

*TRIPLE OXYGEN ISOTOPE
MEASUREMENTS BY TUNABLE INFRARED
LASER DIRECT ABSORPTION
SPECTROSCOPY, AND APPLICATIONS TO
RATITE EGGSHELL*

Drake Yarian

MSc. Thesis

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

Abstract

This project introduces a new sample preparation technique and analytical methodology for high-precision measurement of the three oxygen isotopes directly in CO₂ samples using Tunable Infrared Laser Direct Absorption Spectroscopy (TILDAS). After the introduction and optimization of both the sample preparation and analytical systems, it is applied to fossil ratite eggshell (RES) samples spanning the mid-Miocene through Quaternary Namib environments. A data correction scheme for relating TILDAS measurements to the VSMOW2-SLAP2 scale is also introduced. Applying this correction, analyses of the international CaCO₃ standards IAEA-603, NBS18, and NBS19 are shown to agree well with previously reported values. In addition, $\Delta^{17}\text{O}$ 1 σ reproducibilities of 10, 19, and 60 per meg (1 per meg = 0.001‰) of these standards, respectively, are achieved. Analysis of RES suggests notable environmental differences between the central and southern Namib through time. RES $\Delta^{17}\text{O}$ signal preservation in the context of global climatic and local environmental influences is investigated by relating obtained data to current triple oxygen enabled atmospheric and animal body water models. This project concludes by recommending 3 main points of focus for future research directed towards improving RES data interpretation within the context of (paleo-)environmental reconstruction: (1) adoption of international standards with composition representative of RES, (2) dedicated research into the influence vegetation type has on leaf water $\Delta^{17}\text{O}$, and (3) a community-wide increase in analyses of modern RES $\Delta^{17}\text{O}$ data to provide a better foundation from which paleoclimatic and paleoenvironmental interpretations may be made.

Preface	5
Chapter 1: Introduction and Literature Review	6
1.1) Introduction	6
1.2) Traditional Isotope Terminology and Fractionation	7
1.3) Theme A – CO₂ Isotopologue Measurements: Comparison Between IRMS and TILDAS.....	10
IRMS.....	10
TILDAS	13
Spectroscopic Isotope Terminology	13
Instrumentation.....	15
TILDAS in the Literature	16
1.4) Theme B – Influence and Measurement of Environmental $\Delta^{17}\text{O}$.....	17
Environmental Waters.....	17
$\Delta^{17}\text{O}$ in Natural Materials	18
Soil Carbonates.....	18
Lacustrine Carbonates	19
Others	19
1.5) Theme C – $\Delta^{17}\text{O}$ in Ratite Eggshell: Controls and Applications	19
Ratite Eggshell Synthesis & What it Records.....	20
Body Water Isotopic Influence.....	21
Leaf Water Isotopic Influence	22
Atmospheric O ₂ $\Delta^{17}\text{O}$ – Controls & Significance to Ratite Eggshell	23
pCO ₂ Reconstruction	24
1.6) Project Rationale & Aims	26
Chapter 2: Methods.....	29
2.1) Automated Sample Preparation System.....	29
System Overview	29
AutoCart Valves	31
AutoCart Mixing Volumes	31
Automated Sample Preparation Sequence Summary	31
2.2) Materials	33
International Carbonate Standards.....	33
Internal Standards.....	33

Ratite Eggshell Sample Material.....	35
Collection of Atmospheric Sample.....	35
2.3) Diffusion Theory	36
2.4) Experimental	38
Upstream	39
Overview of Sample Dilution.....	39
Experiment A – Mixing via Passive Diffusion.....	39
Experiment B – Mixing via Turbulent Advection.....	41
Experiment C – Mixing via Circulation	46
Downstream	46
Overview of TILDAS Instrument	46
Experiment D – Gas Introduction and Spectroscopic Measurement.....	48
Experiment E – Assessment of TILDAS Electronics Temperature on $\Delta^{17}\text{O}$	
Precision	50
Experiment F – δ -value Concentration Dependence and Data Correction	51
Chapter 3: Optimization of Automated Sample Preparation System and TILDAS Analyses.....	54
3.1) Experimental	54
Upstream	54
Experiment A – Mixing via Passive Diffusion.....	54
Experiment B – Mixing via Turbulent Advection.....	57
Experiment C – Mixing via Circulation	63
Impact of Mixing via Circulation on Measured δ-values	67
Downstream	69
Experiment D – Gas Introduction and Spectroscopic Measurement.....	69
Experiment E – Assessment of TILDAS Electronics Temperature on $\Delta^{17}\text{O}$	
Precision	74
Experiment F – δ -value Concentration Dependence and Data Correction	80
3.2) TILDAS vs. IRMS Comparison.....	83
Chapter 4: Results & Discussion – Triple Oxygen Isotope Composition of Ratite Eggshell.....	86
4.1) Results	86
Assessment of Measurement Performance & Accuracy	86
Sample Overview	88
Central & Southern Namib Overview	91
Central Namib $\delta^{18}\text{O}$	93
Southern Namib $\delta^{18}\text{O}$	93

Central Namib $\Delta^{17}\text{O}$	94
Southern Namib $\Delta^{17}\text{O}$	94
4.2) Discussion.....	95
Comparison of Body Water Composition with Previously Published Studies	95
Environmental context.....	98
Model Description & Inputs	98
Comparison Between Sample and Modelled RES Values	101
Central Namib	101
Southern Namib.....	103
Main Controls of RES $\Delta^{17}\text{O}$	105
Global vs. Local Scale Influences	105
Effects of Changes in Evaporative Flux & Relative Humidity at the Moisture Source	106
4.3) Future Research	106
Chapter 5: Conclusions.....	108
Works Cited	110
Appendix	119

Preface

In all, this thesis consists of 5 chapters: (1) an introduction to and literature review of the triple oxygen isotope system in regard to terrestrial environments, (2) a description of the new methodology developed and employed in this project, (3) the optimization of the sample preparation and measurement aspects of the novel analytical system, (4) isotopic composition results of fossil ratite eggshell from the Namib Desert, and (5) the project conclusions. This project sets out to introduce a new sample preparation technique and analytical methodology for high-precision measurements of the three oxygen isotopes within CO₂ samples. Following the introduction and optimization of this new methodology, it is applied to fossil ratite eggshell samples roughly spanning the Miocene through Quaternary Namib environments. As very few triple oxygen isotope ratite eggshell data exist globally, these samples greatly increase this data pool and expand our understanding of their recording of (paleo)climatic and (paleo)environmental information. Along these lines, this project suggests a new framework for correlating ratite eggshell triple oxygen isotopic composition to atmospheric pCO₂ and environmental relative humidity at the time of eggshell formation. This framework will also aid in teasing apart the relative broad-scale climate and local environmental influences over ratite eggshell composition.

Chapter 1: Introduction and Literature Review

This chapter examines the existing literature related to the historical relevance of stable oxygen isotopes, the development of various methodologies targeting triple oxygen isotope analysis, and the current and future applications of the rapidly growing field. Following an introduction into stable oxygen isotopes and the traditional isotopic nomenclature, this chapter is organized into 3 “Themes” which encapsulate various aspects of the triple oxygen isotope field. Theme A focuses on methodologies and instrumentation, offering a compare and contrast style narrative between the techniques commonly used to date (isotope-ratio mass spectrometry (IRMS)) and the novel technique introduced in this project (Tunable Infrared Laser Direct Absorption Spectroscopy (TILDAS)). Theme B describes natural influences of the terrestrial triple oxygen isotope system as a function of environmental conditions and the resulting isotopic compositions observed in natural materials which have, to date, been studied with regards to their triple oxygen isotope composition. Theme C extends into the main focus of this project: the triple oxygen isotope composition of ratite eggshells (RES). This theme is further broken into sections regarding the environmental controls over ratite eggshell composition measured by IRMS and TILDAS, and its potential applications. Finally, this chapter concludes with a description of this project’s direction and what can be expected throughout the remainder of this thesis.

1.1) Introduction

The study of stable isotopes have applications in geochemistry, archaeology, hydrology, biology, hydrological and atmospheric sciences, paleoclimate, and many more subfields working in both the modern and the past. Studies of triple oxygen isotopes in modern waters and calcium carbonate (bio)minerals formed from these waters have shown that these isotopes behave in predictable ways and can deliver information about evaporation, aridity, climate, and hydrology (e.g., Aron et al., 2021; Barkan & Luz, 1999; Passey et al., 2014). The isotopic ratios of oxygen in the extremely diverse range of oxygen bearing materials found on Earth offer diagnostic tools for understanding both chemical and physical processes constantly occurring on Earth’s surface. Sometimes these tools can be used to discover fundamental pathways and limitations of a given process (e.g., Horváth et al. (2012) regarding CO₂ triple oxygen composition from various combustion processes). Other times they can be used as hints to infer the processes that drove them to the organization they embody at the time of analysis (e.g., Dansgaard (1964) regarding non-equilibrium evaporation). This latter instance is the approach typically taken when using the distribution of oxygen isotopes to build an understanding of the past. Measuring isotopic ratios in a given material allow us to make statements about the processes that may have been responsible for creating the observed pattern. By narrowing our focus in using these tools to geological, biological, and climatic physical processes we are able to understand in detail past environments (Sarma et al., 2003).

Historically, the abundance ratio of the heaviest isotope of oxygen, ¹⁸O (0.2% of all oxygen), to the (by far) most abundant oxygen isotope, ¹⁶O (99.762%), has been used to study a variety of processes from hydrological cycling to specific chemical reactions to biologic incorporation (e.g., Dansgaard, 1964; Johnson et al., 1998; Luz & Barkan, 2010). The third oxygen isotope, ¹⁷O, (0.038%) was long regarded as an unnecessary addition to the plethora of information measured ¹⁸O/¹⁶O ratios have consistently produced. The reason for this exclusion follows

reasonable logic based on mass-dependent fractionation; processes that influence isotopic ratios do so because ^{18}O has greater mass than ^{16}O . That extra mass is enough to cause the heavier isotopes to respond differently to varying circumstances than the lighter oxygen isotope, ^{16}O . Given that ^{17}O has half the mass difference from ^{16}O as ^{18}O , it stands to reason that the processes that govern $^{18}\text{O}/^{16}\text{O}$ ratios should be reflected half as strongly in $^{17}\text{O}/^{16}\text{O}$ ratios. This logic stood the test of time until researchers were able to look close enough at the differences between expectations and reality [the exception being the study of oxygen isotopic composition of meteorites which display large differences in ^{17}O and ^{18}O , largely pioneered by Robert Clayton and colleagues throughout the 1970s and 1980s].

While this 2:1 influence in isotopic fluxes is nearly the reality, advances in both theory and analytical techniques have allowed us to zoom in on the slight variations from the expected trend (Sharp et al., 2018). In recent years, the true potential of measuring the $^{17}\text{O}/^{16}\text{O}$ ratio alongside sample $^{18}\text{O}/^{16}\text{O}$ has been realized and is quickly becoming one of the more sought-after tools to study the past. What may be referred to as the ‘modern era’ of triple oxygen isotope studies on terrestrial materials began with the work of Barkan & Luz (2005). Improving upon the method of Baker et al. (2002) to fluorinate waters to pure O_2 gas, they were able to produce high-precision measurements of $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ ratios of the initial water samples. The measurements were made using dual-inlet mass spectrometry, which remains the most common analysis today.

The triple oxygen isotope system shows great promise in opening windows into the past. Applied to paleoclimate, implications from triple oxygen studies can include paleo-hydrology (Bergel et al., 2020), reconstruction of past pCO_2 (Pack et al., 2013; Gehler et al., 2016), and also has implications for ecosystem reconstruction. Beyond paleoclimate, these tools may possibly lend themselves useful for paleoaltimetry studies and estimating past global primary production (GPP) (Bao et al., 2008).

As the field of high-precision triple oxygen isotopes is still relatively young, one of the main areas of focus is on materials that are thought to show the largest ^{17}O anomaly. Being highly susceptible to evaporation and their local atmospheric conditions, materials from arid and hyper-arid environments are ideal candidates for testing our assumptions about the isotope systematics at play. Along these lines waters and carbonate (bio)minerals from places such as the Atacama and Sistan Deserts (Surma et al., 2015, 2018; Evans et al., 2018), eastern Africa (Passey et al., 2014; Beverly et al., 2021), the western United States (Passey & Ji, 2019), and similar environments have been collected and analyzed for their triple oxygen isotope compositions.

1.2) Traditional Isotope Terminology and Fractionation

Triple oxygen isotope studies report data with respect to a reference frame bounded by two international primary standards. Vienna Standard Mean Ocean Water (VSMOW) is oceanic in origin and defined as having $\delta^{18}\text{O} = 0\text{‰}$ (permille, where $1\text{‰} = 0.001$). This value is often attributed to the oceans as a whole (Luz & Barkan, 2010). Standard Light Antarctic Precipitation (SLAP) is the second primary reference standard with an assigned $\delta^{18}\text{O} = -55.5\text{‰}$. The vast majority of surface and subsurface waters have $\delta^{18}\text{O}$ between that of SMOW and SLAP, with some heavily evaporated waters, (e.g., leaf waters, closed-basin lakes) having positive $\delta^{18}\text{O}$ values, and some ice/snow reaching even more negative values.

Isotope abundances are reported in delta notation with the unit ‰. An isotopic delta value is defined as:

$$\delta^x = ({}^xR_{\text{sample}}/{}^xR_{\text{standard}}) - 1$$

where x, in the case of oxygen, is either 17 or 18, and R is the ratio of the rare (and heavy) to common (and light) isotope:

$${}^xR = ({}^x\text{O}/{}^{16}\text{O})$$

At equilibrium, isotopic distribution due to fractionation between two substances (A and B) is expressed as a fractionation factor (α) as:

$$\alpha_{A-B} = R_A/R_B$$

Each distinct mass-dependent fractionating processes can be individually described by their respective fractionation factors and fractionate coexisting species accordingly. As mass-dependent fractionation can occur between solids, liquids, and gases, the definition of coexisting species in this case can include things such as a liquid and its related vapor, minerals and their parent waters, and diffused and residual gases. Fractionation factors (α) between different masses of the same species are related by a power law (Matsuhisa et al., 1978; Young et al., 2002) in that:

$${}^{17}\alpha_{A-B} = ({}^{18}\alpha_{A-B})^\theta$$

where θ is the fractionation exponent that defines the relationship between α values (i.e., θ describes how ${}^{17}\text{O}$ and ${}^{18}\text{O}$ will fractionate relative to one another during a given process). As discussed below, θ takes on a different value depending on the specific fractionating process being described. Examples of θ values include: 0.529 (liquid-vapor equilibrium, θ_{eq}) and 0.518 (water vapor diffusion, θ_{diff}).

As mass-dependent fractionation follows a power law theory the need arises, when measuring isotopic ratios of samples across a wide range of isotopic values, to linearize their relationships. There are two technically different, although very similar, definitions of this linearized delta value. The first of which was introduced by Miller et al. (2002) by using 1000 as a scaling factor, taking the natural logarithm of the resulting equation, and including a term to quantify the offset of mass-dependent fractionation lines from the expected terrestrial fractionation slope (a reference line which is no longer in use). Although it was not specifically defined as a new variable, this newly linearized δ value was the basis for early triple oxygen isotope studies (defined as $\Delta^{17}\text{O}$ at the time).

The second linearized δ definition, utilized by Wostbrock et al. (2018), is what is used in this project to keep consistent with the most recent literature with the expectation that this will be the definition adopted by the community in time. Expressed as δ' (“delta-prime”) this is done similarly via scaling and taking the natural logarithm as:

$$\delta' = 1000 \cdot \ln(\delta/1000 + 1)$$

The δ' notation is now the standard isotopic value expression in all triple oxygen isotope studies and is necessary for the calculation of the “capital-delta-prime” ^{17}O value ($\Delta'^{17}\text{O}$), which is the end goal for most triple oxygen isotope studies. Perhaps most easily conveyed visually (Fig. 1.1), the $\Delta'^{17}\text{O}$ value of a given material is calculated as:

$$\Delta'^{17}\text{O} = \delta'^{17}\text{O} - \lambda_{\text{ref}} \cdot \delta'^{18}\text{O}$$

where λ_{ref} is the slope of the reference line relating $\delta'^{17}\text{O}$ and $\delta'^{18}\text{O}$, often taking on the value of 0.528. This is the determined value for the global meteoric water line (GMWL) and is most commonly used for hydrological studies (Meijer & Li, 1998; Luz & Barkan, 2010). Currently recognized by the equation:

$$\delta'^{17}\text{O} = 0.528 \cdot \delta'^{18}\text{O} + 0.000033 \quad (R^2 = 0.99999)$$

the GMWL describes average isotopic composition of globally distributed meteoric waters (Luz & Barkan, 2010). Note that GMWL has a positive (+0.033‰, or +33 per meg) $\Delta'^{17}\text{O}$ value.

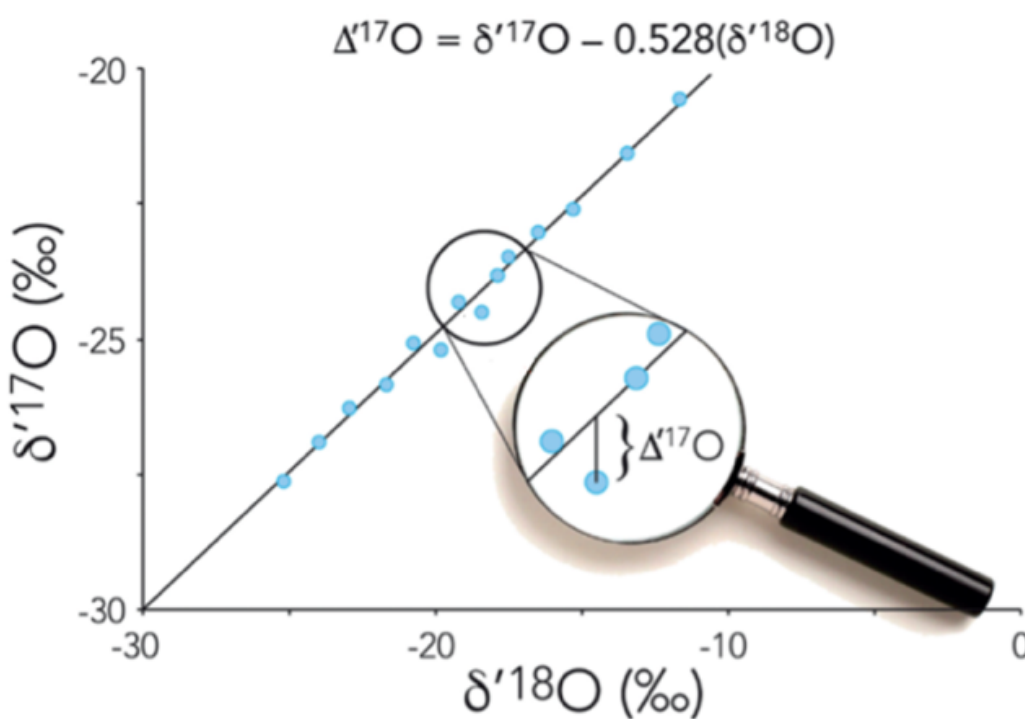


Figure 1.1: Representation of $\Delta'^{17}\text{O}$ value as a deviation from the expected $\delta'^{17}\text{O}$ - $\delta'^{18}\text{O}$ relationship. Solid line has slope 0.528 (GMWL). Image edited from Whiteman et al. (2019), Fig. 1.

When focusing on how different natural processes influence $\Delta'^{17}\text{O}$, the variable θ is typically used in place of λ . The value assigned to θ can vary depending what process is being studied and can be used to compare the isotopic effects of different processes. When using 0.528 as the

constant reference slope any process with a $\theta > 0.528$ will cause waters to have higher, likely positive, $\Delta^{17}\text{O}$ values, and vice versa for $\theta < 0.528$, which will drive $\Delta^{17}\text{O}$ values lower.

