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Freshwater pearl mussels as a stream water stable isotope recorder

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Abstract

For several decades, stable isotopes have been a commonly used and effective tool for flow path analysis, stream water source apportionment, and transit time analysis. The Global Network of Isotopes in Precipitation repository now has monthly precipitation isotope time series extending over several years and even decades in some settings. However, stream water isotope composition time series remain rather short with only very few data sets spanning over more than a few years. A critical challenge in this respect is the collection of stream water isotope data sets across a wide variety of headwater streams and for long durations. We rely on a new approach for stream signal reconstruction based on freshwater mussels, specifically the freshwater pearl mussel *Margaritifera margaritifera*. We use secondary ion mass spectrometry (SIMS) to quantify oxygen isotope ratios in pearl mussel shell growth bands. In our study area, the observed seasonal variability in precipitation $\delta^{18}O$ values ranges between −15‰ and −3‰. This input signal is strongly damped in stream water, where observed values of $\delta^{18}O$ range between −10‰ and −6.5‰. These values are consistent with our measured average shell-derived stream water $\delta^{18}O$ of −7.19‰. Along successive growth bands, SIMS-based stream water $\delta^{18}O_w$ values varied within a seasonal range of −9‰ to −5‰. The proposed SIMS-based shell analysis technique is obviously well suited for analysing isotopic signatures of O in shell material—especially from the perspective of reconstructing historical series of in-stream isotope signatures.

KEYWORDS

freshwater pearl mussel, *Margaritifera margaritifera*, secondary ion mass spectrometry, stream water isotopes

1 | INTRODUCTION

Since early pioneering work by Dinçer, Payne, Florkowski, Martinec, and Tongiorgi (1970), Fritz, Cherry, Weyer, and Sklash (1976), and Merot, Bourguet, and Le Leuch (1981), stable isotopes have been a commonly used and effective tool for flow path analysis and stream water source apportionment (for reviews, see Klaus & McDonnell, 2013; Sprenger, Volkman, Blume, & Weiler, 2015). One area where stable isotopes have been particularly effective has been for transit time analysis (Tetzlaff, Seibert, & Soulsby, 2009). In this application, time series of precipitation isotope composition are compared with time series of stream water isotope composition, and the degree of damping in the seasonal cycle can be quantified via convolution (for review, see McGuire & McDonnell, 2006). Many new model approaches have recently been developed (Harman, 2015; Kirchner, 2016).

But regardless of the approach used, the limiting step in such analyses is the length and spatial completeness of the stream water isotope record. Collecting such information is difficult and time consuming. Monthly precipitation isotope time series now extend several years and even decades in some settings as part of the Global Network of Isotopes in Precipitation (Aggarwal et al., 2007; Dansgaard, 1964). However, stream water isotope composition time series are short with very few data sets spanning over more than a few years as can be seen from the Global Network of Isotopes in Rivers (Halder, Terzer, Wassenaar, Araguás-Araguás, & Aggarwal, 2015). Consequently, we lack information on long-term stream water isotope signature in all but a few highly monitored sites. This is a problem because we do not know how variations in input signatures (related, for example, to changes in atmospheric circulation patterns [Stumpp, Klaus, & Stichler, 2014]) are related to variations in geology and catchment structure (Pfister, Martínez-Carreras, Hissler, & McDonnell, 2017).

A critical challenge going forward is the collection of stream water isotope data sets across a wide variety of headwater streams and for long durations. Despite the advent of field deployable laser spectrometers (Berman, Gupta, Gabrielli, Garland, & McDonnell, 2009) and the deployment of compact environmental laboratories in the field (Floury et al., 2017; Von Freyberg, Studer, & Kirchner, 2017), collection of such data remains prohibitively labour and time intensive. Here, we build on prior work where oxygen stable isotope ratios ($\delta^{18}O$) obtained from mechanically drilled freshwater bivalve shell material have been extensively used for palaeoenvironmental reconstructions (e.g., Helama & Nielsen, 2008; Versteegh, Troelstra, Vonhof, & Kroon, 2009; Versteegh, Vonhof, Troelstra, Kaandorp, & Kroon, 2010), including hydroclimate variables (Kelemen et al., 2017). We show a proof of concept of a new approach to stream signal reconstruction based on freshwater mussels, specifically the freshwater pearl mussel *Margaritifera* (Linnaeus, 1758; Bauer, 1987). We use secondary ion mass spectrometry (SIMS) to quantify oxygen isotope ratios in pearl mussel shell growth lines. To the best of our knowledge, this is the first application of SIMS in the determination of oxygen isotope ratios in freshwater molluscs.

This briefing outlines some of the theory on mollusc shell use in ecology and how it could be applied to stream water isotope reconstruction. We address the following questions in this proof-of-concept analysis:

a. Assuming that we can identify annual sequences in a freshwater pearl mussel shell material for isotope analysis, can we sample this material and analyse isotopic ratios of O with SIMS?
b. Are we able to replicate these SIMS measurements?
c. How does the range, standard deviation, and harmonics of the annual cycle of freshwater pearl mussel shell material relate to precipitation and stream water isotope signals?

