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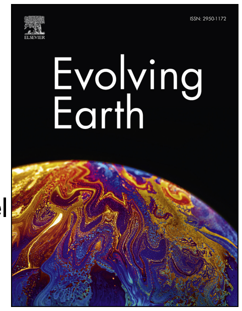

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Emma Samin[1], Bruno Malaizé[1], Émilie P. Dassié[1*], Karine Charlier[1], Dominique Genty[1], Patricia Richard[2], Johan Vieira[3], Magalie Baudrimont[1]

1. Environnements et Paléoenvironnements Océaniques et Continentaux (EPOC), Univ. Bordeaux, CNRS, Bordeaux INP, EPOC, UMR 5805, F-33600 Pessac, France

2. Laboratoire des Sciences du Climat et de l'Environnement (LSCE/IPSL), CEA-CNRS UMR 8212-UVSQ, Université Paris-Saclay, 91191 Gif-sur-Yvette Cedex, France

3. Centre pour l'Aquaculture, la Pêche et l'Environnement de Nouvelle Aquitaine (CAPENA), 33470 Gujan-Mestras, France

* Corresponding author: emilie.dassie@u-bordeaux.fr

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Abstract

The aragonitic shells of freshwater pearl mussels (*Margaritifera margaritifera*) contain annual growth increments, whose composition reflect the geochemistry of the river water and bivalve metabolism. The wide geographic distribution and the long lifespan of *M. margaritifera* coupled with a previously established relationship between the $\delta^{18}O$ values of their shells and river temperature means this taxon is a potentially important environmental archive; such freshwater proxies are currently limited in both space and time. In this paper, we investigate the relationship between $\delta^{18}O$ values and both *in situ* and modeled river temperature (2007 to 2015) for a population of *M. margaritifera* living in the Haute-Dronne River (southwest France). Our $\delta^{18}O$ data permit the reconstruction of seasonal temperature variations in the river. Sclerochronology reveals that shells have also record seasonal patterns and produce winter growth increments, contrary to other investigations carried out on the same mussel species from northern Europe where low winter temperatures (below 5°C) interrupt shell growth. The presented calibration for *M. margaritifera* and host river temperature offers the potential for reconstructing palaeoenvironmental conditions based on fossil specimens of the same species. Such

reconstructions may improve our understanding of past continental climate and help calibrate regional palaeoclimate models.

1. Introduction

Recent climate changes are observed worldwide and request the interest of a large part of the scientific community. Regional expressions of the observed global warming could lead to surprising discrepancies regarding amplitudes and rate of changes. The paleoclimate community benefits from multiple proxies extracted from different records coming from both oceanic and continental areas. However, the limited number of continental proxies, in both space and time, encourages paleoclimatologists to find new local archives to better constrain the regional-scale variability.

The freshwater pearl mussel *Margaritifera margaritifera* (Unionida) has been a subject of interest over the last few decades as an indicator of the regional environmental conditions. *M. margaritifera* lives in the northern hemisphere, particularly in western and central Europe, from the Iberian Peninsula to Scandinavia. Under ideal living conditions, these mussels can reach an impressive lifespan, from a few decades in Southern Europe to a nearly one hundred years in Scandinavia (Bauer, 1992; Mutvei and Westermark, 2001; Schöne et al., 2004) making them a good paleoenvironmental archive.

M. margaritifera is classified as an "endangered" species on a global scale, and a "critically endangered" species on a European scale by the International Union for Conservation of Nature (IUCN). The global population of *M. margaritifera* has suffer a 90% loss during the 20th century. This loss can be linked to both direct and indirect anthropogenic impacts, such as habitat degradation, pearl harvesting, and the depletion of host fish populations (Bertucci et al., 2017, Baudrimont et al., 2020, Life Haute-Dronne, 2021, Baudrimont, 2022). This species is very sensitive to its environment and requires specific conditions such as shallow rivers with high flow velocities, cobble and gravel substrate, low nutrient concentrations, and the presence of *Salmo trutta fario*, as its gills house larvae and allow to disperse juveniles (Life final report, 2021, Bertucci et al., 2017).

The aragonitic shell of these mussels present annual growths increments, visible in both shell surface and cross-secions. A growth slowdown or cessation is marked by the formation of darker layers (Jones and Quitmyer, 1996) or thin opaque and organic scleroprotein-rich ridges (Geist et al., 2005). The darker increments generally correspond, in most bivalve species, to winter shell accretion. Jones and Quitmyer (1996) show, however significant latitudinal differences in the main accretion period, sometimes even within the same species. In the majority of the studies, darker increments form when river water temperature falls below a critical value during winter (designated as winter lines), sometimes corresponding to an interruption in the shell growth. These disturbance lines are due to the stress of the organism in its environment (Jones, 1983, Dunca et al., 2011, Dunca, 2014). Winter lines make it possible to establish a sclerochronology for each individual and then determine their ages (Mutvei and Westermark, 2001; Dunca et al., 2005; Helama et al., 2006). For *M. margaritifera*, the shell growth interrupts when river temperature falls below 5°C, forming these denser and more resistant lines, which delimit two summer growth increments (Dunca, 2014).

Various authors consider that water quality, pH, pollution, and nutrient availability could also influence growth rates (Dunca et al., 2005; Dunca et al., 2011; Dunca, 2014). Helema et al. (2008) also note an imprint of the lunar cycle on micro-growth patterns. However, specific dynamics of each population make inter-comparisons complex (Dunca et al., 2011; Schöne et al., 2004). Besides those controversial factors, the temperature appears to have an undeniable effect on the shell growth, as shown by the distinction between winter lines and summer growth increments in marine and freshwater bivalve shells (Dettman et al., 1998; Goodwin et al., 2001; Schöne et al., 2004; Dunca et al., 2005; Dunca et al., 2011; Verdegaal et al., 2005; Versteegh et al., 2010a; Helama et al., 2014). In their study, Dunca et al. (2005) showed that summer temperature variability can explain 35 % of annual growth variations. Schöne et al. (2004) published a growth-temperature model deduced from increments thickness and has demonstrated a similarity with paleo-temperatures deduced from regional dendrochronology studies. Shell growth rate is therefore a valuable tool for reconstructing the river water paleo-temperatures.

Furthermore, bivalves build their shell by drawing chemical elements that could be used as proxy of environmental variability. Water $\delta^{18}O$ is known to be a good proxy of sources and amount of precipitations. The isotopic signature of rainfall depends on the isotopic composition of the oceanic evaporation area, and the distance between precipitation location and hydrological sources (Dansgaard, 1964). Moreover, the fractionation between water and aragonite, depends on various factors such as temperature (Grossman and Ku, 1985; Dettman et al., 1998) and precipitation regime (Demény et al., 2012, Davis and Muehlenbachs, 2001). Recorded in carbonate shells, oxygen isotope ratio become a valuable tool for paleoclimate reconstructions. Goodwin et al. (2001) and Izumida et al. (2011) have thus conducted river paleo-temperatures reconstructions from bivalves' shells $\delta^{18}O$. Pfister et al. (2018) and Schöne et al. (2020) have reconstructed rivers' $\delta^{18}O$ signal using both shells' isotopic signature of this species and river temperature data. The aim of this study is to reconstruct river temperature using both river and shells' isotopic signature .