While a δ value is a numerical value for a tangible, physical characteristic of a material, $\Delta^{17}\text{O}$ is a numerical representation describing the response of a given material to the fractionating processes occurring directly to, and in the immediate environment around, said material. The Δ symbol represents a variation or change and as such, a $\Delta^{17}\text{O}$ value describes the degree of variation between the actual abundance of heavy isotopes in a material from the expected $\delta^{17}\text{O}$ - $\delta^{18}\text{O}$ relationship. As these variations are quite small $\Delta^{17}\text{O}$ values are measured and reported in terms of per meg (1 per meg = 0.001‰).

1.3) Theme A – CO₂ Isotopologue Measurements: Comparison Between IRMS and TILDAS

IRMS

Isotope-ratio mass spectrometry (IRMS) is a widely used technique for determining isotopic ratios in a range of materials. Most commonly for triple oxygen isotopes, the method of dual-inlet IRMS is employed to allow repeatedly and alternately introducing aliquots of reference and sample gas into the mass spectrometer. To date, the majority of our knowledge and understanding of the triple oxygen isotope system comes directly from data obtained via IRMS based methods (e.g., Passey et al., 2014; Barkan & Luz, 2005).

With a variety of models of isotope-ratio mass spectrometers commercially available, researchers are able to make the decision as to which instrument would best fit their needs. This benefits the researcher in multiple ways. First, from the business standpoint, competition between manufacturers gives motivation for consistent development and improvement of instruments, resulting in a consistent technological progress and opening avenues for collaboration between engineers and researchers.

Another benefit of the existence of multiple instruments that are capable of making the measurements researchers in the field require provides the ability to perform, in addition to intra-laboratory calibration, cross-instrument calibrations. Data discrepancy is nearly a guarantee in a science requiring as high-precision as triple oxygen isotope studies. Having multiple analytical instruments capable of making the same measurements can help in ruling out any suspicion of isotope effects occurring during sample measurement. All this, alongside being the community standard at present, promotes the use of IRMS for triple oxygen isotope research.

However, these instruments also have their drawbacks. The basis of isotope-ratio mass spectrometers is the magnetic field they generate to direct ionized sample beams into assorted collection cups in an exact and predictable way. The creation of this magnetic field requires a large magnet, weighing several hundred kilograms, that can be precisely manipulated and controlled. With the size of the magnet being a physical limitation, isotope-ratio mass spectrometers are quite large in size and require significant dedicated lab space to house them. The instruments also require a significant amount of energy and dedicated power supplies, forcing labs to be specially outfitted to run the equipment. The combination of these factors can

make housing and using an isotope-ratio mass spectrometer difficult, perhaps unrealistic, for many laboratories hoping to study the three-isotope system of oxygen.

For those laboratories that can and do use IRMS there are yet more limitations that must be overcome in order to make the measurements worthwhile. High-precision carbonate analysis via IRMS is limited by the mass resolving power of even the most advanced isotope-ratio mass spectrometers employed today. This mass resolution limitation stems from the release of CO₂ containing both the ¹³C¹⁶O¹⁶O and ¹²C¹⁷O¹⁶O isotopologues during phosphoric acid digestion of carbonates. These two isotopologues both have a nominal mass 45, and therefore are collected in the mass spectrometer's Faraday collection cup for *m/z* 45. However, their true masses are 44.9932 u and 44.9940 u, respectively. Separating this mass difference of 0.0008 u is beyond the capabilities of current isotope-ratio mass spectrometers and prevents triple oxygen isotope measurements from being made directly on the CO₂ produced from the sample.

This leads to what is likely the biggest challenge in using IRMS to make triple oxygen isotope measurements. The inability to use carbonate originating CO₂ forces researchers to convert the CO₂ into a different form that avoids the problematic isotopologue mass overlap. The most favored product is pure O₂ gas, which has no interference on the ¹⁷O¹⁶O isotopologue and therefore allows for a true determination of the ¹⁷O/¹⁶O ratio during the same measurement as the ¹⁸O/¹⁶O ratio. Triple oxygen IRMS measurements have been made with varying degrees of reproducibility (reported standard deviations for studies referenced below listed as 1σ, unless stated otherwise). At present, the goal for high-precision Δ¹⁷O measure is 1σ ≤ 0.010‰ (10 per meg).

Several methods have been developed and adopted to convert sample CO₂ evolved from carbonates to O₂ in the laboratory. In general, the various sample preparation methods currently in use can be grouped into 2 categories: the “exchange methods” and the “conversion methods” (Passey & Levin, 2021).

The exchange methods all hinge on promoting controlled isotopic exchange of sample CO₂ with another, isotopically known, oxygen bearing compound (e.g., H₂O, CuO, CeO₂) and back-calculating the Δ¹⁷O of the original sample. The exchange methods can take on several forms. Some techniques require analyzing the sample CO₂ both before and after exchange with an isotopically known species, allowing one to back-calculate the Δ¹⁷O of the original sample (e.g., Assonov & Brenninkmeijer, 2001, 1σ = 0.140‰; Kawagucci et al., 2005, 1σ = 0.35‰; Mrozek et al., 2015, 1σ = 0.6‰). Others perform similar isotopic exchange followed by the fluorination of the second species (e.g., H₂O, CeO₂) to O₂, the analysis of which provides the base for back-calculating the Δ¹⁷O of the sample CO₂ (Hoffman & Pack, 2010, 1σ = 0.21‰; Barkan & Luz, 2012, 1σ = 0.005‰).

The most widely adopted exchange method for high-precision analysis has come to be Pt-catalyzed exchange (e.g., Mahata et al., 2013, 1σ estimated as 0.045‰; Barkan et al., 2015, 2019, 1σ = 0.006‰; Fosu et al., 2020, 1σ = 0.007‰). This method uses heated platinum (~750 °C) to catalyze isotopic exchange of subequal amounts of CO₂ and O₂, typically over the span of about 1 hour. Both the CO₂ and O₂ must be analyzed before the exchange. After exchange, the gases are separated and each re-analyzed, allowing for the back-calculation of the original CO₂.

This method is dependent on having a solid grasp of the exchange characterizing $^{18}\alpha$ and $^{17}\alpha$ values, both of which are used to calculate θ_{Pt} . Calculating $^{18}\alpha$ and θ_{Pt} requires analysis of paired CO_2 and O_2 with known $\Delta^{17}O$ values. For the Pt-exchange method, this calculation causes the method to create its own limitation. This method inherently does not allow for the direct measurement of $\Delta^{17}O$ CO_2 (the determination of which is, after all, the reason for the development of this method), and therefore stops itself short of being a fully independent/isolated analytical technique.

The conversion methods avoid the need of analyzing sample gases multiple times and only require a single, definitive measurement to determine the $\Delta^{17}O$ of the sample originating O_2 . Compared to the exchange methods, the conversion methods are relatively assumption free. By quantitatively converting sample CO_2 to the O_2 introduced into the mass spectrometer, a single set of measurements accurately returns the sample $\delta^{18}O$ and $\delta^{17}O$ (and therefore the $\Delta^{17}O$). In a perfect system, the method of choice would always retain 100% of the oxygen in the original carbonate, eliminating any observable isotopes effects that may occur during the process by guaranteeing analysis of the entirety of the sample.

Converting sample $CaCO_3$ or CO_2 to O_2 is done via fluorination by various means. Fluorination can be done either directly on the sample (e.g., Miller et al., 2002; Wostbrock et al., 2020), or indirectly as a conversion step in a longer process of sample conversion and preparation (e.g., Passey et al., 2014). Some groups have accomplished direct fluorination of bioapatite using lasers (Gehler et al., 2011, 2016, $1\sigma = 0.020\text{‰}$; Pack et al., 2013, external reproducibility $1\sigma = 0.030\text{‰}$), a technique primarily used in studying phosphates in tooth enamel, with good precision. By directly measuring the $\delta^{18}O$ and $\delta^{17}O$ of the O_2 released from the original sample, the $\Delta^{17}O$ measured is the true $\Delta^{17}O$ of the sample and no back-calculations are necessary. The only calculation necessary in this method is calibrating to the VSMOW-SLAP reference frame, a standard reference frame for all laboratories, using known standards.

At present, the Wostbrock group is working towards reviving direct fluorination using “Ni-bombs” in which sample $CaCO_3/CO_2$ in the presence of BrF_5 is contained in sealed Ni capsules and reacted at 750 °C for 4 days (Wostbrock et al., 2020). The released O_2 gas then goes through a series of intense cryogenic cleaning and purification steps before being admitted to the mass-spectrometer. Using this method, the group has measured the three main international triple oxygen isotope calcite standards (NBS18, NBS19, IAEA-603), and, separately, their corresponding extracted CO_2 with reported 1σ precisions varying from 0.003‰ to 0.011‰ , (average $1\sigma = 0.008\text{‰}$).

Passey et al. (2014) introduced a method in which sample $CaCO_3$ is converted to CO_2 via phosphoric acid digestion, the CO_2 is then reduced to H_2O by reaction with excess H_2 in the presence of an Fe catalyst, and the resulting H_2O is fluorinated using CoF_3 to pure O_2 . The resulting O_2 is then subjected to extensive cryogenic cleaning before analysis in the mass-spectrometer. Measuring two international standards (NBS18, NBS19), an internal standard, and tank CO_2 , they report 1σ reproducibility ranging from 0.006‰ to 0.014‰ with an average $1\sigma = 0.010\text{‰}$ ($n = 10, 13, \text{ and } 13$, respectively).

A commonality among all the successful conversion methods with the intention of high-precision triple oxygen isotope analysis is the use of a molecular sieve filled/lined GC column in the O₂ cleaning/purification process. The specific parameters, i.e., length, diameter, temperature, etc., of the GC column have varied across studies (e.g., Passey et al., 2014; Pack & Herwartz, 2014; Wostbrock et al., 2020), but it seems the key lies in the presence of a 5Å molecular sieve providing ample trapping sites for unwanted contaminants.

TILDAS

Optical spectroscopy is a widely used measurement technique across many scientific disciplines. There is a long, although sporadic, history of using infrared spectroscopy to measure isotopologue abundances in CO₂, the vast majority focusing on ¹³C/¹²C ratios. Milatz et al. (1951) first constructed a dual beam infrared absorption instrument to measure ¹³C/¹²C ratios in CO₂. While the measurement errors were extremely high ($1\sigma > 20\%$), their instrument's essential features were similar to infrared instruments in use today (Kerstel, 2004). Infrared spectroscopy to measure ¹⁸O/¹⁶O ratios in CO₂ was first done by Lehmann et al. (1977) with an estimated accuracy of $\pm 10\%$. Comparing the measurement precisions of these early works to the estimated potential of today's infrared spectroscopy instruments lends perspective to the degree of technological advancement of the last several decades. Modern technology is now enabling infrared spectroscopy to work its way into the increasingly precise field of triple oxygen isotopes.

McManus et al. (2015) introduced a dual infrared laser instrument designed to measure 3 isotopologues of H₂O and 4 of CO₂ simultaneously and with high-precision. Tunable Infrared Laser Direct Absorption Spectroscopy (TILDAS) has since emerged as the present product of this initial introduction. To date TILDAS has shown great promise in matching, if not approving upon, analytical precision of IRMS systems for $\Delta^{17}\text{O}$ measurements. Recently, Perdue et al. (2022) reported the first $\Delta^{17}\text{O}$ measurements using a TILDAS instrument, with very good precision ($1\sigma = 5\text{-}10$ per meg). Conversely, Wang et al. (2020) achieved precisions of 0.01‰ (10 per meg) on measurements of the doubly substituted isotopologue ¹³C¹⁸O¹⁶O (the species of interest for CO₂ clumped isotope (Δ_{47}) measurements). As this species is 16 times less abundant than the ¹²C¹⁷O¹⁶O isotopologue of interest to triple oxygen isotope studies, it is realistic to expect TILDAS to achieve the same precision benchmark for triple oxygen isotope studies (Passey & Levin, 2021).

Spectroscopic Isotope Terminology

In spectroscopy, isotopologues take on a shorthand labeling for easy relation to the high-resolution transmission molecular absorption database (HITRAN) database (Rothman et al., 2005; Gordon et al., 2022). Table 1.1 gives the HITRAN notation and abundances of the 4 CO₂ isotopologues measured by the instrument used in this project (i.e., ¹²C¹⁶O¹⁶O, ¹²C¹⁸O¹⁶O, ¹²C¹⁷O¹⁶O, ¹³C¹⁶O¹⁶O). It is necessary to note that spectroscopic isotopic delta values are, by definition, slightly different than those calculated for mass spectrometric measurements. IRMS (and other mass spectrometry instruments) measure and report isotopic delta values on the basis of atomic abundance ratios (e.g., ¹⁸O/¹⁶O, ¹⁷O/¹⁶O), while laser spectroscopy makes measurements based on molecular isotopologue abundances (e.g., ¹²C¹⁸O¹⁶O vs. ¹²C¹⁷O¹⁶O vs. ¹²C¹⁶O¹⁶O). While technically different, this project will treat atomic isotopic delta values and molecular isotopologue delta values as functionally equivalent. As such, the traditional atomic

isotope notation (e.g., $\delta^{18}\text{O}$) is retained, except when describing δ -value calculation and correction.

Isotopologue	HITRAN Notation	HITRAN Abundance (X)
$^{12}\text{C}^{16}\text{O}^{16}\text{O}$	626	0.98420
$^{12}\text{C}^{17}\text{O}^{16}\text{O}$	627	0.000734
$^{12}\text{C}^{18}\text{O}^{16}\text{O}$	628	0.0039471
$^{13}\text{C}^{16}\text{O}^{16}\text{O}$	636	0.01106

Table 1.1: HITRAN notation and relative abundances of the 4 main CO_2 isotopologues. Of interest to this project are 626, 627, and 628.

As with atomic isotope calculations, the fundamental measure of defining isotopic composition is the isotopic ratio of the minor to major isotopes. The following equations use the oxygen isotope system as the example, but the same formatting and logic applies to the carbon isotopes (^{12}C & ^{13}C) which are simultaneously measured by the instrument. Adopting the notation of Griffith et al. (2018):

$$^x\text{r} = n(^x\text{O})/n(^{16}\text{O})$$

where, again, x is either 17 or 18 and n is the amount (in moles or atoms) of the isotope. Using the isotope ratios here, we can extrapolate to the isotopic abundance or fraction of each isotope:

$$\begin{aligned} ^{16}\text{x} &= 1/(1 + ^{17}\text{r} + ^{18}\text{r}) \\ ^{17}\text{x} &= ^{17}\text{r}/(1 + ^{17}\text{r} + ^{18}\text{r}) \\ ^{18}\text{x} &= ^{18}\text{r}/(1 + ^{17}\text{r} + ^{18}\text{r}). \end{aligned}$$

Isotopic abundances are fractional such that, by definition, the sum of which is $^{16}\text{x} + ^{17}\text{x} + ^{18}\text{x} = 1$. Similar to the atomic isotope abundances above, the molecular isotopologue abundances are calculated as:

$$\begin{aligned} \chi_{626} &= ^{16}\text{x} \cdot ^{12}\text{x} \cdot ^{16}\text{x} \\ \chi_{627} &= 2 \cdot ^{16}\text{x} \cdot ^{12}\text{x} \cdot ^{17}\text{x} \\ \chi_{628} &= 2 \cdot ^{16}\text{x} \cdot ^{12}\text{x} \cdot ^{18}\text{x} \end{aligned}$$

with the multiplication factor 2 arising for the heavy isotopologues as a result of a heavy oxygen having 2 possible positions in an individual CO_2 molecule. The calculation for a spectroscopic delta value is calculated from these molecular isotopologue fractions (χ) and their natural abundances (X). The spectroscopic $\delta^{18}\text{O}$ equivalent can be calculated as:

$$\delta^{18}\text{O} = \delta(628) = \frac{\chi'_{628}}{\chi'_{626}} - 1 = \frac{\chi_{628}/X_{626}}{X_{628}/X_{626}} - 1$$

and similarly for other isotopologues, where χ_{628}/χ_{626} is equivalent to the ^{18}R ($^{18}\text{R} = ^{18}\text{O}/^{16}\text{O}$) of the sample, and X_{628}/X_{626} is the ratio of HITRAN abundances of the minor to major isotope –

functionally equivalent to the atomic isotope ratio, ^{18}R , of the standard. HITRAN abundances differ from the actual abundances found in reference frame standards (e.g., VSMOW2-SLAP2, VPDB). As a result, both scale and concentration dependence corrections must be applied. The scheme for such corrections has been laid out in Griffith et al. (2012) and elaborated on further by several other groups (e.g., Wen et al., 2013; Flores et al., 2017, Griffith et al., 2018). Combining all of this, spectroscopic delta values can be viewed as an isotopologue mole fraction count normalized by HITRAN defined natural abundances, scaled to absolute concentration and then the applied standard reference frame.

Instrumentation

When directly comparing the systems as a whole for IRMS and TILDAS based approaches, several stark differences immediately appear. Among these, TILDAS instruments, and likely future laser-based systems in general, require smaller sample sizes and shorter analysis times, have a lesser laboratory footprint (both in terms of physical space and power consumption), are nondestructive to sample gas, and, perhaps most significantly, avoid much of the elaborate and intensive sample preparation required by IRMS techniques. As the TILDAS instrument measures isotopologue abundance on the sample CO_2 directly, there is no need to convert sample gas to O_2 for analysis. This is not to say that careful sample preparation will not be required for TILDAS systems, but rather that new methods focusing on CO_2 will need to be developed for doing so.

Avoiding the need for comprehensive sample conversion to O_2 , TILDAS based systems are not subject to many of the complications involved in the IRMS based techniques. The presumed carbonate analysis method will share the same first step of CaCO_3 digestion in phosphoric acid to produce the desired CO_2 . Similarly, the following cryogenic and GC column cleaning and purification of the CO_2 will also follow along comparable, if not the same, techniques. The divergence of sample preparation steps between IRMS and TILDAS based measurements will occur from this point of having prepared clean, dry CO_2 .

One emerging requirement for optimal TILDAS triple oxygen targeted measurements on CO_2 is the sample being accurately and precisely diluted to near atmospheric concentrations. High-purity N_2 stands out as a candidate as the dilution medium in that it is inert, has negligible infrared absorption, and is relatively cheap. Complications arise here in that it is essential to have a truly well-mixed gas as the analyte introduced into the TILDAS optical cell to avoid isotopic fractionations caused by diffusion. As diffusion of CO_2 in N_2 is a slow process, the development of systems that force mixing via turbulence will be a high priority for increasing sample throughput. Concentration variations between measured aliquots of the analyte similar to those discussed in Wang et al. (2020) for Δ_{47} measurements may also cause poor measurement repeatability. Fortunately, diffusion fractionation and concentration variations are two observations of the same limitation and should be able to be solved simultaneously with the development of a suitable sampling system.

The dual laser system of the TILDAS employed in this project is fitted with two interband cascade lasers (ICLs) which are temperature controlled by a liquid chiller with temperature precision in the milli-Kelvin range (1 milli-Kelvin = 0.001 Kelvin). The two lasers operate at different temperatures (approximately 17.9C and 9.5C for laser 1 and laser 2, respectively), and can be adjusted independently. ICLs have been specifically selected for this instrument as they

allow for measuring on a very narrow linewidth in the mid-IR, necessary for separating the absorption peaks of the isotopologues of interest, while providing exceptional stability.

Exhibiting highly characteristic absorption in the mid-infrared region of wavenumber 2310 cm^{-1} , the 4 most abundant isotopologues of CO_2 can be clearly resolved. With only minor overlap between $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ and $^{12}\text{C}^{17}\text{O}^{16}\text{O}$ (2310.00 cm^{-1} and 2309.98 cm^{-1} , respectively), improvements in laser and semiconductor technology have allowed for the separation of these absorption peaks, and therefore a true measurement of their abundances simultaneously.

