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EVALUATION OF BIOMEDIATED FRACTIONATION OF 13C AND 18O ISOTOPES: AQUATIC GASTROPOD PHYSIOLOGY AND GEOCHEMISTRY

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Abstract

Stable carbon and oxygen (^{13}C and ^{18}O) are widely used paleoenvironmental proxies within aquatic carbonate systems. This study characterizes fractionation from the incorporation of ^{13}C and ^{18}O from water into gastropod shells from spring locations in the West Desert region of Utah. Water-to-shell differences of stable isotopes between sites, species (of which there were six), intrashell variations, species physiology (gill and lung breathing), and shell geochemistry were measured. Water temperature, salinity, pH, and alkalinity were monitored over a four-year period at each of the locations and water isotope and major ion chemistry was characterized. Gastropod shells were isolated from the spring sediment and were cleaned using a combination of manual and chemical methods. The shells were separated by site and species and then crushed into homogenized powders using a mortar and pestle. For some shells, intrashell transects were taken using a microdrill. Samples were analyzed for $\delta^{13}C$ and $\delta^{18}O$ at the SIRFER lab at the University of Utah. Thin sections were made and examined using microscopy for three of the species. The results demonstrate variations in isotopic fractionation between species, sites, and physiology. The intrashell variation had slight statistical significance indicating variation throughout the shells. This study contributes to a larger study on the use of aquatic gastropod shells as paleoenvironmental proxies and helps to constrain stable isotope fractionation due to vital effects in physiology and mineralogy.

Positionality Statement

The first author of this study is a white cis female, who identifies as queer. She is an undergraduate student with a background in geology, geography, and sustainability studies. Both coauthors also identify as female. One is a recent Ph.D. graduate, and the other is a tenured professor. There is a unique age spread among the authors that provide a balance of experience and fresh ideas. All three authors focus on bringing intersectionality into their geoscience research along with accessibility and comprehensibility for scientists and non-scientists.

Introduction

Stable isotopes are important paleoclimate and paleoenvironmental proxies. Many calcareous organisms such as diatoms, foraminifera, ostracods, and gastropods capture these isotopes while they build their shells. Oxygen-18 (^{18}O) and carbon-13 (^{13}C) are two major

isotopes commonly used with calcareous organisms due to their incorporation into the matrix of the shells from the water they live in.

When these isotopes are analyzed, there are oftentimes discrepancies in isotope values, these are lumped together in the nebulous term vital effects (Shanahan). Being able to detangle and quantify the biomediated isotope fractionation values is valuable when using calcareous organisms for paleoclimate and paleo-hydrology proxies.

Without being able to calibrate the gastropod species isotopes, including the vital effects, to the present conditions we cannot fully test hypotheses of what isotopes "should" look like in various climate scenarios, especially accounting for shifts in reservation processes. Therefore, it is important to isolate the amount of vital effect cause by a gastropods physiology and its shell's mineralogy.

Physiology

As noted, carbonate shelled organisms such as Gastropods are widely used as geochemical proxies in paleo-reconstructions. Gastropods are specifically good proxies because they are often larger in size than other calcareous organisms (diatoms, ostracods, or foraminifera) and can be found in a range of environments from freshwater to saline water, rivers, lakes, or the oceans (Fortunato, 2016). Gastropods are excellent proxies for aquatic geochemical environments because they take the carbon and oxygen atoms directly from the water into their shells, and preserve them over time in sediments, essentially trapping the oxygen and carbon isotopes of the conditions when the shell formed.

To build their shell gastropods use calcium and bicarbonate which is taken up through their gills, transported throughout tissues and blood. They are then stored in extrapallial fluid just below the nacreous shell layer (hard outer shell) and above the outer epithelium or mantle (soft inner tissue) (Wilbur, 1964) (Figure 1). This pocket of fluid serves as a staging ground for the ions that will then be used to for the conical spirals, or whorls making up the shell. In this fluid there can be higher concentrations of magnesium which can induce aragonite growth conditions despite aragonite being a less stable form of calcium carbonate. We did find that metabolic carbonate can supply up to 10% of the carbon used to build the shell of some aquatic species (Wilbur and Saleuddin, 1983; McConnaughey, 2003; Shanahan et al., 2005). In addition to this there is a evidence that lung breathing gastropods use more metabolic carbon in their shell, which can alter their isotope values creating a less ideal proxies for water chemistry (Goodfriend, 1992; Goodfriend and Ellis, 2002; Stott, 2002; Copeland et al., 2012; Hill et al., 2017; Padgett et al., 2019).