France counts around a hundred thousand of *M. margaritifera* individuals, with the largest population located in the Haute-Dronne River, Dordogne (SW of France, Figure 1) and reaching 15,000 specimens according to a count carried out in 2003 by Patrice Cholet for the *Association Patrimoine Halieutique Limousin Périgord*. The population of mussels living in the Haute-Dronne River thus represents 15% of the national population; the species has disappeared from 60% of France's rivers in which it has been identified at the beginning of the 20th century (Life Haute-Dronne, 2021). The Haute-Dronne River is qualified as a remarkable area in France for the conservation of the species by the 'Museum National d'Histoire Naturelle'. Thus, regarding the various hazards and ecological pressures on the species, the population of Haute-Dronne River has been chosen for a special European Life + Nature program (2014 to 2021), to ensure the production and protection of this species (Life final report, 2021). This Life + Nature program (2014-2021) aimed to rehabilitate the quality of the Haute-Dronne River to offer adequate habitats to the population of pearl mussels and its host fish. During this project, territorial planning has been developed to restore river natural stream. The *Salmo trutta fario* was granted protected status, and an aquaculture farm has been created to support the population. In addition, exceptional authorizations have been delivered to collect several specimens for shell and soft tissue analyses to understand the interaction with the environment and the impact of potential pollution (Bertucci et al., 2017, Baudrimont et al., 2020). The present study aims to re-use the collected shells, given that specimens from this population are difficult to obtain due to their protected status, and to exploit the potential of the species as paleo-marker. Indeed, shells of *M. margaritifera* are renowned to be good archives due to (i) their long lifespan (ii) their quantifiable growth increments acting as mussel's life chronology (iii) their ability to capture chemical elements from surrounding water, especially oxygen isotopes whose proportion varies with seasonality, and (iv) their sentinel species status, testifying to sustainable environmental conditions in the river during their life.

Shells collected as part of this project gave us the rare opportunity to conduct isotopic analyses on this population. This study aims to evaluate the relationship between *M. margaritifera* shell $\delta^{18}O$ signature and Haute-Dronne River temperature variations. This is a preliminary study including available regional environmental data to assess the possibility of reconstructing the river temperature evolution through

M. margaritifera shells. The aim is to compare water temperature computed from aragonite oxygen isotope fractionation with *in situ* and modeled river temperatures. Results from this study could motivate further research with additional environmental data from river monitoring.

2. Material and methods

2.1 Study area and instrumental datasets

Empty shells have been collected on the riverbanks of the Haute-Dronne River in September 2015, near the village of Saint-Saud Lacoussière, located in the northern part of Dordogne (SW-France, Figure 1), authorization of sampling given by the DIREN (French Ministry of the Environment, Agreement N°39/2016 in 2008). The area, located 200km from the Atlantic Ocean, is influenced by temperate marine climate and Atlantic fluxes, with mild winters (average January-Frebruary-March = 6.5°C for 1984-2007) and relatively cool summers (July-August-September = 18.7°C) (Genty, 2008).

Over the last decades, the area climate parameters such as the air temperature (T_{air}), the amount of precipitation (accuracy ± 0.2mm), the rainfall isotopic signal ($\delta^{18}O_{precip.}$, measured using CO_2 equilibrium on a Finnigan MAT 252, with an analytical error of ± 0.05 ‰), and the river temperature were monitored (supp. mat. Table 1). For precipitation, both rainfall amount and isotopic signal have been measured monthly since 1998, with rain gauges above the Villars cave, located twelve kilometres south of the shell collection area (Figure 1). These data have been collected as part of other studies in the area conducted on Villars cave's speleothems, renowned to be reference archives of regional paleoclimate (Genty, 2008; Genty et al., 2014).

Local river temperatures have been measured punctually from 2006 to 2019 by the *Laboratoire d'Analyse et de Recherche de Dordogne (LDAR24)* but unfortunately without a regular sampling interval. Therefore, there is no monthly record available for the river temperature. However, a monthly air temperature record since 1950 exists for the area, provided by the European Environment Agency (E-OBS gridded dataset, https://surfobs.climate.copernicus.eu/dataaccess/access_eobs.php, last access spring 2023). By comparing river and air temperature datasets, we found a significant linear correlation ($r^2 = 0.81$, p-value<0.01) between both signals (Figure 2). We therefore decided to derive river temperatures from air temperatures and create a monthly modelled river temperature dataset (T_{Dronne}) (supp. mat. Table 2). For the purpose of our study, we have arbitrarily created seasonally resolved datasets for which seasons are defined as December-January-February interval for winter, March-April-May interval for spring, June-July-August interval for summer, and September-October-November interval for autumn.

2.2 Shells collection and preparation

Four *M. margaritifera* shells have been selected for our study, referenced as S144, S185, S187, and S194. Given their position and preservation state, the mussels were dead a few weeks before being collected, probably killed by a predator (nutria, Pichon, 2017).

The shells were embedded at the EPOC Laboratory (Environnements et Paléoenvironnements Océaniques et Continentaux, UMR5805, University of Bordeaux, France) in Epoxy resin Araldite 2020 as recommended by Schöne et al. (2017) to avoid geochemical contamination between carbonates and Epoxy. Three-to-four-millimetre width sections were cut, cross-sectioning the shell in the direction of minimum length, from the umbo (juvenile material) to the ventral margin (recent material) (Figure 3). Shells' cross-section pictures have been taken using a stereo microscope Kern OZO553 coupled with a camera Kern ODC825 supported by Microscope VIS© software.

2.3 Micro-drilling and oxygen stable isotope analyses

Shell sampling has been carried out at LSCE laboratory (Laboratoire des Sciences du Climat et de l'Environnement, UMR8212, Gif-sur-Yvette, France) using a Micromill PXC14 (New wave Research, with Komet drills H99 104 008) coupled with MEO© software. The furrows have been drilled into the prismatic layer of the shells, following the inclination of the dark and light increments (Figure 4). We configured the device to drill a furrow with a depth of 100 µm, across the whole width of the prismatic layer. The furrow width is estimated around 250 µm from the camera images. Due to the size of the drill bit, we were able to only sample one or two furrows on each increment (two to four values per year), which allowed to reach bi-annual resolution (Figure 4). Between 50 and 150µg of powder have been collected for each furrow.

Powder samples have been analysed by Isotope Ratio Mass Spectrometry (IRMS) at the EPOC laboratory. Samples were dissolved with an acid solution of 103% H_3PO_4 at 90°C in a Kiel IV-carbonate sample preparation device linked to the IRMS. The $\delta^{18}O$ signature of the generated CO_2 gas was analysed in a Mat253 ThermoFisher IRMS. $\delta^{18}O$ values are missing for some increments because we were not able to collect enough powder. The international standard NBS19, referenced to Vienna Pee Dee Belemnite (VPDB) has been analysed in the same conditions as the shells' samples. Over the duration of the shells' analyses, the average standard deviation of the NBS19 was 0.03‰ (N= 23).

2.4 Shell curvature correction

To be able to visually compare growth increments aspect and spatial variation of stable isotopes values, we corrected the shell curvature (Figure 5). We pointed the XY coordinates of each furrow on cross-section pictures with ImageJ© software, to measure segment lengths between nearby points and thus sample spacing. We summed segments to compute the distance of each sample from the ventral margin. For the same purpose, cross-section pictures were adjusted to correct the shell curvature by flattening the prismatic layer along the horizontal axis. These two stages allowed us to directly compare shells pictures and growth increments with isotopic values variation, aligned on a horizontal axis (Figure 5).

3. Results

3.1 Shells' increments and $\delta^{18}O$ variability

The shells' pictures taken with the stereo microscope show dark and light growth increments, probably related to seasonal accretion (Figure 4 and Figure 5). This assumption is supported by the difference between the isotopic values of dark and light increments. As shown in Figure 5, shells' $\delta^{18}O$ signatures present a range of variability similar to each other's, with values ranging between -3.7 and -5.4‰. Maxima occur for dark increments and minima for the light ones. The amplitude of the $\delta^{18}O$ signal reduces towards the ventral margin, especially visible for S144 and S185. Increment widths also decrease throughout mussel life, with larger increments corresponding to the first years of the organism life.