Of crucial importance to instrument performance and analytical precision are the physical operating conditions of the various parts of the instrument. Laser temperature, cell pressure and temperature and ambient room temperature are but a few of the operating conditions that have clear and immediate impacts on measurement precision. While the high sensitivity of a TILDAS instrument to its local environment makes it susceptible to a suite of potential precision-worsening operating condition fluctuations, it also opens the gateway for extremely precise measurements once placed in a suitable environment. While the true potential has yet to be demonstrated, TILDAS may provide several benefits over the traditional IRMS approach to measuring triple oxygen isotopes. Perhaps the most significant of these benefits are drastically smaller sample sizes and the avoiding of several phases of sample preparation necessary for IRMS studies.

TILDAS in the Literature

To date, TILDAS and other laser instruments have been used several times for isotopologue measurements of multiple species (e.g., Ono et al., 2014; Sakai et al., 2017; Stoltmann et al., 2017; Prokhorov et al., 2019; Steur et al., 2021; Wang et al., 2020), several of which are briefly introduced below.

Sakai et al. (2017) used a TILDAS instrument equipped with a single quantum cascade laser to measure $^{18}\text{O}/^{16}\text{O}$, $^{17}\text{O}/^{16}\text{O}$, and $^{13}\text{C}/^{12}\text{C}$ ratios in CO_2 released from CaCO_3 and reported measurement repeatability of $<0.1\text{‰}$ and 0.069‰ for $^{18}\text{O}/^{16}\text{O}$ ($n = 10$) and $^{17}\text{O}/^{16}\text{O}$ ($n = 10$), respectively. While the study stopped short of calculating and reporting $\Delta^{17}\text{O}$ values, they show that high-precision measurement and quantification of CO_2 isotopologue ratios using infrared laser spectroscopy is well within reach.

Prokhorov et al. (2019) employed a dual interband cascade laser (ICL) system to measure 6 isotopologues of pure CO_2 gas, sourced both from tank CO_2 and CaCO_3 . This study also did not go as far as reporting $\Delta^{17}\text{O}$ values of their sample, but they report the instrument is capable of $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ measurements with precisions of 0.05‰ and 0.15‰ , respectively. The study offers insights into the comparability of direct laser absorption spectroscopy (DLAS) and IRMS and shows that the two methods are extremely comparable in their measured delta values. This suggestion lends support to community acceptance and implementation of DLAS systems for high-precision isotopologue measurements in CO_2 .

Steur et al. (2021) presented a performance assessment of a dual ICL TILDAS instrument for a series of three separate experiments spanning a two-year period. Measuring the triple oxygen isotope composition of CO_2 in three different prepared mixtures of CO_2 in dry air alongside a

known quality control gas they were able to demonstrate long-term stability of the system. The experimental prepared samples had an overall pCO₂ range from 313 to 1025 ppm. Using the wide range of pCO₂ in the sample gases, the study put forth two different methods for calibrating the CO₂ concentration dependence (referred to as the CO₂ mole fraction dependence (CMFD)) observed in spectroscopic systems as discussed above.

Beyond isotopologues of CO₂, Ono et al. (2014) used a TILDAS instrument equipped with two continuous wave quantum cascade lasers (QCLs) to measure several isotopologues of methane, focusing on the doubly substituted (clumped) ¹³CH₃D (where methane, CH₄, has both a heavy carbon and one heavy hydrogen (deuterium, D)). For pure CH₄, the natural statistical clumped ¹³CH₃D concentration is about 5.3 ppmV (Ma et al., 2008), about 1.3% that of bulk atmospheric CO₂ (~420 ppmV). Using this system, they were able to achieve precisions of <0.04‰ (2σ) for the singly substituted isotopologues ¹³CH₄ and ¹²CH₃D. For the doubly substituted ¹³CH₃D, a precision of 0.2‰ (2σ) and an accuracy of 0.7‰ (2σ) were achieved.

1.4) Theme B – Influence and Measurement of Environmental Δ¹⁷O

Environmental Waters

Of importance to triple oxygen isotopes of carbonates are the various processes that influence the isotopic composition of the water that they precipitate from. An accurate interpretation of carbonate isotopic compositions requires a good understanding of the drivers of isotopic variation of their parent waters.

The oceans are the dominant source of water vapor to the atmosphere. Following evaporation from the oceans, water vapor will undergo Rayleigh distillation. This process describes the fractionation of water (for both H and O) as water vapor condenses to liquid water in an air parcel (Craig, 1961; Dansgaard, 1964). This condensing water mass is the production of continental precipitation which, after falling to the surface, may be altered further by subsequent evaporation and diffusion, among other (sub)surface processes. Surface waters that are taken up by plants and animals for sustenance are then incorporated into their structures (e.g., leaf and stem water for plants, body water for animals) until they reenter the hydrological cycle. Surface waters containing dissolved ions may precipitate out minerals, as is the case of lacustrine and soil carbonates.

Each of these steps in the formation and distributing of environmental waters is enacted by a single or combination of processes, each of which fractionates ¹⁷O and ¹⁸O according to its own fractionation exponent (θ). In cases of multiple processes acting on a given unit of water, the fractionation effects are shared and the observed θ will trend towards whichever process is exerting a greater influence. For example: molecular diffusion of water vapor will fractionate oxygen along a slope of θ = 0.5185 (Barkan & Luz, 2007), whereas equilibrium exchange between liquid water and water vapor occurs along a slope of θ = 0.529 (Barkan & Luz, 2005). A mass of water vapor that has diffused some distance, then come to isotopic equilibrium with its new surroundings will have an observed fractionation along a slope at some θ value between these two.

In general, global precipitation has positive $\Delta^{17}\text{O}$ values. This can largely be attributed to two things: the influence of molecular diffusion from the oceans, and condensation fractionating along a slope of $\theta = 0.529$ (Passey & Levin, 2021). As the condensation θ is greater than λ_{ref} , the resulting condensates will have a slightly higher $\Delta^{17}\text{O}$ value than the vapor it was formed from.

Once on the surface, waters are free to interact with their local environments. Evaporation is perhaps the most obvious consequence of being deposited on the surface. With few exceptions, evaporation nearly always causes a decrease in the $\Delta^{17}\text{O}$ value of the remaining surface water (Passey & Levin, 2021). Evaporated waters may include both natural and artificial closed-lake basins, leaf waters, insect body waters, and perhaps even late-stage seasonal watering holes. As such, evaporated waters are among the earliest subjects of hydrological triple oxygen isotope studies. Most relevant to this project are leaf waters that are highly evaporated, possessing isotopic compositions higher in $\delta^{18}\text{O}$ and proportionally lower in both $\delta^{17}\text{O}$ and $\Delta^{17}\text{O}$, and which are the main source of oxygen to body waters of ratite.

$\Delta^{17}\text{O}$ in Natural Materials

The concept of measuring carbonates for triple oxygen isotopes rests on two main ideas: that $\Delta^{17}\text{O}$ values of meteoric, evaporated, and animal body waters are measurably different, and that carbonates of various origins retain information about their parent waters during formation (Bao et al., 2016). Passey et al. (2014) presented the first high-precision $\Delta^{17}\text{O}$ dataset for terrestrial carbonates and were able to determine the fractionation factors for both relating the O_2 analyte to the original carbonate material as well as the carbonates to their respective parent waters. This newly informed view of the carbonate-parent water relationship has been the basis for carbonate triple oxygen isotope studies since.

Building on this foundation, studies have since reported triple oxygen isotope values on a variety of carbonate materials; soil and lacustrine carbonates, modern and fossil eggshells, bioapatite, and marine and lacustrine shells are among the presently studied carbonate materials. Of significant interest are carbonates from arid regions where evaporation is expected to be high. Examples of soil and lacustrine carbonates, and other non-carbonate materials are briefly presented below. Examples specific to ratite eggshells, and their environmental influences, are discussed in detail in Section 1.5, below.

Soil Carbonates

Soil carbonate $\delta^{18}\text{O}$ values have long been used to infer that soil waters are evaporated (e.g., Quade et al., 1989). Further research, now invoking the use of $\Delta^{17}\text{O}$, continues to support this hypothesis. Recent work has continued the suggestion that soil carbonates form from soil waters that are, at least moderately, evaporated (Passey et al., 2014; Beverly et al., 2021). Beverly et al. (2021) sampled soil carbonates from the Serengeti ecosystem across a range of aridity and precipitation regimes with the goal of reconstructing soil water isotopic composition. They found a trend of decreasing $\Delta^{17}\text{O}$ values with increasing aridity and $\delta^{18}\text{O}$ values, further supporting this hypothesis.

By combining the use of triple oxygen isotopes with traditional carbon isotope measurements on carbonate minerals found in paleosols, the potential for detailed ecosystem reconstruction arises.

Together, these tools provide the means to constrain vegetation type, aridity, and, to a lesser degree, moisture source.

Lacustrine Carbonates

Especially pronounced in closed-basin lakes, evaporation drives an increase in $\delta^{18}\text{O}$ and corresponding decrease in $\Delta^{17}\text{O}$ values of the lake water, often reaching negative values (Luz & Barkan, 2010; Passey et al., 2014, Passey & Levin, 2021). The enriched isotopic composition of these waters is then recorded in authigenic lake and biogenic carbonates as they precipitate, fractionating along their respective fractionation slopes. Given knowledge of the material specific α values, the isotopic composition of the carbonate parent waters at the time of formation can be both modeled to within $\sim 3\%$ (e.g., Passey & Ji, 2019, Table 5).

Others

Several other materials, both carbonate and non-carbonate, have also been subject to triple oxygen isotope studies. Speleothems (Sha et al., 2020), carbonatites (Fosu et al., 2021), silicates and phosphates (e.g., Cowie & Johnston, 2016; Wostbrock et al., 2018, 2020), sulfates (Bao et al., 2008), and nitrates (Bao & Gu, 2004) are all examples of the increasing variety of solid materials which are actively the subjects of triple oxygen isotope analyses. In addition to these, several species of atmospheric gases have also been analyzed including O_2 , O_3 , CO_2 , H_2O , and N_2O (Bao et al., 2016).

As expanded upon below, the eggshells of various avian species have also been suggested to retain potentially useful information. Another biomineral that has gained relevance in (paleo)climate studies is bioapatite. Typically studied in the form of tooth enamel, this material can give insights into environmental parameters similarly to eggshell carbonates. Levin et al. (2006) suggested that, given a constrained area, higher bioapatite $\delta^{18}\text{O}$ values in evaporation sensitive animals (e.g., ostrich) indicate a combination of both an emphasis by the animal on plant waters as their primary source of dietary water and lower relative humidity conditions.

As the isotopic composition of terrestrial biominerals is directly related to the animals' body water isotopic composition, studies of animal body water have also recently gained relevance. Body water composition can be either directly measured or reconstructed from biominerals. Examples of such studies are Pack et al. (2013), Passey et al. (2014), and Whiteman et al. (2019), which are all presented and discussed below.

1.5) Theme C – $\Delta^{17}\text{O}$ in Ratite Eggshell: Controls and Applications

Of significant interest for terrestrial carbonate triple oxygen isotope studies are ratite eggshells (RES). Offering both climatic and archaeological insights, modern and fossil ratite eggshells hold a wealth of potential knowledge. Isotopic studies of ratite eggshells gain information of their formation environment including local vegetation patterns, seasonal precipitation events, and evapotranspiration (Niespolo et al., 2020). Several factors contribute to making ratite eggshell an excellent study material. Their accumulation at many archaeological sites makes them relatively readily available, and because they are suitable to radiometric dating, they can be used to place dates on their interpreted formation environment. Throughout this thesis, modern

ostrich (*Struthio camelus*) is used as a modern analog for broader (fossil) ratite eggshell synthesis, isotopic influence, and expected isotopic composition interpretation.

Ostriches are largely unselective feeders and therefore will isotopically record the conditions of their local feeding environments (Milton et al., 1994). Being nonobligate drinkers, most of their dietary water, and therefore body water, is sourced from the food they eat. Much of their diet consists of green plants (Williams et al., 1993) and showing little to no preference with regards to C3, C4, and CAM plants, the overriding factor for food preference appears to be plant tenderness (Ségalen & Lee-Thorp, 2009; Lee-Thorp & Ecker, 2015). With leaves being highly susceptible to evapotranspiration, they inherit evaporatively-enriched-isotopic signatures (i.e., the absolute amounts of $^{18}\text{O} > ^{17}\text{O} > ^{16}\text{O}$). As body water is the source of O in the formation of eggshells, this unlocks a, albeit quantitatively complex, qualitatively fairly straight forward chain of isotopic partitioning with fewer assumptions as compared to other biominerals.

The vast majority of an ostrich's body water oxygen is sourced from atmospheric oxygen and leaf waters. Atmospheric O_2 $\Delta'^{17}\text{O}$ is, while observationally variable, fairly well documented with an average reported value of -434 per meg (Passey & Levin, 2021). Leaf waters also can be quite variable in $\Delta'^{17}\text{O}$ as they are subject to the conditions of their local environments. In tandem with the occurring evaporation driven enrichment in $\delta^{18}\text{O}$, enrichment of ^{17}O relative to ^{16}O ($\delta'^{17}\text{O}$) also occurs. The relative fractionation between ^{18}O and ^{17}O in the leaf water happens in accordance with the fractionation exponent λ_{transp} . As $\lambda_{\text{transp}} < \lambda_{\text{ref}}$ (when the assigned λ_{ref} value is 0.528) ^{17}O is preferentially lost relative to ^{18}O and the leaf water would have an observed negative $\Delta'^{17}\text{O}$ value. As the largest contributor of oxygen to ratite body water, the negative $\Delta'^{17}\text{O}$ value observed in ratite eggshells can be largely attributed to the evaporation of leaf waters.

As both of these reservoirs have negative $\Delta'^{17}\text{O}$ values, we expect ratite eggshell to have negative $\Delta'^{17}\text{O}$ values of similar scale. Atmospheric O_2 sets the lower limit of eggshell $\Delta'^{17}\text{O}$ (i.e., all of animal's body water oxygen comes from the atmosphere), while leaf water sets the effective upper bound (i.e., all body water oxygen comes from leaves), with lesser influence from other, minor water sources.

Ratite Eggshell Synthesis & What it Records

Using modern ostrich (*Struthio camelus*) as the analog for ratite eggshell formation, we can infer the formation and isotopic recording of ratite eggshells of both extant and extinct species. Ostrich breeding season corresponds with the rainy season of the region, even in areas subject to a bimodal precipitation regime (Niespolo et al., 2020). Dependent on sufficient buildup of nutrients, ostriches typically lay eggs over a period a couple months after the rainy season (Leuthold, 1977; Sinclair, 1978; Johnson et al., 1998). The actual formation of the eggshell is rapid, happening in <24h (Nys et al., 2004) and is presumed to reflect the previous 3-5 days of the ostriches' diet and behavior (Johnson et al., 1998).

As an isotopic pathway, several steps are required to trace the origins of oxygen bound in the eggshell CaCO_3 back and balance the accumulated effects of contributing sources. Starting with the eggshell itself and working backwards, the only isotopic preservation that can be directly calculated is that of body water from which the eggshell is precipitated. Passey et al. (2014)

presented the fractionation factor (α) between ostrich body water and eggshell calcite as: $\alpha_{\text{calcite-bw}} = 1.0379 \pm 0.002$. This value allows for the back-calculation of the body water $\delta^{18}\text{O}$ value from the measured isotope values of the eggshell calcite.

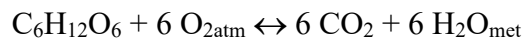
Body Water Isotopic Influence

With the ability to reliably reconstruct body water isotopic values, the various influences of environmental factors must be differentiated. Nonobligate drinkers such as ostrich get their dietary oxygen from two main sources: leaves and the atmosphere. Using a modified version of the Kohn (1996) model for relating biomineral $\delta^{18}\text{O}$ values to body water $\delta^{18}\text{O}$ values, Niespolo et al. (2020) suggest that foliar water and leaf cellulose oxygen contribute ~66% of dietary oxygen (57% and ~9%, respectively), and atmospheric oxygen intake during respiration and oxygen from atmospheric water vapor combine for the remaining ~34% (~30% and ~4%, respectively). As nearly 90% of ostrich body oxygen is sourced from two main reservoirs (i.e., foliar water and atmospheric oxygen), triple oxygen isotope studies of ratite eggshell is naturally directed towards these two areas of interest. For a complete examination of inputs and outputs contributing to ostrich body water make-up, see Passey & Levin (2021; Fig. 9).

In constructing a visual for the relative contributions of oxygen to body water it's helpful to lay the inclusions out in the style of chemical reaction formulas. Given the estimated contributions listed above, paying attention only to the oxygen contributions, we could write out the expected formula as:

$$\text{H}_2\text{O}_{\text{bw}} \leftarrow 0.57(\text{H}_2\text{O}_{\text{foliar}}) + 0.09(\text{O}_{2 \text{ cellulose}}) + 0.3(\text{O}_{2 \text{ atm}}) + 0.04(\text{H}_2\text{O}_{\text{vap}})$$

where $\text{H}_2\text{O}_{\text{bw}}$ is the animal's body water – the source of oxygen in the eggshell. Of the four inputs represented in this equation, two contribute oxygen via water ($\text{H}_2\text{O}_{\text{foliar}}$ & $\text{H}_2\text{O}_{\text{vap}}$) and two contribute molecular oxygen ($\text{O}_{2 \text{ cellulose}}$ & $\text{O}_{2 \text{ atm}}$). As water is the species of significance in eggshell synthesis, the two molecular O_2 contributors must be transformed into water before they can be incorporated into the eggshell. This transformation is done via the basic metabolic pathway:



where $\text{H}_2\text{O}_{\text{met}}$ is metabolic water, and $\text{C}_6\text{H}_{12}\text{O}_6$, the carbohydrates of the leaf itself, is representative of the leaf cellulose and its structural oxygen contributing to the animal's body water. Note that in this balanced equation atmospheric O_2 ($\text{O}_{2\text{atm}}$) contributes 12 oxygen atoms compared to the 6 contributed via carbohydrates, suggesting that during the metabolizing of food stuffs $\text{O}_{2\text{atm}}$ exerts the greater influence on the isotopic composition inherited by the animal than the food material itself. The leaf carbohydrate oxygen here is not to be confused with foliar water oxygen. Carbohydrates are the leaf structure, the solid material from which the leaf is built. Foliar water is the water that is contained within the macroscopic leaf structure. Consuming leaves provides both as sources of body water oxygen to the animal. Taking this $\text{H}_2\text{O}_{\text{met}}$ and combining it with the other significant contributors to body water, the pathway for body water oxygen via water species can be represented as approximately:

$$\text{H}_2\text{O}_{\text{bw}} \leftarrow 0.57(\text{H}_2\text{O}_{\text{foliar}}) + 0.39(\text{H}_2\text{O}_{\text{met}}) + 0.04(\text{H}_2\text{O}_{\text{vap}})$$

In the following text the isotopic influences of each of these oxygen reservoirs are expanded upon, focusing mainly on leaf water and atmospheric O₂. Water vapor will be discussed largely in the context of relative humidity and the influences it exerts over evaporation, transpiration, and, ultimately, on ratite eggshell $\Delta^{17}\text{O}$.

Leaf Water Isotopic Influence

Several factors influence isotopic compositions of leaf water. While mainly observed and expressed in terms of their impact on $\delta^{18}\text{O}$ values, each of these factors also influences $\delta^{17}\text{O}$ values, and therefore $\Delta^{17}\text{O}$. As in the case of the origin of leaf and body waters, meteoric water isotope values mark the starting point from which mass-dependent fractionating processes exert their effects on all environmental water compositions. Variation in regional meteoric water isotope values alone can be observed in leaf and body water, and biomineral isotopic compositions. A notable example of this is presented in Ségalen et al. (2006) and further expanded upon by Senut et al. (2009), where eggshell carbonates from the southern and central Namib display $\delta^{18}\text{O}$ values that are offset by an average ~ 5 per mil, with the former having the lower value consistently over the last ~ 20 Ma. This offset is interpreted as being likely related to the difference in moisture source.