The gastropods that were collected in this study include *Melanoides tuberculata* (Mt)*, Tryonia porrecta* (Tp)*, Pyrgulopsis pilsbryana (*Pp)*, Physella gyrina* (Pg)*, Planorbella sp. (oregonensis)* (PSP)*,* and *Succinea rusticana* (Sc). This group represents a mixture of endemic and invasive species, gill and lung physiologies, and varying calcite and aragonite compositions (Figure 3). *Melanoides* are an invasive species to Utah, and distributed globally (Facon et al., 2003; Leng et al., 1999). This species occupies both fresh and brackish water as well as a range of temperatures

(Raw et al., 2016). *Tryonia* species is endemic to Utah, Nevada, California, and Arizona (Hovingh 2018). It prefers spring-fed water systems that range in temperature from 22-28°C (Oliver and Bosworth 1999). *Pyrgulopsis* species is found in Idaho, Utah, and Nevada (Hershler, 1994; Liu et al., 2017). They are of the subclass Hydrobiidae, and are mostly found in water temperatures from 22-35°C (Hershler, 1998). *Physella* is widely distributed throughout the United States (Newman et al., 1996). This study examined a subspecies (utahensis) that is endemic to Utah, Nevada, Colorado, and Wyoming. *Physella utahensi*s inhabit shallow spring-fed pools and eat detritus, diatoms, fungi, and microscopic living organisms (Newman et al., 1996). *Planorbella* is a highly endemic species that is only found at three spring locations in Oregon, Nevada, and Utah (Blevins et al. 2017, Duncan 2008, Frest and Johannes 1995; Furnish et al. 2002). This is one of the aquatic lung breathing species, it inhabits hard substrates or submerged vegetation., but breaths in carbon dioxide (CO_2) rather than bicarbonate (HCO_3).

Methods

There were six site locations all within the west desert region of Utah. Four of the sites were near Blue Lake, the fifth site was in Skull Valley at Horseshoe Springs, and the sixth was Red Butte Springs which is located in the Wasatch Foothills (Figure 2). These sites were chosen due to their environmental stability. Their temperatures and isotopic hydrogen and oxygen levels were constant over the two-year monitoring period (Figure 4). Across these six sites, six aquatic gastropod species were studied:

The samples were collected from the different locations by taking scoops of the sediment and placing it into Tupperware containers. During this time multiparameter water measurements were taken. Once back at the lab the sediment was air dried and restored.

Picking the shells began by boiling DI water to pour into a 500mL beaker that had roughly 100mL of dry sediment. After the boiling water was added to the sediment, I added 10mL of Calgon to act as a cleaning agent. The sediment, soap, and water mixture would sit and be gently stirred every 5 minutes until all the clumps had broken up. This mixture was them poured through a three sieved system (256-106 µm (top-bottom) and rinse with DI water. After this the first and second sieves were dumped out onto a paper towel to dry. Once dry the shells were picked out from each layer and placed into containers, labeled, and stored. I did this for each site.

Once the shells were picked roughly 1g worth of shells was picked out to be cleaned. To clean the shells, I would submerge them in 3% hydrogen peroxide and sonicate them for five minutes. The content would then be emptied from the vial into a small tupperwear. The shells would be picked out of the Tupperware placed back into the rinse vial an filled with DI water and sonicated again. This step would be repeated until the water was clean. Once the water was cleaned the shells were set out to dry in the laminar flow hood.

After the shells were finished drying, I took them to the SPATIAL Lab in FASB to prepare and weight the samples to be run for their stable oxygen and carbon. In the lab I needed a mortar and pestle, glass vials with PTFE plastic screw lids, microbalance, micro scoop, weighing paper, L-shaped tweezers, straight tweezers, compressed air, ethanol, KimWipes, gloves. To powder the samples, I would place the shells into a ceramic mortar and pestle and gently crush and powdered the sample. I would then wipe the clean area and all tools that will be used with

ethanol and gently spray out microbalance with compressed air. Then I cut a multitude of 1cm X 1cm squares of weighing paper. Using the tweezers to grab a square of weighing paper I crimped it into 4ths on the clean area. Making sure the microbalance is on, I hit the 'Tare' button, and placed the weighting paper onto the balance. I then opened the sample (on the clean area) and use the micro scoop to transfer sample onto the crimped weighing paper. I then weighted the sample and weight paper on the microbalance. The target mass is **125-175µg.** Once the target mass was attained the sample was carefully remove the weight paper and delicately inserted into the glass the vial. Gently shake the paper while removing it to ensure all the sample has transferred into the vial. This procedure was repeated for each shell sample rinsing the mortar and pestle with DI water and wiping them down with ethanol.

Once all the samples were weighted into the glass vials, they were sent to the SIRFER Lab and the University of Utah's Biology Department. The results were then cleaned in excel and analyzed in R Studio.

Results

After running the shell samples the results of the elemental analysis can be seen in Table1. Figures 4-7 are graphs of these values plotted just for the Pp species. Compared to the Tp species the Pp magnesium levels are much lower. It is good to see that all the shells, including both species, are showing similar amounts of Sr. If Sr isotopes were to be run on these shells it would be hypothesized that they're ratios would also be similar. The white Pp shell does appear to have a much lower calcium content compared to the other shells. An interesting note on iron is that the blue shell has significantly larger amounts than the white and tan ones do. Unfortunately, copper was unable to be detected due to machine error.

