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EVALUATION OF AQUEOUS GASTROPOD SHELLS AS GROUNDWATER RADIOCARBON PROXIES ACROSS SPECIES AND SITES

J C Lerback^{1,2*}  • S Bagge¹ • B B Bowen^{1,3}

¹Department of Geology and Geophysics, University of Utah, 115 South 1460 East, Room 383, Salt Lake City, UT 84112, USA

²Department of Earth, Planetary and Space Sciences, University of California, Los Angeles, 595 Charles Young Drive East, Box 951567, Los Angeles, CA 90095, USA

³Global Change and Sustainability Center, University of Utah, 115 South 1460 East, Room 234, Salt Lake City, UT 84112, USA

ABSTRACT. We evaluate carbonate gastropod shells as ¹⁴C proxies for groundwater discharge at springs. Groundwater ¹⁴C is commonly used to estimate groundwater transit times, and a carbonate shell proxy would present a different way of collecting groundwater ¹⁴C data. Specifically, we test the hypothesis that in exclusively groundwater-fed spring systems, water ¹⁴C is preserved in carbonate shells at multiple sites, species, and water ¹⁴C. We first present isotopic and water temperature variability over several years at three spring sites in Utah. We then compare the ¹⁴C of contemporaneously collected water, sediment, and shells of benthic gastropods (*Melanoides tuberculata*, *Pyrgulopsis pilsbryana*, and *Physella gyrina*). We show that water and shell ¹⁴C activities at each site are correlated (slope = 1.00, R² = 0.999, n = 22). These results support the hypothesis that ¹⁴C from groundwater is preserved in carbonate shells, and that aqueous gastropods a viable groundwater ¹⁴C proxy. Finally, we describe the utility and limitations of using gastropod shells as a groundwater ¹⁴C proxy.

KEYWORDS: gastropods, groundwater, hydrology, isotopes.

INTRODUCTION

Radiocarbon (¹⁴C) can be used to estimate apparent transit times in groundwater aquifers (also referred to as residence times and groundwater “ages” in the literature) (Ingerson and Pearson 1964; Mook et al. 1974; Tamers 1975; Fontes and Garnier 1979; Fontes 1992; Kalin 2000; Geyh 2000; Plummer and Glynn 2013; Han and Plummer 2016; Cartwright et al. 2020; and others). Modeling ¹⁴C depletion in groundwater must account for the variation of atmospheric or initial ¹⁴C activities and for soil-gas exchange and C dissolution in both open and closed systems. These processes are commonly accounted for using stable carbon isotopes (δ¹³C), dissolved inorganic carbon (DIC) concentration, and knowledge of the aquifer system mineralogy and flow geometry (with a variety of methods reviewed in Clark and Fritz 2013; Han and Plummer 2016; Cartwright et al. 2020 and references therein). Because of these considerations, ¹⁴C is useful to estimate groundwater mean transit times of 100–30,000 years and are particularly useful (if imprecise) in arid and semi-arid regional aquifers (Cartwright et al. 2020). Calculation of these averaged transit times are critical for understanding groundwater flow rates, which in turn inform groundwater recharge rates and management strategies for sustainable resource use (Cook and Böhlke 2000).

Gastropods and other mollusks build carbonate shells, the chemistry of which have been used as a proxy for a variety of paleoenvironmental conditions. Gastropods are excellent proxies for aquatic geochemical environments because they are globally distributed and live in a wide variety of environments from hypersaline and marine, to freshwater environments (Fortunado 2016). Many studies show that aqueous δ¹³C signatures are retained in carbonate shells are preserved in the sedimentary record (Keith et al. 1964; Mook and

*Corresponding author. Email: jlerback@ucla.edu

Vogel 1968; Fritz and Poplawski 1974; von Grafenstein et al. 1992; Tevesz et al. 1997; Balakrishnan et al. 2005; Shanahan et al. 2005; Salvador 2018; and others). This paper addresses the idea that gastropod shells may provide material which records ^{14}C of groundwater, a proxy for groundwater transit times. The collection of spring-dwelling gastropod shells requires little equipment (a shovel and a container) and the ^{14}C analysis is a simple and cost-effective laboratory procedure.

Previous studies have suggested this direct relationship between the ^{14}C activity of springwaters and shells. Riggs (1984) described the springwater at two sites with very depleted water ^{14}C activities (11.6 percent modern carbon, or pMC, at Crystal Pool and 3.9 pMC Big Springs in Ash Meadows, Nevada) measured in 1973 (Pearson and Bodden 1975; Winograd and Pearson 1976) and two shells collected the same sites at an unspecified later date (10.6 pMC and 3.3 pMC for Big Spring and Crystal Pool, respectively). Riggs reports a third shell and water at King Spring with a 4.3 pMC difference (where water is 1.6 pMC and shell is 5.9 pMC), which they attribute to partial water ^{14}C reequilibration with the atmosphere due to the shallow flow from the discharge are to the shell collection pool. Notably, Brennan and Quade (1997) use Riggs' (1984) correlation to apply this ^{14}C depletion in sedimentary records as a for past groundwater transit times. However, they suggest but do not model the potential effect of springwater reequilibration with air as it flows away from the discharge points, nor do they investigate a numerical relationship between modern waters and modern shells at the study sites (although these sites are in the same general region of southern Nevada, U.S.A. as the Riggs 1984 study). Lastly, Copeland et al. (2012) suggest that the ^{14}C depletion due to groundwater chemistry described by Riggs (1984) and Brennan and Quade (1997) may explain their observed ^{14}C depletion of aquatic snails relative to stratigraphic age markers in Late Pleistocene archaeological deposits, but do not argue that the shells are directly representing groundwater transit times.

To add to these previous studies, this study includes (1) a seasonal evaluation of water isotopic variation through time, (2) contemporaneously collected water and shell samples, (3) shells collected directly at spring discharge sites, (4) with a range of groundwater transit times (modern and Holocene) to derive a robust correlation at different spring types, (5) shells of several genera (including endemic genera) to constrain inter-species variation of $\Delta^{14}\text{C}_{s-gw}$, (6) a comparison of ^{14}C of organic C in detritus to shell and water ^{14}C , and (7) a transect of ^{14}C in shells with increasing distance from the subsurface discharge point at one site.

We measure the relationship between modern water and modern shells, testing the viability of gastropod shells as a proxy for groundwater ^{14}C . Specifically, we test the hypothesis that groundwater and shell ^{14}C activities (C_{gw} and C_s , respectively) are equal across gastropod genera, sites, and at different ^{14}C activities. We propose the notation of $\Delta^{14}\text{C}_{s-gw}$ to represent this comparison calculated by the following equation:

$$\Delta^{14}\text{C}_{s-gw} = {}^{14}\text{C}_s - {}^{14}\text{C}_{gw} \quad (1)$$

where $\Delta^{14}\text{C}_{s-gw}$ is 0 if the hypothesis is supported.