If the isotopic composition of meteoric water is held constant, leaf $\delta^{18}\text{O}$ values will generally increase with decreasing relative humidity (Forstel, 1978; Ferhi & Letolle, 1977) and increasing temperature (Gonfiantini et al., 1965; Flanagan et al., 1991). Lower relative humidity promotes evapotranspiration – leading to the enrichment of leaf water ^{18}O (Ségalen & Lee-Thorp, 2009). Similar to initial vapor formation from the oceans, the Rayleigh distillation model also closely works for leaf water isotopic compositions due to the relatively infinite volume of the atmosphere versus the small pool of water held in a leaf (Li et al., 2017). Not subject to the initial isotopic compositions of the regional meteoric waters that are being taken up by plants, these processes will have the same influence trends on plant and leaf water isotopic compositions, and therefore the end compositions of the eggshells of interest in this project.

The most important controls on leaf water $\Delta^{17}\text{O}$ values are the relative humidity and the isotopic composition of water vapor in a leaf's immediate surroundings during transpiration (Li et al., 2017). These two factors are inversely related in the magnitude of their effects as water vapor composition is thought to have greater impacts when relative humidity is high and a lesser impact under lower relative humidity (Landais et al., 2006; Li et al., 2017). The transpiration process fractionates oxygen along its own slope (λ_{transp}), which has been observed to vary between 0.511 and 0.534 (Landais et al., 2006; Li et al., 2017). This large range of fractionation slopes can be attributed to variations in relative humidity throughout the sampling timeline as relative humidity varies widely throughout the day (Li et al., 2017). Note once again that any fractionation slope value (θ or λ) greater than the reference slope (in this project $\lambda_{\text{ref}} = 0.528$) will result in a positive $\Delta^{17}\text{O}$ value, and vice versa for fractionation slopes less than 0.528.

As transpiration is effectively evaporation from the leaf surface, the lower relative humidity in arid environments promotes fractionation from transpiration in a similar way to evaporation from other surfaces. In the arid environments of interest in this project, λ_{transp} is assumed to always be less than 0.528, driving leaf water $\Delta^{17}\text{O}$ values negative. Lower relative humidity also lends to

minimizing the influence of surrounding vapors, aiding in dictating negative leaf water $\Delta^{17}\text{O}$ values. The consumption of these $\Delta^{17}\text{O}$ -depleted leaf waters should provide the largest lever in the concentrating of ratite eggshell $\Delta^{17}\text{O}$ values negatively to, expectedly, several hundred per meg.

Atmospheric O_2 $\Delta^{17}\text{O}$ – Controls & Significance to Ratite Eggshell

Young et al. (2014) presented 10 controls on $\Delta^{17}\text{O}$ of atmospheric O_2 ($\Delta^{17}\text{O}(\text{O}_2)$). These include numerous reaction rates, fluxes between oxygen reservoirs, and the isotope fraction factors associated with the many steps. While many of them are beyond the scope of this project, a good understanding of the more significant controls aids in predicting the downstream effects observed in $\Delta^{17}\text{O}$ of ratite eggshell.

Stratospheric ozone (O_3) is highly enriched in ^{17}O due to the photochemical reactions responsible for its production and destruction, the rates of which we assume to be constant. This is reflected in the extremely high $\Delta^{17}\text{O}$ anomaly (on the order of 10^5 per meg) of stratospheric O_3 . As ozone is unstable and photolyzes into O_2 and monatomic oxygen (O) (see Young et al., 2014, Eq. R3a-R3j), the high ^{17}O isotopic signature of the ozone is imparted largely on the monatomic oxygen. This excess monatomic ^{17}O readily exchanges with CO_2 in the stratosphere, which is then transported down into the troposphere during atmospheric mixing events. The net result of this reaction and exchange pathway is the formation of high- $\delta^{18}\text{O}$ - positive $\Delta^{17}\text{O}$ CO_2 , and low- $\delta^{18}\text{O}$ - negative $\Delta^{17}\text{O}$ O_2 (Bao et al., 2008). It is here where the sensitivity of atmospheric O_2 to CO_2 has its origins. The $\Delta^{17}\text{O}$ of atmospheric O_2 is subject to the absolute amount of ^{17}O - CO_2 exchange occurring because the monatomic ^{17}O is preferentially exchanged with CO_2 . An increase in stratospheric pCO_2 means there is more CO_2 to exchange with the ^{17}O -enriched monatomic oxygen, therefore increasing the $\Delta^{17}\text{O}$ of CO_2 and decreasing the $\Delta^{17}\text{O}$ of atmospheric O_2 . Combining this relationship with stratosphere-troposphere mixing gives rise to the decidedly depleted $\Delta^{17}\text{O}(\text{O}_2)$ value (-434 ± 22 per meg; average of currently reported values, Passey & Levin, 2021) observed today.

We can take this relationship between pCO_2 and $\Delta^{17}\text{O}(\text{O}_2)$ and use it at the basis of a quite simple thought experiment regarding its applications to paleoclimate. Let us imagine a time in the past where, all else unchanged, pCO_2 was increased to 600 ppm, similar to current estimates for the Miocene. Based on the behavior described above, we would expect the $\Delta^{17}\text{O}$ of atmospheric O_2 to be more negative relative to today's value. This would be the case as higher pCO_2 would entail a greater absolute amount of equilibrium exchange with stratospheric ^{17}O . With more of the available ^{17}O being included in CO_2 , there is less ^{17}O available to be included in atmospheric O_2 , therefore a more depleted $\Delta^{17}\text{O}(\text{O}_2)$ value would be the result. This thought experiment can be reversed for times where pCO_2 would be expectedly lower relative to today (e.g., during strong glacial intervals). Less CO_2 to exchange with stratospheric ^{17}O would allow for more ^{17}O to be included in atmospheric O_2 , giving rise to higher (less negative) $\Delta^{17}\text{O}(\text{O}_2)$ values.

Atmospheric pCO_2 is intimately related to global gross primary production (GPP). For the purposes of this project, GPP can be thought of as photosynthetic drawdown of CO_2 . Variations in global GPP are therefore inversely related to atmospheric pCO_2 , meaning higher productivity leads to lower pCO_2 and vice versa. Variations in GPP therefore should have clear effects on

$\Delta^{17}\text{O}(\text{O}_2)$ based on the framework described above. Exerting its greatest influence over long timescales, atmospheric pCO_2 can be expected to reflect in the $\Delta^{17}\text{O}$ values measured in ratite eggshells spanning times of known or suspected pCO_2 change.

pCO₂ Reconstruction

An exciting potential use of triple oxygen isotopes in terrestrial vertebrates is the reconstruction of paleo- pCO_2 in the time and place of biomineral formation. Atmospheric O_2 $\Delta^{17}\text{O}$ is sensitive to pCO_2 as described above (Luz & Barkan, 1999; Bao et al., 2008; Young et al., 2014). Given that atmospheric oxygen can contribute a substantial portion of an animal's body water oxygen, especially in evaporation sensitive animals such as ostrich, this sensitivity can theoretically be used as a proxy for atmospheric pCO_2 .

Pack et al. (2013) modeled expected $\Delta^{17}\text{O}$ values of bioapatite as a function of body mass over a wide range of atmospheric pCO_2 and showed that lower body mass is correlated with lower $\Delta^{17}\text{O}$ values, and vice versa. Similar model developments Combining this relationship with the sensitivity of atmospheric O_2 $\Delta^{17}\text{O}$ to pCO_2 , it may be possible to infer past primary productivity.

Reconstructing pCO_2 at the time and place of ratite eggshell formation requires the constraining of relative humidity (Rh). This constraining is necessary on the basis that both relative humidity, by extension of evaporation, and pCO_2 drive isotopic changes in the oxygen reservoirs available for eggshell formation. While it will not be possible to calculate the exact relative humidity of the environment at the time of eggshell formation, ruling out certain “regimes” where a given relative humidity would not allow for distinct ranges of eggshell $\Delta^{17}\text{O}$ will be reasonably achievable.

As part of this project, a framework for teasing apart the relative influences of relative humidity and pCO_2 is proposed (Fig. 1.2). This framework lays out the expected relationships between $\Delta^{17}\text{O}$ of atmospheric O_2 , pCO_2 , and Rh (combining aspects of the relationships of Passey et al. (2014), Young et al. (2014), and Cao & Bao (2013)). As shown, decreasing relative humidity is correlated with higher $\delta^{18}\text{O}$ and lower $\Delta^{17}\text{O}$ values, consistent with our understanding that lower relative humidity promotes evaporation, and therefore more negative $\Delta^{17}\text{O}$ values as discussed above.

The framework extends to characterize the changes in magnitude of the atmospheric O_2 $\Delta^{17}\text{O}$ - pCO_2 -Rh relationship under higher and lower pCO_2 conditions. With higher pCO_2 , where the $\Delta^{17}\text{O}$ of atmospheric O_2 is more negative than today, changes in relative humidity will have a relatively larger impact on eggshell $\Delta^{17}\text{O}$, giving rise to greater variation in eggshell $\Delta^{17}\text{O}$ values (Fig. 1.2). This is due to there being an increased absolute amount of ^{17}O in CO_2 available for photosynthesis, and therefore a greater absolute amount of ^{17}O contained in leaves (and leaf waters) consumed by ratite birds. This higher abundance of ^{17}O allows for increased evaporative fractionation in the leaf, driving leaf water $\Delta^{17}\text{O}$ lower as more ^{17}O is lost to evaporation relative to what would be the case under today's atmosphere. Therefore, a steeper $\delta^{18}\text{O}$ – $\Delta^{17}\text{O}$ slope is observed for changes in Rh under higher pCO_2 . This relationship is flipped for times of low pCO_2 , where changes in Rh maintain their influence on $\delta^{18}\text{O}$, but due to the higher $\Delta^{17}\text{O}$ of

atmospheric O_2 (a function of decreased pCO_2), the absolute spread of eggshell $\Delta'^{17}O$ values is severely truncated.

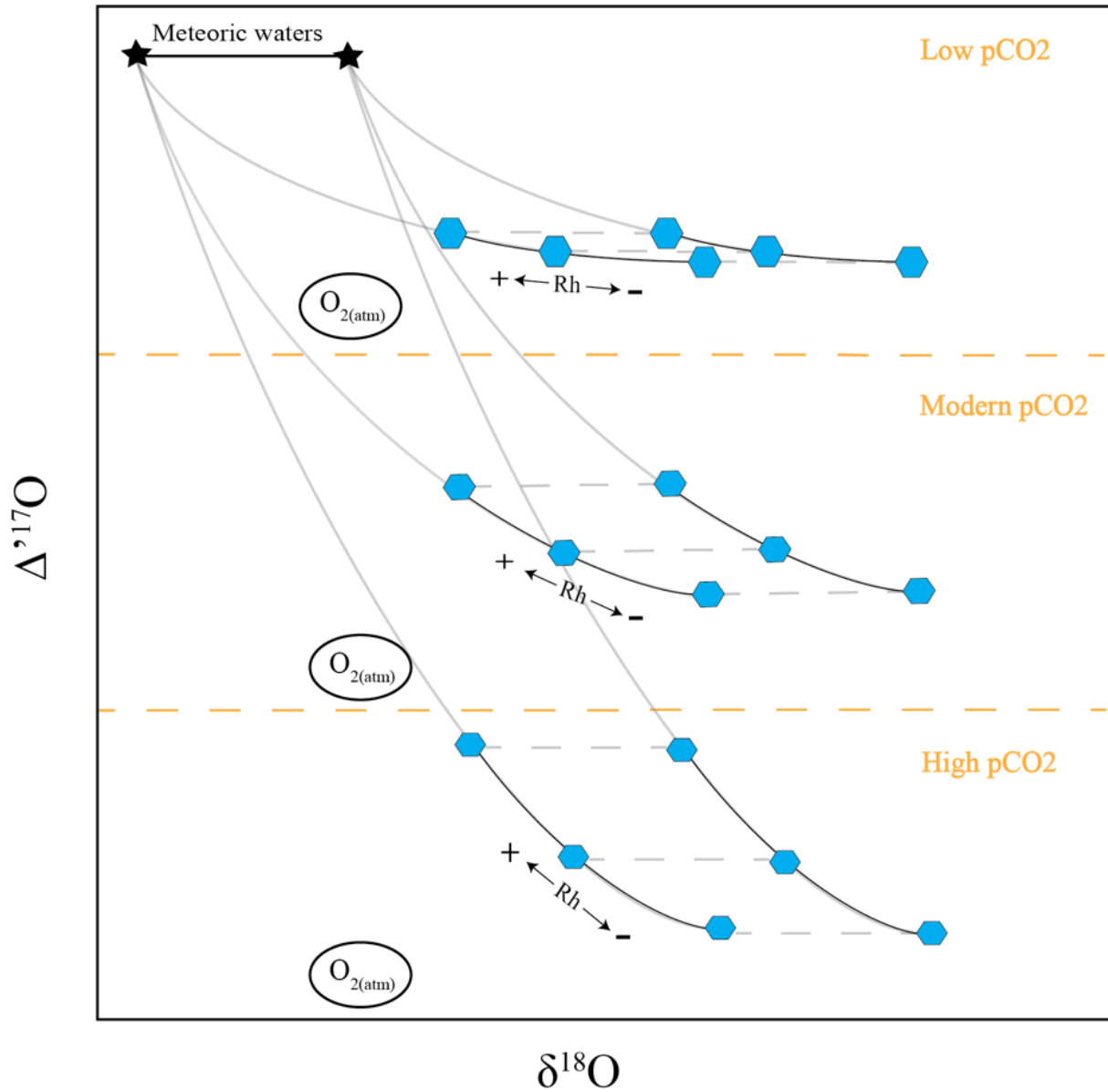


Figure 1.2: Suggested framework for expected “regimes” of ratite eggshell isotopic composition under varying atmospheric pCO_2 and relative humidity (Rh). $O_{2(atm)}$ is atmospheric oxygen, the $\Delta'^{17}O$ value of which is a function of atmospheric pCO_2 (as discussed in the text). Black stars and solid connecting line represent possible meteoric water isotopic compositions. Blue hexagons are potential resulting ratite eggshell isotopic compositions. The curves (light gray and black) represent fractionation between meteoric waters and ratite eggshell. Fractionation extent is a function of local relative humidity at the time of eggshell formation. No variables are to scale.

1.6) Project Rationale & Aims

This project introduces a novel optical spectroscopic method for measuring the triple oxygen isotope composition of CO₂ directly and provides the groundwork for future studies following with similar techniques. The application of TILDAS to quantify the variation of triple oxygen isotopes across a range of location, time, and materials has the potential to allow us to be more specific in our dissecting of modern and past environments than ever before.

The usefulness of triple oxygen isotopes to interpret the formation environments of oxygen bearing minerals is becoming increasingly apparent. Over the past ~2 decades, technological advancements have provided us with the ability to probe deeper into the dynamic systems responsible for the minute differences in triple oxygen isotope distribution observed by an increasing number of laboratories and scientists. The continued improvement of methodology and technology has lit the way for realizing the depth of questions about the past we are not just capable of asking but are working towards being able to answer. More specific questions require more precise, more certain measurements. In working towards these more precise measurements we must be open to new ways of thinking and analysis.

Optical spectroscopy is an extremely well-studied and catalogued field that has applications in perhaps the widest range of scientific fields of any currently used measurement techniques. Mid-infrared spectroscopy alone has applications ranging from environmental monitoring and emissions measurements, to manufacturing chemical analysis and medically important molecule detection, to atmospheric and planetary sciences (Tittel et al., 2008). With applications to trace gas analysis, advancements in laser and semiconductor technology have promoted the development of extremely precise spectrometers capable of resolving the absorption differences of the several isotopologues of CO₂ simultaneously. More specifically, this newest generation of spectrometers allows us to resolve the different maximum absorption frequencies of specific molecular isotopologues within a bulk CO₂ sample. In the practical sense, being able to quantify the relative abundances of the 3 oxygen isotopes while keeping the sample in CO₂ form avoids many of the complications involved in converting samples to the, at present, more readily measurable form of O₂ (see Section 1.3, above). Limiting sample species conversions eliminates several potential sources of error, promoting more repeatable and replicable measurements.

As has been the case for the triple oxygen isotope field since the beginning of its ‘modern era’, the technology for making the desired measurements exists and is ready to be optimized and put to use. The technological advance in analytic instruments often precedes the question of: “how do we maximize its potential?” Perhaps worded more precisely: “how do we make the error introduced by our methods less than the inherent error of the analytical instrument?” In isotope geochemistry, the difficulties in improving data quality often lie in the front-end work of sample handling and preparation. Sample material is a one-time consumable, necessitating confidence in the used analytical methodology.

Despite recent advances, IRMS remains limited in its ability to resolve the tiny mass differences necessary for distinguishing the ¹²C¹⁷O¹⁶O isotopologue from more dominant species of the same nominal mass (e.g., ¹³C¹⁶O¹⁶O). Given adequate sample preparation, TILDAS, and other advanced optical spectroscopic instruments, circumvent this issue by measuring the gas directly, as opposed to relying on ion collectors. A similar version to the TILDAS system used in this

project was first introduced by McManus et al. (2015) with the goal of measuring several isotopologues of H₂O and CO₂ simultaneously. While the TILDAS employed in this project focuses only on the CO₂ isotopologues, the dual-laser system as a whole has been subject to improvements since. In catching up to these advancements, we are just now developing and optimizing custom-built sample handling systems to allow us to use it to its full potential.

The driving force behind this project is the development of a novel CO₂ sample preparation technique with the intention of making the sample suitable for TILDAS measurements (Section 1.3). Preliminary testing has shown that, once the requirements for sample preparation are achieved, TILDAS measurements are capable of entering the community of high-precision (<10 per meg) triple oxygen isotope analyses, which thus far have been limited to those using IRMS techniques (e.g., Passey et al. (2014); Wostbrock et al. (2020)). TILDAS measurements of the clumped ¹³C¹⁸O¹⁶O isotopologue (a species an order of magnitude less abundant than the ¹²C¹⁷O¹⁶O isotopologue of focus in this project) have already been made with 10 per meg precision (Wang et al., 2020). As such, it is reasonable to expect TILDAS to achieve the same precision benchmark for triple oxygen isotope studies (Passey & Levin, 2021).

Our understanding of triple oxygen isotope systematics in the natural world is yet improving. Most present work focuses on materials expected to reflect the largest variation from the GMWL slope (λ_{ref}) of 0.528 in $\delta^{17}\text{O} - \delta^{18}\text{O}$ space. As evaporation and aridity are known to result in a strong divergence from this reference line, materials from (hyper)arid environments (e.g., ratite eggshell) are ideal for testing our present interpretations. From the starting point of evaporation giving rise to the source vapors of meteoric waters and ending with the material of interest, $\Delta^{17}\text{O}$ can be measured and the individual fractionating effects inferred. In a vacuum, individual processes have isotopic influences in a consistent direction along the range of δ values observed in nature. While these influences have typically been observed in the $\delta^{18}\text{O}$ values of the sample material (e.g., water, carbonates), they also exert their influences on $\delta^{17}\text{O}$ values in accordance with the true fractionation exponent (θ) value for that given process. We now have the power to go beyond single statement causes of isotopically enriched vs. depleted samples and can differentiate the relative impacts of various processes acting in tandem.