For the isotope analysis Figure 5 shows the distribution of carbon (A) and oxygen (B) isotopes across the different sites. Water isotope values are accurately represented the snail shells' isotope values for both carbon and oxygen. All of the species' isotope values are all similar across species and site for carbon however there is significant variance within the oxygen isotope values across species and site. The transects of the Mt shells show a general decrease in carbon isotope value while the oxygen are more scattered (Fig. 6). The physiology of the shells is only reflected in one species of lung breathing snail, Pg, (Fig. 7). The carbon isotopes don't vary based on physiology

Discussion

There does not appear to be a significant reason for why the shells are varying colors. This is also probably due to a massive error on my part for only running one sample of each shell. Having a larger data set would have hopefully highlighted more trends within the species. Not surprisingly calcium is one of the varying elements due to it being the matrix of the shell ($CaCO_3$) (Table3). Strontium is lower than calcium which again makes sense with the incorporation of it into the shell and how it is widely used as a highly reliable isotope for creating isoscapes. Noting that the magnesium levels were higher in the Tp species than in the Pp species could potentially indicate that its shell has a higher aragonite content. In addition to that the white shell had much lower levels of calcium that the tan and blue ones did. This could be telling of age, time in water, or changes in pH throughout the spring.

The stable isotopes indicate that the aquatic gill-breathing snail shells capture carbon isotopes in a way that can be used to recreate the water chemistry of groundwater since they accurately reflect the water isotopes using fractionation factors. Figure 7 shows that the only vital effects to appear are in one species of lung breathing snail in oxygen isotopes. Across all species the carbon isotopes do not vary by physiology. These finding are important because they have the potential to allow us to determine past water chemistries calculated from the isotopes from shells in sediment cores using lab determined fractionation factors. This is pending our further finding of how shell mineralogy impact isotope values. Looking at the intrashell transects of the Mt shells there appears to be little variation across the shell for the oxygen isotopes, but the carbon isotopes have a slightly negative value trend towards the tip of the shell. This value was found to not be statistically significant meaning that for methodology purposes shells are ok to be homogenized regardless of study. This also means that the shell would not be a good proxy for recording short-term time periods over the life of the snail.

Conclusions

Overall, there was a lot learned from this study and as it usually happens more questions arise. The trace element analysis gave good information that can give us clues as to what the mineralogy of the shells might be as well as how they reflect the water trace elements. The stable carbon and oxygen isotopes gave good information on how the physiology of a snail can or doesn't impact their value and thus fractionation factor. As it turns out oxygen isotopes are more impacted by the physiology than the carbon isotopes are. As it stands now it appears that the vital effects discrepancy may lie in the mineralogy of the shells. This is where we would like to see further research go. In the larger research question, we would like to be able to quantify these vital effect discrepancies to obtain accurate fractionation fators that can be used in equations to model the dissolved carbonate system and the pCO_2 of the atmosphere at the time the snails were living. This can help inform us as to how groundwater systems change over time and in response to changing climates and carbon levels.

Acknowledgments

I, Sam Bagge, would like to thank Dr. Brenda Bowen and Dr. Jory Lerback for bringing me on to their research team to work on this project for the past 3 years. They both taught me more than just skills regarding field and lab work. They went the extra mile to make sure they were being effective and involved mentors and helping set me up for success. I would also like to thank the Office of Undergraduate Research for the UROP funding I received that allowed me to continue working on this project through COVID. I would also like to thank the SIRFER, SPATIAL, and Earth-Core ICP-MP labs for helping me and teaching me to run and process my samples. Seeing this side of research improved my ability to design more efficient and thoughtful sampling plans as well as how to approach the varying research questions I had.