3.2 Seasonal variation

3.2.1 Age Model

To built our age model, we counted annual layers in both the corrected images and isotopic measurement records; the highest $\delta^{18}O$ values corresponding to darker increments, (Figure 5). Assuming that mussels died shortly before being collected in September 2015, the ventral margin is used as a time marker (summer period). Therefore, we used light and dark increments, also corresponding to $\delta^{18}O$ minima and maxima values, as a seasonal signal with a combination of two successive increments (one light with one dark) corresponding to one year of accretion, to count backward from 2015. Using this method, we counted around eight to ten years for each. This age corresponds to a minimum age since there was a loss of the early stages of life due to erosion of the umbo. Once established, those age models allowed us to compare individuals' shell $\delta^{18}O$ records to one another.

While for most of the records, high $\delta^{18}O$ values correspond to dark increments, and low $\delta^{18}O$ values to light increments, some measurements do not fit this pattern. Regarding points closed to increment transitions, we can suspect that the constrain on the sampling resolution may be the source of this discrepancy. However, we cannot assume this hypothesis for points located in the centre of increments, as for the fifth light increment from the ventral margin, with high values comparable to values related to dark increments, and corresponding to summer 2010 in all shells (Figure 5).

3.2.2 Water temperature computation from shells' $\delta^{18}O$ signature

In 1985, Grossman and Ku published an equation describing the fractionation and the incorporation of oxygen isotopes in aragonite deposition, as a function of the temperature and the $\delta^{18}O$ composition of the surrounding water. The equation corrected by Dettman et al. (1998) is as follows (Equation 1):

$$1000 * \ln(\alpha) = 2.559(10^6 * T_{W(K)}^{-2}) + 0.715 \qquad \text{Eq. (1)}$$

Where $T_{W(K)}$ is the river temperature, expressed in Kelvin, and α is the fractionation between water and aragonite, computed as:

$$\alpha_{arag.}^{water} = \frac{1000 + \delta^{18}O_{arag.\ (SMOW)}}{1000 + \delta^{18}O_{water\ (SMOW)}} \qquad \text{Eq. (2)}$$

To convert our isotopic values relative to the Vienna Pee Dee Belemnite (VPDB) reference to values relative to the Vienna Standard Mean Ocean Water (VSMOW), we applied the Eq. (3) from Gonfiantini et al. (1995):

$$\delta^{18}O_{arag.\ (SMOW)} = 1.03091(1000 + \delta^{18}O_{arag.\ (VPDB)}) - 1000 \qquad \text{Eq. (3)}$$

Unfortunately, $\delta^{18}O_{water}$ has never been measured in the river. To conduct our study, we must assume that the $\delta^{18}O_{water}$ of the Haute-Dronne River might be close to the rainfall isotopic composition ($\delta^{18}O_{precip.}$) measured at Villars' cave. We are conscious of the uncertainties linked with this hypothesis, which constitutes a weakness in this study. This work is a preliminary study that use previously collected specimens to look at their potential for environmental reconstructions in the region. Therefore, we can only reconstruct semi quantitative temperature timeseries and we will therefore solely focus our interpretation on temperature variability. Meanwhile we think that this assumption remains realistic, considering the relatively small size of the Haute-Dronne River watershed (Figure 1) and the metamorphic and granitic riverbed which limits water infiltration (D. Genty, per communication). Both factors, added to *M. margaritifera* host rivers characteristics (low depth, high velocities), suppose a fast response time of river runoff to rainfall, with efficient water drainage on the watershed.

Chronology based on the distinction of dark-light increments gives a nearly seasonal resolution for our shells' $\delta^{18}O$ records. Both seasonal shells' $\delta^{18}O$ values and precipitation are implemented into Eq. (2) to compute theoretical river temperatures records (Eq. (1)) deduced from each shell (T_w^{S144}, T_w^{S185}, T_w^{S187}, T_w^{S194}). The average reconstructed temperature record T_w shows a similar range of variability with T_{Dronne} for the 2007-2015 time period (Figure 6).

Figure 6 presents the equation components, with shells' $\delta^{18}O$ (Fig 6a), river's $\delta^{18}O$ derived from $\delta^{18}O_{precip}$ (Fig 6b), and both river-modelled and reconstructed water temperatures from the two previous parameters (Fig 6c). $\delta^{18}O_{precip}$ maxima and shells' $\delta^{18}O$ minima are in phase with temperature maxima.

4. Discussion

4.1 Shells' $\delta^{18}O$ variability

In our study, the succession of light and dark increments, suggested that this population is undergoing continuous shell growth with winter material deposition, contrary to northern populations whose growth interrupts for water coldest temperatures (Mutvei and Westermark, 2001; Schöne et al., 2004b; Schöne et al., 2005; Dunca et al., 2005; Helama et al., 2006; Helama et al., 2008; Dunca et al., 2011; Dunca, 2014; Helama et al., 2014; Pfister et al., 2018; Schöne et al., 2020). Dark increments, with higher $\delta^{18}O$ values, might be related to winter growth. Indeed, previous authors have demonstrated that bivalves' aragonite precipitation near winter lines incorporates less negative $\delta^{18}O$ values than for the constitution of summer growth increments (Goodwin et al., 2001; Schöne et al., 2005; Izumida et al., 2011; Pfister et al., 2018; Schöne et al., 2020). According to *in situ* and modelled data, the river temperature rarely falls below the mussels' minimum growth temperature (5°C according to Dunca, 2014), preserving them from complete growth cessation during this cold season. Furthermore, population monitoring in Portugal, living in relatively warmer waters, also clearly shows continuous growth in winter (Joaquim Reis, University of Lisbon, Marine and Environmental Sciences Centre, per communication). The Haute-Dronne *M. margaritifera* population is therefore capable of recording both summer and winter variability, reinforcing its potential as a climate proxy.

The Haute-Dronne temperatures reconstructed from *M. margaritifera* shells are in good agreement with *in situ* and modelled temperature data. This procedure has already been carried out in other regions on marine (*Chione cortezi*) and freshwater (*Hyriopsis* sp.) aragonitic shells by Goodwin et al. (2001,2003) and Izumida et al. (2011) respectively and has given reliable river paleo-temperature reconstructions. Here we do not pretend to compute extreme river temperature, conscious of the uncertainties linked to our study, (i) the use of average values of water isotopic composition and mixed material for each increment due to furrows width), (ii) the use of river $\delta^{18}O$ values derived from precipitation $\delta^{18}O$. However, we clearly show the possibility of reconstructing the annual river temperature cycle.

Regarding increment widths, the observed narrowing near the ventral margin agrees with previous studies (Hastie et al., 2000; Helama et al., 2008; Dunca et al., 2011; Dunca,2014). The authors presented variable growth curves between populations, all showing significant thinning of increments after the first years of mussel life due to growth slowdown.

In our study, aragonite $\delta^{18}O$ values are higher than what was found in previous studies on *M. margaritifera*. For populations in Sweden, Schöne et al. (2005, 2020) showed values ranging from -9.32 to -13.89‰. For populations in Luxembourg, Pfister et al. (2018) presented values between -6 and -7‰ depending on seasonality. On Unionids bivalves in Rhine and Meuse, Verdegaal et al. (2005) and Versteegh et al. (2010a, 2010b) determined mean values of -9‰ and -6.5‰, respectively. This feature is in accordance with the isotopic gradient of meteoric waters observed over Europe (Langebroek et al., 2011). Moreover, compared to northern sites, the population from the Haute-Dronne River is far away from glacier meltwater influence and under the fluxes of Atlantic moisture with rare solid precipitation (snow) which could explain the observed less negative values (Dansgaard, 1964).

