

HW5: Capstone Project Proposal

Stable isotope analysis (SIA) is a powerful tool that has been a staple of the fields of oceanography, geochemistry, and forensic sciences (Wostbrock et al. 2018; Landais et al. 2012; Ehleringer et al. 2008). Recently, the application of SIA has expanded to other fields such as ecology and animal biology (Newsome et al. 2007; Cucherousset & Villéger, 2015; Whiteman et al. 2019a). However, the incorporation of SIA to ecology and animal biology is often limited to carbon and nitrogen isotopes (Vander Zanden et al. 2016). In addition, many researchers in the fields of ecology and animal biology choose to send their samples to other institutions for analysis instead of completing their analyses in-house because of the expenses and logistics related to traditional isotope analysis (Speakman, 1997). The development of advanced instrumentation such as cavity ring-down spectroscopy (CRDS) and off-axis integrated cavity output spectroscopy provide a potential solution to these setbacks (Thorsen et al. 2011; Melanson et al. 2018). In particular, CRDS is an affordable alternative to the long-standing traditional reliance on isotope ratio mass spectrometry (IRMS; Schauer et al. 2016). CRDS instruments, such as the Picarro L2140-*i*, provide high-precision measurements of hydrogen and oxygen stable isotopes in a fraction of the time required for IRMS analysis, and CRDS instruments possess automated functionality, allowing for a less labor-intensive process compared to IRMS (Schauer et al. 2016).

The capability of expanding the application of SIA to more frequently include oxygen and hydrogen would be highly beneficial to the fields of ecology and animal biology (Vander Zanden et al. 2016). Stable isotopes of oxygen and hydrogen ($\delta^{17}\text{O}$, $\delta^{18}\text{O}$, and $\delta^2\text{H}$) can provide information related to the sources of environmental water intake and to the animal's body water pool (Vander Zanden et al. 2016; Whiteman et al. 2019b). For example, measurements of injected ^2H and ^{18}O tracers can reflect the size of the body water pool (Andrews et al. 1997) and metabolic rate (Speakman, 1997), natural abundance of ^{18}O can reflect environmental water sources (Bryant and Froelich, 1995; Kohn, 1996), and a new application that simultaneously measures natural abundance of ^{16}O , ^{17}O , and ^{18}O (i.e., $\Delta^{17}\text{O}$) can be used to infer relative changes in metabolic rate and water intake (Pack et al. 2013; Whiteman et al. 2019b; Sabat et al. 2021). Most hydrogen and oxygen analyses use blood plasma or serum samples (Speakman, 1997), but under the right circumstances (e.g., a trained animal in captivity) saliva and urine samples can be collected as well (Fancy et al. 1986). A central premise for all these methods is that water is critical to animal biology (Hill et al. 2008). Most terrestrial animals are ~60-70% water by mass, and this body water comes from a combination of environmental sources (i.e., ingestion of preformed water by drinking or eating) and endogenous processes (e.g., newly-synthesized metabolic water, a byproduct of metabolic pathways; Kohn 1996; Hill et al. 2008).

For my capstone project, I will develop methodology for a data pipeline that will allow users to efficiently design CRDS analysis runs, export their raw data, and correct their raw data so that it is publishable. While the Picarro L2140-*i* is a user-friendly instrument (Hutchings & Konecky – Accepted), the concepts related to SIA are still daunting and may likely intimidate many ecologists or animal biologists who may consider incorporating this type of instrumentation into their lab. For example, when I first joined John Whiteman's Lab, I was able to figure out the technology related to the Picarro in less than a month. However, the complex concepts related to designing effective analysis runs which would produce precise and accurate

measurements (at the $\Delta^{17}\text{O}$ level), and correcting raw values via internationally accepted water standards so that data is publishable, took well over 12 months to master. As previously mentioned, while the application of SIA of oxygen and hydrogen isotopes is expanding within the fields of ecology and animal biology, many researchers continue to rely on shipping their samples to outside institutions for analysis. A major goal of this capstone project and of my dissertation research is to demonstrate that equipping a lab for this type of research is affordable and relatively straightforward. Therefore, developing and providing my code and text documents via Github so that researchers at other institutions can access these resources is critical. However, another major goal is to improve the workflow within my own lab so that the next group of graduate students can more easily transition to this type of work.

While the raw datasheet exported from the Picarro provides data related to >25 variables, the variables of interest are as follows: 1) $\delta^{17}\text{O}$, $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$ (calculated from $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$), $\delta^2\text{H}$, deuterium (^2H) excess (calculated from $\delta^2\text{H}$ and $\delta^{18}\text{O}$), and average water concentration. I plan to use 6 datasets for this project: 3 datasets related to 'standards runs' and 3 datasets related to 'analysis runs'. Each standards run dataset is composed of ~600 measurements, while analysis runs typically are composed of 300-450 measurements. The standards run datasets involve measurements of internationally accepted water standards, along with other in-house waters. The standards run is designed so that isotope values of these waters are measured and established for the next set of analysis runs which contain samples from different animals with unknown values. For example, a 3-day standards run will be conducted that will influence the next 2-months of analysis runs. At the end of these two months, another standards run will be conducted and the cycle repeats. Once a standards run is completed, the raw data is exported and imported into python. The code then builds a correction equation from a regression comparing the known and measured values of the different internationally accepted water standards for $\delta^{17}\text{O}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$. The individual correction equations are then applied to the $\delta^{17}\text{O}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$ values for each water included in the standards run, allowing for generation of corrected values for $\delta^{17}\text{O}$, $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$, $\delta^2\text{H}$, and deuterium excess. The corrected values are then compared to known and established in-house values to ensure that the run was successful. As such, I will demonstrate that the code I'm developing effectively cycles through a standards run, produces comparable isotope values to the known and in-house established values, and that these corrected measured values can be used to successfully inform proceeding analysis runs. I will repeat this three times (6 datasets in total) to increase validity.

This project is relevant to my dissertation research because this will improve the current workflow for CRDS analysis in my lab. My dissertation research focuses on estimating metabolism and water intake of captive deer mice; free-ranging mule deer in Utah; and introduced, free-ranging South African oryx in New Mexico. As such, I have ~125 samples remaining to analyze for my research. Each of these samples will be run across multiple analysis runs, so completing these analyses will take a considerable amount of time. Therefore, this project will be vital to improving my ability to efficiently analyze samples. While I currently have developed methodology for analysis runs, this methodology is lacking in several areas: 1) the code is only about 25% automated; 2) the standards run and analysis run codes are currently separate which causes significant delays; and 3) both standards and analysis run code is bulky and not presentable to a wider audience. By the end of this project, I hope to have a nearly fully-

automated code that is designed to use the measurements from the standards run to inform the forthcoming analysis runs. In addition, this code will be annotated and explained with markdown via a Jupyter Notebook to help new users with transitioning to this type of work. Finally, text documents will be included to facilitate incorporation of this code into different institution's workflow.

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