In comparison, stable carbon isotopes ($\delta^{13}\text{C}$) have been shown to be generally retained in carbonate shells but also exhibit measurable variations with shell mineralogy, species, growth season, temperature, salinity, or other environmental parameters often described as “vital effects” (Balakrishnan et al. 2005; Shanahan et al. 2005; Salvador 2018). We consider

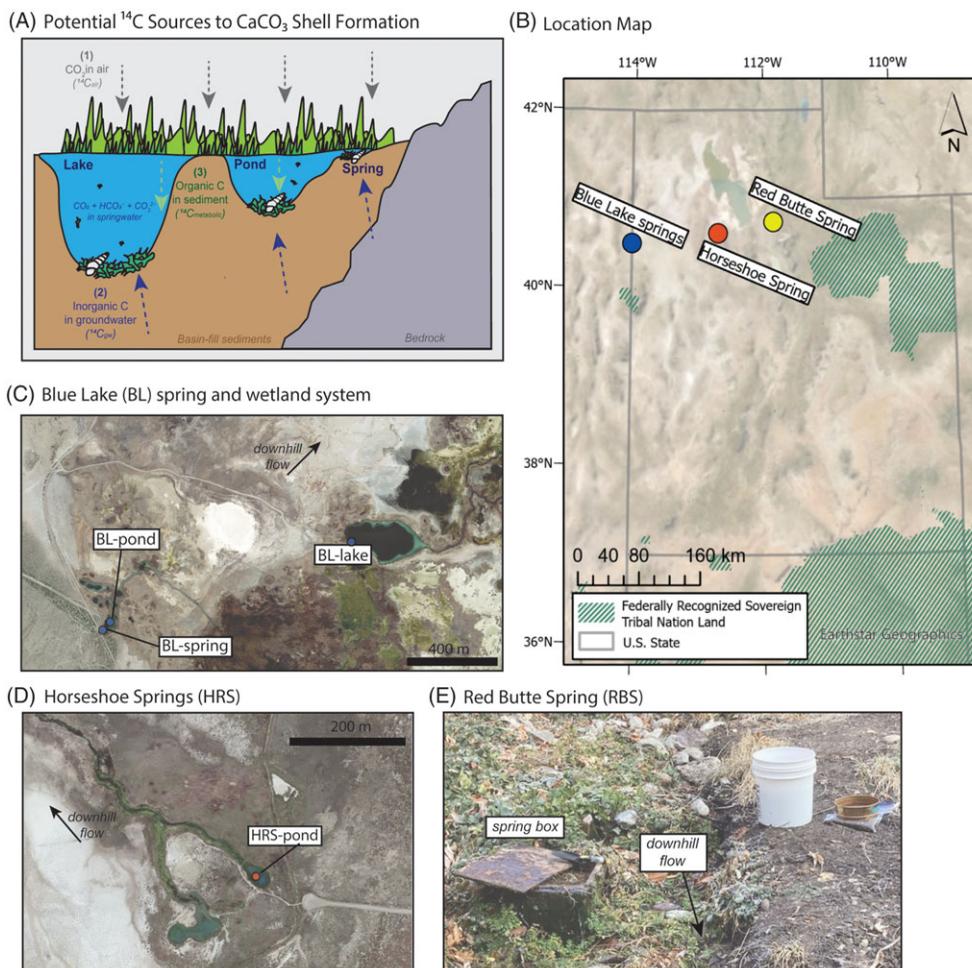


Figure 1 Site information. (A) A schematic cross-section (not to scale) of spring with potential carbon sources that contribute to the formation of carbonate shells. (B) Sample sites are shown as dots on a map of Utah. The purple cross represents a weather station. (C) Sampling sites within the Blue Lake (BL) spring system. (D) Sampling site from Horseshoe Spring (HRS). (E) Field photo from Red Butte Spring.

the following three C sources that contribute to carbonate shell formation: (1) Carbon dioxide in the atmosphere equilibrates with dissolved gasses in the water at the air-water interface, where $^{14}\text{C}_{\text{air}}$ (or “modern carbon”) is approximately 100 pMC. (2) Inorganic carbon dissolved in groundwater seeps into the spring through the bottom sediments or fractured bedrock. This course of DIC is likely depleted in ^{14}C due to the transit time in the aquifer saturated zone, with contributions from other DIC sources such as dissolution of carbonate aquifer material, among others (for more information, please see a more thorough examination of these considerations in the discussion). (3) Organic carbon in detritus from plant, algal, or microbial material either falls into the spring or grows in the water column. The detritus is consumed by gastropods and used to form shell carbonate. We measure the organic ^{14}C in sediment which we treat as a relative proxy for metabolic ^{14}C . These C fluxes are presented in Figure 1A and described further by McConnaughey and Gillikin (2008).

Overall, this study examines the relationship between groundwater and aquatic gastropod shells as an alternative sampling material for researchers investigating groundwater transit times.

Site Description

We sampled three spring sites in northwestern Utah, on traditional and ancestral lands of the Newe/Western Shoshone, Goshute, and Ute peoples (Figure 1B). Blue Lake (BL) and Horseshoe (HRS) springs are large wetland spring systems in Utah's West Desert. The third site is Red Butte Spring (RBS): an urban spring in Salt Lake City that runs down a hillside into Red Butte Creek. All three spring sites support perennial wetland ecosystems and were selected as they represent a range of groundwater transit times, spanning the Holocene (BL) to modern (RBS) (Fitzgerald 2019; Lerback et al. 2019).

BL is in the Salt Spring Waterfowl Management Area, near the Utah-Nevada border and HRS is just south of the Great Salt Lake (Figure 1C). Neither BL nor HRS are fed by surface water runoff as there is no visual evidence of streams directed to the springs, and both springs occur near fractured limestone outcrops and flow along a slight topographic slope towards the basinal playa deposits, remnants of glacial Lake Bonneville (Louderback and Rhode 2009; Lerback et al. 2019). BL and HRS are fed by groundwater discharge from the bottom of the springs. Prior investigation at BL-spring modeled ^{14}C apparent groundwater transit times of Holocene ages (Lerback et al. 2019). BL is the largest of the spring systems sampled and was sampled from three sites: (1) a shallow (0.5 m depth) spring (called "BL-spring"), which seeps from fractured limestone a meter above the other pools, (2) a pond ("BL-pond") 2–3 m in depth just below the BL-Spring, and (3) the main lake bottom, about 17 m in depth ("BL-lake").

HRS has two primary ponds with perennial active discharge that converge as the water runs west towards the playa sediments (Figure 1D).

RBS discharge had a tritium activity over 5 TU, representing modern groundwater recharge, and a modeled apparent transit time between 0 and 5 years using ^3H - ^3He dating methods (Solomon et al. 1993; Fitzgerald 2019). This site is on a slope such that water pools only to about 5 cm depth. The springwater discharges from a piped spring box, which is where aquatic gastropods were found (Figure 1E).