Stable isotope measurements of ostrich eggshell have been made for at least 5 decades (e.g., von Schirnding et al., 1982; Johnson et al., 1993; Ségalen & Lee-Thorp, 2009; Passey et al., 2014). The focus initially being on carbon isotopes, ostrich (and ratite in general) eggshell has become a crucial material for measuring and understanding the isotopes of oxygen in situ. While only limited (4 samples, all of which are modern ostrich) wild RES $\Delta^{17}\text{O}$ data have thus far been published, the foundation for doing so has been laid. Pack et al. (2013), Passey et al. (2014), and Whiteman et al. (2019) all report body water $\Delta^{17}\text{O}$ data for a variety of mammals and birds, as summarized and shown in Passey & Levin (2021; Fig. 10). The two former present reconstructed body waters from biogenic apatite and biogenic carbonate measured values. With the insights of Passey et al. (2014), it can be expected that future studies will use similar techniques to assess environmental evolution of, most notably, arid regions via fossil biogenic carbonates.

The ratite eggshell samples studied in this project were previously collected for study by Ségalen et al. (2002; 2006). Initially analyzed for their $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ composition, the authors used the results to infer the strong pattern of seasonality of rainfall in the (proto-)Namib Desert similar to

the dichotomy that exists today, as well as dismiss early expansion of C4 plants into the region. The samples used in this project are subset of the samples presented in these previous studies, spanning the mid-Miocene through the Quaternary, and are intentionally selected to provide the widest possible ranges in $\delta^{18}\text{O}$ composition within each available time period. Intentionally selecting samples in this way provides this project several advantages: (1) coverage of a wide range of $p\text{CO}_2$, GPP, and other global climatic characteristics (see Steinthorsdottir et al. (2021)), (2) considerable saving of time and materials by pre-screening samples for $\delta^{18}\text{O}$ composition, and (3) the ability to verify the accuracy of TILDAS measurements by comparing measured $\delta^{18}\text{O}$ values with the original study.

Explicitly, the aims for this project are:

- Develop and construct a sampling system to prepare samples to be amenable to TILDAS measurements.
- Optimize TILDAS spectroscopic parameters and interrogate instrumental limitations for achieving 10 per meg $\Delta^{17}\text{O}$ precision.
- Analyze select fossil RES samples to map the $\delta^{18}\text{O} - \Delta^{17}\text{O}$ space of the central and southern Namib Desert since the mid-Miocene.
- Interpret the RES data alongside current models to assess the influences of $p\text{CO}_2$, GPP, and regional environmental conditions.

Chapter 2: Methods[†]

Historically, the triple oxygen isotope composition ($\Delta^{17}\text{O}$) of sample CO_2 has typically been measured via isotope-ratio mass spectrometry (IRMS). These previous methods rely heavily on complicated sample preparation procedures in which CO_2 is transformed into a more easily measurable form, most often pure O_2 . Such techniques for these analytical methods range from converting sample CO_2 to O_2 via a series of chemical reactions to controllably mediating isotopic exchange between sample CO_2 and another oxygen bearing species (e.g., O_2 , H_2O). These preparation procedures often require multiple assumptions in substantiating their efficacy, however relatively assumption free exceptions do exist (see Wostbrock et al., 2020).

This project introduces a new methodology for the high-precision $\Delta^{17}\text{O}$ measurements directly in CO_2 using absorption spectroscopy. By diluting sample CO_2 to trace concentrations, a laser based instrument, namely Tunable Infrared Laser Direct Absorption Spectroscopy (TILDAS), is able to measure the abundance of distinct CO_2 isotopologues in the sample simultaneously. Recent advances in spectrometer technology have the potential to revolutionize the study of triple oxygen isotopes, as well as other notoriously difficult to measure isotopic compositions (e.g., clumped CO_2 (Δ_{47})). Ultimately, the success of the spectrometer hinges on the ability to accurately and repeatedly prepare CO_2 samples to trace concentrations optimal for the spectroscopic measurement.

This project has focused on both aspects of this greater analytical system. First, a novel sample preparation system was designed and created to meet the constraints inherent to the TILDAS instrument. Second, the TILDAS instrument was thoroughly investigated, and resultingly optimized, to allow achievement of the benchmark analytical precision typically identified as the objective for $\Delta^{17}\text{O}$ measurements via IRMS. As such, this chapter is structured into 4 main sections: introducing the novel preparation system, description of the standard materials used for calibrating and optimizing both ends of the greater analytical system, description of the diffusion theory limiting sample preparation efficacy, and the experimental evolution of both the sample preparation system and the TILDAS instrument. The “Experimental” section is divided into 2 main subsections: “Upstream” and “Downstream”, which refer to the sample preparation system and the TILDAS instrument, respectively. Each of these subsections are further divided into 3 “Experiments” which describe the evolution of both the “Upstream” and “Downstream” components referenced.

2.1) Automated Sample Preparation System

System Overview

The automated CO_2 sample preparation system introduced in this project (previously described in an associated publication by Hare et al. (2022)) was designed and constructed in house. The purpose of this system is to cryogenically purify, dilute, and mix sample CO_2 with N_2 to a

[†] Aspects of this research have been previously published in Hare et al. (2022). This chapter goes into more detail regarding the development of the sampling system and experimental identification of TILDAS operating conditions and limitations, conducted by the candidate. Said publication is cited several times throughout this chapter.

nominal $400 \mu\text{mol mol}^{-1}$ in a highly repeatable manner, where each sample is $\sim 9 \mu\text{mol CO}_2$ ($\sim 0.9 \text{ mg CaCO}_3$ equivalent). The system is comprised of 4 main features: (1) a break-seal manifold (valves 1-6), (2) liquid N_2 cryogenic trap (MV2), (3) 3 mixing volumes (MV1, MV2, MV3 - combined volume 687 mL), and (4) a circulation loop with a diaphragm pump inline (CTS Series, Parker Hannifin Corp., USA) (Fig. 2.1). The system is evacuated using a connected Edwards RV5 rough vacuum pump. Valves 1-7, 16-21, and those on either end of the circulation loop are pneumatic and bellows-sealed (Swagelok SS4-BK-VA-1C). Valve 8 is a three-way solenoid valve (P/N 009-0294-900, Parker Hannifin Corp., USA). Non-alphanumerically identified valves are bellows sealed and manually toggled. Data from the two pressure gauges (Agilent Varian CDG-500, 0-10 Torr and WIKA S-20, 0-3 bar) is monitored by a compact data acquisition unit at 0.5 Hz (cDAQ-9171, National Instruments Corp., USA). MV1 is a custom made sampling flask (GlassChem CC, South Africa, 576 mL) designed to maximize turbulent mixing. The system can accommodate gaseous samples introduced by any of 3 means: pure CO_2 loaded in break-seal tubes, CO_2 evolved from carbonates via acid digestion (from an accessory Thermo GasBench II), or bulk atmospheric samples directly from the removable atmospheric sampling flask. Each sample type is prepared via its specific preparation sequence, all of which are based in a single custom LabVIEW program.

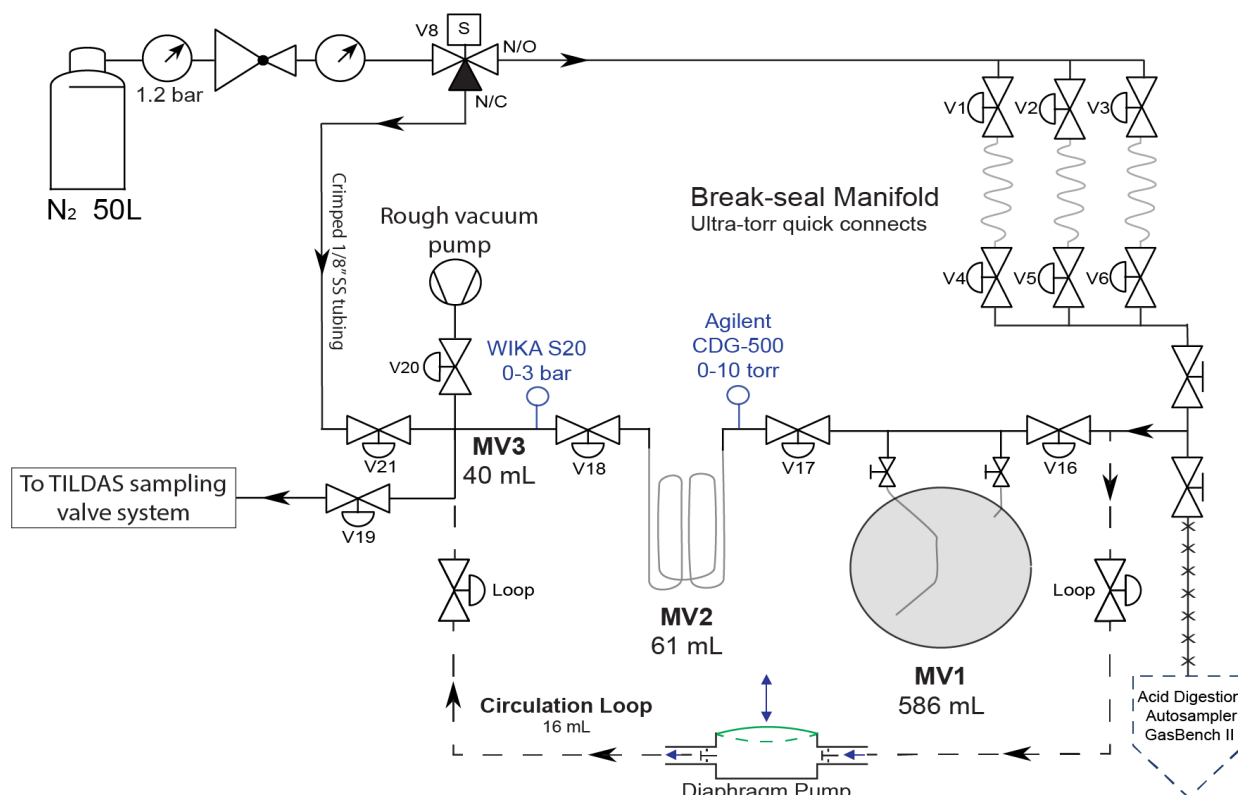


Figure 2.2: Schematic of AutoCart designed and constructed for this project. Automated CO_2 sample preparation for high-precision $\Delta^{17}\text{O}$ measurements. MV1, MV2, and MV3 are the mixing volumes. Samples can be introduced via the break-seal manifold, by acid digestion of carbonates via a Thermo GasBench II, or directly via the removable atmospheric sampling flask which doubles as MV1. System dilutes CO_2 in ultra-high purity N_2 to $\sim 400 \mu\text{mol mol}^{-1}$. Samples are mixed using a diaphragm pump to circulate sample gas through the combined mixing volumes for 2.5 minutes.

AutoCart Valves

V1 (diaphragm valve) – Up-stream end of break-seal 1
V2 (diaphragm valve) – Up-stream end of break-seal 2
V3 (diaphragm valve) – Up-stream end of break-seal 3
V4 (diaphragm valve) – Down-stream end of break-seal 1
V5 (diaphragm valve) – Down-stream end of break-seal 2
V6 (diaphragm valve) – Down-stream end of break-seal 3
V7 (diaphragm valve) – Inlet for N₂ supply
V8 (3-way solenoid valve) – N₂ supply director (normally open to break-seal manifold, normally closed to V21/MV3)
V16 (diaphragm valve) – Separates sample inlet side from preparation side of AutoCart
V17 (diaphragm valve) – Separates flask volume from liquid N₂ trap
V18 (diaphragm valve) – Separates liquid N₂ trap from MV3
V19 (diaphragm valve) – Inlet from AutoCart to TILDAS switching valve system
V20 (diaphragm valve) – To vacuum pump
V21 (diaphragm valve) – Dilution N₂ shut-off
Circulation Loop (2x diaphragm valve) – 2 pneumatically connected valves at either end of the circulation loop
Manual toggle valve separating GasBench system & AutoCart
Manual toggle valve separating break-seal manifold & AutoCart

AutoCart Mixing Volumes

Mixing volume 1 (MV1) – 586 mL (flask + V16-V17 volume)
Mixing volume 2 (MV2) – 61 mL (liquid N₂ trap)
Mixing volume 3 (MV3) – 40 mL
Circulation Loop – 16 mL

Automated Sample Preparation Sequence Summary

Here, a summary of the currently utilized sample preparation sequence is given. The sequence described here was first implemented on 22 March 2022 and has been used to prepared all standards since. Experiments A, B, and C presented below describe the evolution of applied sample preparation techniques, largely in the context of sample mixing extent, mixing times, and the role of diffusion.

Each of the 3 sequence types handled by the LabVIEW code can be summarized by being split into three parts. For each of them, the first part is preparation of the circulation loop later used for mixing the diluted sample gas, the second part cryo-trapping and pumping over of the sample gas, and the third part, which is identical for all sequences and sample types, is the thawing, diluting, and mixing of the sample gas. Following is a summary of the sample preparation sequences currently incorporated in the LabVIEW code.

All sample preparations happen in an identical manner. First, the circulation loop is manually evacuated then filled with high purity N₂ to 1100 mbar. The sequence is then started, the first steps being the re-evacuation of the loop and subsequent pressurizing to 1300 mbar of the same high-purity N₂. The inline diaphragm pump is then briefly activated to cycle gas through the loop, moving any potential atmosphere leak during evacuation into a more easily evacuated

volume and recharging the loop with N₂. The circulation loop is then closed off on both ends and allowed to slowly leak N₂ during the duration of the respective sample preparation sequences.

The preparation sequences differ in the sample transfer, cryo-trapping, and post-trapping cleaning steps. Carbonate samples introduced via the GasBench II are manually sampled via the sampling needle and directed into the AutoCart upstream of MV1. The CO₂ passes over the flask via a 1/8" o.d. stainless steel tubing bypass as the flask valves are closed at this point and is cryo-trapped in MV2. A transit time of 40 minutes is allotted for comprehensive transfer and collection of sample CO₂ from the sampling vials. MV2 is the vacuum pumped over the frozen sample gas to 75 mTorr before sample thaw.

Samples introduced via break-seals via the incorporated manifold are cryo-pulled under static vacuum into MV2 for 10 minutes, passing over the flask via the same bypass. After 10 minutes, the full volume is vacuum pumped over the frozen sample gas to 75 mTorr before sample thaw.

Atmospheric samples introduced by connecting the sampling flask to the AutoCart as MV1 are handled initially by evacuating the MV1 head-space created. Once evacuated, MV1 is closed off at valves 16 and 17 and the flask valves opened. The sample is then restrictively vacuum pumped through the cryo-trap on MV2 to 90 mTorr. Once achieved, the sample is thawed in MV2. This process typically takes ~50 minutes. It is of suspicion that a small amount of atmospheric N₂ condenses in the cryo-trap during this process. This excess gas is accounted for by a small offset in the sample yield when calculating dilution specifications.

For all sequences samples are allowed to thaw for 6 minutes in MV2 before the yield is measured. Measured yields are then used to calculate sample dilution requirements including amount of N₂ to be added and the number of turbulent mixing steps to be performed. The sample CO₂ is expanded into MV1 and signifies the beginning of the dilution and mixing process.

Sample dilution and initial mixing takes place in MV1 and is done by repeatedly pressurizing MV3 and MV2 to 1450 mbar of N₂ and subsequently expanding into MV1. The large pressure change combined with the flask's specific design to maximize turbulence promote even sample dilution. After pressure equilibration, MV1 is isolated and MV3 and MV2 repressurized to 1450 mbar. These steps are repeated n times as determined by measured sample yield (typically 4-5). n is calculated according to the curve:

$$n = 3e^{-6}x^2 + 0.0031x + 0.0261$$

where n is the expansion number and x is the target dilution pressure. n need not strictly be rounded to a whole number but can be a decimal under the condition that the fraction of n multiplied by 1450 mbar is greater than the pressure already contained in MV1 after the previous expansion. For example, when $n = 4.872$, $0.872 \times 1450 \text{ mbar} = 1264.4 \text{ mbar}$. Typical MV1 pressure after 4 expansions is ~765 mbar, so an expansion of 1264.4 mbar would occur to complete sample dilution. In the event that a partial expansion cannot occur (e.g., when $n = 4.123$, $0.123 \times 1450 \text{ mbar} = 178.4 \text{ mbar}$, less than MV1 pressure), n is rounded down to the nearest whole number and N₂ is then non-turbulently added to MV1 via valve V21 until the calculated dilution pressure is reached.

The direction of N₂ flow from MV3 through MV2 and into MV1, combined with the earlier expansion of the sample CO₂, concentrates the sample in MV1. This causes an excess of N₂ in MV2 and MV3 at the end of the dilution process. To overcome this, dilution requirements are calculated with respect to MV1 (586 mL) rather than the combined volume of MV1,2,3 (687 mL). After the dilution process is complete, V17 closes, isolating MV1, and MV2 and MV3 are evacuated. MV1 is then expanded to MV1,2,3, thereby achieving accurate sample dilution throughout the entirety of the mixing volumes where true dilution pressure and accuracy are recorded.

Further mixing occurs as the circulation loop is opened to the full mixing volume and the diaphragm pump activated. The diaphragm pump circulates at 750mL/min for 2.5 minutes, allowing sample gas to circulate through the entirety of the cart ~3 times. After 2.5 minutes of circulating the diaphragm pump is switched off, the loop is closed, and sample preparation is considered complete.

2.2) Materials

During the development of this method, three international carbonate standards, three internal carbonate standards, and dry atmospheric samples were used for testing, calibration, and assessment of the system. International carbonate standards used include NBS18, NBS19, and IAEA-603. The internal standards are Cavendish Marble (CM) and two modern ostrich eggshells (OES).

International Carbonate Standards

NBS18, NBS19, and IAEA-603 are internationally recognized and agreed upon carbonate standards used for, among other things, triple oxygen isotope analysis (Table 2.1). NBS18 is an igneous carbonatite of mantle origin. As such, it has lower $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values and a higher $\Delta^{17}\text{O}$ value than its sedimentary carbonate counterpart, NBS19 (Sharp et al., 2018). NBS19 is a marble consisting of the mineral calcite, however the material has been exhausted and will soon no longer be used and/or reported. IAEA-603 comes from a single slab of Carrara marble and, similarly to NBS19, consists of calcite. This standard has been created with the intention of replacing the exhausted NBS19 and accordingly is very similar in isotopic composition.

Standard	$\delta^{17}\text{O}$	$\delta^{18}\text{O}$	$\Delta^{17}\text{O} (\pm 1\sigma) (\theta = 0.528)$
NBS18	3.636	6.99	-0.048 (± 0.009)
NBS19	14.923	28.65	-0.102 (± 0.010)
IAEA-603	14.831	28.47	-0.100 (± 0.007)

Table 2.1: Values for NBS18, NBS19, IAEA-603 as given by the IAEA datasheet (https://nucleus.iaea.org/rpst/ReferenceProducts/ReferenceMaterials/Stable_Isotopes/13C18and7Li/NBS_18.htm)

Internal Standards

The standard Cavendish Marble (CM) is an inhouse carbonate standard used by the Stable Light Isotope Laboratory for measurements of $\delta^{18}\text{O}$. Collected from marble building materials sourced

from the Widouw Formation, its $\delta^{18}\text{O}$ has been well characterized by years of measurements using several mass-spectrometers and now TILDAS. This project has further characterized this material via high-precision measurements of $\delta^{17}\text{O}$ alongside $\delta^{18}\text{O}$, ultimately resulting in the ascertainment of its $\Delta^{17}\text{O}$.

The OES standards prepared for this project were a modern ostrich eggshell collected from a nature reserve near Etosha, Namibia (UCT-Et-1), and a modern ostrich eggshell collected near West Coast National Park, Western Cape, South Africa (UCT-WC-1). The two internal standards were prepared from shell fragments of each in April 2022. Eggshell fragments were first sanded using a handheld Dremel drill to remove bulk organic material from exposed surfaces. To prevent contamination, the Dremel drill was air blasted and wiped clean with ethanol between standards, and new sanding faces and diamond tipped drill bits were used for each. Shell fragments were then treated with 1% bleach solution for 8 hours to remove organic material still present, rinsed with DI water, and dried in a furnace at 70°C. They were then ground and sorted by sieves to obtain grain sizes between 180 and 600 μm . This grain size range was chosen to closely match that of the IAEA-603 standard (200-500 μm). A large amount of both samples with grain sizes of <180 μm was also collected but has thus far not been characterized.