4.2 Water and shells' $\delta^{18}O$ contributions to temperature reconstruction

To determine the weighting of each factor ($\delta^{18}O_{water}$ and shells' $\delta^{18}O$) on the river temperature reconstruction, we first computed theoretical water temperature only from shells' $\delta^{18}O$ values by fixing the $\delta^{18}O_{water}$ to its mean value to negate the influence of its variations. The seasonal amplitude of this newly shells' $\delta^{18}O$-derived river temperature ($T_{w\text{-}arag}$), represents 30% of the total signal amplitude (Figure 7), and is significantly correlated to *in situ* and modelled river temperatures (Pearson linear correlation, $R^2 = 0.62$, p-value = 0.0003). Additionally, $T_{w\text{-}precip}$ determined secondly by fixing shells' $\delta^{18}O$ to its mean value, presents a seasonal amplitude close to the total signal amplitude (80%). Mean T_w, $T_{w\text{-}precip}$, and $T_{w\text{-}arg}$ are not statistically different (p-value = 0.97 with Kruskal-Wallis test).

We can conclude that the shells' $\delta^{18}O$ signature would not be sufficient in itself to reconstruct the full amplitude of T_w, but this proxy provides a valuable information on river mean temperature and seasonality. Shells' $\delta^{18}O$ can therefore be a powerful tool to reconstruct river temperature variability even if reconstructed signal is an attenuated one.

4.3 Remarkable summers

4.3.1 Summer 2010

A plateau is observed for summer 2010 in all selected shells' $\delta^{18}O$ measurements (Figure 6). As the plateau is observed for the four records, we can exclude the hypothesis of mussels' independent behaviour. This event might refer to a local or regional climatic influence. However, no anomaly can be detected neither on both rainfall amount and $\delta^{18}O_{precip.}$ signal (Figures 7 and 6) nor in river *in situ* and modelled temperatures (Figure 6). We suppose that another environmental factor, not related to the river temperature (T_{Dronne}) or $\delta^{18}O_{precip.}$ signal, could be responsible for this anomaly. It would be interesting to complete this study with additional measurements such as trace elements analyses that are known proxies of environmental changes.

The reconstructed temperature T_w for summer 2010 is only slightly lower than mean reconstructed temperature values over the entire length of the record, showing that the unexpectedly high $\delta^{18}O$ values induce only a minor effect on the river temperature amplitude reconstruction. This is certainly due to the limited (around 30%) influence of shells' $\delta^{18}O$ variability, as explained in Section 4.2. Likewise, winter 2009 and 2012 also show the dominance of the $\delta^{18}O_{water}$ factor in reconstructed temperature signal amplitude (Figure 6).

4.3.2 Summer 2011

A large discrepancy can be observed between reconstructed and measured temperature data during summer 2011 (Figure 6). T_w is computed from T_w^{S144}, T_w^{S185}, T_w^{S187}, and T_w^{S194} and a sampling offset repeated on the four shells is unlikely. The observed deviation could be explained by the approximation of the river water $\delta^{18}O$ signal, which we have associated with $\delta^{18}O_{precip}$, due to the lack of data on the river. Nevertheless, regarding the amount of precipitation measured at Villars' cave (Genty, per communication), a significant rainfall deficit is observed in 2011 (Figure 8). A deficit in rainwater supply to the river would logically involve a river $\delta^{18}O$ signal linked with other sources further upstream or underground. Moreover, considering the large weight of this factor in water temperature reconstruction, an incorrect estimation of the water $\delta^{18}O$ value could fully explain the discrepancy. Unfortunately, further data on the river are lacking to test this hypothesis. Complementary shells trace elements analyses might also help constrain this deviation.

5. Conclusion

This study aimed to assess the potential of *M. Margaritifera* shells from the Haute-Dronne River population as a proxy for regional paleo-environmental reconstructions. We show that this population provides an annual record of river conditions, covering each season (winter included) suggesting a nearly continuous growth over the year. Additionally, $\delta^{18}O$ recorded in shells allowed to reconstruct river paleo-temperatures during the organism's life from 2007 to 2015. We use rainfall isotopic d18O composition to derived river d18O values since no measurements of river $\delta^{18}0$ are available for the time interval covered by our shells. While this method seems convincing, it might be interesting to measure the annual variability of this parameter in the river for future studies. Meanwhile this work clearly demonstrates the possibility of reconstructing the seasonal variability of the Haute-Dronne River temperature by using the isotopic signature of *M. margaritifera* shells regarding the clear difference of $\delta^{18}O$ values between dark and light growth increments. According to these promising results, we could now consider conducting a similar study on longer time scales with older or fossilized shells, as some authors have done with fossilized freshwater bivalves shells in other regions to reconstruct precipitations (Davis and Muehlenbachs, 2001, with *Margaritifera facalta*), global environmental changes (Demény et al., 2012, with *Unio* sp.) and river $\delta^{18}O$ signals (Versteegh et al., 2010b, with *Unio* sp.). To fine-tune our study on this population, we could carry out trace elements analyses to better understand river's physicochemical cycles and physiological process. *M. margaritifera* shells are certainly inadequate to fully reconstitute the amplitude of temperature seasonal variations, but still provide a valuable water temperature average and constitute good river archives when no information exists on watercourses. Moreover, these shells can highlight multi decadal trends, recorded throughout the mussels' long life. We could thus consider recovering temperature trends over the last decades with an annual resolution, which is remarkable for continental archives under temperate climate.

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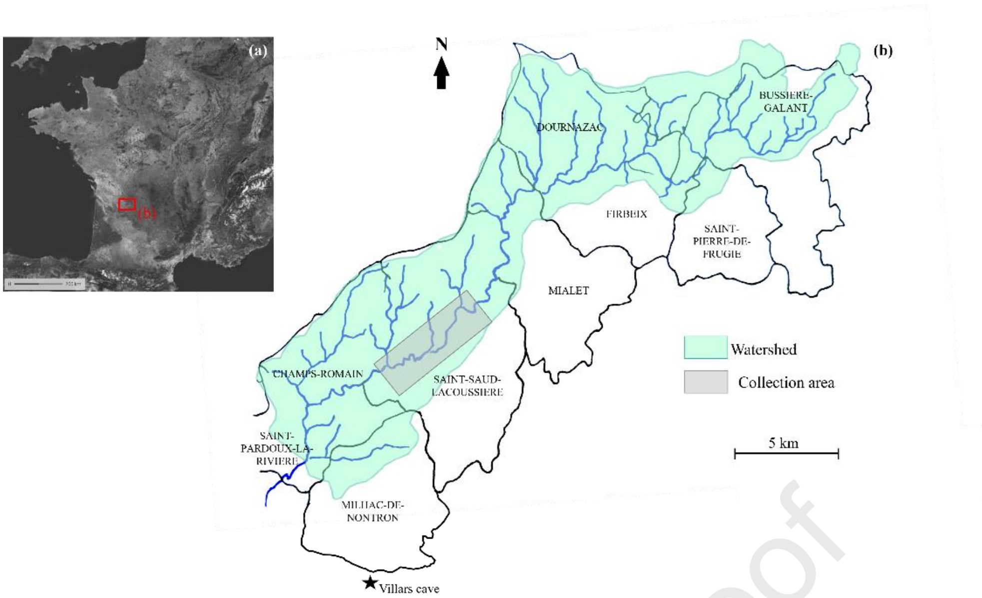
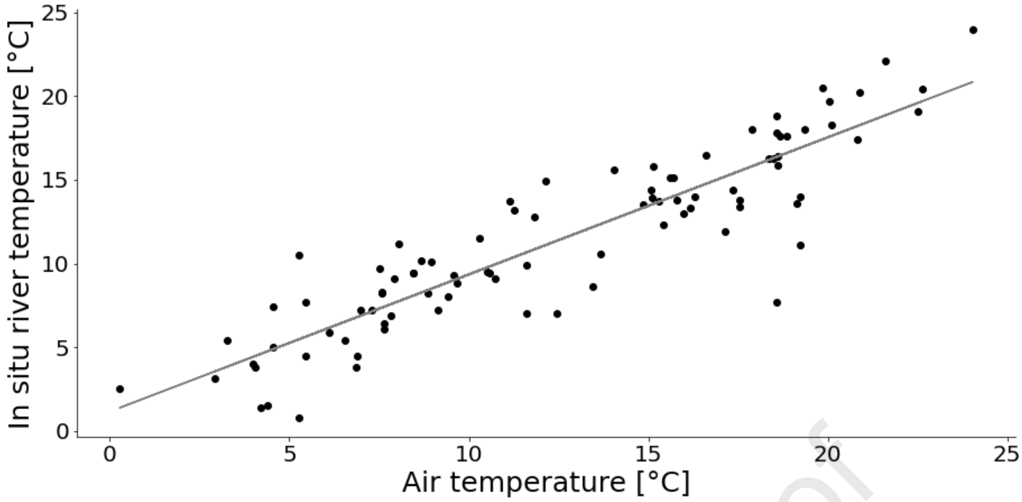