Gastropod Physiology

Gastropods build their shells in a series of conical spirals, or whorls. Calcium and CO_2 -bicarbonate ions are primarily supplied by direct uptake from the surrounding environment, although in some species metabolic carbonate can supply up to 10% of the carbon (Wilbur and Saleuddin 1983; McConnaughey 2003; Shanahan et al. 2005). Shell carbonate is precipitated in the extrapallial fluid or extracellular calcifying fluid between the mantle (soft inner tissues) and the solid shell. Calcareous material (either calcite or aragonite) is fixed in layers with an organic matrix to extend or expand the shell whorls (Wilbur 1964). We note that lung-breathing gastropods may use more metabolic C in their shells, making them less ideal representatives of water chemistry (Goodfriend 1992; Goodfriend and Ellis 2002; Stott 2002; Copeland et al. 2012; Hill et al. 2017; Padgett et al. 2019).

MATERIALS AND METHODS

Water and sediments (containing gastropod shells) were collected at BL, HRS, and RBS spring sites in Utah's West Desert to understand spatial and temporal variation in spring chemistry. Exact locations are provided in the data hosted under the doi:10.5281/zenodo.5587202. Gastropods were collected under a research agreement with Utah Division of Wildlife Resources (4COLL10642).

Spring Environmental Characteristics

Spring environmental characteristics were sampled through multiple seasons from 2018–2020. Sample types and locations are summarized in Table 1. A Hydrolab® MS5 multiparameter Mini Sonde (OTT Hydromet, Loveland, Colorado) or a YSI® multiparameter probe (YSI Inc, Yellow Springs, OH) was used for in-situ measurements of water temperature and specific conductance with a measurement error of less than 0.5°C and 10 µs/cm, respectively. Water was collected in high-density polyethylene (HDPE) bottles that were washed with 5% HCl stable hydrogen and oxygen isotope composition analyses ($\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively). Measurements of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ were performed with a Laser Water Isotope Analyzer Picarro L2130i at the SIRFER laboratory at the University of Utah, and the reported measurement error for these is less than 0.1‰. Weather station data from station DPG25 from 2016 to 2021 (located at latitude 40.52 and longitude -113.75) was accessed through MesoWest. This data provides air temperatures measured from January 2016 to April 2021. Measured and modeled isotopes of precipitation from the University of Utah were collected from 2016 to 2022 from the Online Isotopes in Precipitation Calculator (OIPC; Bowen et al. 2005; West et al. 2009; Bowen et al. 2021).

Carbon Isotope Measurements

Water samples to be analyzed for C isotopes were collected from several points where temperature and flow rates indicated spring discharge. Samples were collected in 250-mL glass bottles with solid glass stoppers and cleaned according to Woods Hole Oceanographic Institution specifications. Sample water collected was pumped into the bottle, which was held underwater and overflowed to prevent air equilibration and bubbles within the container. The stopper was then coated with Apiezon-M high vacuum grease and twisted into the bottleneck to ensure the seal.

The top 5 cm of sediment at the spring bottom was collected in clean plastic containers. Sediment for organic carbon analysis was transferred to a clean glass vial and freeze-dried within 48 hours of sample collection. Stored, dried sediments were disaggregated using Calgon and deionized (DI) water following Forester (1999).

Bulk sediment was processed at the University of Utah to disaggregate and sort gastropod shells. Shells collected for isotopic analysis were culled and stored in 90% ethanol. Gastropods analyzed for ^{14}C in this study include *Melanooides tuberculata* and *Pyrgulopsis pilsbryana*, and *Physella gyrina* (Hovingh 2018). All are aquatic, benthic, bottom-feeding genera, which we chose to study aquatic, gill-breathing taxa because it is likely the C used to build shells is from the aqueous environment (Fritz and Poplawski 1974; Rhoads and Lutz 1980; Wilbur and Saleuddin 1983). *Melanooides* are an invasive species to Utah and distributed globally (Facon et al. 2003). This species on average lives from 1 to 5 years (Dudgeon et al. 1985; Pointier et al. 1992). This species lives both in brackish and

Table 1 Summary of samples and analyses in study.

Site	Lab	Water	Sediment	Shell- <i>Melanoides</i>	Shell- <i>Pyrgulopsis</i>	Shell- <i>Physella</i>	Total
BL-Lake	NOSAMS ^a	1	0	4	0	0	5
BL-Lake	Time-series field parameters	6	0	0	0	0	6
BL-Pond	NOSAMS ^a	1	1	4	0	0	6
BL-Spring	NOSAMS ^a	2	0	4	4	0	10
BL-Spring	Time-series field parameters	9	0	0	0	0	9
HRS	NOSAMS ^a	2	1	0	0	8	11
HRS	Time-series field parameters	11	0	0	0	0	11
RBS	NOSAMS ^a	2	0	0	0	2	4
RBS	Time-series field parameters	5	0	0	0	0	5
Total carbon isotope measurements	7	2	12	4	6	36	
Total time series field parameter measurements	31	0	0	0	0	31	

^aNational Ocean Sciences Accelerator Mass Spectrometer is abbreviated as NOSAMS.

freshwater, as well as at a wide range of water temperatures (Raw et al. 2016). *Pyrgulopsis* species is found in the Idaho, Utah, and Nevada (Hershler 1994; Liu et al. 2017). *Pyrgulopsis* are of the subclass *Hydrobiidae* and are mostly found in water temperatures from 22–35°C (Hershler 1998). Finally, *Physella* is a widely distributed aquatic species found throughout the United States (Newman et al. 1996). The *Physella* subspecies sampled in this study (*utahensis*) is endemic to Utah, Nevada, Colorado, and Wyoming. They are found in vegetated shallow spring-fed pools. This species is categorized as a generalist with a diet that consists of detritus, diatoms, fungi, and microscopic living organisms.

Sieves were used to separate size fractions of sediments, from which gastropods were collected and sorted by genus and photographed. Carbonate shells were then cleaned for chemical analysis using the following practices, also detailed in Caporaletti (2011) and described here. To remove organic material, we soaked samples for 10 minutes with 3% hydrogen peroxide (Xia and Engstrom 1997; Janz and Vennemann 2005). Shells were then rinsed with deionized water and stored in glass vials. Vials with shells and deionized water were sonicated in 10-min increments and rinsed until water remained clear after sonication (Lawrence 2008). Shells were then rinsed with ethanol and dried in a dust-free environment (Mischke 2007). Bulk shells were homogenized using a clean mortar and pestle, and smaller species were combined to acquire an adequate sample size.

Dissolved inorganic carbon in water, inorganic carbonate, and organic carbon in sediment were analyzed for ^{14}C and $\delta^{13}\text{C}$ at the National Ocean Sciences Accelerator Mass Spectrometer facility at Woods Hole Oceanographic Institution (NOSAMS). ^{14}C to ^{12}C in an unknown sample relative to the known ratio in appropriate measured standards. ^{14}C was reported as fraction modern C, which we discuss in the text as percent modern C (pMC), as specified by Stuiver and Polach (1977). Modern ^{14}C is defined at NOSAMS as 95% of the ^{14}C concentration of NBS Oxalic Acid I, normalized to $\delta^{13}\text{C}_{\text{VPDB}} = -19\text{‰}$ (Olsson 1970). The average reported error is 0.12 pMC. $\delta^{13}\text{C}$ was reported as per mil (‰) relative to Vienna Pee Dee Belemnite (subscript VPDB), where error was not reported but NOSAMS lab accession number is provided. Data processing and statistical analyses were performed using R v3.6 in RStudio.