The UCT-Et-1 eggshell was formed in a pan environment in a summer rainfall regime, where local mean annual rainfall ranges from 250 mm/yr in the west to 550 mm/yr in the east (Wanke et al., 2018) and local vegetation is dominantly grassland (Hipondoka et al., 2013) with interspersed stands of woody plant communities (Le Roux et al., 1988). However, this specific eggshell was collected from a nearby nature reserve with an artificial, year-round water hole. As a result, this sample is not likely to accurately represent wild ratite eggshell isotopic composition as consistent water availability diminishes the expected $\Delta^{17}\text{O}$ signal characteristic of high aridity environments where surface waters are generally highly evaporated. Groundwater in the region has been sampled via boreholes and has been reported to have $\delta^{18}\text{O}$ values between $\sim -3\text{‰}$ and -6‰ (Riddell et al., 2016). Riddell et al. (2016) also report accompanying $\delta^2\text{H}$ composition as between $\sim -32\text{‰}$ and -45‰ . (Note: $\delta^2\text{H}$ is not a focus of this project but is often reported alongside $\delta^{18}\text{O}$ in hydrological studies as correlated indicators of evaporation). The exact $\delta^{18}\text{O}$ value of local groundwater is dependent on groundwater recharge rates and the isotopic composition of the recharging meteoric waters. During the rainy season (Oct-Apr) local meteoric water $\delta^{18}\text{O}$ is typically between $\sim -4\text{‰}$ and -8‰ (Wanke et al., 2018).

The UCT-WC-1 eggshell was collected from a semi-arid environment near the Atlantic coast. Characterized by a winter rainfall regime, the West Coast National Park receives an average annual rainfall of $\sim 265\text{mm/yr}$ (Hanekom et al., 2009). Isotopic composition data of local precipitation is not available. However, West et al. (2014) report modeled precipitation in the area as having $\delta^{18}\text{O}$ of between -4.1‰ and -1.7‰ . Local vegetation is classified as Langebaan Fynbos/Thicket (Hanekom et al., 2009), largely consisting of C3 plants. This sample is expected to accurately portray the isotopic composition of local wild ostriches during the winter months, as eggs are typically laid shortly after the rainy season (Leuthold, 1977; Sinclair, 1978; Johnson et al., 1998).

Ratite Eggshell Sample Material

The ratite eggshell (RES) samples analyzed in this project are a subset of samples originally studied by Ségalen et al. (2002;2006) for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$. These studies suggest strongly season rainfall regimes in the proto-Namib ($\delta^{18}\text{O}$) and rule out the early expansion of C4 plants into the region ($\delta^{13}\text{C}$), arguing instead this expansion occurred much later (in the Pliocene from around 5 Ma) (Ségalen et al., 2006). The subset of samples analyzed in this project includes samples from both the central and southern Namib dating to the Quaternary, Miocene Climatic Transition (MCT), and the Mid-Miocene Climatic Optimum (MMCO). Also included are two samples from the Pliocene southern Namib, however central Namib counterparts representing this time period were not available. The species are *Namornis oshanai* (15–16 My, MMCO), *Diamantornis laini* (8–10 My, MCT), *Struthio daberensis* (2–5 My, Pliocene), and *S. camelus* (0–2 My, Quaternary). The complete sample list, description of stratigraphic position, details regarding potential diagenetic aspects and sample preparation, and the basis for species identification can be found in Ségalen et al. (2006).

Collection of Atmospheric Sample

The atmospheric sample presented here, UCT-CPT-1, was collected from Cape Point, South Africa on the morning of 14 February 2022. The sample was collected at the Global Atmosphere Watch (GAW) station which has a 30-meter tall atmosphere sampling tower onsite with sampling lines running the height of the tower and into the laboratory at its base. GAW real-time monitoring of CO and radon showed no terrestrial (anthropogenic) influence on collected air. The collection setup consisted of a length of ¼” o.d. stainless steel tubing with a U-trap bent into it, the 576mL atmospheric sampling flask, and a LI-COR CO₂/H₂O analyzer (LI-850, LI-COR Inc., USA). For sample collection, the tower’s sampling line was connected to the s.s. tubing and associated U-trap. This was then plumbed (crucially) into the siphon attached inlet of the sampling flask (Fig. 2.2). The flask outlet was connected to the LI-850 analyzer. Prior to sampling, the flask had been pre-evacuated, flushed with dry N₂ (99.999%), and pressurized to ~1100 mbar of N₂. Super-ambient pressurization of the flask allowed the opening of the inlet stopcock after connection to the rest of the sampling line without contaminating the flask volume.

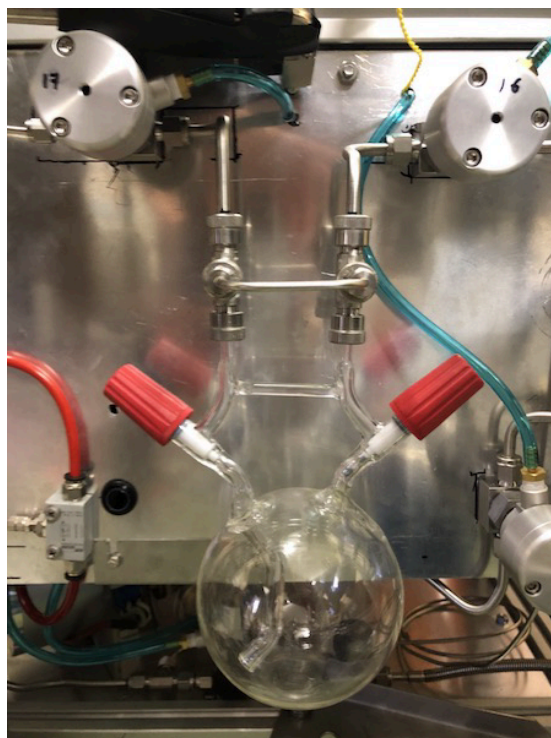


Figure 2.2: Photograph of the removable atmospheric sampling flask which doubles as MV1. Teflon stopcocks are Schott Produran. Flask volume is 576 mL. Flask is custom made (GlassChem CC, South Africa) of borosilicate glass. Siphon extending downwards from the left stopcock promotes mixing as sample circulates through the AutoCart. Design inspired by atmospheric sampling flasks used by National Oceanic and Atmospheric Administration (NOAA).

For sample collection, the U-trap was lowered into a dewar containing $-50\text{ }^{\circ}\text{C}$ ethanol to freeze out water vapor in situ. The height of ethanol in the dewar was slowly raised over the course of sample collection to increase trapping surface area and prevent blockage in the tubing due to ice buildup. Sampling began by opening the flask's inlet and outlet stopcocks and activating the diaphragm pump within the LI-850 (CTS Series, Parker Hannifin Corp., USA). After water was removed the air sample flowed directly into the flask, the geometry of which promotes mixing. As the outlet of the flask was connected to the LI-850, CO_2 and H_2O concentrations were monitored in real time by dedicated software (LI-COR LI-8x0). The LI-850 pump operates at 750 mL min^{-1} , meaning that the full flask volume is circulated through the LI-850 every ~ 40 seconds. Sampling was performed for 10 minutes to ensure complete flask circulation. After 10 minutes sampling was concluded by turning off the LI-850 diaphragm pump and closing both the inlet and outlet stopcocks on the flask. The sample was transported back to UCT and prepared on the AutoCart the same day.

2.3) Diffusion Theory

A consequence of diffusion occurring within the prepared sample gas is the asymmetrical movements of the $^{12}\text{C}^{17}\text{O}^{16}\text{O}$ and $^{12}\text{C}^{18}\text{O}^{16}\text{O}$ isotopologues as the lighter isotopologue diffuses more quickly than the heavier, resulting in mass-dependent fractionation. In the triple oxygen isotope system, fractionating processes are described by fractionation factors (α), which vary between isotope ratios. In conventional atomic isotope notation, the relationship between the

fractionation factors, of any process, for $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ is defined as: $\alpha^{17/16} = (\alpha^{18/16})^\theta$. After rearranging, this can be written:

$$\theta_{\text{diff}} = \left(\frac{\ln(\alpha^{17/16})}{\ln(\alpha^{18/16})} \right) \quad (2.1)$$

where the subscript “diff” denotes diffusive fractionation. For the diffusion of CO_2 in N_2 , the process relevant to the system introduced in this project, θ_{diff} can be calculated as the ratio of the diffusivities of the various CO_2 isotopologues (e.g., of nominal mass 44, 45, 46) in N_2 , which in turn is simply the ratio of fractionation factors of the CO_2 isotopologues. At 700 mbar and 21°C , typical conditions during sample preparation, it can be shown that $\theta_{\text{diff}} = 0.509$ (Hare et al., 2022).

All $\Delta^{17}\text{O}$ values reported in this project are calculated by the equation:

$$\Delta^{17}\text{O} = \delta'^{17}\text{O} - \theta(\delta'^{18}\text{O}) \quad (2.2)$$

where $\theta = 0.528$ (the global reference line) and $\delta'^{17}\text{O} = 1000\ln(\delta^{17}\text{O}/1000 + 1)$, and similar for $\delta'^{18}\text{O}$. Mass-dependent fractionating processes are reflected in measured $\Delta^{17}\text{O}$ values as a result of subequal effects on the δ -values and can be highly consequential when performing triple oxygen isotope measurements. Notably, the system specific calculated $\theta_{\text{diff}} (= 0.509)$ is less than that of the global reference line ($\theta = 0.528$) used for calculating $\Delta^{17}\text{O}$ values. As such, a given parcel of CO_2 that has diffused through N_2 will have lower $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values, and erroneously high $\Delta^{17}\text{O}$ ($\epsilon(\text{diff})$), as compared to the initial, undiffused CO_2 (Fig. 2.3). Mass-balance dictates that the remaining, thus far undiffused, CO_2 will have consequentially higher $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values, and significantly more negative $\Delta^{17}\text{O}$, than the initial CO_2 gas.

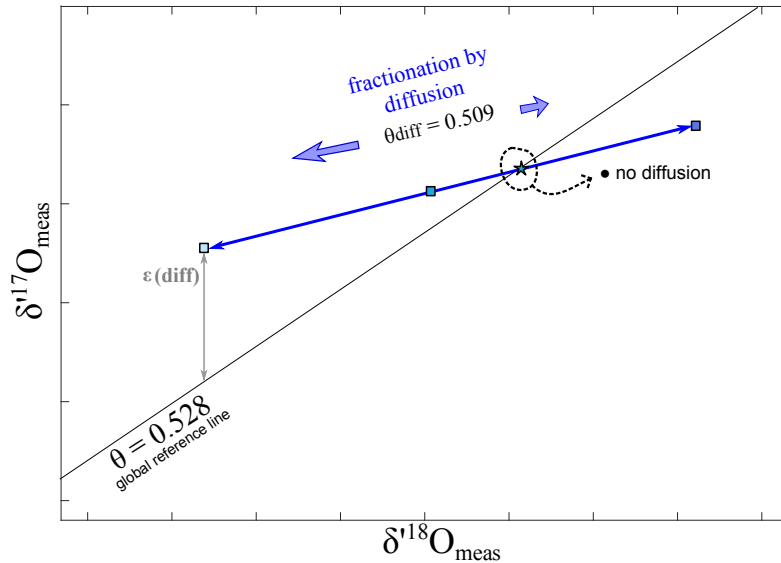


Figure 2.3: Figure adapted from Hare et al. (2022). Framework showing the effects of diffusion on measured $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values. Axes not to scale. Aliquots of sample gas that have undergone partial diffusion will trend lighter in measured $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values. Remaining CO_2 will, by mass-balance, trend more enriched.

The diffusive influence on triple oxygen isotope measurements can be conceptualized as Figure 2.4. For an incompletely mixed gas, which has undergone some amount of diffusion, sequential measured aliquots will have varying relative amounts of the $^{12}\text{C}^{17}\text{O}^{16}\text{O}$ and $^{12}\text{C}^{18}\text{O}^{16}\text{O}$ isotopologues, causing asymmetrical drift in the $\delta'^{17}\text{O}_{\text{meas}}$ and $\delta'^{18}\text{O}_{\text{meas}}$ values. As a byproduct of this asymmetry and the mathematical definition of a $\Delta'^{17}\text{O}$ value, large variations in $\Delta'^{17}\text{O}_{\text{meas}}$ are observed upon analysis. This effect is eliminated when sample gas is completely mixed, allowing subsequent aliquots to have identical relative amounts of the $^{12}\text{C}^{17}\text{O}^{16}\text{O}$ and $^{12}\text{C}^{18}\text{O}^{16}\text{O}$ isotopologues. Drift in $\delta'^{17}\text{O}_{\text{meas}}$ and $\delta'^{18}\text{O}_{\text{meas}}$, as represented by the curves, may occur over the course of an analysis as a result of TILDAS laser drift and slight temperature, pressure, and CO_2 concentration effects, among other factors. The stipulation for performing high-precision spectroscopic $\Delta'^{17}\text{O}$ measurements in a mixed sample gas is that this drift in $\delta'^{17}\text{O}_{\text{meas}}$ and $\delta'^{18}\text{O}_{\text{meas}}$ occurs in sync, a condition that cannot be met when diffusive fractionation occurs or is incomplete. Diffusive fractionation effects can be overcome, however, in the event that a sample is completely mixed, such that unequal distribution of the various isotopologues can no longer be observed. Complete mixing can be accomplished passively by simply allowing sample gas to diffuse to completion, or actively by turbulence, circulation, or a combination thereof.

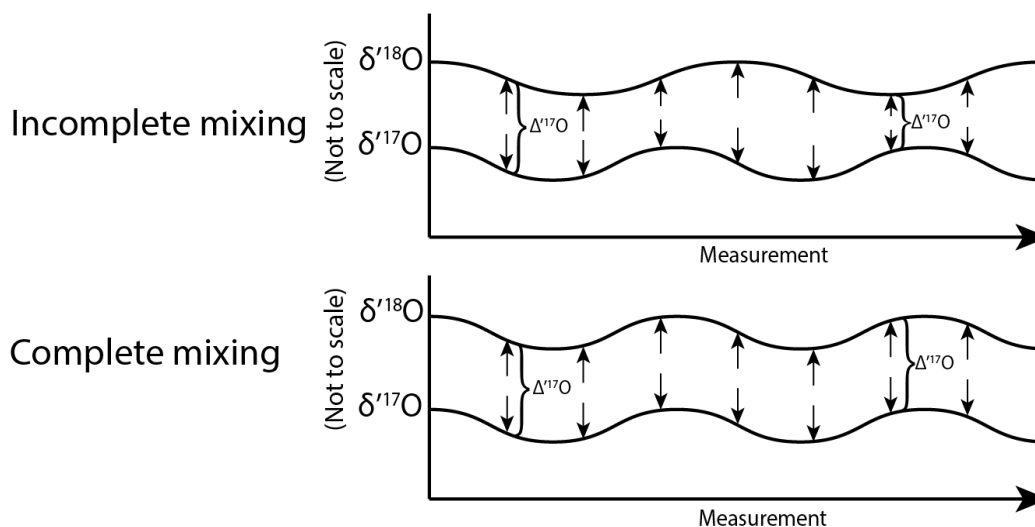


Figure 2.4: Conceptualization of impact of diffusion on measured $\Delta'^{17}\text{O}$ values. Curves represent instrumental drift over the course of an analysis. In an incompletely mixed gas, drift in $\delta'^{17}\text{O}$ and $\delta'^{18}\text{O}$ occur out of phase, resulting in large measured $\Delta'^{17}\text{O}$ variation. In a completely mixed gas, instrumental drift in $\delta'^{17}\text{O}$ and $\delta'^{18}\text{O}$ occurs in phase and measured $\Delta'^{17}\text{O}$ is unaffected.

2.4) Experimental

The following is an encapsulation of all methodological and analytical procedures that have been developed and applied in this project. The “Experimental” section of this chapter is divided into two main subsections: Upstream and Downstream. The Upstream subsection focuses on the design and evolution of the Automated Sample Preparation System, in regard to sample mixing, introduced above. The Downstream subsection focuses on advancements in the application of TILDAS for performing high-precision $\Delta'^{17}\text{O}$ measurements in CO_2 . The subsections are each divided into 3 “Experiments”, each with a specific aim. This section/subsection hierarchy is

maintained in the following chapter of this thesis: “Optimization of Analytical Procedure: Results and Discussion” where the results of all Experiments shown here are presented and discussed.

Upstream

Overview of Sample Dilution

All samples presented in this project were prepared in similar fashion by a preparation sequence that can be broadly summarized as:

- 1) Cryogenic trapping of sample CO₂ in MV2
- 2) Thawing of the trapped CO₂ in MV2 and yield measurement
- 3) Calculation of dilution specifications
- 4) Dilution of CO₂ with ultra-high purity N₂ in MV1
- 5) Expansion of diluted bulk sample through MV1,2,3 volume where final sample pressure was measured, and the dilution accuracy quantified.

While lacking in detail, these steps provide a basis for qualitative comparison and quantitative assessment of the evolution of the methodology introduced in this project. Several iterations of the sample preparation sequence are presented below. To aid in clarifying calculations and procedures regarding sample dilution and mixing, images of the annotated custom LabVIEW code created for automation of the preparation sequence are included. Unfortunately, however, LabVIEW does not always lend itself well to screenshots as a result of lacking a zoom feature. As such, some instances of the program presented here have been manually divided into separate pieces despite being a part of the same function. These instances are noted clearly when shown.

The TILDAS employed in this project is specifically designed to measure several isotopologues of CO₂ at a mole ratio of $\sim 400 \text{ mmol mol}^{-1}$. This requires CO₂ sample gas to be diluted in another gas, in this case ultra-high purity N₂. The analytical method of measuring many discrete aliquots of the sample implies that not only does the bulk sample need to have a CO₂ concentration of $\sim 400 \text{ mmol mol}^{-1}$, but every measured aliquot must also. As such, a key aspect of sample preparation is creating a bulk sample with minimal concentration gradients (i.e., a well-mixed gas). Several physical processes are capable of mixing gas, with varying effectiveness and time requirements. Evaluation of the mixing effectiveness for each of the presented dilution and mixing methods was performed by measuring the abundance of the ¹²C¹⁶O¹⁶O isotopologue (χ_{626}) in discrete aliquots of the prepared sample. To date, 68 standards have been prepared on the AutoCart. Average dilution accuracy of all standards, as determined by the ratio of actual dilution pressure to target dilution pressure, is 100.08% ($1\sigma = 1.27\%$, $n = 68$). This corresponds to an average concentration of $400.3 \pm 5.1 \text{ mmol mol}^{-1}$ (1σ , $n = 68$). The results of all experiments presented here are reported in the following chapter of this thesis.

Experiment A – Mixing via Passive Diffusion

The first trial of preparing an accurately diluted and well-mixed sample focused on mixing solely by diffusion. Diffusion causes mass-dependent fractionation as the lighter isotopologues diffuse through the medium, in this case N₂, more quickly than the heavier isotopologues. Because diffusion affects both concentration and isotopologue distribution, variations in CO₂ concentration within the bulk sample gas will be reflected in the isotopologue abundance ratios,

and therefore in the measured δ -values. Due to this, samples mixed by diffusion must be completely mixed, and diffusion a terminated process, prior to sample measurement. Ultimately, diffusion is only an issue because the system used in this project does not measure 100% of the prepared sample gas. If 100% of it was measured, the sample would be completely characterized, and diffusive effects would cancel themselves out. However, because diffusion was expected to be an exceptionally slow process, adoption of diffusive sample mixing was likely to limit sample throughput and render itself impractical. Nonetheless, early experiments focused on diffusion allowed for fine-tuning of the dilution aspect of the AutoCart and provided valuable insight into mixing rates.