Figure 1 – Maps of the location of the M. margaritifera population (a) Map of France (source: https://www.geoportail.gouv.fr/) with study area location in SW of France (b) Map of the Haute-Dronne river (blue) and corresponding watershed (green) (source: Life final report, modified). Shells have been collected close to the village of Saint-Saud Lacoussière, in the area in grey. The Villars cave where have been collected precipitation data is located a few kilometers southern the collection area.



523 *Figure 2 - Comparison between in situ river temperature measurements (provided by the Laboratoire d'Analyse et de*
524 *Recherche de Dordogne LDAR24) and corresponding air temperature at Villars (provided by the European Environment*
525 *Agency). A linear correlation is found between the two parameters (Pearson correlation, R^2 = 0.81, p-value<0.01).*

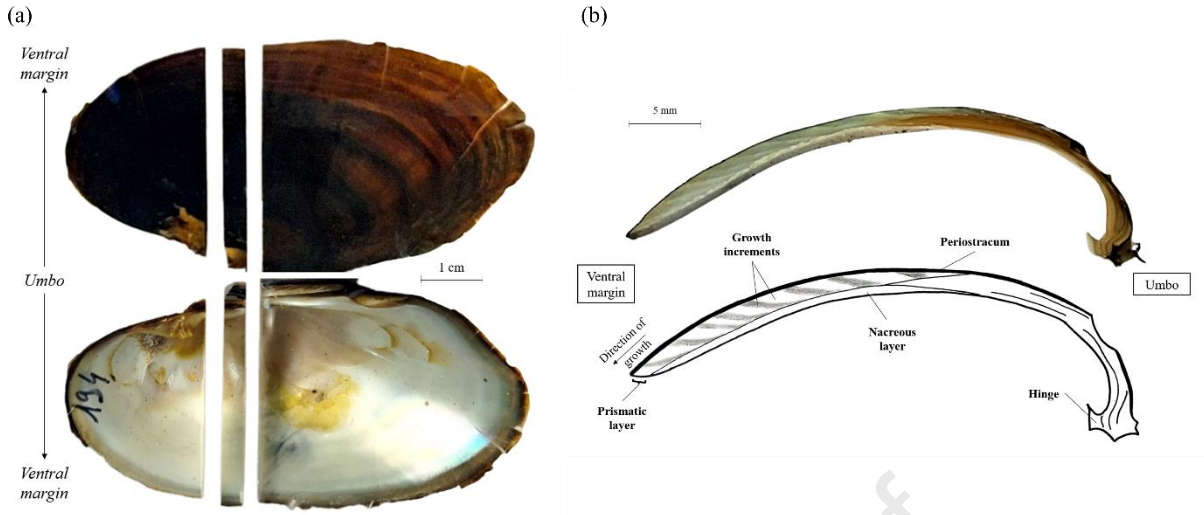


Figure 3 – M. Margaritifera *shells (a) Outer and inner sides of shells, with visible increments on the outer side. Shells cross-sections have been cut from the umbo to the ventral margin, perpendicular to growth increments, in the direction of minimum length. (b) Cross-section scanner imagery (up) and schematic reproduction of main structures (down), including dark and light growth increments in the prismatic layer.*