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Tables and Figures

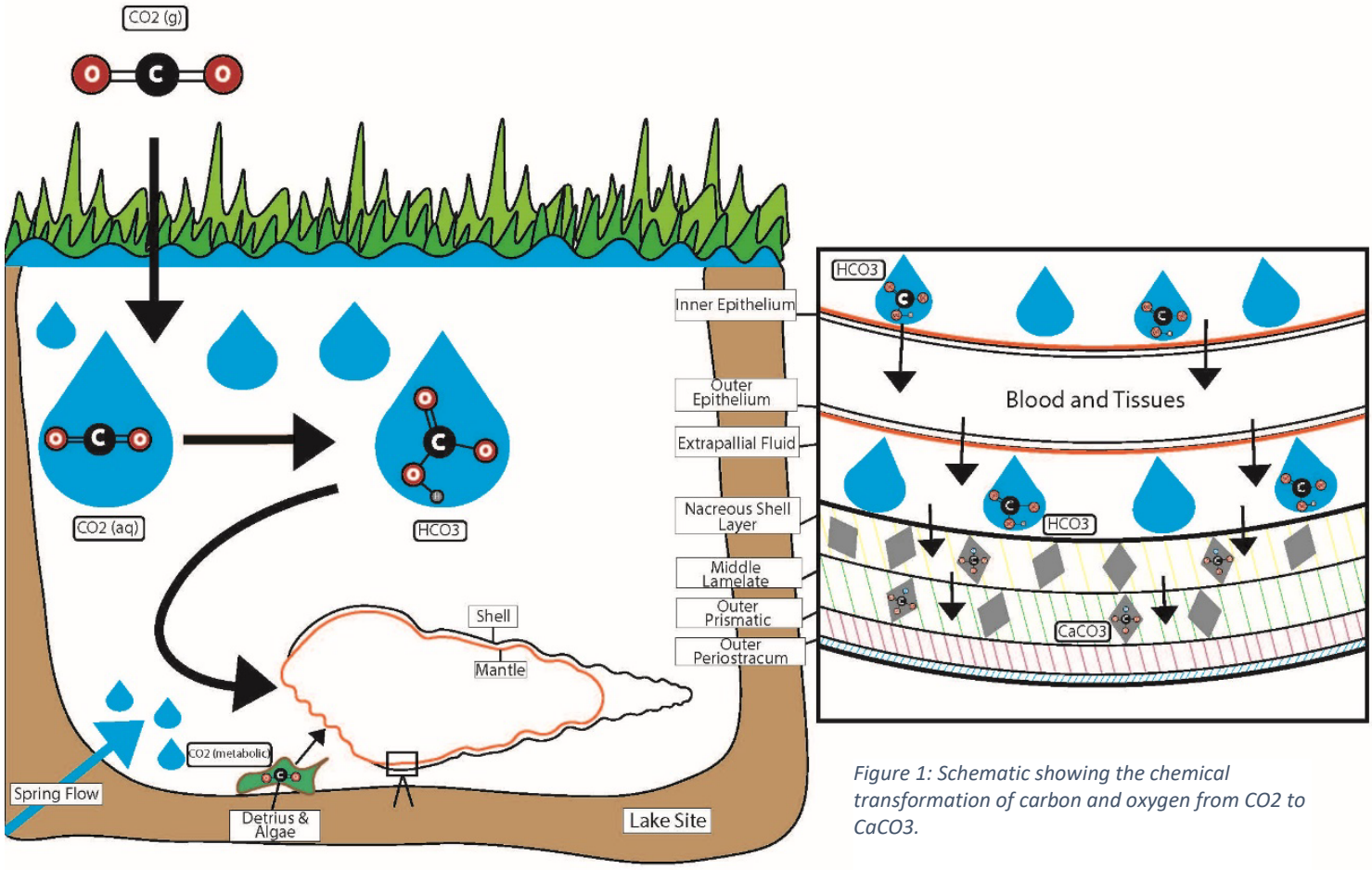

Figure 1: Schematic showing the chemical transformation of carbon and oxygen from CO2 to CaCO3.

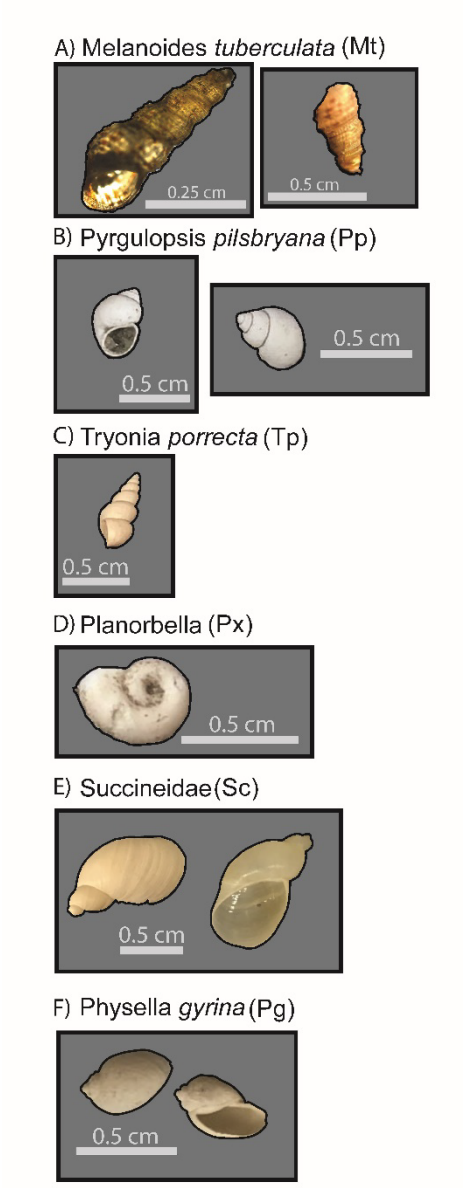

Figure 3: A) Invasive, gill breathing, aragonite. B) Endemic, gill breathing, unknown. C) Endemic, gill breathing, aragonite. D) Endemic, lung breathing, aragonite. E) Endemic, lung breathing, unknown. F) Endemic, gill breathing, unknown.

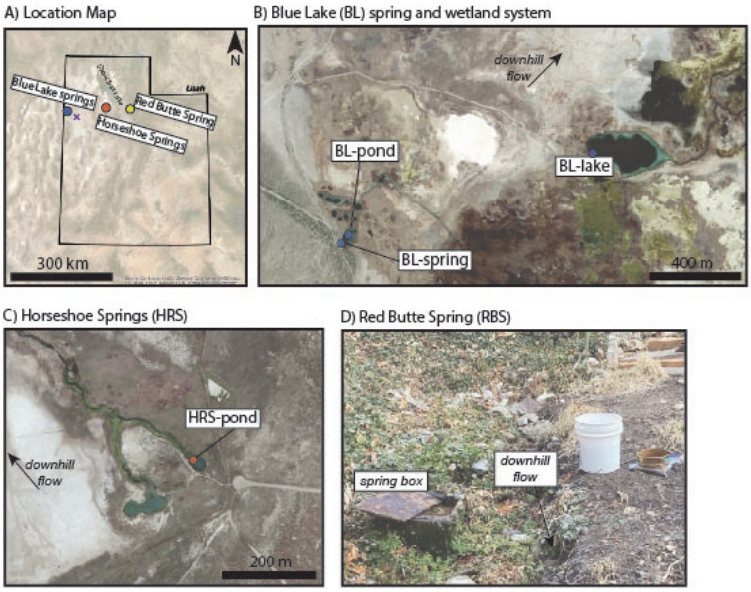

Figure 2: A) Shows all three groundwater spring sites. B) Shows the sites located at Blue Lake Springs and the groundwater flow. C) Horseshoe Springs and the site location with the groundwater flow. D) Red Butte Springs spring box and the groundwater flow direction.