RESULTS

Spring Environmental Characteristics

BL, HRS, and RBS exhibit relatively constant characteristics through seasons as sampled from 2016 to 2021 (Table 2 and Figure 2; Lerback et al. 2019). BL and HRS are mesothermal spring systems, whose average temperatures are 10 above the mean annual air temperature of 12°C. BL-Lake, BL-Spring, and HRS have average water temperatures of 23°C (max = 24°C, min = 22°C, n = 6), 28°C (max = 31°C, min = 26°C, n = 9), and 21°C (max = 28°C, min = 17°C, n = 10); respectively. RBS has an average temperature of 11°C (max = 12°C, min = 10°C, n = 5), which is lower than the mean annual air temperature because it is approximately 100 m higher in elevation (1372 m MSL) than the weather station (1286 m MSL). The air temperature monthly averages range from 28°C in July to -3°C in December (see boxplots in Figure 2A).

As shown in Figure 2B and Table 2, $\delta^2\text{H}$ isotope composition of springwaters varied by a maximum of 6.4‰ (at RBS) and less than 2‰ at BL and HRS. BL-Lake and BL-Spring

Table 2 Spring environmental data. N.D. means no data.

Site	Collection date	Water temp (°C)	$\delta^2\text{H}_{\text{VSMOW}}$ (‰)	$\delta^{18}\text{O}_{\text{VSMOW}}$ (‰)	SpC (uS/cm)	Data source
BL-Spring	3/26/2017	25.6	-123.89	-15.90	7556	Lerback et al. 2019
BL-Spring	4/26/2017	27.0	-124.85	-16.05	8946	Lerback et al. 2019
BL-Spring	6/2/2017	26.3	N.D.	N.D.	9131	Lerback et al. 2019
BL-Spring	7/31/2017	27.0	-124.78	-15.92	N.D.	Lerback et al. 2019
BL-Spring	9/15/2017	28.7	-125.01	-15.99	8710	Lerback et al. 2019
BL-Spring	11/18/2017	28.3	-125.00	-16.00	9306	Lerback et al. 2019
BL-Spring	3/9/2018	29.0	N.D.	N.D.	8388	Lerback et al. 2019
BL-Spring	5/3/2018	28.7	-124.50	-16.10	8891	Lerback et al. 2019
BL-Lake	1/16/2017	22.6	-123.70	-15.86	8956	Lerback et al. 2019
BL-Lake	9/15/2017	23.2	-124.01	-15.74	9135	Lerback et al. 2019
BL-Lake	11/18/2017	21.9	N.D.	N.D.	9623	Lerback et al. 2019
BL-Lake	3/9/2018	22.3	N.D.	N.D.	8702	Lerback et al. 2019
BL-Lake	5/3/2018	24.2	N.D.	N.D.	9272	Lerback et al. 2019
HRS	11/14/2016	20.2	-123.16	-16.23	N.D.	This study
HRS	2/26/2017	17.0	-122.19	-16.06	N.D.	This study
HRS	3/26/2017	21.2	-121.58	-15.98	N.D.	This study
HRS	4/30/2017		-121.98	-16.04	N.D.	This study
HRS	5/31/2017	22.2	-121.50	-16.00	9635	This study
HRS	7/31/2017	28.2	-121.66	-15.89	N.D.	This study
HRS	9/15/2017	21.2	-122.41	-16.01	9871	This study
HRS	11/18/2017	17.2	N.D.	N.D.	9828	This study
HRS	1/8/2018	21.0	N.D.	N.D.	N.D.	This study
HRS	3/3/2018	19.7	N.D.	N.D.	10085	This study
BL-Lake	11/17/2018	24.0	N.D.	N.D.	8734	This study
BL-Spring	7/28/2019	30.8	N.D.	N.D.	8886	This study
HRS	8/20/2020	20.7	N.D.	N.D.	12115	This study
RBS	3/20/2018	10.6	-120.8	-16.0	1823	Fitzgerald 2019
RBS	7/11/2018	11.22	-118.3	-15.5	1143	Fitzgerald 2019
RBS	10/22/2018	12.35	-118.7	-15.5	1084	Fitzgerald 2019
RBS	1/9/2019	11.45	-114.4	-13.8	2106	Fitzgerald 2019
RBS	3/26/2021	9.8	N.D.	N.D.	N.D.	This study

yielded an average $\delta^2\text{H}$ isotope composition of -123.8‰ (max = -123.7‰ , min = -124.0‰ , $n = 6$), and -124.7‰ (max = -123.9‰ , min = -125.0‰ , $n = 9$). The $\delta^2\text{H}$ isotope compositions of HRS and RBS had averages of -122.1‰ (max = -121.5‰ , min = -123.2‰ , $n = 11$), and -118.1‰ (max = -114.4‰ , min = -120.8‰ , $n = 4$). The measured $\delta^2\text{H}$ isotope composition of precipitation varied from -17.0‰ in May to -193.3‰ in January whereas the modeled $\delta^2\text{H}$ isotope composition of precipitation varied from -63.0‰ in August to -193.0‰ in January. The annual weighted average (by average monthly precipitation) is -107.3‰ and -142.5‰ for measured and modeled $\delta^2\text{H}$ isotope composition of precipitation, respectively.

As shown in Figure 2C and Table 2, $\delta^{18}\text{O}$ isotope composition of springwaters varied by a maximum of 2.2‰ at RBS and less than 0.5‰ at BL and HRS. BL-Lake and BL-Spring yielded an average $\delta^{18}\text{O}$ isotope composition of -15.8‰ (max = -15.7‰ , min = -15.9‰ ,