2 | THEORY

The basic principle of tracing isotopic signatures in mollusc shells relates to the geochemical information that is recorded as calcium carbonate precipitate (i.e., layers of calcite or aragonite) during the shell growth process. This leads to successive growth bands of variable shading—commonly interpreted as annual or seasonal bands (Wurster & Patterson, 2000). The isotopic composition of shell carbonate is influenced by isotopic composition of seawater and water temperature (Epstein, Buchsbaum, Lowenstam, & Urey, 1953; Epstein, Buchsbaum, Lowenstam, & Urey, 1951; Wefer & Berger, 1991). Oxygen isotopic composition of calcium carbonate deposited by marine molluscs is temperature dependent and therefore of great value as a palaeothermometer (Urey, 1947). The amount of added material in each band depends on the bivalve growth rate, itself a function of water temperature; the specimen's age and reproductive cycle; and nutrient availability (Goodwin, Schöne, & Dettman, 2003). Yearly growth is mostly affected by water temperature (Goodwin et al., 2003).

Carbon and oxygen isotopic signatures in freshwater mussel shells are related directly to their surrounding water chemistry and temperature. Thus, the ratio of ^{18}O and ^{16}O in shells is influenced by the isotopic signature of surrounding water and temperature-controlled fractionation effects. Several studies have shown that mollusc shells forming in isotopic equilibrium with the surrounding water become lighter in their $\delta^{18}O$ signatures during cold months (Dettman, Reische, & Lohmann, 1999; Goodwin et al., 2003; Schöne, Goodwin, Flessa, Dettman, & Roopnarine, 2002). In ecology, several studies have now used these simple patterns to reconstruct reliable chronologies of $\delta^{18}O$ from fossil specimens (Helama & Nielsen, 2008). Along similar lines, stable isotopes (C and N) in (soft tissues of) molluscs have been used in a variety of studies on food web ecology (Delong & Thorp, 2009).

Versteegh et al. (2009, 2010) have documented strong seasonal correlations between shell stable isotope ratios and surrounding river water for unionid freshwater bivalves in the Meuse and Rhine basins. They found that shell growth rates were strongly related to food availability on the one hand, whereas the onset and cessation of shell growth lines were linked to stream water temperature. In two rivers located in Sweden, Dunca and Mutvei (2001) have documented a minimum threshold of 5°C in water temperature for the onset of shell growth in *M. margaritifera*. Versteegh, Vonhof, Troelstra, and Kroon (2012) were able to relate growth band $\delta^{18}O$ in *Mytilus edulis* shells to glacier meltwater dynamics over several years. They also used shell $\delta^{18}O$ of *Unio pictorum* and *Unio tumidus* as a proxy of historical discharge of the river Meuse in the Netherlands. Kelemen et al. (2017) have documented the potential for shell $\delta^{18}O$ values

FIGURE 1 Freshwater pearl mussel (*Margaritifera margaritifera*) specimen in their natural habitat (picture: Alexandra Arendt; http://www.margaritifera.eu/de/)

in three species of unionid shells (*Chambardia wissmanni*, *Aspatharia dahomeyensis*, and *Aspatharia chaiziana*) to reconstruct past $\delta^{18}O$ signatures in stream water of the Oubangui and Niger Rivers in Central and West Africa.

Our SIMS-based proof-of-concept work for exploring a long-term stream water stable isotope recorder focuses on the pearl mussel, *M. margaritifera*, a freshwater mussel of the Order Unionida (Figure 1). They are found mostly in cool upland streams with bedrock, cobble and gravel substratum, moderate flow velocities, low nutrient concentrations, and low carbonate content, with salmonid hosts being present (Geist, 2010). They are widespread across Europe including Austria, Belarus, Belgium, Czech Republic, Finland, France, Germany, Great Britain, Ireland, Latvia, Lithuania, Norway, Northern Ireland, Portugal, Poland, Russia, Spain, and Sweden (Lopes-Lima et al., 2017).

Freshwater pearl mussels (*M. margaritifera*) are bivalve molluscs. The periostracum corresponds to the outermost layer of their shell, essentially composed of organic material (Figure 2). Below the periostracum, elongated calcium carbonate crystals develop perpendicularly to the shell's surface and form the prismatic layer. The inner layer of the shell is composed of nacre.

The pearl mussel is the freshwater bivalve with the longest lifespan in western-central Europe, living typically for more than 80 years and reaching reproductive maturity at approximately 10–14 years of age (Lopes-Lima et al., 2017).

3 | METHODS

3.1 | Precipitation and stream water isotope sampling

Precipitation samples for liquid stable isotope analysis were collected fortnightly from 2011 to 2015 in the Weierbach experimental catchment (Luxembourg; Figure 3). The bedrock geology of this site is dominated by shale, and land use is characterized by forest, grassland, and arable land. Since 2015, a sequential precipitation sampling device is operated in the catchment. Grab samples of stream water were taken fortnightly at the Weierbach catchment outlet (0.45km^2). Additionally, event scale measurements were taken at hourly time scale for selected storm events. All precipitation and stream water samples were analysed for δD and $\delta^{18}O$ composition with a Los Gatos DLT100 off-axis integrated cavity output spectroscopy laser spectrometer. The values are reported in ‰ relative to Vienna Standard Mean Ocean Water 2 standards (International Atomic Energy Agency, 2009) with an accuracy of 0.21‰ for $\delta^{18}O$ and 0.34‰ for $\delta^{2}H$.