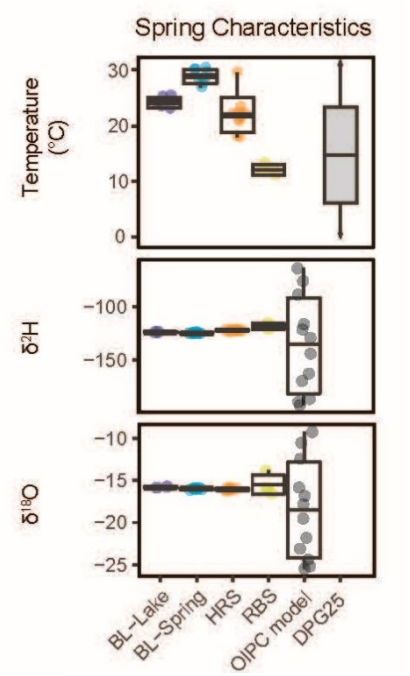

Figure 4: Three stability parameters showing measurements taken constantly at each spring site.

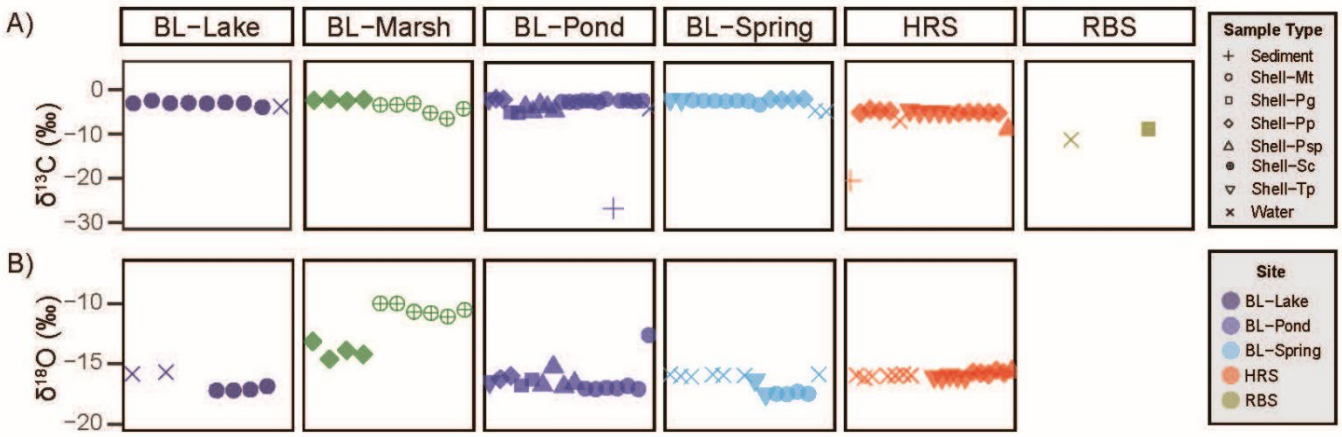

Figure 5: A) Shows the various carbon-13 isotopes across the six sites and six species. Sediment and water isotopes are also included. B) Shows the oxygen-18 isotopes across sites, species, water, and sediment.