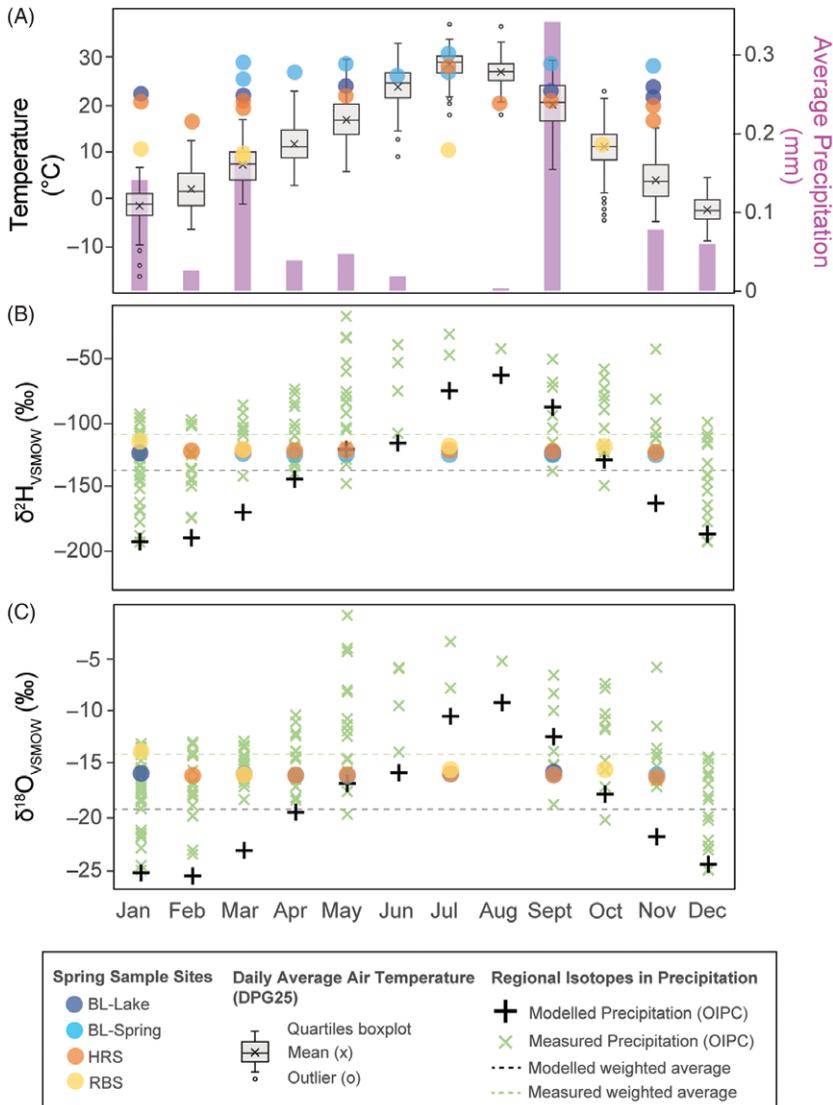


Figure 2 Seasonal variation in spring characteristics. (A) Water temperature from spring sites and air temperature from weather station DPG25 are shown by month. Daily average air temperature data are represented by monthly boxplots representing four quartiles, mean, and outliers. Temperature Monthly averages of precipitation from 2016-2021 are shown as pink bars, with the axis labeled on the left of the figure. (B) The $\delta^2\text{H}$ isotope composition of springwaters and regional precipitation is shown by month. The isotopic composition of precipitation is represented by measured and modeled data from OIPC. The dashed horizontal lines represent the annual average of precipitation weighted by precipitation amount. (C) The $\delta^{18}\text{O}$ isotope composition of springwaters and regional precipitation is shown by month. The isotopic composition of precipitation is represented by measured and modeled data from OIPC. The dashed horizontal lines represent the annual average of precipitation weighted by precipitation amount.

$n = 6$), and -16.0‰ (max = -15.9‰ , min = -16.1‰ , $n = 9$). The $\delta^{18}\text{O}$ isotope compositions of HRS and RBS had averages of -16.0‰ (max = -15.9‰ , min = -16.2‰ , $n = 11$), and -15.2‰ (max = -13.8‰ , min = -16.0‰ , $n = 4$). The measured $\delta^{18}\text{O}$ isotope composition of precipitation varied from -1.0‰ in May to -25.0‰ in December. The modeled $\delta^{18}\text{O}$ isotope composition of precipitation varied from -9.2‰ in July to -25.2 in January. The annual weighted average (by average monthly precipitation) is -14.5‰ and -19.3‰ for measured and modeled $\delta^2\text{H}$ isotope composition of precipitation, respectively.

In addition, the specific conductance (SpC) of BL-Lake is $9070 \mu\text{S}/\text{cm}$ (max = $9620 \mu\text{S}/\text{cm}$, min = $8700 \mu\text{S}/\text{cm}$, $n = 6$), BL-Spring is $873 \mu\text{S}/\text{cm}$, (max = $9310 \mu\text{S}/\text{cm}$, min = $7560 \mu\text{S}/\text{cm}$, $n = 9$), and HRS is $10310 \mu\text{S}/\text{cm}$ (max = $12120 \mu\text{S}/\text{cm}$, min = $9640 \mu\text{S}/\text{cm}$, $n = 11$). RBS had an average of $1540 \mu\text{S}/\text{cm}$ (max = $2110 \mu\text{S}/\text{cm}$, min = $1080 \mu\text{S}/\text{cm}$, $n = 5$). We did not observe a seasonal variation in SpC at these sites despite rainfall events, likely because there is less than $30 \text{ mm}/\text{year}$ on average, and we did not observe evidence of surface water runoff entering the spring systems. Runoff from snowmelt and rain events is not channeled directly to these spring systems.

Carbon Isotope Measurements

The measured radiocarbon activity of water, shell, and sediment are reported in Table 3 and discussed in the following subsections.

^{14}C values from shells and water, range from 7.9 pMC at BL-Spring to 81.8 pMC at RBS, and the ^{14}C of bulk organic C in sediment was 29.5 pMC at BL-Pond and 42.0 pMC at HRS. The average $\Delta^{14}\text{C}_{s-gw}$ across gastropod genera and site is 0.27 pMC (SD = 0.77 , $n = 22$). A paired t-test for all shell and water samples resulted in a small, statistically insignificant difference where $p > 0.05$ (mean difference = 0.27 pMC , $t = 1.65$, $df = 21$, $p = 0.11$). This close relationship does not appear to vary by $^{14}\text{C}_{gw}$; a linear model between $^{14}\text{C}_s$ and $^{14}\text{C}_{gw}$ yielded a slope of 1.002 with an R^2 of 0.999 and an RMSE of 0.751 (Figure 3A). The difference between $^{14}\text{C}_s$ and $^{14}\text{C}_{metabolic}$ is more than 20 pMC at BL-Pond and 5 pMC at HRS.

Table 4 provides summarize this studies' sample $\Delta^{14}\text{C}_{s-gw}$ by mean, sd, and n , and divided by species and site. An analysis of variance test was run to understand how $\Delta^{14}\text{C}_{s-gw}$ varies as a function of species and as a function of site. We found slight differences in average $\Delta^{14}\text{C}_{s-gw}$ by both genera ($f(2) = 25.35$, $p < 0.001$) and by site ($f(4) = 18.5$, $p < 0.001$). Separated by genera, the mean $\Delta^{14}\text{C}_{s-gw}$ for *Melanoides* and *Physella* shells is below 0.25 pMC , whereas the *Pyrgulopsis* shells had a $\Delta^{14}\text{C}_{s-gw}$ greater than 1 . Excluding the data from *Pyrgulopsis* shells, a paired t-test for shell $\Delta^{14}\text{C}_{s-gw}$ (*Melanoides* and *Physella*) resulted in a small difference at $p < 0.05$ (mean difference = -0.21 pMC , $t = -2.53$, $df = 13$, $p = 0.02$). Separated by site, HRS had $\Delta^{14}\text{C}_{s-gw}$ greater than 1 , whereas the other sites yielded $\Delta^{14}\text{C}_{s-gw}$ less than 0.3 pMC .