3.2 | Shell collection and preparation

The studied specimen (*M. margaritifera*) was taken from the river Our (Luxembourg; Eybe, Thielen, Bohn, & Sures, 2013), approximately 40 km north-east of the Weierbach catchment (Figure 3). Because no stable isotope data are available for the Our river, we had to rely on stable isotope data for O and H in water taken from the nearby Weierbach location. Although it does not host any pearl mussels, the

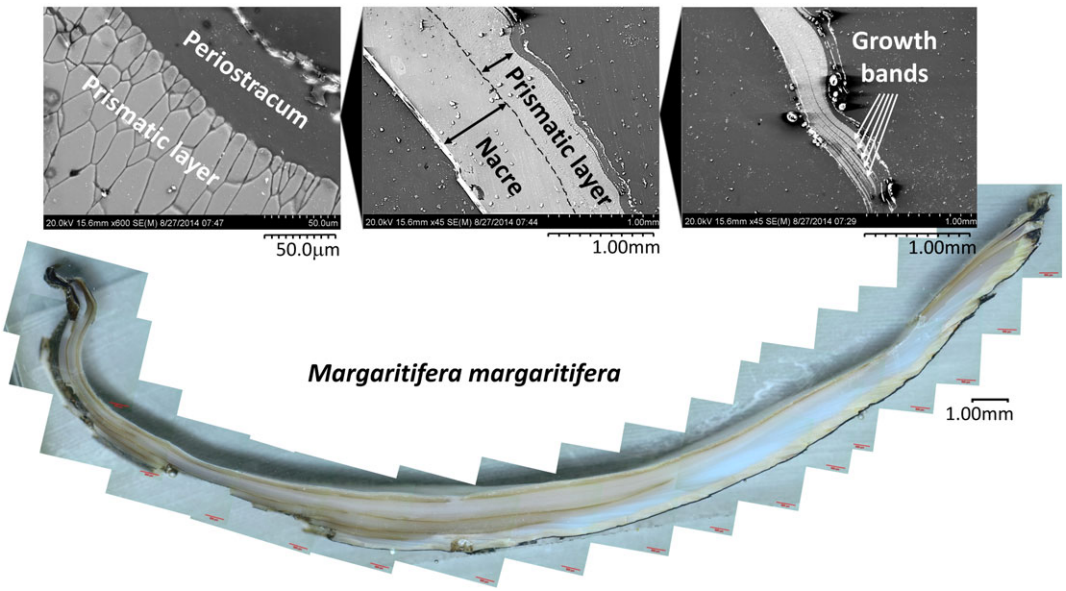

FIGURE 2 Optical (bottom) and scanning electron microscope (top) imagery of a ~3 mm slice of a pearl mussel (*Margaritifera margaritifera*) valve. Scanning electron microscope images: successive growth bands (top right), prismatic layer and nacre (top centre), and details of calcium carbonate prisms and periostracum (top left)

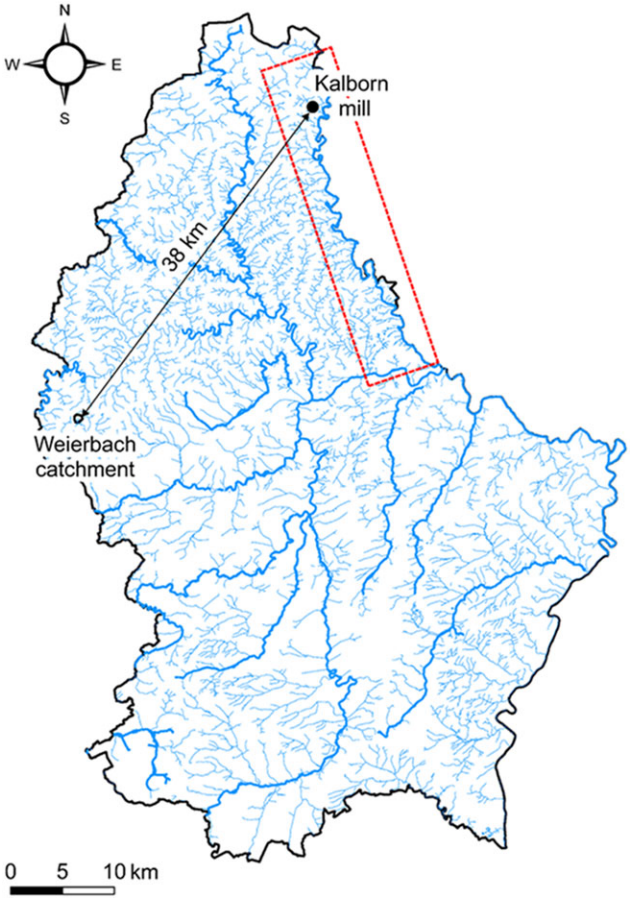

FIGURE 3 Location map of the Weierbach catchment and shell collection site near the Kalborn mill on the Our River (delimited by red box)

TABLE 1 Water temperature, pH, and conductivity in the Weierbach creek and the Our River (average values for the period 2008–2010)

	Weierbach	Our River
Water temperature, °C	9.0 (σ = 4.5)	10.0 (σ = 5.9)
pH	7.04 (σ = 0.7)	7.61 (σ = 0.6)
Cond., µS/m	~54 (σ = 4.4)	~138 (σ = 14.6)

Note. Our river data taken from http://www.margaritifera.eu/de/.