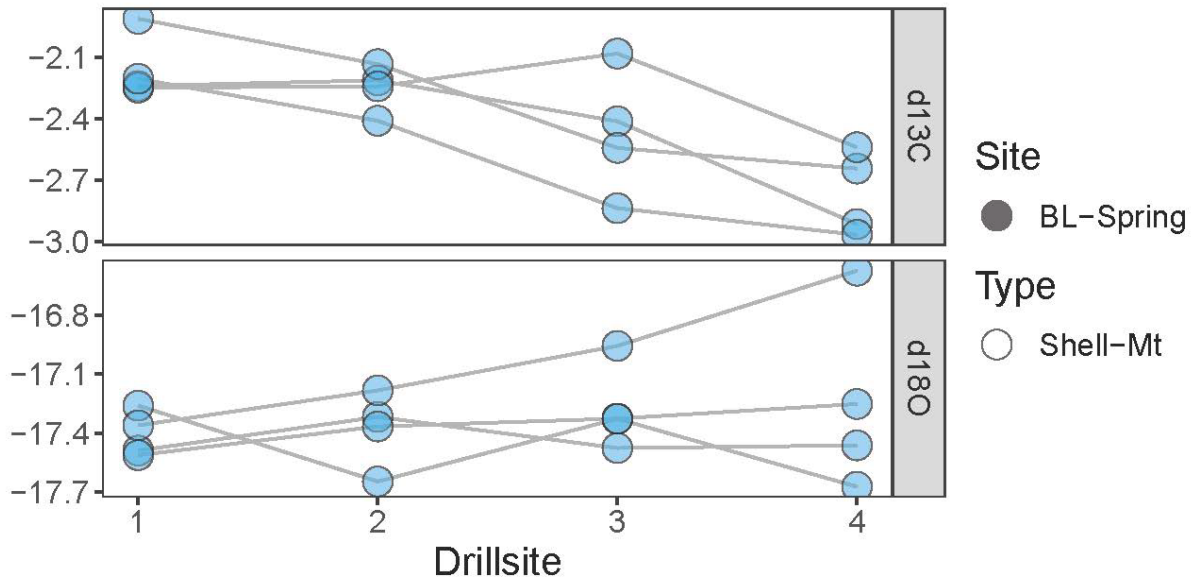

Figure 6: Figures showing the carbon-13 (top) and oxygen-18 (bottom) isotope values at 4 drill sites along the Melanoides tuberculata shells.

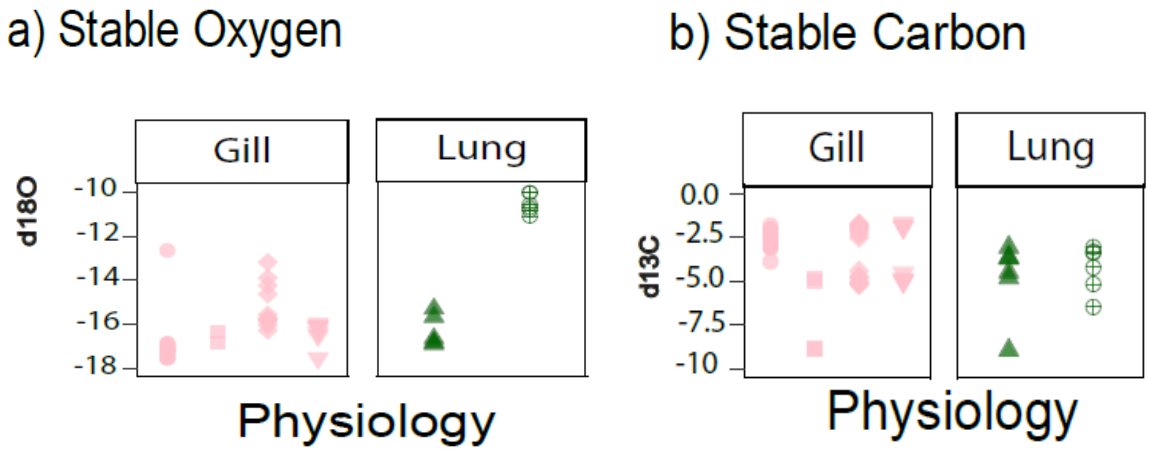

Figure 7: a) Shows the distribution of oxygen isotopes verses the snails' physiology. b) Shows the carbon isotope distribution versus the snails' physiology.

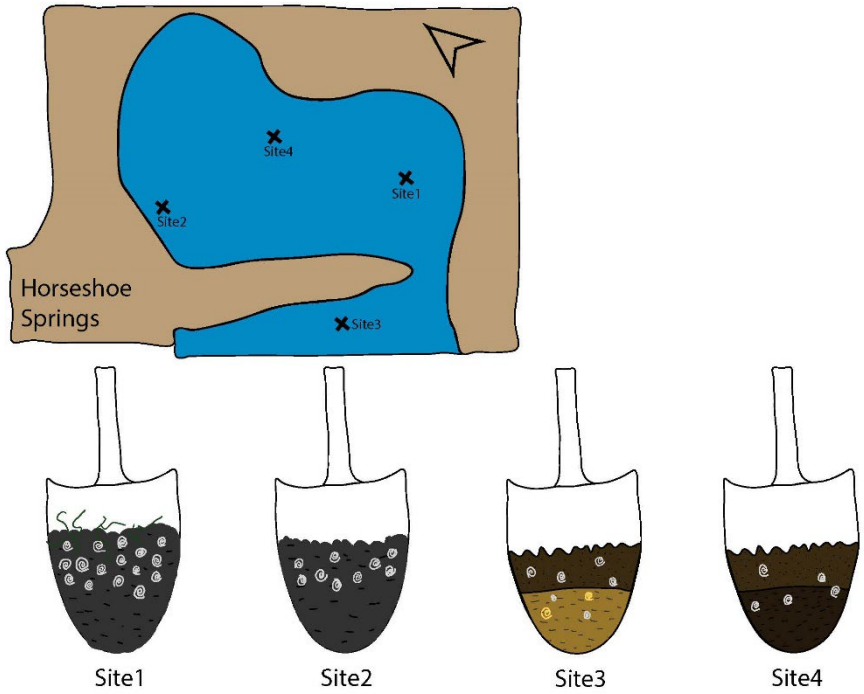

Figure 8: Figure showing the four different site locations in Horseshoe Springs as well as their observed lithologies.