Sediment, shells, and water were analyzed for $\delta^{13}\text{C}$ to understand potential effects of environment-related and/or biomediated carbon isotope fractionation. $\delta^{13}\text{C}_{\text{VPDB}}$ values range between -11.3‰ to -1.8‰ for water and shell samples whereas the sediment $\delta^{13}\text{C}_{\text{VPDB}}$ is -26.6‰ and 20.6‰ at BL-pond and HRS, respectively, which are within the range of values expected of plant material in the region (Hart et al. 2010). A linear model between $\delta^{13}\text{C}_{\text{shells}}$ and $\delta^{13}\text{C}_{\text{water}}$ yielded a slope of 0.897 and an R^2 of 0.926 (Figure 3B).

Table 3 Measured carbon isotope data. N.D. means no data.

Site	Sample type	Shell species	¹⁴ C (pMC)	¹⁴ C error (pMC)	δ ¹³ C _{VPDB} (‰)	Collection date	NOSAMS accession #
BL-Spring	Shell	<i>Melanoides</i>	8.91	0.16	-2.21	7/28/2019	OS-154092
BL-Spring	Shell	<i>Melanoides</i>	8.23	0.17	-2.26	7/28/2019	OS-154093
BL-Spring	Shell	<i>Melanoides</i>	8.52	0.17	N.D.	7/28/2019	OS-154094
BL-Spring	Shell	<i>Melanoides</i>	7.93	0.17	-3.08	7/28/2019	OS-154095
BL-Pond	Shell	<i>Melanoides</i>	8.78	0.1	-2.17	7/28/2019	OS-155385
BL-Pond	Shell	<i>Melanoides</i>	9.05	0.1	-2.09	7/28/2019	OS-155386
BL-Pond	Shell	<i>Melanoides</i>	9.22	0.1	-2.37	7/28/2019	OS-155387
BL-Pond	Shell	<i>Melanoides</i>	8.91	0.1	-2.14	7/28/2019	OS-155388
BL-Lake	Shell	<i>Melanoides</i>	8.38	0.1	-3.06	11/17/2018	OS-155389
BL-Lake	Shell	<i>Melanoides</i>	8.32	0.1	-2.85	11/17/2018	OS-155390
BL-Lake	Shell	<i>Melanoides</i>	8.5	0.1	-2.98	11/17/2018	OS-155391
BL-Lake	Shell	<i>Melanoides</i>	8.66	0.1	-3.89	11/17/2018	OS-155392
HRS	Shell	<i>Pyrgulopsis</i>	36.86	0.12	-5.18	8/20/2020	OS-157606
HRS	Shell	<i>Pyrgulopsis</i>	36.61	0.11	-4.43	8/20/2020	OS-157607
HRS	Shell	<i>Pyrgulopsis</i>	36.08	0.14	-4.77	8/20/2020	OS-157608
HRS	Shell	<i>Pyrgulopsis</i>	36.2	0.13	-4.79	8/20/2020	OS-157609
BL-Spring	Shell	<i>Pyrgulopsis</i>	9.13	0.1	-1.82	8/20/2020	OS-157610
BL-Spring	Shell	<i>Pyrgulopsis</i>	9.35	0.09	-1.97	8/20/2020	OS-157611
BL-Spring	Shell	<i>Pyrgulopsis</i>	9.19	0.09	-1.94	8/20/2020	OS-157612
BL-Spring	Shell	<i>Pyrgulopsis</i>	8.99	0.09	-1.83	8/20/2020	OS-157613
RBS	Shell	<i>Physella</i>	81.81	0.21	-26.61	3/26/2021	OS-157515
RBS	Shell	<i>Physella</i>	81.83	0.19	-20.65	3/26/2021	OS-157516
BL-Pond	Sediment		29.5	0.16	-8.86	8/20/2020	OS-162412
HRS	Sediment		42.04	0.15	-8.88	8/20/2020	USGS 404442111511001 (D- 1- 1) 9cbc-S1
BL-Lake	Water		8.51	0.18	-3.73	11/17/2018	OS-154600
BL-Pond	Water		9.53	0.18	-4.00	7/28/2019	OS-154599
BL-Spring	Water		8.5	0.07	-4.50	6/2/2017	(data from Lerback et al. 2019)

(Continued)

Table 3 (Continued)

Site	Sample type	Shell species	^{14}C (pMC)	^{14}C error (pMC)	$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)	Collection date	NOSAMS accession #
BL-Spring	Water		7.82	0.18	-4.65	7/28/2019	OS-154598
HRS	Water		34.86	0.14	-7.02	8/20/2020	OS-157425
RBS	Water		81.5	0.16	-11.29	3/26/2021	OS-162396
RBS	Water		82.39	0.24	-10.88	3/26/2021	USGS 404442111511001 (D- 1- 1) 9cbc-S1
HRS-subsite1	Water		33.74	0.13	-6.54	4/9/2022	OS-167940
HRS-subsite0	Shell	<i>Pyrgulopsis</i>	36.74	0.14	-4.89	4/9/2022	OS-167940
HRS-subsite2	Shell	<i>Pyrgulopsis</i>	41.71	0.15	-5.05	4/10/2022	OS-167885
HRS-subsite3	Shell	<i>Pyrgulopsis</i>	37.65	0.14	-5.06	4/11/2022	OS-167886
HRS-subsite4	Shell	<i>Pyrgulopsis</i>	36.52	0.14	-5.15	4/12/2022	OS-167888