Weierbach creek was used as our representative stream sampling site because it exhibits nearly identical physiographic characteristics and latitude and elevation characteristics to the Our River. Sine wave inferred catchment mean transit time from the Weierbach creek is ~1.7 years (Pfister et al., 2017). Both the Our and the Weierbach sites are underlain by shale bedrock and exhibit similar basic water chemistry characteristics, with average annual water temperature of 9°C to 10°C and average annual pH values of ~7 (Table 1). Conductivity is somewhat higher in the Our River (annual average = 138 µS/m) in comparison with the Weierbach creek (annual average = 54 µS/m).

One valve of the pearl mussel was embedded in an epoxy resin, before being cut along its axis of maximum growth and then polished. One half was treated with Mutvei's solution (Schöne, Dunca, Mutvei, & Norlund, 2005) to visualize its growth structures (Figure 4). Mutvei's solution consists of 500-ml 1% acetic acid, 500-ml 25% glutaraldehyde, and approximately 5- to 10-g alcian blue powder. This is a simple and fast technique for staining in shadings of blue annual and subannual growth structures in biogenic carbonates, allowing for microgrowth structures to be observed with optical light microscopy and scanning electron microscopy. Indentation marks were made on the second half of the valve to better locate areas of interest during SIMS measurements (Figure 5). After polishing, the major part of the resin was removed to limit its degassing during SIMS analyses. Next, the sample was embedded in a 2-cm-diameter aluminium ring by using Wood's alloy (a low melting fusible Bi-base alloy). The surface of the sample was coated with a thin gold layer to prevent any charge effect.

The specimen that we had at our disposal for this exploratory work is undated (i.e., date of death is unknown). We inferred an age of 42 years from the number of winter lines that we were able to discern. This is in agreement with the 45 years inferred from the longitudinal length of the shell (as per Dunca, Söderberg, & Norrgrann, 2011).

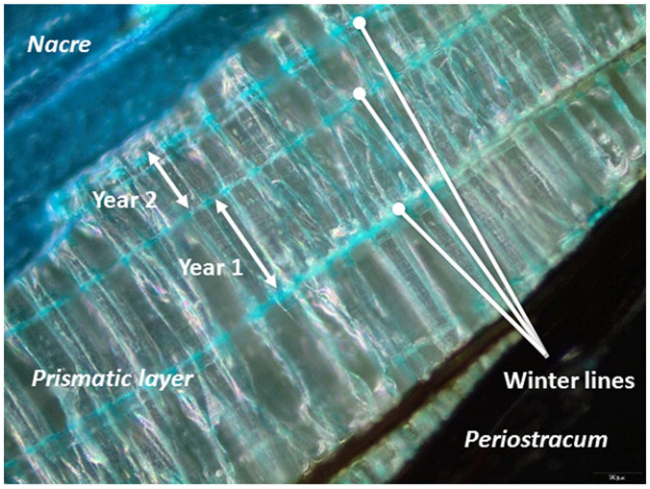

FIGURE 4 Pearl mussel growth structures stained with Mutvei's solution (shadings of blue). Two successive growth bands shown as examples

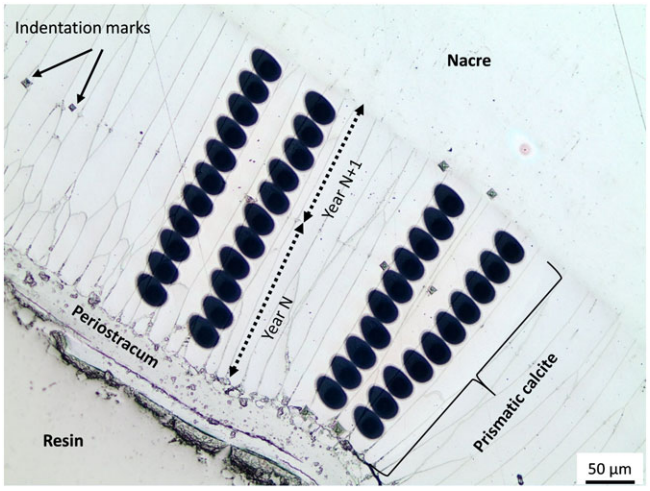

FIGURE 5 Example of an optical image showing SIMS analysis pits and their localisation. Replication of analytical profiles on two growth bands (year N and year N + 1) on the studied pearl mussel valve. The four profiles are oriented perpendicular to the general orientation of the mollusc's growth bands

3.3 | Analysis

We used SIMS to measure $\delta^{18}O$ signature in pearl mussel shell growth bands (for more details on SIMS, see Sangely et al., 2015). SIMS is a useful alternative to older protocols for stable isotope analyses of molluscs that previously relied on local (e.g., scratching the surface or applying a dental drill) or massive (i.e., crushing of the entire shell) mechanical treatment of the specimen. Linzmeier, Kozdon, Peters, and Valley (2016) recently used SIMS with 10-µm beam-spot size for investigating oxygen isotope variability within Nautilus shell growth bands (with the objective to study depth migration behaviour). The same analytical approach was also used to study seasonal growth in Arctic bivalves (Vihtakari et al., 2016). To the best of our knowledge, SIMS has not yet been applied to freshwater molluscs for determining stable isotope ratios of O in shell material.

