sub>w</sub>), we used the following equation described by Poulain (2010):  $\delta^{18}$ O<sub>w</sub> = 0.144 x Salinity – 4.707 (N=35, R<sup>2</sup>=0.97, P<sub>V</sub> < 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<sub>w</sub> 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  $\delta^{18}O_{\text{shell}}$  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).

260 III.4. Catch seasonality

BAD-5 shows its 4<sup>th</sup> and last annual notch very close to the ventral margin (11 days). On the other hand, 261 the BAD-18 shell shows 6 weeks of growth from the 4<sup>th</sup> annual break to the ventral margin. Thus BAD-262 263 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  $\delta^{18}$ O variation over the course of a year of growth and the  $\delta^{18}$ O at the 266 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 267 268 BAD-5 and BAD-18 respectively). The last weeks of growth of BAD-5 and BAD-18 shells displayed  $\delta^{18}$ 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  $\delta^{18}$ 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  $\delta^{18}$ O shell value (highest SST). From this day, a calendar date for each couple increment/isotope sample was 280 generated (forwards and backwards). This work has been applied to the 3<sup>rd</sup> and 4<sup>th</sup> year of growth of 281 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 282 of May and mid-November; the following year (4<sup>th</sup>) the growth starts at mid-march and stops at the 283 284 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

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 estimated to 4 months which, with the duration of growth of the 3<sup>rd</sup> year of BAD-5 (7 months), allow one to assess the complete growth activity pattern of BAD-5 during that year.

#### IV. Discussion

#### 292 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.

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.

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).

However, restart of growth after the winter break is still not easy to determine in archaeological shells as the duration of growth break is age-dependent and can also vary from year to year due to energy reserves kept from the previous year (Chauvaud et al. 2012). That could be particularly the case during the third year of growth of BAD-5 with a growing season of ten months suggesting that some extra energy from the second year may have been used at the beginning of the third years and that a higher part of the third year energy reserved may have been redirected for shell growth activity leading to a 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 growth rate later catching up with the modern ones during the 3<sup>rd</sup> and 4<sup>th</sup> years. 341

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

The latitudinal factor acts as a driver of growth heterogeneity in bivalves (Sato 1999). Indeed Chauvaud 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.

#### 360 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 paleotemperature 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.).

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-gathererfishers 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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#### 409 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.

#### 414 VIII. Declaration of interest

415 Declarations of interest: none.

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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).



А

В

С

560

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).



Figure 3: Map of the Mesolithic shell-middens along the European Atlantic coast with the modern and 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  $\mu$ m). C. Growth increment thickness variation. D.  $\delta^{18}O_{shell}$  isotopic values (Red spots). Annual notches (N1-N4 and vertical solid lines).



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

- 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
595 BAD-1	8 657	15.07	7.03	4.8	47.5	5.98	11.9	21.55	27.51	27.72

598 Table 2:  $\delta^{18}O_{\text{Shell}}$  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	
600	BAD-18	20	-0.09	0.41	-0.66	0.55	-0.08	

- Table 3: Quartile method to assess the season of shellfish collection through the study of the annual
- for a 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 δ <sup>18</sup> Ο (‰)	BAD-18 δ <sup>18</sup> Ο (‰)	Inferred season	
	Maximun value	0.68	0.547	winter	
Quartila	75th percentile	-0.015	0.238	winter	
Quartile	Median	-0.25	-0.19	Autumn/Spring	
woder	25th percentile	-0.505	-0.573	Summer	
	Minimun value	-0.78	-0.663	Summer	
Time of	Last wooks of growth	0 190	0 202	Late Winter /	
collection	Last weeks of growth	0.189	0.303	Early Spring	

- 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

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).
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