	Element								
Shell	**Li**	**Be**	**Na**	**Mg**	**Al**	**K**	**Ca**	**Fe**	**Sr**
Pp Blue	1.597256	0.006959	2170.257	80.59641	5.946195	20.85199	398182.4	100.0258	1263.86
Pp White	1.469738	0.007691	1960.424	63.91406	12.28618	17.68818	347402.3	67.23353	1131.32
Pp Tan	2.00035	0.010529	2666.641	51.14775	7.120932	22.44113	415211.2	14.2352	1200.77
Tp	1.444302	0.013381	2878.581	230.9201	81.87697	45.00295	425228.9	64.69341	1317.47
Deep Water	0.003631	3.82E-05	13.73596	11.8183	0.955221	31.74669	14673.51	0.558817	13.93732
Shallow Water	0.043009	0.000192	204.521	7.72862	0.508837	2.14963	12395.67	3.456159	47.99967

Table 1:Table showing the different elements in ppm within the differing HRS shells.

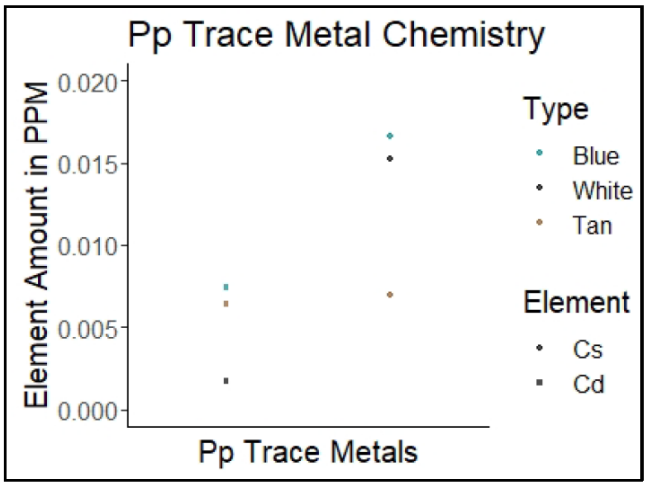

Figure 8: Scatter plot showing the Trace metal amounts in ppm in the Pp shells from HRS.

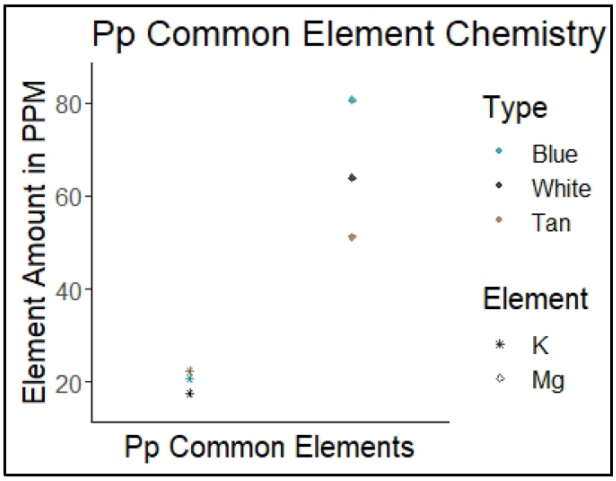

Figure 9: Scatter plot showing the common elements in the Pp shells from HRS.

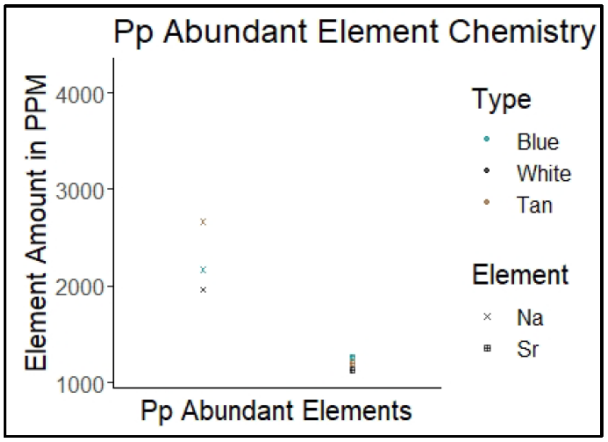

Figure 10: Scatter plot showing the abundant elements in the Pp shells from HRS.

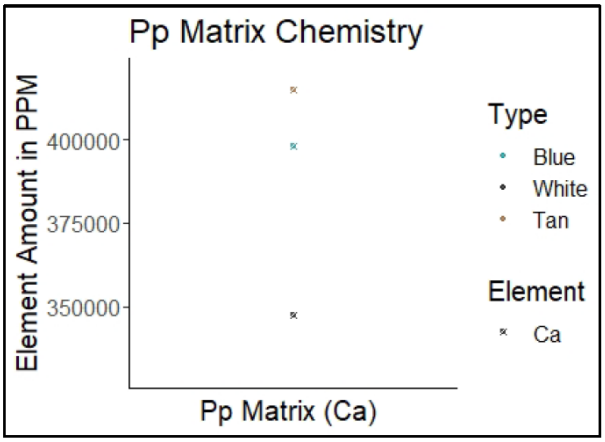

Figure 11: Scatter plot showing the matrix (Ca) elements in the Pp shells from HRS.