The SIMS measurements were carried out on a CAMECA IMS 1280 ion microprobe at the CRPG-CNRS in Vandœuvre-lès-Nancy, France. The analytical conditions were similar to those used by Rollion-Bard, Mangin, and Champenois (2007). The instrument was run with a Cs^+ bombardment (~3 nA) at 20 keV and a normal incidence electron flood gun. For each measurement, a 15-µm-diameter focused beam was scanned over a surface of 15 × 15 μm^2. The mass resolving power (M/ΔM) was adjusted at 4500 to eliminate isobaric interferences ($^{16}OH_2^-$, $^{17}OH^-$, and $^{16}OD^-$). The energy band was limited to 35 eV. The intensities of $^{18}O^-$ and $^{16}O^-$ were simultaneously recorded using two off-axis Faraday cups (multicollection system) over 2.5 min. Analyses were repeated along profiles oriented perpendicular to the growth lines of the shell (Figure 5).

Prior to the SIMS measurements, a short presputtering of the surface was done to remove oxygen contamination on the sample surface. The chamber pressure was maintained at 3–4 × 10^{-9} mbar by using a liquid nitrogen cold trap.

The instrumental mass fractionation $\delta^{18}O$ was determined using the calcium carbonate standard CCcigA with a measured value of 18.94. The instrumental fractionation was −5.4‰. The external precision on our standard sample was 0.1‰ (1σ). The internal precision for CAMECA IMS 1280 ion microprobe was better than 0.1‰ (2σ).

Measurements were carried out in two zones, covering six successive growth bands. The first two growth bands (Years 1 and 2) were located in the first zone. The other four growth bands (Years 3 to 6) were located in an adjacent second zone. Replicate measurements were carried out along four and three profiles in the first and second zone, respectively (Figure 6).

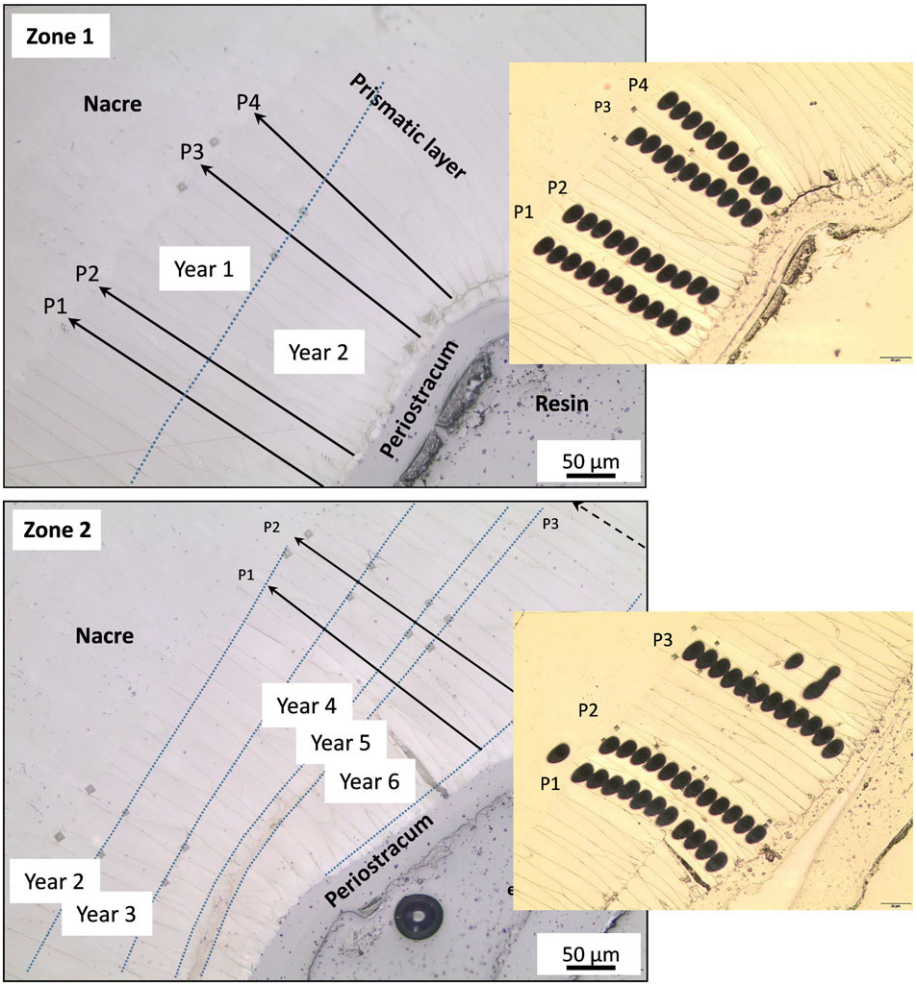

FIGURE 6 Localization of the ion microprobe measurements in Zones 1 (top) and 2 (bottom) of the studied freshwater pearl mussel valve (note that all profiles are oriented along individual calcite prisms in order to maximize measurement consistency). The different profiles (P1, P2, P3, and P4) are oriented perpendicular to the general orientation of the mollusc's growth bands

The areas of interest were carefully chosen to avoid interfaces between calcium carbonate prisms that might induce an additional isotopic fractionation. Thus, the SIMS analyses were carried out along single prisms over several years.