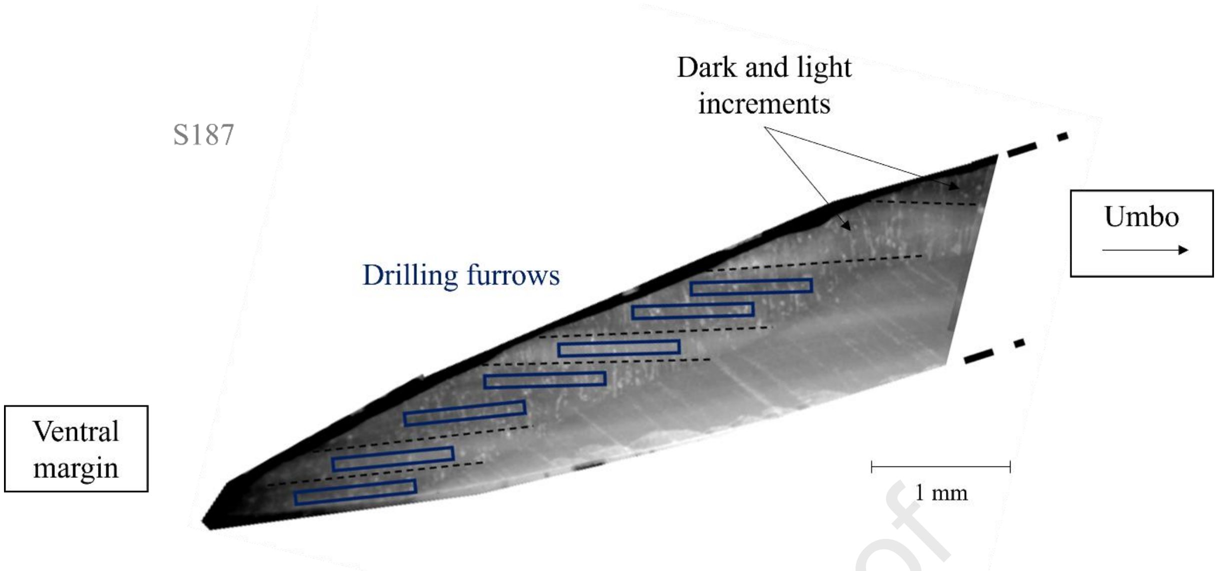
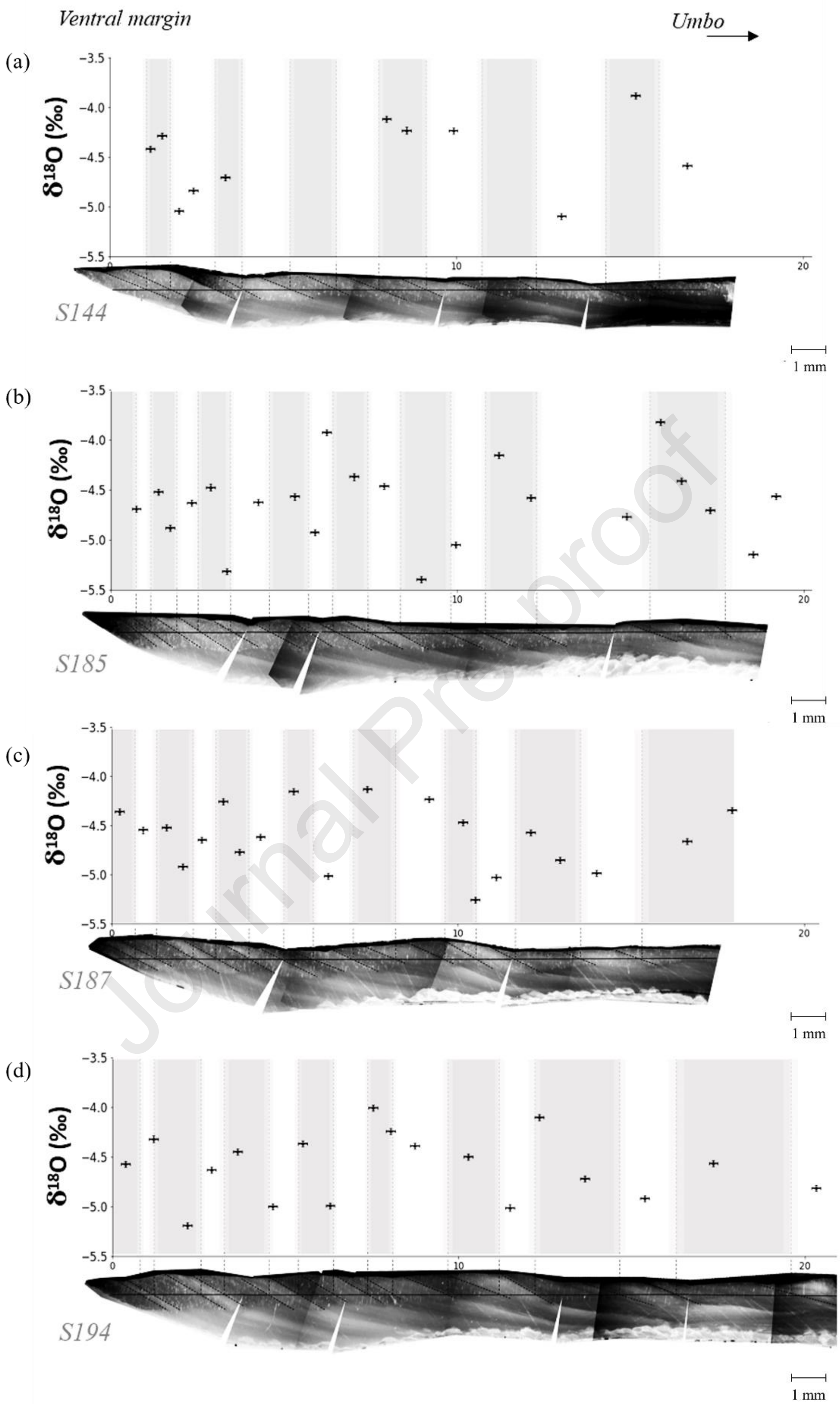


Figure 4 – Part shells' dark and light growth increments sampling, located on the ventral margin. Drilling paths are limited to the prismatic layer and are chosen in regards to the distinction of dark and light increments on stereo microscope pictures, by accentuating contrasts. One or two furrows are drilled on each depending on increments width.



537

538 *Figure 5 – $\delta^{18}O$ records for the four selected shells (a) S144 (b) S185 (c) S187 (d) S194. Measurements have been carried out*
539 *with an IRMS device on powder samples collected for each furrow drilled in growth increments. Dark and light increments*
540 *were differentiated by dark and light gradients on charts. Stereo microscope pictures have been planarized along the charts'*
541 *x-axis to enable the comparison between shells' structures and $\delta^{18}O$ values.*

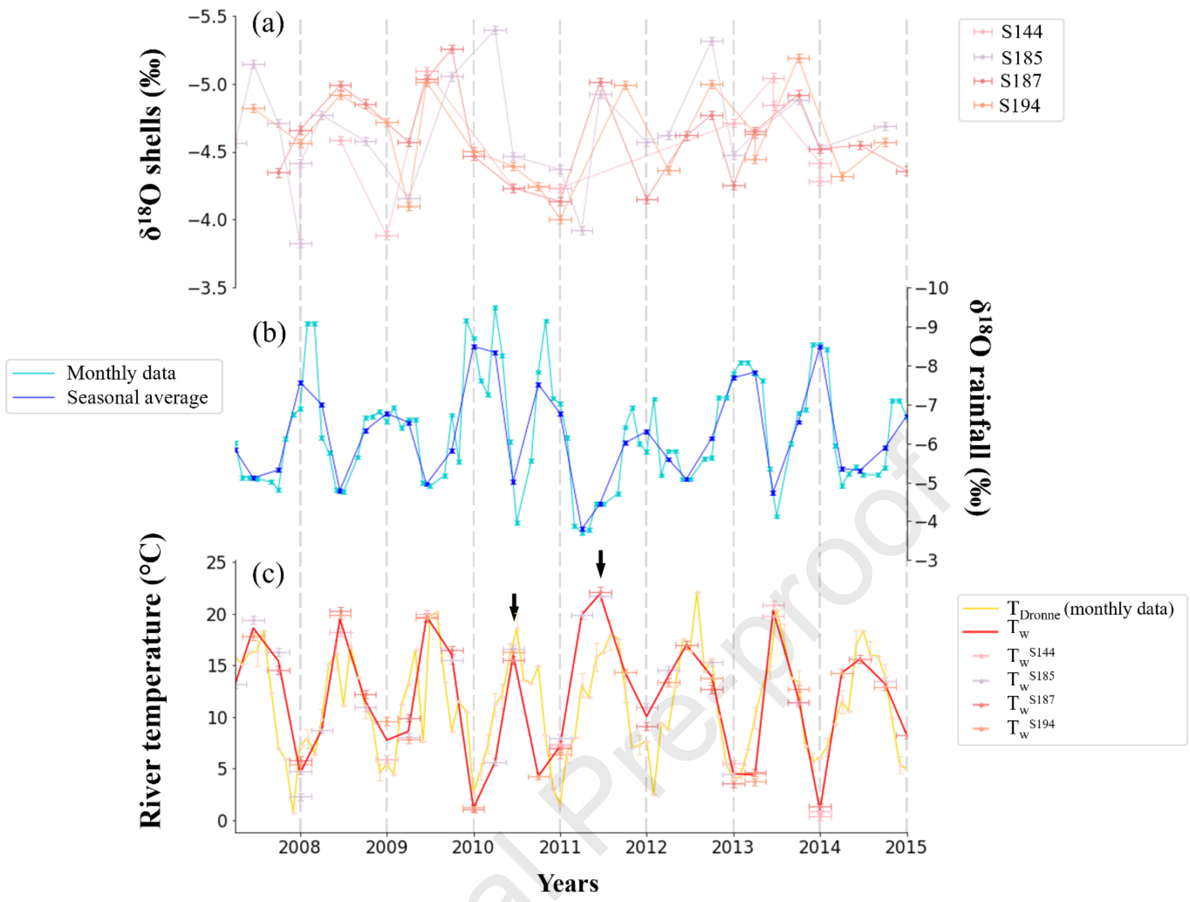


Figure 6 – (a) Shells' $\delta^{18}O$ records aligned on age model (reversed y-axis) (b) $\delta^{18}O$ record of rainfall, monthly measured above the Villars cave (light blue), close to the mussels' living area (data provided by D. Genty) (reversed y-axis). The seasonal average has been computed (dark blue) as DJF = winter, MAM = spring, JJA = summer, SON = autumn (c) Monthly T_{Dronne} record (yellow) compared with reconstructed temperature average Tw (red), computed from Grossman and Ku equation (1986), corrected by Dettman et al. (1999). Grey dashed lines indicate winters and black arrows point to remarkable summers.