Sample	Mg/Ca	Sr/Ca
Pp Blue	0.00020	0.00317
Pp White	0.00018	0.00326
Pp Tan	0.00012	0.00289
Tp	0.00054	0.00310
Deep Water	0.00081	0.00095
Shallow Water	0.00062	0.00387

Table 2: Shows the different ratios of magnesium and strontium to calcium,

Element	Average	Standard Deviation	Coefficient of Variation	Sample Variance
Cd	0.0052	0.0030	58.02	9.063E-06
Cs	0.0129	0.0052	40.31	5.740E-05
As	0.59	0.39	66.21	5.645E-05
K	20.33	2.42	11.90	2.710E-05
Mg	65.22	14.77	22.64	0.225
Sr	1198.65	66.30	5.53	0.194
Na	2265.77	362.67	16.01	0.154
Ca	386931.97	35276.62	9.12	138.543

Table 3: Different statistical analysis for each element of the Pp shells from HRS.

Site	d18O-Difference	d13C-Difference
BL-Lake	-12.79	13.40
BL-Pond	N/A	12.41
BL-Spring	-13.82	12.73
HRS	-10.81	8.87
RBS	-6.33	N/A

Table 4: The difference between shell and water isotopes at each site for both carbon and oxygen.

Species	d18O-Difference	d13C-Difference
Mt	-13.20	12.81
Pg	8.39	12.58
Pp	-7.27	9.71
Psp	-7.07	11.67
Tp	-11.97	10.33

Table 5: The difference between shell and water isotopes for each species for both carbon and oxygen.

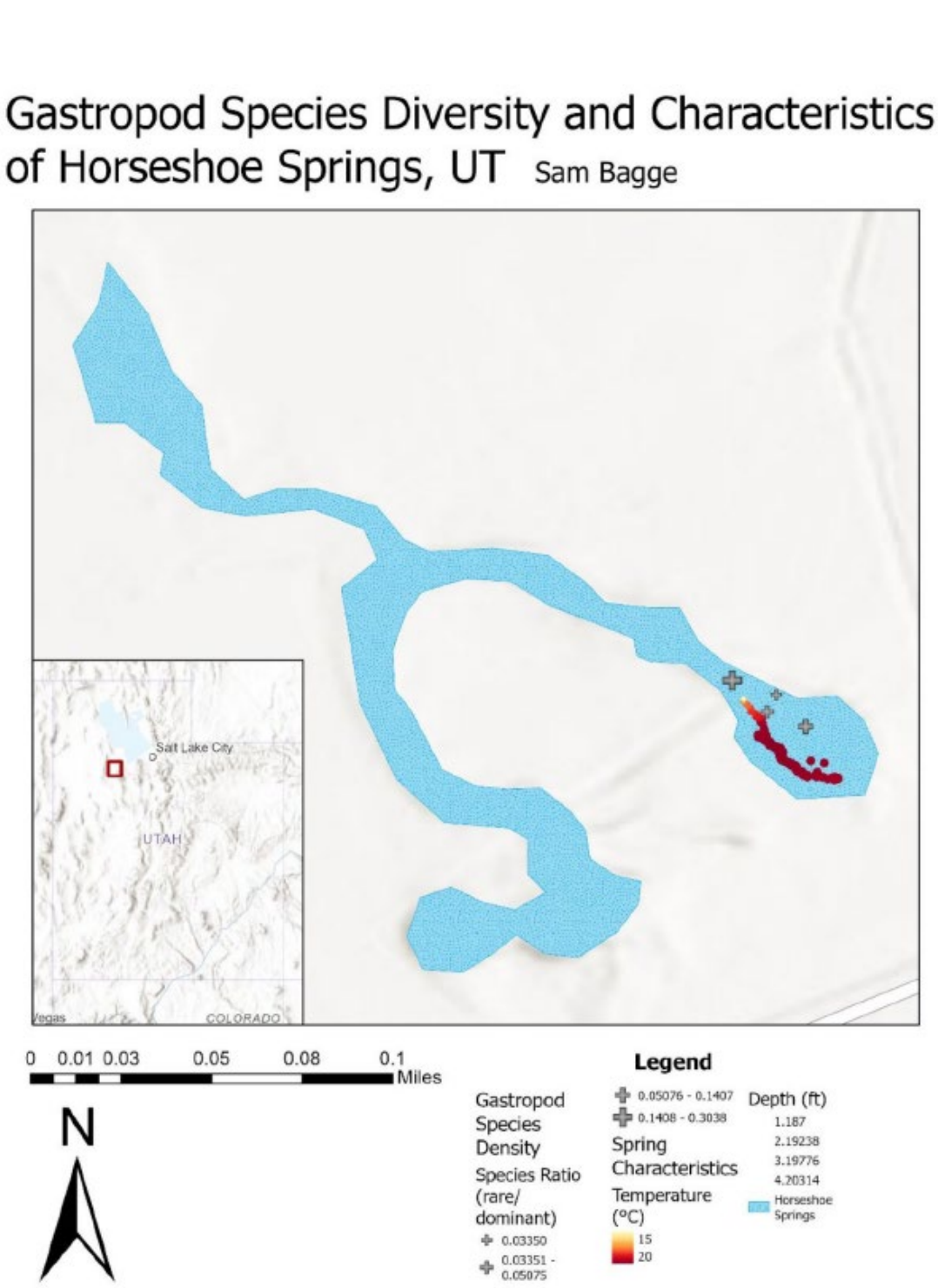

Figure 12: Figure 4: A map showing the extent of Horseshoe Springs, the relative species ratios, depth, and temperature profiles of a transect within the spring (ArcGIS).