3.4 | Inferring shell $\delta^{18}O$ values in water from $\delta^{18}O$ values in shell material

We followed Friedman and O'Neil (1977) for inferring $\delta^{18}O$ ratios in water from $\delta^{18}O$ ratios in shell material:

$$1000 \ln\alpha = 2.78 \left(10^6 T^{-2}\right) - 2.89, \quad (1)$$

where T = stream water temperature (in °K) and α = fractionation between water and calcite.

$$\alpha_{water}^{calcite} = \frac{\left[1000 + \delta^{18}O_{ca}(VSMOW)\right]}{\left[1000 + \delta^{18}O_{w}(VSMOW)\right]}, \quad (2)$$

where ca is shell calcite and w is water. Note that $\delta^{18}O_{ca}$ values were initially relative to the Vienna Pee Dee Belemnite (VPDB) reference. They were converted to the Vienna Standard Mean Ocean Water (VSMOW) as per Gonfiantini, Stichler, and Rozanski (1995):

$$\delta^{18}O_{ca}\ (VSMOW) = \alpha_{water}^{calcite}\left(1000 + \delta^{18}O_{ca}\ (VPDB)\right) - 1000. \quad (3)$$

Ultimately, $\delta^{18}O$ ratios in water were obtained via

$$\delta^{18}O_{w}(VSMOW) = \frac{\left[1000 + \delta^{18}O_{ca}(VSMOW)\right]}{\alpha} - 1000. \quad (4)$$

4 | RESULTS

The indentation marks in the nonstained sample allowed us to target analytical sites across six growth years. In order to assess repeatability, we carried out additional replicate measurements along several parallel lines within the individual annual growth increments (Figure 6).

For all 79 spots analysed by SIMS, the standard deviation of the measurements ranged between 0.13‰ and 0.20‰ (Pee Dee Belemnite). We relied on an average water temperature in the Our River of 15°C (April–September growth period; time span 2008–2010) for inferring stream water isotope values $\delta^{18}O_w$ from pearl mussel shell SIMS measurements (Equation (1)). For growth bands 1 and 2, average stream water isotope values inferred from pearl mussel shell SIMS analysis ($\delta^{18}O_w$) along the four investigated profiles ranged from −7.45‰ to −6.34‰ (Table 2). For the three profiles investigated along growth bands 3 to 6, average isotope values ($\delta^{18}O_w$) ranged from −8.83‰ to −5.99‰ (Table 2). For the six growth bands, the average stream water $\delta^{18}O_w$ signature, based on all SIMS-based measurements, was −7.19‰ (with a median value of −7.14‰; Table 3).

The measurements suggest an intra-annual and interannual variability in stream water $\delta^{18}O_w$ values (Table 2; Figure 7). Along successive bands, SIMS-based stream water $\delta^{18}O_w$ values alternatively tend to increase and decrease, mostly within a range of −9‰ to −5‰ (Figure 7). Note that a change in stream water temperature of 1°C applied to Equation (1) for the determination of the fractionation factor α will ultimately lead to a change in ~0.2‰ in stream water $\delta^{18}O_w$.

Previous work in our region of interest has shown a strong seasonality in isotope signatures of O and H in precipitation (Pfister et al., 2017), as shown by $\delta^{18}O$ data from 2011 to 2016 at the Roodt meteorological station (Figure 7). Long-term observations of $\delta^{18}O$ in precipitation range between −15‰ and −3‰. Isotopic depletion is most pronounced in winter precipitation, whereas summer rainfall is on average significantly enriched—with an average $\delta^{18}O$ value of −7.73‰ and a median value of −7.25‰ (Figures 7 and 8; Table 3).

The Weierbach stream water $\delta^{18}O$ is a damped reflection of the precipitation input (Figures 7 [top] and 8). Observed values of $\delta^{18}O$ in stream water between 2010 and 2015 range between −10‰ and −6.5‰. Stream water has an average $\delta^{18}O$ value of −7.90‰ and a median value of −7.85‰ (Figure 8; Table 3).

In the freshwater pearl mussel growth bands, our SIMS-based estimations of stream water $\delta^{18}O$ revealed a strong seasonality, with an average $\delta^{18}O$ value of −7.19 and a median value of −7.14‰ (Figures 7 and 8; Table 3). Due to the fact that the SIMS analysis was restricted to the much larger summer growth lines (winter lines being substantially smaller due to limited growth of the pearl mussels in winter), it is likely that the full range in $\delta^{18}O$ values may not be entirely covered by the data series at hand.

5 | DISCUSSION

5.1 | On the technical aspects of shell material sampling and isotope analysis

To the best of our knowledge, this is the first application of the SIMS technique for freshwater pearl mussel shell analysis intended to reconstruction of stream water $\delta^{18}O$ time series. In comparison with the vast majority of other more conventional protocols used in mass spectrometry (e.g., drilling and shell crushing), the SIMS technique allows in situ high accuracy isotopic measurements (including replication) along growth lines: for example, 15-μm SIMS beam spot size versus several hundreds of micrometre for a drill bit diameter. Its spatial resolution is large enough to observe isotopic variations within one growth year. It is well suited for analysing isotopic signatures of O in shell material—especially from the perspective of reconstructing historical series of in-stream isotope signatures.

5.2 | Is the mussel shell material a faithful reconstruction of the stream signal?