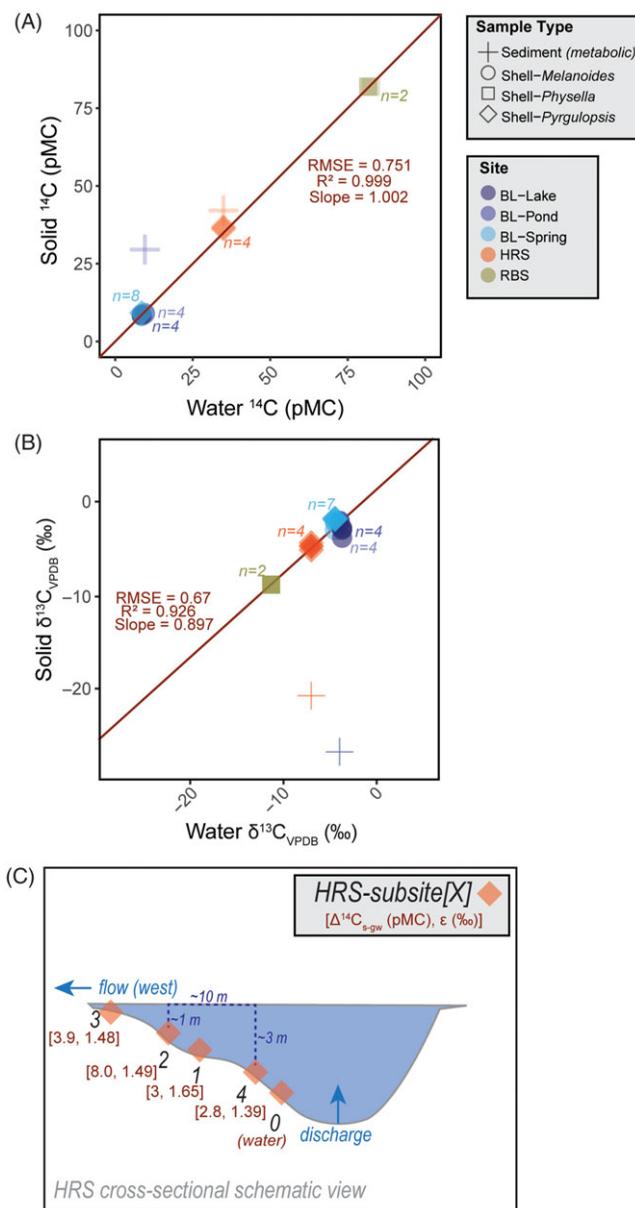


Figure 3 C isotope analyses. (A) Water ^{14}C activity is plotted against the ^{14}C activity of solid (shell and sediment) samples collected at the same place and same date. The red solid line represents a linear regression between water and shell samples, and regression statistics are annotated on the plot. Analytical error is smaller than the points shown. (B) Water $\delta^{13}\text{C}$ isotope composition is plotted against the $\delta^{13}\text{C}$ isotope composition of solid (shell and sediment) samples collected at the same place. The red solid line represents a linear regression between water and shell samples, and regression statistics are annotated on the plot. Analytical error is smaller than the points shown. (C) A schematic diagram of the bathymetry at HRS. Shells and water were collected at the four marked HRS subsites with increasing distance from the primary HRS discharge point. $\Delta^{14}\text{C}_{\text{s-gw}}$ and ϵ are annotated for each sample.

Table 4 Summary of ^{14}C isotopic differences measured between water and shells ($\Delta^{14}\text{C}_{\text{s-gw}}$). Analyses do not include data from HRS subsites. N.A. indicates that the statistic is not applicable.

Division	Data summarized by	$\Delta^{14}\text{C}_{\text{s-gw}}$			ϵ		
		Mean (pMC)	SD (pMC)	n	Mean (‰)	SD (‰)	n
None	Total	0.27	0.77	22	1.88	0.77	21
Species	Mt	-0.23	0.34	12	1.39	0.77	11
Species	Pg	N.A.	N.A.	2	N.A.	N.A.	2
Species	Pp	1.12	0.55	8	2.42	0.29	8
Site	BL-Lake	-0.04	0.15	4	0.54	0.47	4
Site	BL-Pond	-0.54	0.19	4	1.81	0.12	4
Site	BL-Spring	0.28	0.50	8	2.34	0.44	7
Site	HRS	1.58	0.36	4	2.23	0.31	4
Site	RBS	N.A.	N.A.	2	N.A.	N.A.	2

To directly compare shell $\delta^{13}\text{C}$ and water $\delta^{13}\text{C}$, we calculated the difference between shell and water, and plotted the average and SD by species represented by ϵ (Table 4). The ϵ of all samples was -1.88‰ ($n=21$, $\text{sd}=0.77\text{‰}$). A paired t-test resulted in a low p-value, where we see there is a small difference in means ($t=11.14$, $\text{df}=20$, $p<0.01$). An analysis of variance test was run to understand how ϵ varies as a function of species and as a function of site. We found a statistically-significant difference in ϵ by both genera ($f(2)=7.7$, $p<0.001$) and by site ($f(4)=18.2$, $p<0.001$).

While the ^{14}C activity of modern shells is similar to the ^{14}C activity of water, it is apparent that the ^{14}C of sediment is higher in both locations; 29.50 pMC at BL-Pond and 42.04 pMC at HRS. Due to the small sample size, we do not share statistical observations. The observed difference could be due to a fraction of the organic carbon in the sediment deriving from vascular plant material, which would reflect an atmospheric ^{14}C signal (Pigati 2002; Shanahan et al. 2005).

We note that half (4) of the *Pyrgulopsis* shells were found at the HRS site. At BL-Spring, where *Pyrgulopsis* and *Melanoïdes* shells were collected from the same site, we see a difference in average $\Delta^{14}\text{C}_{\text{s-gw}}$ by genera ($f(1)=11.96$, $p=0.014$). At this site, *Pyrgulopsis* shells had an $\Delta^{14}\text{C}_{\text{s-gw}}$ of 0.67 pMC ($\text{SD}=0.15$, $n=4$) and the *Melanoïdes* shells had an $\Delta^{14}\text{C}_{\text{s-gw}}$ of -0.10 pMC ($\text{SD}=0.42$, $n=4$). This $\Delta^{14}\text{C}_{\text{s-gw}}$ difference of 0.76 pMC between species indicates that there may be genera-dependent biomediated fractionation or differences from gastropod shell deposition (if the shell grew in a more shallow environment than where it was deposited).

Potential explanations for the observed $\Delta^{14}\text{C}_{\text{s-gw}}$ include incorporation of metabolic carbon, live gastropod mobility, or movement after initial shell deposition by the pond bathymetry, circulation, or other sediment disturbance. Previous studies show that it is possible for shells to incorporate up to 10% of their ^{14}C from metabolic C from consumed detritus (Wilbur and Saleuddin 1983; Raw et al. 2016). The $\delta^{13}\text{C}$ of a shell sample may be used to estimate how much of the ^{14}C is derived from atmospheric and metabolic C sources. However, this may not be appropriate in all cases, as we show that the $\delta^{13}\text{C}$ composition of shells is less negative than both measured water and sediment sources and that other

spring-dwelling gastropod shells been reported to demonstrate some vital effect offsets (Shanahan et al. 2005). It is also possible the shells formed at a shallower area of the pond, where modern ^{14}C from the air (around 100 pMC) was equilibrating with the water and raising the average ^{14}C activity in the water. The shells would have grown in an aqueous environment with a slightly elevated ^{14}C activity, and moved, fell, or were moved by fish or circulation to the deeper sampling point.

To address potential groundwater reequilibration with air as springwater moves away from the discharge point, we provide ^{14}C data from four subsites at HRS and a contemporaneously collected water sample from the discharge point. These samples have increasing the distance from the bottom discharge site and decreasing depth, which are shown schematically in Figure 3C. The shell and water data are provided in Table 3 and annotated Figure 3C, where the water at HRS-subsite0 had 33.7 pMC, and *Pyrgulopsis* shells ranges from 36.5 pMC at HRS-subsite4 (within a meter of the discharge) to a high 41.7 pMC at HRS-subsite2 (approximately 10 meters from the discharge area). Although there is only one sample at each subsite, these data indicate the distance from the discharge source, pond circulation patterns, and potential for gastropod movement after shell formation should be considered when using this proxy, as has been suggested by Riggs (1984) and Copeland et al. (2012).