Automated dilution and diffusive mixing was first tested on two samples of the internal laboratory standard Cavendish Marble (CM). Given precise determination of the 3 mixing volumes (MV's) integrated into the AutoCart, the amount of N_2 required for diluting sample CO_2 to a nominal $400 \text{ mmol mol}^{-1}$ was calculated from measured CO_2 yield after thawing, as measured by an Agilent Varian CDG-500, 0-10 Torr range pressure gauge (Fig. 2.5A). Sample CO_2 was then expanded from MV2 into MV1, a $\sim 10x$ increase in volume, to concentrate CO_2 in the flask. After CO_2 expansion, N_2 diluent was added via V21. The direction of flow from V21 towards MV1 was intentional to promote complete collection of CO_2 in MV1 as $\sim 10\%$ of total CO_2 remains in MV2 after the initial expansion. When the required amount of N_2 diluent to create a $400 \text{ mmol mol}^{-1}$ CO_2 in N_2 bulk sample was introduced, V21 closed, and the dilution ended.

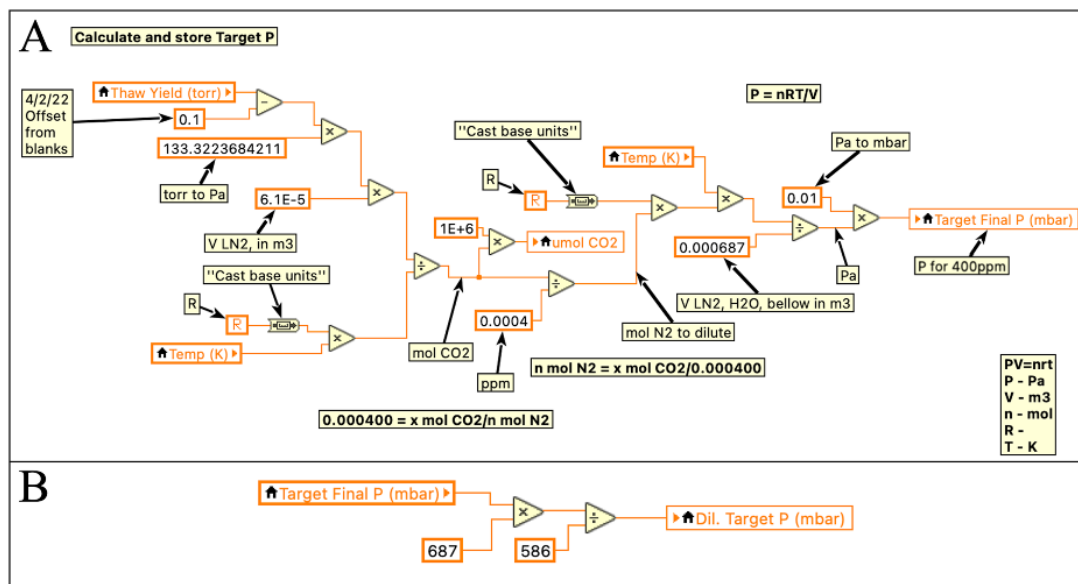


Figure 2.5: **A)** Dilution requirements are calculated on a per sample basis according to the ideal gas law: $PV = nRT$, where P is pressure in pascal, V is volume in cubic meters, n is the amount of gas in moles, R is the universal gas constant, and T is temperature in Kelvin. Inputs to the calculation include sample yield, volume in which yield was measured (MV2, 61mL), and temperature (arbitrarily set to 294 K). The variable “Target Final P (mbar)” is the pressure at which when reached V21 closes, and sample dilution is terminated. **B)** Scaling dilution to MV1 volume to overcome excess N_2 in MV2 and MV3, following Boyle’s Law: $P_1 V_1 = P_2 V_2$.

Both CM samples, CM-1 & CM-2, were allowed to mix in MV1 for 4.5 hours and 10 minutes, respectively. This large range of diffusion times was intended to examine potential end-member

mixing times and aid in characterizing the rate of diffusion in the closed system. The spherical shape of MV1 was intentionally chosen to promote rapid and even diffusion. By its nature, a sphere has the lowest characteristic length (typically defined as the ratio between volume and surface area; $L_C = V_{\text{body}}/A_{\text{surface}}$) of any shape, thus minimizing the distance a given parcel of CO_2 must travel as diffusion proceeds.

The direction of N_2 flow from MV3 through MV2 and into MV1 caused an excess of N_2 to remain in MV2 and MV3 after dilution was complete. This was overcome by calculating initial dilution requirements with respect to MV1 (586 mL) rather than the combined MV1,2,3 volume (687 mL) (Fig. 2.5B). After the sample dilution had completed, V17 closed to isolate MV1, and MV2 and MV3 were evacuated. After evacuation, MV1 was expanded back into the full MV1,2,3 volume, and thereby achieved accurate sample dilution throughout the entirety of the mixing volumes. After MV1 expansion, final sample pressure (WIKA S-20, 0-3 bar) and dilution accuracy was determined.

Experiment B – Mixing via Turbulent Advection

Experiment B is the aggregation of several attempted techniques for turbulently mixing sample gas and limiting the role of diffusion. The commonality for the techniques developed and tested here was the repeated over pressurization of MV3 and MV2 to a predetermined pressure followed by rapid expansion into MV1 (Fig. 2.1). The flask constituting MV1 is designed to promote turbulence by the addition of a siphon from one of the mounted stopcocks leading to the bottom of the volume, directly opposite the other stopcock (Fig. 2.2). This design was inspired by atmospheric sampling flasks used by the U.S. based National Oceanic and Atmospheric Administration (NOAA). The efforts of this Experiment were made to investigate the effect of mixing via turbulent advection on bulk sample mixing times and isotope ratios of sequentially measured aliquots of the resultingly prepared sample. This experiment presents the characterization of resulting mixing volume pressures from the tested advection techniques as a function of varying initial pressures.

Details of dilution procedure

1100 mbar N_2 Expansions

Diluting and mixing sample gas by repeated advection, colloquially “sloshing”, was intended to maximize turbulence encouraged by the design of the MV1 flask. The number of turbulent N_2 expansions (“sloshes”) into MV1 required to achieve target dilution pressures was determined by a series of automated tests in which MV2 and MV3 were filled to arbitrary pressures and expanded n times. The resulting pressure of the combined MV1,2,3 volume was recorded after each expansion. Initially, expansions were performed at an MV2,3 pressure of 1100 mbar. Repeated 1100 mbar expansions of MV2,3 into MV1 were characterized by the curve:

$$n = 1e^{-5}x^2 + 0.0002x + 0.3427$$

where n is the number of expansions, and x is the resulting pressure distributed throughout MV1,2,3. It is worth noting to assure clarity that this curve, and similar later presented in this chapter, were calculated with respect to the “Target Final Pressure” (see Fig. 2.5A) – the pressure within MV1,2,3 before which, during a sample preparation run, V17 would close to isolate MV1 and MV2,3 would be evacuated to clear excess N_2 . The presented curves do not

directly represent the final pressure at which the sample was prepared, but rather the pressure at which it must be diluted to in order to achieve a sample with a bulk CO₂ concentration of 400 mmol mol⁻¹.

Using this curve, the first series of samples mixed via repeated advection was performed on 3 samples of the internal standard Cavendish Marble (CM-3, CM-4, CM-5), as well as on 1 sample each of the international carbonate standards NBS19 (NBS19-1), and NBS18 (NBS18-1). For each of the samples, 5 expansions were performed regardless of sample yield and the targeted dilution pressure. Diluting strictly by 5 expansions was not expected to achieve accurate sample dilutions, and as such, following completion of the expansions N₂ was non-turbulently added to MV1 via V21 until the targeted dilution pressure was reached. After advection and final dilution was completed, each sample was allowed to diffuse for varying amounts of time, ranging from 10 minutes to overnight, to allow comparison to the sample mixing extent by diffusion only observed in samples CM-1 and CM-2.

The atmospheric sample UCT-CPT-1 was prepared using the same repeated 1100 mbar expansion technique applied to the samples described above with the exception that 10 expansions were applied. Greater theoretical yield, as calculated from CO₂ concentration given by the LI-850 during collection, required more N₂ diluent to achieve the targeting 400 mmol mol⁻¹ sample dilution, thus permitting more expansions without over-diluting the sample. After dilution the sample was allowed to diffuse in MV1 overnight before TILDAS analysis the following morning revealed excellent mixing of the bulk sample.

High Pressure N₂ Expansions

Despite success in achieving complete mixing by a combination of advection and extended diffusion times, realistic adoption of the sample preparation method introduced in this project relied on high sample throughput (i.e., shorter sample preparation times). To achieve further improved sample mixing more quickly, the mixing via turbulent advection technique was altered to use fewer, higher pressure expansions than had been applied thus far. To this end, automated tests of repeated expansions of 1400 mbar and 1500 mbar were performed, similar to that done for the 1100 mbar expansions discussed above.

Repeated 1400 mbar expansions of MV2,3 into MV1 were characterized by the curve:

$$n = 3e^{-6}x^2 + 0.0033x + 0.0268$$

while repeated 1500 mbar expansions resulted in the curve:

$$n = 3e^{-6}x^2 + 0.003x + 0.0254$$

where, again, n is the expansion number, and x is the resulting pressure distributed throughout MV1,2,3. As expected, both functions resulted in significantly decreasing the number of expansions required to achieve realistic sample dilution pressures. MV1,2,3 pressure for n expansions of both curves are shown in Table 2.2.

	1400 mbar Expansions	1500 mbar Expansions
<i>n</i>	<i>x</i>	<i>x</i>
0	0	0
1	232	250
2	430	460
3	596	639
4	733	786
5	847	907

Table 2.2: Empirically derived MV1,2,3 pressure after *n* turbulent N₂ expansions from MV2,3 to include MV1.

NBS18-5 was the first sample to be prepared using expansions of 1400 mbar. This sample was committed to being prepared by 5 expansions, regardless of sample yield and to be calculated dilution requirements. This sample had a target dilution pressure of 877.740, fortuitously close to the projected MV1,2,3 pressure after 5 1400 mbar expansions. Similar to the samples presented above, the remaining N₂ required to achieve the target dilution pressure, in this case ~30 mbar, was added non-turbulently. NBS18-5 was allowed to diffuse in MV1 for 40 minutes prior to TILDAS analysis. Measurements of *c*₆₂₆ revealed that this sample was, excluding those allowed to diffuse overnight, by far the best mixed sample to date.

The near over dilution of NBS18-5 prompted a further evolution of the turbulent advection strategy that had thus far been applied. In order to control the amount of N₂ diluent turbulently added to the sample more precisely, a method of partial expansions was developed in which the final expansion of any given sample could be truncated to a MV2,3 pressure which would expand to exactly the pressure required to achieve bulk sample CO₂ concentration of 400 mmol mol⁻¹, as determined based on sample yield. Figure 2.6 shows an example of this when using expansions of 1450 mbar and the curve representative thereof (given below). However, the calculation method is the same for other expansion pressures and can be applied simply by updating the necessary values. (Note: Figure 2.6 is divided into panels A and B here for formatting purposes but exists as a single sequence of calculations in the LabVIEW code).

Calculation of the partial expansions was done on a per sample basis by solving the respective curve applied to the sample dilution (e.g., that of 1400 or 1500 mbar expansions) for *n* by inputting the “Dil. Target P” (i.e., “Dilution Target Pressure”) as *x*. *n* was then rounded down to the nearest whole number, “Slosh to do” (Fig. 2.6A), which becomes the minimum number of expansions that will be performed. The remainder of *n* above the nearest whole number, “Rem. slosh frac.” (“Remaining slosh fraction”), is then multiplied by the full applied expansion pressure (e.g., 1450 mbar) (Fig. 2.6B). The result of this multiplication is the pressure at which the final expansion occurs (“P. frac. slosh” – “Pressure of fractional slosh”), with the condition that this pressure is greater than the pressure contained in MV1 after the previous expansion. For example, typical MV1 pressure after 4 expansions of 1400 mbar is ~733 mbar. When, for a given dilution target pressure, *n* = 4.872, 0.872 * 1400 mbar = 1220.8 mbar. This hypothetical sample is diluted by 4 expansions at 1400 mbar and, because 1220.8 mbar > 733 mbar, a final expansion of 1220.8 mbar is done to achieve exact dilution pressure without the non-turbulent

addition of N₂. In the contrasting event that a partial expansion cannot occur (e.g., when $n = 4.123$, $0.123 * 1400 \text{ mbar} = 172.2 \text{ mbar}$, which is less than MV1 pressure after 4 expansions), n is rounded down to the nearest whole number, here 4, and N₂ is then non-turbulently added to MV1 via valve V21 until the calculated dilution pressure is reached. While this written example assumes a MV1 pressure after 4 expansions, no assumptions of such pressure are made during sample preparation as the resulting pressure of each expansion is stored after it's occurrence. This stored pressure is later compared against the "P. frac. slosh" when determining the logic of applying a partial expansion, guaranteeing correct handling of the advection application.

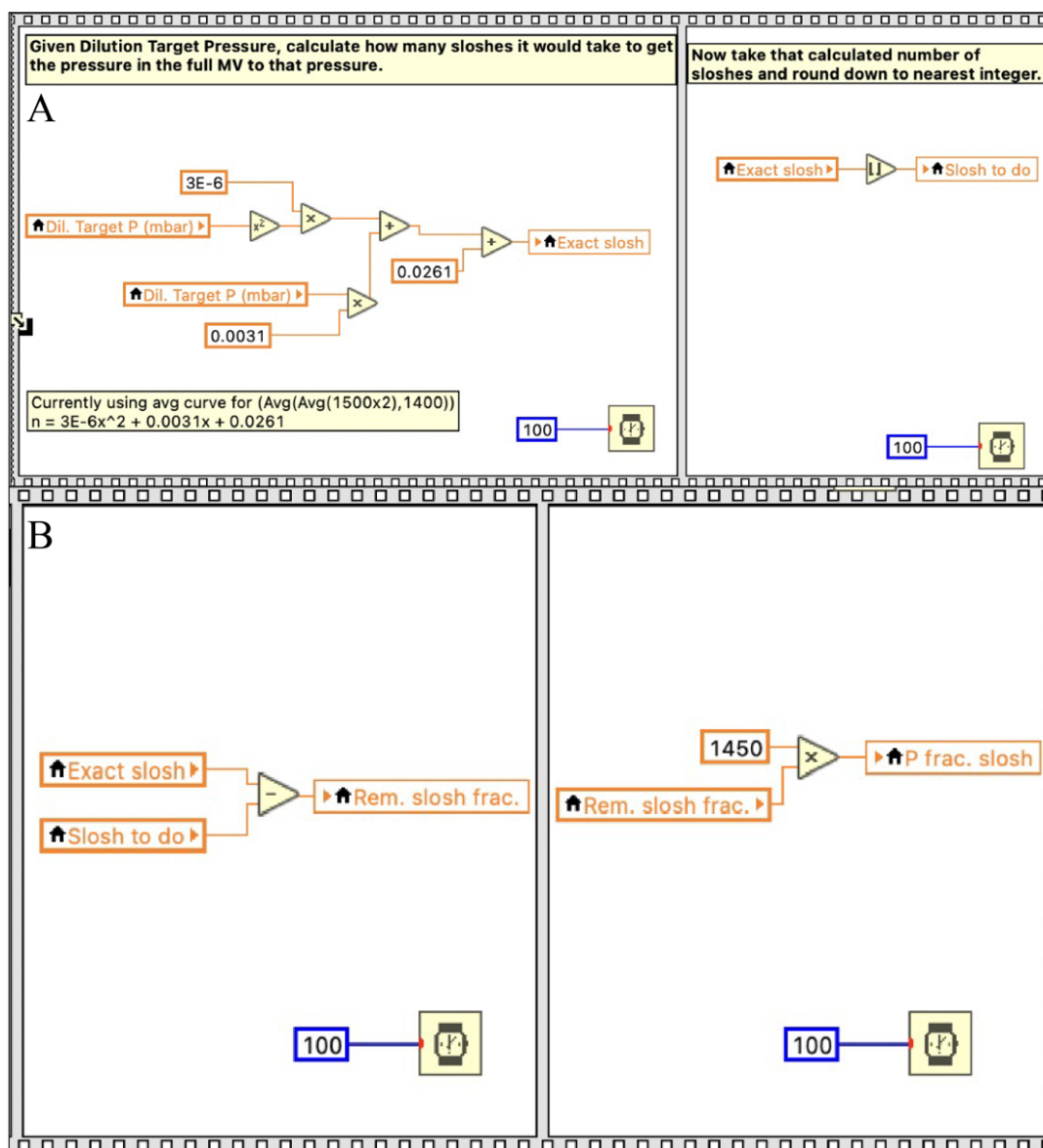


Figure 2.6: **A)** Calculation of sample specific number of turbulent expansions to perform for diluting sample CO₂ with N₂. The initial input "Dil. Target P (mbar)" is calculated according to Figure 2.5. The curve used for calculation of "Exact slosh" is that determined for expansions of MV2,3 pressure 1450 mbar. **B)** Determination of the final partial expansion pressure, "P. frac. slosh", if necessary to complete sample dilution by repeated advection only.

This fractional expansion method was applied to 4 CM samples (CM-7, CM-8, CM-9, CM-10), as summarized in Table 2.3 with expansions of varying pressures to test both functionality and forced mixing extent. These samples were prepared using the previously calculated curves for expansions of 1400 and 1500 mbar, as well as one for expansions of 1450 mbar. The curve for expansions of 1450 mbar was determined as:

$$n = 3e^{-6}x^2 + 0.0031x + 0.0261$$

where, again, n is the expansion number, and x is the resulting pressure distributed throughout MV1,2,3. Curve fitting for repeated expansions of 1400, 1450, and 1500 mbar, along with samples diluted by each of them, are shown in Figure 2.7. Diluting samples in this methods eliminates the need to non-turbulently add N_2 diluent to the sample, thus minimizing the extent diffusion the sample is subject to. TILDAS measurement of this set of samples revealed an expected trend; those with longer diffusion times were more completely mixed than those with shorter diffusion times, despite the limitation of non-turbulently added N_2 . Samples that were allowed to diffuse for 40 minutes had achieved the expected required mixing extent for TILDAS $\Delta^{17}O$ analysis. However, those samples allowed to diffuse for 10 minutes were not yet completely mixed, and the requirement of wait times of 40 minutes were a large hinderance to sample throughput.