The reconstructed stream water $\delta^{18}O$ isotopic signature from shell material is dependent on stream water temperature. Because the date of death of the studied pearl mussel is unknown, we had to rely on average stream water temperature (April to September), applied to all SIMS-based measurements for inferring stream water $\delta^{18}O_w$ values. Independently from this temperature effect, our measured shell-derived stream water $\delta^{18}O$ exhibited a strong damping of the

TABLE 2 Measured $\delta^{18}O$ values (PDB and VSMOW) in a freshwater pearl mussel (*Margaritifera margaritifera*) shell and estimated stream water $\delta^{18}O_w$ values for six growth bands and four replicate lines

Growth band	Line 1	Line 1	Line 1	Line 2	Line 2	Line 2	Line 3	Line 3	Line 3	Line 4	Line 4	Line 4
	PDB	VSMOW	$\delta^{18}O_w$	PDB	VSMOW	$\delta^{18}O_w$	PDB	VSMOW	$\delta^{18}O_w$	PDB	VSMOW	$\delta^{18}O_w$
1				−6.65	24.06	−6.79	−7.60	23.08	−7.74	−6.16	24.56	−6.30
1	−6.56	24.15	−6.70	−6.39	24.32	−6.53	−5.83	24.90	−5.97	−5.77	24.96	−5.91
1	−6.65	24.05	−6.79	−6.75	23.96	−6.89	−5.95	24.78	−6.09	−6.58	24.12	−6.72
1	−7.13	23.56	−7.27	−5.85	24.87	−6.00	−5.63	25.11	−5.77	−5.82	24.91	−5.96
1	−5.97	24.76	−6.11	−5.45	25.29	−5.59	−6.96	23.74	−7.1	−6.64	24.07	−6.78
Average			−6.72			−6.36			−6.53			−6.34
2	−6.66	24.04	−6.80	−5.88	24.85	−6.02	−7.00	23.69	−7.14	−4.95	25.81	−5.09
2	−6.98	23.71	−7.12	−5.61	25.13	−5.75	−7.00	23.69	−7.14	−6.05	24.68	−6.19
2	−7.14	23.55	−7.28	−7.64	23.03	−7.78	−7.04	23.66	−7.18	−6.98	23.72	−7.12
2	−7.10	23.59	−7.24	−7.32	23.36	−7.46	−7.37	23.31	−7.51	−7.14	23.55	−7.28
2	−7.92	22.75	−8.06	−7.23	23.45	−7.37	−7.69	22.99	−7.82	−7.69	22.99	−7.83
2	−8.04	22.62	−8.18	−7.55	23.13	−7.69	−7.49	23.19	−7.63	−8.53	22.12	−8.67
Average			−7.45			−7.01			−7.40			−7.03
3	−8.22	22.44	−8.36	−8.33	22.32	−8.47	−8.75	21.89	−8.89			
3	−7.98	22.68	−8.12	−8.99	21.64	−9.13	−6.65	24.06	−6.79			
3	−7.03	23.66	−7.17	−7.58	23.10	−7.72	−6.62	24.09	−6.76			
Average			−7.88			−8.44			−7.48			
4	−6.41	24.30	−6.55	−6.50	24.21	−6.64	−6.71	23.99	−6.85			
4	−7.91	22.75	−8.05	−7.52	23.15	−7.66	−5.26	25.49	−5.4			
4	−7.50	23.18	−7.64	−6.79	23.91	−6.93	−5.57	25.17	−5.71			
Average			−7.41			−7.08			−5.99			
5	−7.03	23.66	−7.17	−5.90	24.83	−6.04	−7.04	23.66	−7.18			
Average			/			/			/			
6	−7.71	22.97	−7.84	−7.86	22.81	−8.00	−7.81	22.86	−7.95			
6	−8.37	22.28	−8.51	−8.01	22.66	−8.15	−6.66	24.04	−6.80			
6	−8.25	22.40	−8.39	−6.80	23.90	−6.94	−6.99	23.71	−7.13			
6	−9.09	21.54	−9.23	−6.36	24.36	−6.5	−6.61	24.09	−6.75			
6	−10.06	20.54	−10.19	−7.23	23.45	−7.37	−8.32	22.33	−8.46			
Average			−8.83			−7.39			−7.42			

Note. PDB: Pee Dee Belemnite; VSMOW: Vienna Standard Mean Ocean Water.

TABLE 3 Statistics of $\delta^{18}O$ signatures (average, median, and total range) in precipitation (5 years), stream water (5 years), and the stream water inferred from pearl mussel shell analyses (~5 years)

	Precipitation (‰)	Stream water (‰)	Stream water (inferred from pearl mussel shell analyses; ‰)
Average	−7.73	−7.90	−7.19
Median	−7.25	−7.85	−7.14
Range	11.57	3.34	5.10

precipitation signal, consistent with our stream water signal from a nearby catchment.

Our preliminary results suggest that the range in the shell $\delta^{18}O$ signal seems to be slightly larger than in stream flow. This needs to be confirmed by further investigations. The fact that the pearl mussel specimen has been collected in a different catchment, of different size, and at a different time than water samples used for stream isotope signature analysis certainly is a source of uncertainty in this respect. Also, the fact that our SIMS measurements were carried out on a single shell may not allow to account for the full range of natural variability in $\delta^{18}O$ signals recorded by the pearl mussels. For this proof-of-concept work, we focused mainly on multiple measurement profiles along the growth bands of a single shell to verify the replicability of the analytical protocol. Future SIMS-based work will therefore have to rely on larger numbers of investigated shells.