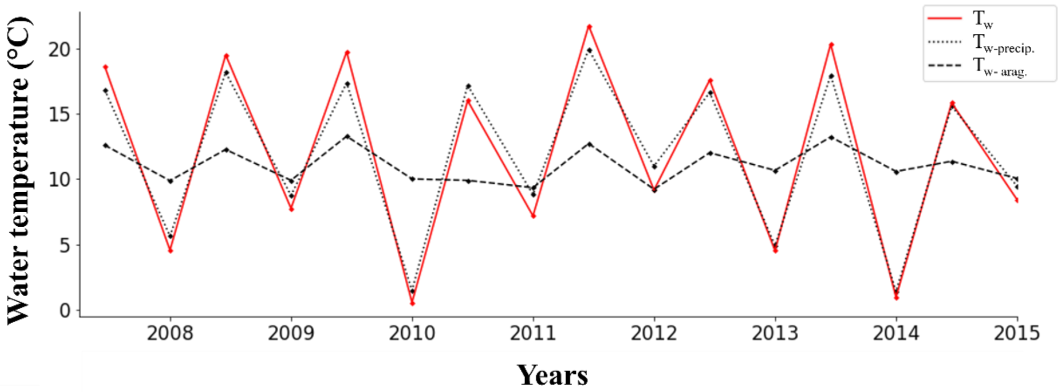


Figure 7 – Theoretical winter and summer temperature reconstructions only considering rainfall $\delta^{18}O$ variation ($T_{w\text{-precip}}$ thin black dashed line) and shells $\delta^{18}O$ variation ($T_{w\text{-arag}}$ bold black dashed line). Only winter and summer T_w are represented to compare $T_{w\text{-precip}}$ and $T_{w\text{-arag}}$ amplitudes with the expected signal.

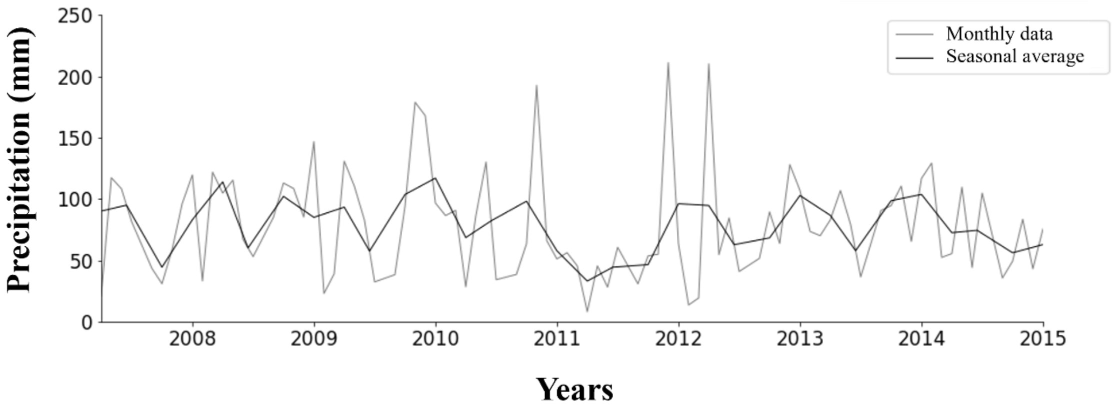


Figure 8 – Monthly record of precipitation amount measured above the Villars cave (grey) (data provided by D. Genty), and seasonal average (black). The year 2011 shows an annual rainfall deficit compared to the other years over the considered period in the region.

Supplements

Table 1 – Monthly amount of precipitations (mm) and isotopic composition $\delta^{18}O_{precip.}$ (‰) measured above the Villars cave from 2007 to 2015 (data provided by D. Genty). Monthly modeled air temperature at Villars T_{air} (°C) (data provided by E-OBS, https://surfobs.climate.copernicus.eu/dataaccess/access_eobs.php)

Date	Precipitation ± 0.2 [mm]	Villars rainfall $\delta^{18}O$ ± 0.05 [‰]	Villars air temperature [°C]
15/01/2007	87.5	-6.41	6.85
15/02/2007	141.6	-6.41	8.44
15/03/2007	132.3	-6.34	8.46
15/04/2007	20.8	-6.02	15.15
15/05/2007	117.3	-5.13	15.71
15/06/2007	108.3	-5.13	18.36
15/07/2007	81.6	-5.09	18.54
15/08/2007			18.57
15/09/2007	43.5	-5.01	15.43
15/10/2007	30.9	-4.81	12.46
15/11/2007	58.6	-6.13	6.10
15/12/2007	96.3	-6.73	5.28
15/01/2008	119.6	-6.89	6.98
15/02/2008	33.2	-9.08	8.27
15/03/2008	121.8	-9.08	7.63
15/04/2008	104.8	-6.15	10.50
15/05/2008	115.3	-5.76	15.62
15/06/2008	67.1	-4.81	18.24
15/07/2008	53.0	-4.76	19.22
15/08/2008			19.10
15/09/2008	84.9	-5.65	15.10
15/10/2008	113.0	-6.65	12.04
15/11/2008	108.5	-6.68	7.52
15/12/2008	85.3	-6.82	4.27
15/01/2009	146.7	-6.55	3.27
15/02/2009	23.0	-6.93	5.45
15/03/2009	38.9	-6.40	8.06
15/04/2009	130.8	-6.62	11.27
15/05/2009	110.4	-6.62	16.60
15/06/2009	82.6	-5.00	18.59
15/07/2009	32.4	-4.90	20.04
15/08/2009			20.90
15/09/2009	38.3	-5.18	17.55
15/10/2009	93.8	-6.73	13.44
15/11/2009	178.8	-5.53	10.30
15/12/2009	167.9	-9.16	5.26
15/01/2010	96.7	-8.71	1.89
15/02/2010	86.5	-7.61	4.37
15/03/2010	90.7	-7.26	7.61
15/04/2010	28.4	-9.49	12.29
15/05/2010	86.5	-8.27	13.29