DISCUSSION

The data presented in this study support the hypothesis that the $^{14}\text{C}_s$ represents $^{14}\text{C}_{gw}$ across three species and three spring wetlands (five sites). The data presented in this study demonstrate that modern shells do not reflect modern air ^{14}C activities nor bulk sediment organic ^{14}C (as a proxy for metabolic ^{14}C). We note that only one site had ^{14}C data for more than one species.

The $^{14}\text{C}_s$ proxy described in this study can be used to model groundwater transit times rather than direct measurement of $^{14}\text{C}_{gw}$. Measurements of $^{14}\text{C}_{gw}$ are commonly used to estimate aquifer transit times, and many methods have been developed to do this by not only considering the radioactive decay of ^{14}C along the flowpath, but also corrections including the distribution of flowpath lengths sampled (e.g., Jurgens et al. 2016; Broers et al. 2021) and the input of ^{14}C in pre-recharge atmosphere, DIC from the dissolution of aquifer materials, or soil CO_2 (Fontes and Garnier 1979; Kalin 2000; Clark and Fritz 2013; Clark 2015; Han and Plummer 2016; Cartwright et al. 2020; Wang et al. 2020 and references therein). Models of groundwater transit time demonstrate relatively small sensitivity to errors in ^{14}C input values compared with flowpath geometries, model type, and other associated uncertainties (e.g., initial $\delta^{13}\text{C}$, heterogeneity in the stable isotope ratios of matrix calcite, open-system carbonate dissolution, methanogenesis; Cartwright et al. 2020). For example, the apparent ^{14}C transit time at BL-Spring was modelled by Lerback et al. (2019) to be on average Holocene recharge. They used the measured ^{14}C of 8.5 pMC, $\delta^{13}\text{C}$ of -4.5‰ , and alkalinity of 226 mg/L as CaCO_3 and pH of 7.3, with an initial ^{14}C of 100 pMC and $\delta^{13}\text{C}$ of -22‰ . DIC from limestone was modelled to have a ^{14}C activity of 0 pMC and $\delta^{13}\text{C}$ of 0‰ , following Gardner and Heilweil (2014) transit times models of waters in the same greater aquifer system (Kennedy and Genereaux 2007). The model developed by Han and Plummer (2013) as an update to the Fontes and Garnier (1979) model uses these inputs to estimate the contribution of carbonate rock dissolution, leading Lerback et al. (2019) to calculate an apparent transit time of 5600 years. In comparison, the International Atomic Energy Agency model (Gonfiantini 1972) focuses on the potential

mixing of different flowpaths, which Lerback et al. (2019) used to calculate an apparent transit time of 10,800 years, which aligns with their modelled transit times of 11,000 in a particle simulation using the regional steady-state numerical groundwater model (Brooks et al. 2014). The difference between models is consistent with the observed model difference reviewed by Cartwright et al. (2020). Using these BL-Spring parameters and the Han and Plummer (2016) model through NETPATH, a 0.27 pMC (the average $\Delta^{14}C_{s-gw}$) change to measured ^{14}C the modeled transit time by approximately 250 years, whereas the difference between models was several thousand years (Plummer et al. 1994; Parkhurst et al. 2008; El-Kadi et al. 2010; Lerback et al. 2019).

This study builds upon three studies which touch upon this idea of shells as groundwater transit time proxies: Riggs' (1984), Brennan and Quade (1997), and Copeland et al. (2012). To directly compare our data with that of Riggs (1984), we calculate that their three sites reported $\Delta^{14}C_{s-gw}$ of 0.6, 1.0, and 4.2 pMC, where the latter (higher) $\Delta^{14}C_{s-gw}$ is associated with some potential water reequilibration with atmosphere. Building upon these initial measurements, this study reports shell and water samples collected at the same time, adds an understanding of seasonal springwater variability, and investigate the change in $\Delta^{14}C_{s-gw}$ as distance increases from the discharge source.

In addition, Brennan and Quade (1997) apply the $\Delta^{14}C_{s-gw}$ proxy to the fossil record in Nevada, USA. This paper reports the ^{14}C -ages in years B.P. rather than ^{14}C activities as pMC or fraction modern C, and as such, they report aquatic shell ^{14}C -age deficiencies (Δt) of 0–10,000 years (recognize that these ages would need other corrections for groundwater transit times as is described previously). Visual interpretation from their Figure 3 shows that this is a ^{14}C activity depletion of 0–4 pMC. While Brennan and Quade (1997) discuss the impact of springwater reequilibration with air as a function of springwater circulation or morphology, they do not assess this impact or validate this $\Delta^{14}C_{s-gw}$ proxy in modern settings. They note that to apply this proxy to the fossil record it is important to select shells without evidence of overgrowth and diagenesis to account for potential post-depositional C-exchange, and they also emphasize the importance of reliable depositional age controls.

Copeland et al. (2012) suggest the $\Delta^{14}C_{s-gw}$ proxy to explain the apparent ^{14}C depletion of shells in the archeological record at a cultural site in Sonora, Mexico. They identify an approximately 2000 ^{14}C -year lag for the aquatic shells (*Planorbella* sp.). Their data provided for Stratigraphic Unit B3 has an average charcoal ^{14}C activity of 67.7 pMC ($n = 3$) and two aquatic shells to have 51.2 pMC and 55.8 pMC. Stratigraphic Unit B5 has an average charcoal ^{14}C activity of 79.1 pMC ($n = 8$) and the co-occurring aquatic shell to have 58.1 pMC. Copeland et al. (2012) explain that the depletion is likely due to surface water chemistry and suggest that it may in part be due to groundwater depletion (citing Brennan and Quade 1997), but do not describe the depositional environment as directly groundwater-fed, recognizing that it might be an anthropogenic, managed wetland environment.

In the application of this proxy, researchers should consider the following site characteristics:

- (1) Morphology: the spring should have a pool directly fed by groundwater discharge. The pool shape and circulation should contain carbonate shells that live in springs whose flow rate is such that the advective transport of C is greater than the downward diffusion of atmospheric C, or flowing water such that the water that has not re-equilibrated with air.
- (2) Temporality: these springs should be persistent over several months (hillside springs that

go dry seasonally may not harbor aquatic snails). (3) C inputs: the pond recognizably spring-fed without significant water inputs from rain, ephemeral streams, rivers or the ocean. This is most likely in arid regions without storm runoff or river flooding that might carry modern carbon into the pool.

In sum, we test the hypothesis that in exclusively groundwater-fed spring systems, water ^{14}C is preserved in carbonate shells. Water, sediment, and shells of benthic gastropods (*Melanoides*, *Pyrgulopsis*, and *Physella*) from three sites in Utah were analyzed for ^{14}C . We show that water ($n = 6$) and shell ($n = 22$) ^{14}C activities at each site are correlated ($R^2 = 0.999$). These results support the hypothesis that ^{14}C from groundwater is preserved in bicarbonate shells, and future studies should expand upon potential species- or site- specific vital effects.

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