Nevertheless, our findings are encouraging and consistent with earlier work (based on mechanic drilling and isotope ratio mass spectrometers), where glacier meltwater dynamics have been reconstructed over several years in a Greenland fjord from oxygen isotope ratios determined from growth rings in shells of *M. edulis* (Versteegh et al., 2012). Along similar lines, Versteegh et al. (2009, 2010) have documented for two rivers in the Netherlands a clear relationship between stable isotope signatures in growth rates of unionid freshwater bivalves and isotope signatures in ambient river

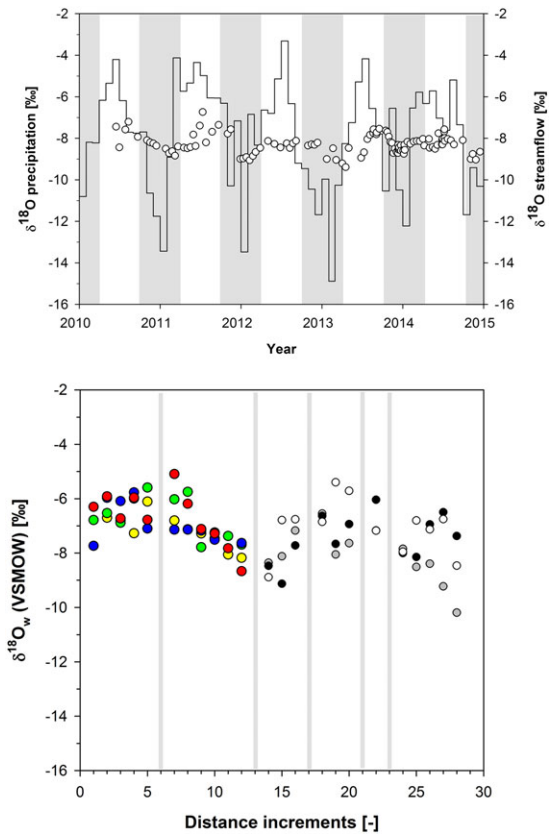

FIGURE 7 Time series of $\delta^{18}O$ signatures in precipitation (top lines), stream water (top dots) and stream water (as inferred from pearl mussel shell along secondary ion mass spectrometry analysis path; bottom). Grey bars indicate winter season. Blue, red, green, and yellow dots represent four profiles in Zone 1 (Bands 1 and 2). White, grey, and black dots represent three profiles in Zone 3 (Bands 3 to 6)

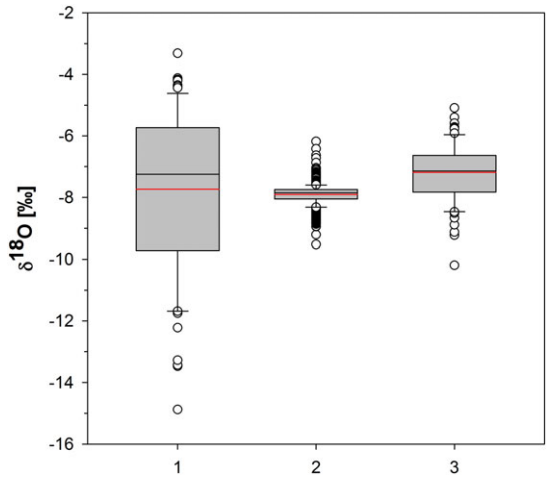

FIGURE 8 Box plots of $\delta^{18}O$ signatures in (1) precipitation, (2) stream water, and (3) stream water inferred from pearl mussel shell analyses. Horizontal black line: median. Horizontal red line: average

water. They reported the fastest growth rates for spring and early summer—coinciding with largest food availability in stream water. Shell growth onset and cessation were largely controlled by water temperature.

6 | CONCLUSIONS

Mussels are living archives of past environmental conditions in stream water. Pearl mussels—or any other freshwater mussel—offer considerable potential for providing complementary data to the IAEA GNIR (International Atomic Energy Agency Global Network of Isotopes in Rivers) network. They may serve to both extend existing records of stream water isotope data and provide stream water isotope data for previously nonmonitored streams.

Our proof-of-concept work shows that identification of seasonality in annual sequences in pearl mussel shell material is possible for *M. margaritifera*. The SIMS technique provided consistent $\delta^{18}O$ signatures in six successive growth lines and along parallel replication profiles. We found similar average and median $\delta^{18}O$ values for precipitation, stream water, and shell material. Isotope signal amplitudes were highest in precipitation, with a significant damping characterizing both the $\delta^{18}O$ signatures in stream water and shell material. Because the studied pearl mussel is found in a wide array of geographical settings where precipitation and stream water have been sampled in previous isotope hydrology studies, this finding can be confirmed by others and with further analyses.

More experimental work is needed under controlled laboratory conditions to better understand the links between stream water isotope signatures, water temperature, and shell material. Experiments are needed to show mechanistically how living specimens (e.g., submerged in water with artificially enriched water) eventually assimilate into their shell material the isotopic signatures in stream water. Further work should also focus on the potential for SIMS analysis to determine $\delta^{2}H$ signatures in stream water from shell material.

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