15/06/2010	130.1	-6.04	17.93
15/07/2010	34.2	-3.97	21.31
15/08/2010			19.13
15/09/2010	38.5	-5.55	16.18
15/10/2010	63.5	-7.83	12.13
15/11/2010	192.6	-9.15	7.58
15/12/2010	66.0	-7.16	2.92
15/01/2011	51.1	-7.01	4.40
15/02/2011	56.2	-6.15	6.55
15/03/2011	45.6	-3.89	9.43
15/04/2011	8.1	-3.71	14.57
15/05/2011	45.5	-3.78	17.13
15/06/2011	28.1	-4.44	17.87
15/07/2011	60.6	-4.43	18.48
15/08/2011			20.50
15/09/2011	30.8	-4.71	18.86
15/10/2011	53.6	-6.42	13.98
15/11/2011	55.0	-6.91	11.61
15/12/2011	211.1	-5.99	7.50
15/01/2012	63.6	-5.79	5.44
15/02/2012	13.6	-7.14	0.27
15/03/2012	19.3	-5.18	10.57
15/04/2012	210.1	-5.80	9.68
15/05/2012	54.6	-5.80	15.99
15/06/2012	84.6	-5.08	18.68
16/07/2012	40.9	-5.08	18.60
16/08/2012	14.4		21.62
15/09/2012	51.6	-5.61	17.35
16/10/2012	89.5	-5.63	14.04
15/11/2012	63.7	-7.17	8.95
16/12/2012	128.0	-7.17	6.54
16/01/2013	107.0	-7.80	3.99
13/02/2013	73.5	-8.08	3.78
16/03/2013	70.1	-8.08	7.82
15/04/2013	83.0	-7.80	10.72
16/05/2013	106.9	-7.61	11.84
15/06/2013	79.2	-5.34	16.81
16/07/2013	36.5	-4.12	22.65
16/08/2013	50.3		20.05
15/09/2013	90.6	-5.99	17.55
16/10/2013	94.6	-6.78	14.91
15/11/2013	110.5	-6.87	7.31
16/12/2013	65.4	-8.53	5.51
16/01/2014	116.7	-8.53	7.63
13/02/2014	129.2	-8.42	7.20
16/03/2014	52.4	-5.94	9.59
15/04/2014	55.5	-4.90	12.64
16/05/2014	109.6	-5.22	13.67

15/06/2014	44.1	-5.40	19.39
16/07/2014	104.8	-5.20	20.10
16/08/2014	72.1		18.10
15/09/2014	35.6	-5.19	18.61
16/10/2014	49.3	-5.38	15.50
15/11/2014	83.5	-7.09	11.60
16/12/2014	43.1	-7.09	5.15
16/01/2015	75.6	-6.70	4.56
13/02/2015	69.8	-6.31	4.29
16/03/2015	48.0	-5.67	9.14
15/04/2015	47.7	-4.66	13.00
16/05/2015	46.8	-5.56	15.31
15/06/2015	52.3	-4.43	20.23
16/07/2015	9.2	-5.48	22.52
16/08/2015	101.3		20.91
15/09/2015	62.0	-5.08	15.80
16/10/2015	57.5	-5.08	12.18
15/11/2015	51.2	-4.81	10.74
16/12/2015	10.5	-3.28	8.72

Table 2 - Monthly Haute-Dronne temperature T_{Dronne} from 2007 to 2015, including in situ measurements (provided by the Laboratoire d'Analyse et de Recherche de Dordogne) and additional values modeled from the linear relationship between the air temperature at Villars (provided by E-OBS, https://surfobs.climate.copernicus.eu/dataaccess/access_eobs.php) and the river temperature. The uncertainty on the in situ river temperatures are not provided and consequently considered as negligeable. The uncertainty on the modeled temperatures is computed from the model parameters.

Date	River temperature [°C]	Uncertainty	Data type
01/2007	3.8	0.0	*in situ*
02/2007	9.4	0.0	*in situ*
03/2007	9.4	0.0	*in situ*
04/2007	15.8	0.0	*in situ*
05/2007	15.1	0.0	*in situ*
06/2007	16.3	0.0	*in situ*
07/2007	16.3	1.4	modeled
08/2007	18.3	0.0	*in situ*
09/2007	12.3	0.0	*in situ*
10/2007	7.0	0.0	*in situ*
11/2007	5.9	0.0	*in situ*
12/2007	0.8	0.0	*in situ*
01/2008	7.2	0.0	*in situ*
02/2008	7.9	0.9	modeled
03/2008	6.4	0.0	*in situ*
04/2008	9.8	1.0	modeled

05/2008	15.1	0.0	*in situ*
06/2008	16.1	1.4	modeled
07/2008	11.1	0.0	*in situ*
08/2008	16.8	1.4	modeled
09/2008	13.9	0.0	*in situ*
10/2008	11.0	1.1	modeled
11/2008	9.7	0.0	*in situ*
12/2008	4.7	0.8	modeled
01/2009	5.4	0.0	*in situ*
02/2009	4.5	0.0	*in situ*
03/2009	11.2	0.0	*in situ*
04/2009	13.2	0.0	*in situ*
05/2009	16.5	0.0	*in situ*
06/2009	7.7	0.0	*in situ*
07/2009	19.7	0.0	*in situ*
08/2009	20.2	0.0	*in situ*
09/2009	13.4	0.0	*in situ*
10/2009	8.6	0.0	*in situ*
11/2009	11.5	0.0	*in situ*
12/2009	10.5	0.0	*in situ*
01/2010	2.7	0.7	modeled
02/2010	4.7	0.8	modeled
03/2010	7.4	0.9	modeled
04/2010	11.2	1.1	modeled
05/2010	12.0	1.1	modeled
06/2010	15.8	1.3	modeled
07/2010	18.6	1.5	modeled
08/2010	13.6	0.0	*in situ*
09/2010	13.3	0.0	*in situ*
10/2010	14.9	0.0	*in situ*
11/2010	8.3	0.0	*in situ*
12/2010	3.1	0.0	*in situ*
01/2011	1.5	0.0	*in situ*
02/2011	6.5	0.9	modeled
03/2011	8.0	0.0	*in situ*
04/2011	13.1	1.2	modeled
05/2011	11.9	0.0	*in situ*
06/2011	15.8	1.3	modeled
07/2011	16.3	0.0	*in situ*
08/2011	18.0	1.4	modeled

09/2011	17.6	0.0	*in situ*
10/2011	12.6	1.2	modeled
11/2011	7.1	0.0	*in situ*
12/2011	7.3	0.9	modeled
01/2012	7.7	0.0	*in situ*
02/2012	2.5	0.0	*in situ*
03/2012	9.4	0.0	*in situ*
04/2012	8.8	0.0	*in situ*
05/2012	13.0	0.0	*in situ*
06/2012	17.6	0.0	*in situ*
07/2012	16.4	0.0	*in situ*
08/2012	22.1	0.0	*in situ*
09/2012	14.4	0.0	*in situ*
10/2012	15.6	0.0	*in situ*
11/2012	10.1	0.0	*in situ*
12/2012	5.4	0.0	*in situ*
01/2013	4.0	0.0	*in situ*
02/2013	4.2	0.7	modeled
03/2013	6.9	0.0	*in situ*
04/2013	9.9	1.0	modeled
05/2013	12.8	0.0	*in situ*
06/2013	14.9	1.3	modeled
07/2013	20.4	0.0	*in situ*
08/2013	17.6	1.4	modeled
09/2013	13.8	0.0	*in situ*
10/2013	13.4	1.2	modeled
11/2013	7.2	0.0	*in situ*
12/2013	5.7	0.8	modeled
01/2014	6.1	0.0	*in situ*
02/2014	7.1	0.9	modeled
03/2014	9.3	0.0	*in situ*
04/2014	11.5	1.1	modeled
05/2014	10.6	0.0	*in situ*
06/2014	17.0	1.4	modeled
07/2014	18.3	0.0	*in situ*
08/2014	16.0	1.3	modeled
09/2014	15.9	0.0	*in situ*
10/2014	13.9	1.2	modeled
11/2014	9.9	0.0	*in situ*
12/2014	5.4	0.8	modeled

01/2015	5.0	0.0	*in situ*
02/2015	4.7	0.8	modeled
03/2015	7.2	0.0	*in situ*
04/2015	11.8	1.1	modeled
05/2015	13.7	0.0	*in situ*
06/2015	17.7	1.4	modeled
07/2015	19.1	0.0	*in situ*
08/2015	18.3	1.5	modeled
09/2015	13.8	0.0	*in situ*
10/2015	11.1	1.1	modeled
11/2015	9.1	0.0	*in situ*
12/2015	8.3	1.0	modeled

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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