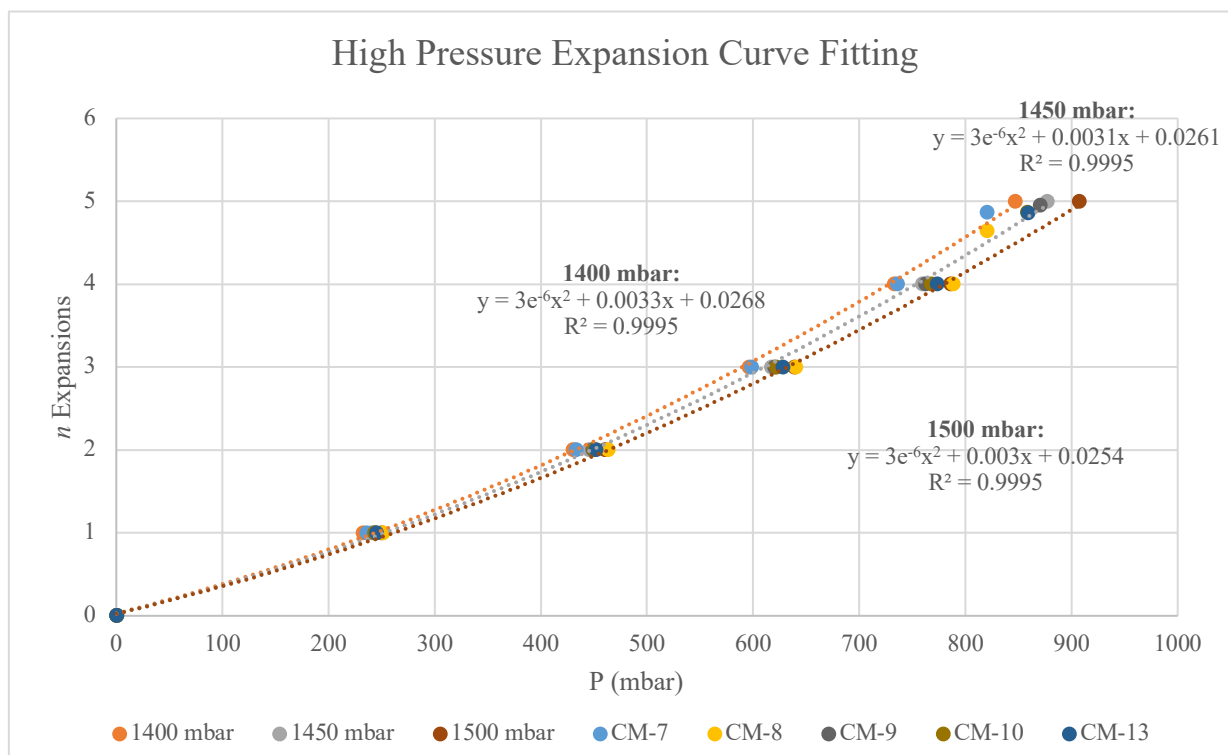


Figure 2.7: Visualization of empirical functions determined for MV1,2,3 pressure after n expansions of the MV2,3 volume of varying initial pressures: 1400, 1450, 1500 mbar. Overlaid are 5 Cavendish Marble (CM) samples prepared by a combination of the modeled expansion pressures. Characterization of these expansion curves allowed for precise sample preparation by repeated advection only, avoiding the need to non-turbulently dose N_2 into the AutoCart mixing volume.

Sample	Expansion P (mbar)	<i>n</i> Expansions	t diffusion
CM-7	1400	4.868	40 min
CM-8	1500	4.643	10 min
CM-9	1450	4.951	10 min
CM-10	1450	4.870	40 min

Table 2.3: Dilution and advective mixing parameters for the first samples prepared using the partial expansion technique. CM is Cavendish Marble.

Experiment C – Mixing via Circulation

In addition to Experiments A and B above, Experiment C was performed with the goal of increasing sample throughput while simultaneously improving repeatability of bulk sample mixing. To this end, a circulation loop was added to the AutoCart in expectance that forcing the sample gas through the full MV1,2,3 volume repeatedly would encourage mixing on shorter timescales than either repeated advection or diffusion alone.

The circulation loop consists of 1/4" o.d. stainless steel tubing with an inline diaphragm pump (CTS Series, Parker Hannifin Corp., USA) (Fig. 2.1). The circulation loop, when open and activated, directs sample gas in a clockwise direction from upstream of MV1 and into MV3. Sample gas then flows through MV3, MV2, and into MV1. Crucially, the direction of flow forces sample gas to enter MV1 via the flask's siphon, enhancing mixing. After installation, the loop was leak tested and it was realized that the diaphragm pump was not gas tight when the circulation loop was pumped to vacuum. Pump leakage was also noted to exist in the opposite scenario when the loop was pressurized to super-ambient pressures with N₂. It was discovered that the leak rate could be slowed by briefly activating the diaphragm pump after pressurizing the circulation loop. By filling the internal volume of the diaphragm pump to super-ambient pressures, any leaks through the diaphragm seals were directed outwards to the environment, thereby maintaining purity of the loop and internal pump volume. This, however, posed risk to sample gas when circulation is active as samples are always diluted to sub-ambient pressures, implying the risk of sample contamination by atmospheric leaks into the diaphragm pump.

After the circulation loop and diaphragm pump was installed, 5 CM samples (CM-13, CM-14, CM-15, CM-16, CM-17) were prepared using the newly adapted automated preparation sequence. This sequence consisted of the fractional expansions turbulent advection technique described above, combined with subsequent circulation via the loop. The flow rate of the inline diaphragm pump (750 mL min⁻¹) causes sample gas to circuit the entire mixing volume of the AutoCart (687 mL) in under a minute. Using this as a reference frame, circulation times of 1, 2, and 3 minutes were initially tested.

Downstream

Overview of TILDAS Instrument

The TILDAS system is a commercially available infrared laser spectroscopy instrument developed and sold by Aerodyne Research Inc. (ARI). The instrument employed in this project, also described by Hare et al. (2022) is fitted with two co-aligned interband-cascade lasers (DFB-

ICLs, nanoplus Nanosystems and Technology GmbH); customized to provide optimal performance at CO₂ mixing ratios around 400 mmol mol⁻¹ (in N₂). Each laser targets a specific wavelength, each chosen to provide strong and well-balanced absorption signals of the individual isotopologues of interest (Table 2.4). This targeting allows for simultaneous measurements of the ¹²C¹⁶O¹⁶O, ¹²C¹⁷O¹⁶O, ¹²C¹⁸O¹⁶O, and ¹³C¹⁶O¹⁶O isotopologues. All aspects of the TILDAS measurement system (e.g., valve timings, laser control, signal processing, data acquisition, etc.) are controlled by a TILDAS dedicated software (TDLWintel).

	Wavenumber (cm ⁻¹)	Wavelength (nm)	Isotopologues of Interest
Laser 1	2310	4329	¹² C ¹⁶ O ¹⁶ O, ¹² C ¹⁸ O ¹⁶ O, ¹³ C ¹⁶ O ¹⁶ O
Laser 2	2349	4257	¹² C ¹⁷ O ¹⁶ O

Table 2.4: Targeted wavenumber, wavelength, and isotopologues of the dual-laser TILDAS instrument employed in this project. Both lasers are interband-cascade lasers. Note: the ¹²C¹⁶O¹⁶O and ¹²C¹⁸O¹⁶O isotopologues also exhibit absorption peaks in the region targeted by Laser 2, however the stronger absorption signals for these isotopologues identified in the region of Laser 1 are used for isotopologue abundance calculations. The inverse for the ¹²C¹⁷O¹⁶O isotopologue is true; a small absorption peak is identified in the region of Laser 1 but is not used for abundance calculations.

The instrument housing is continually purged with dry N₂ at a rate of ~1 L min⁻¹. As N₂ alone commits no infrared absorption and is inert, it provides the means for tightly controlling the instrument's internal environment without impacting analysis. Purging occurs as N₂ is regulated into the non-leak-tight housing where it is allowed to evenly leak outwards along the edges, thereby preventing back-diffusion of lab air into the system. The housing walls are thermally regulated to provide a highly stable internal environment.

A liquid chiller is used for regulating the temperature maintaining the thermal stability of the greater instrument housing as well as both of the lasers. Liquid coolant temperature is maintained by combination of a Peltier thermoelectric element and a closed loop temperature controller. The chiller maintains a setpoint at ~23 K and has been observed, over the course of 12 hours of near continuous measurement, to be stable to 0.0023 K (1σ, *n* = 33798). Coolant flows first into Laser 1, followed by Laser 2, and finally circulates along the inner walls of the instrument housing, before returning to the chiller reservoir. Lasers 1 and 2 operate at ~18 C and ~9.5°C, respectively, and are stable to 0.004 and 0.005 K, respectively (1σ, *n* = 33798), and are not observably influenced by external factors such as housing N₂ purge rate or lab aircon settings.

During measurement, the lasers are sequentially scanned at a frequency of 1500 Hz (i.e., measuring 1500 spectra sec⁻¹). At analysis onset, TILDAS measures and averages 1500 spectra, creating a 1-second average spectrum. This average spectrum is fit individually to each laser and plays the role of improving the signal-to-noise ratio during actual sample measurement. Measured spectra must be normalized to laser transmission and require scaling to end member absorption fits to do so. To this end, a zero-light signal is measured and interpreted, acting effectively as a measurement with complete absorption. For the other end member scenario, a polynomial form baseline is applied and acts as a zero absorption (total transmission) spectrum. Together, these end member absorption fits and the laser specific absorption lines of the

expected isotopologues present in the relevant spectral region, combine to provide the basis for normalizing measured sample spectra to laser transmission.

The lasers scan individually, such that only one is scanning at a time. In the TILDAS instrument housing the lasers reflect off a series of highly polished mirrors prior to entering a leak-tight multipass absorption cell. Once within the cell, the beams reflect off of two more mirrors in a pattern such that 194 passes are accumulated. These accumulations amount to create an optical pathlength of 36 m, providing sufficient absorption for high-precision measurements. After exiting the optical cell, the laser beams are focused on a thermoelectrically cooled HgCdTe detector (J19, Teledyne Judson), where the remaining (not absorbed) light is quantified.

Experiment D – Gas Introduction and Spectroscopic Measurement

For this experiment, analyses were performed by alternately introducing discrete aliquots of sample and reference gas into the TILDAS optical cell. Each aliquot is measured in the optical cell for ~80 seconds, during which V1 is evacuated and the next aliquot introduced to V1 (20 mL). After measurement, the optical cell is evacuated and the pre-filled V1 gas (either sample or reference) is expanded into the optical cell (200 mL). A measurement cycle, consisting of the measurement of subsequent aliquots of sample and reference gas, takes ~3 minutes, with a full analysis typically consisting of 16-20 cycles and taking ~50 minutes. Each measurement cycle produces a $\Delta^{17}\text{O}$ value as calculated from $\delta^{17}\text{O}_{\text{meas}}$ and $\delta^{18}\text{O}_{\text{meas}}$ values of the corresponding sample and reference gas aliquots. For all analyses, the first 3 measurement cycles and their calculated $\Delta^{17}\text{O}$ values are ignored to avoid thermal effects as the optical cell achieves thermal stability early in the analysis. This repeated, alternating aliquot measurement procedure is analogous to traditional dual-inlet IRMS techniques.

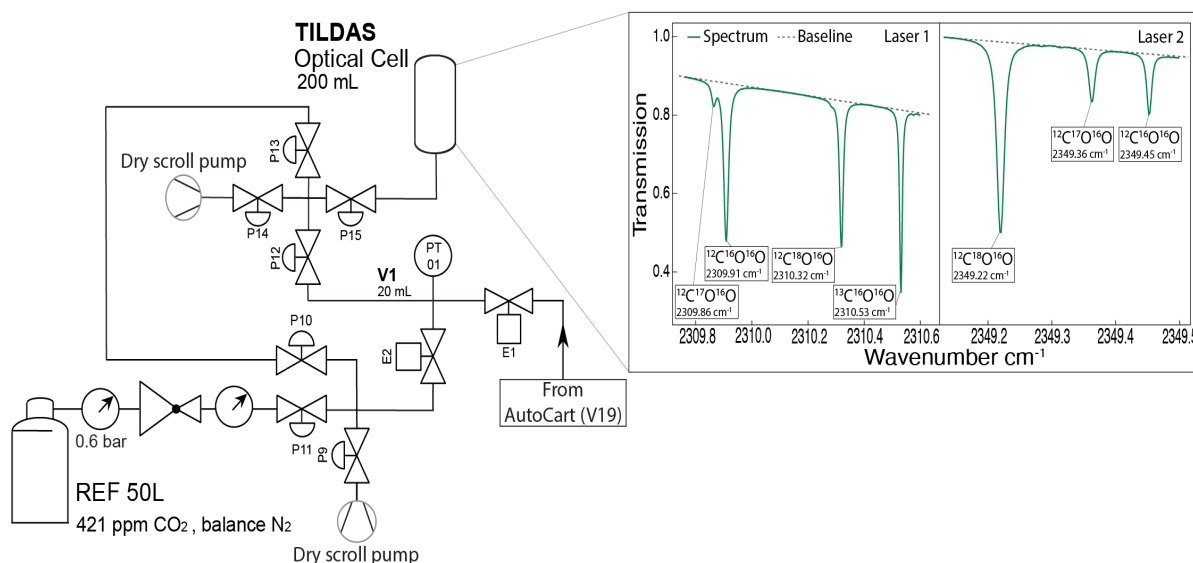


Figure 2.8: Schematic of TILDAS switching valve system, identical to that previously described elsewhere (Wang et al., 2020). Valves P9-15 are pneumatic bellows sealed valves. Valves E1,2 are electronic valves. Not shown is a critical orifice in line between P11 and E2 which slows V1 filling of reference gas. Reference gas tank supplied by prepared by Air Liquide South Africa (Pty) in July 2021. Tank was allowed to sit undisturbed for several months before initial measurements were made. Spectrum inlay is a typical absorption spectrum for reference gas as optical cell pressure ~30 Torr. CO₂ isotopologues and associated wavenumbers are given.

Sample gas is introduced to the TILDAS sampling valve system, identical to that previously described by Wang et al. (2020), from the AutoCart via valve V19, a critical orifice and valve E1 (Fig. 2.8). A consequence of filling V1 by sample gas expansion from the AutoCart mixing volumes as described above is that the time it takes to fill V1 to 300 Torr increases slightly over the course of an analysis as a result of decreasing sample supply pressure. Samples prepared on the AutoCart are typically prepared to ~ 700 - 800 mbar and decrease to ~ 450 - 500 mbar over the course of an analysis. The lengthening of aliquot filling time applies only to aliquots of sample gas, as reference gas is supplied at a constant 0.6 bar by a sub-ambient regulator (3396 series, Matheson Tri-gas Inc., USA). 0.6 bar was chosen as the reference gas supply pressure to most accurately parallel sample supply pressure as an analysis progresses. Matching of sample and reference gas supply pressure allows for similar V1 filling accuracy and timing between respective aliquots. Reference gas is introduced to the sampling valve system via valve P11, a critical orifice, and valve E2.

Spectroscopic isotopologue analyses are subject to a variety of instrumental conditions including optical cell pressure and temperature as well as various laser parameters (e.g., temperature, current offset), amongst others. The TILDAS employed in this project was specifically designed for low pressure measurements of CO_2 diluted to ~ 400 $\mu\text{mol mol}^{-1}$. Reduced pressure within the optical cell allowed for sharpening spectral absorption lines, which can also be described as reducing spectral pressure broadening effects, allowing for highly specific absorption lines to be targeted. During a typical analysis, optical cell pressure is ~ 28 Torr, and generally stable to <300 mTorr (Fig. 2.9). Measurement precision is also subject to the thermal stability of the optical cell. Cell temperature during any given analysis is ~ 297 K and is typically stable to <0.1 K.

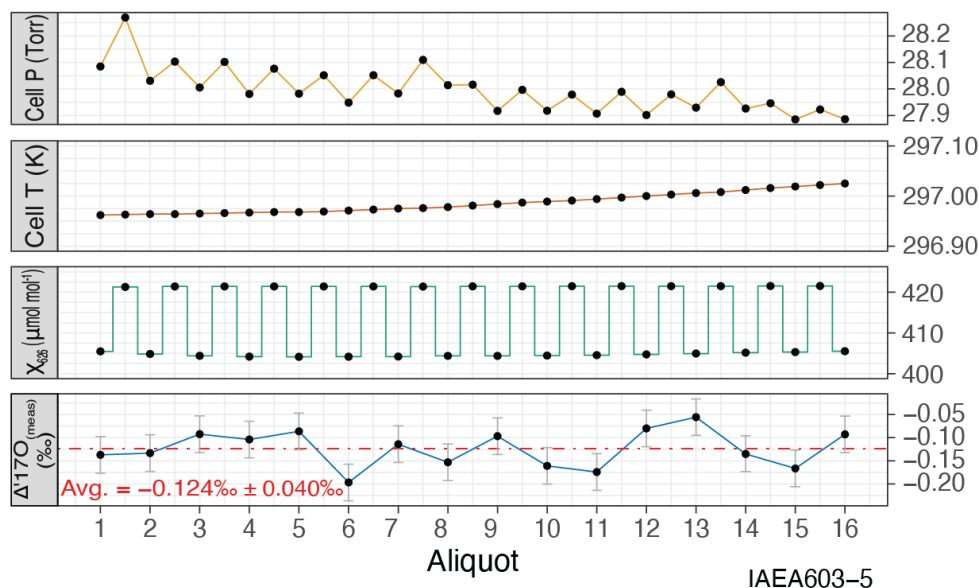


Figure 2.9: Figure from Hare et al. (2022). Example of IAEA-603 standard measurement and instrument conditions of a typical analysis. Sample gas was $8.645 \mu\text{mol CO}_2$ evolved by phosphoric acid digestion (70°C for 2 hours) of 0.914 mg of IAEA-603 carbonate standard. Sample was mixed to $404.8 \mu\text{mol mol}^{-1} \text{CO}_2$ in N_2 with initial sample pressure 703.53 mbar. Analysis was performed by repeated measurement of subsequent aliquots of prepared sample gas and reference gas (50 L high-pressure tank, $421 \mu\text{mol mol}^{-1}$). During analysis, optical cell temperature was stable to within <0.1 K, and cell pressure within <300 mTorr. χ_{626} is the concentration of the $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue. Error bars for $\Delta^{17}\text{O}_{\text{meas}}$ are 1σ .

Experiment E – Assessment of TILDAS Electronics Temperature on $\Delta^{17}\text{O}$ Precision

To address the effects of temperature on TILDAS analytical precision a series of reference gas vs. reference gas experiments were conducted. Over the course of a day, repeated analyses (each with a duration between 38 and 65 minutes) of reference gas against itself were performed while varying multiple aspects of the TILDAS operational environment. Tested variables were internal N_2 purge rate, lab aircon temperature setpoint, and the absence altogether of aircon temperature control, as summarized in Table 2.5. Variety in the internal purge rate was not expected to have an influence on measurement precision. Other than a potential slight impact on the thermal stability of the TILDAS housing, purge rate is taken to be inconsequential providing that it is sufficient to maintain a dry, N_2 dominated internal environment. As was observed, the purge N_2 flow rate had no definitive effect on analytical performance over the course of the experiments.

All sub-experiments presented here represent varying combinations of the intentionally targeted variables and, on occasion, other analytical intrusions. Sub-experiment E4 was unique in that 10 people spent notable amounts of time in the instrument room over the course of the analysis, as compared to 1-3 for all others. The drastically increased number of heat sources and thermal mass in the room resultingly caused the lab aircon units to activate more frequently than other sub-experiments.

Sub-Experiment	Time started	Duration (min)	N_2 purge (L min^{-1})	Aircon setpoint ($^{\circ}\text{C}$)
E1	6:31	65	1.5	23
E2	7:38	60	1	23
E3	8:59	38	1.5	23
E4	10:02	60	1	23
E5	11:11	45	1	23
E6	12:11	57	1.5	23
E7	13:31	55	1	23
E8	14:34	47	1	23
E9	15:30	45	1	OFF
E10	16:39	41	1	24
E11	17:44	52	1	OFF

Table 2.5: Summary of parameters regarding sub-experiments performed for investigation of the effect of TILDAS electronics temperature on measured $\Delta^{17}\text{O}$ precision.

Sub-experiment E5 was intentionally manipulated for ~6 minutes during the analysis by covering the intake fan on the electronics box with a piece of paper. Covering the intake resulted in drastic instantaneous heating of the internal electronics. Removing the cover caused cooling of similar nature as the inrush of laboratory air cooled the system. This action was intended to exaggerate any impact on measurement precision caused by electronics thermal changes.

Sub-experiment E10 was unique by virtue of its laboratory aircon setpoint of 24°C. This was intended to observe the effects of stifling the cooling ability of the liquid chiller as lab air was warmer than the chiller's setpoint.

Sub-experiments E9 and E11 were performed to assess the avoidance of aircon induced air temperature changes altogether. Shutting off of the aircon units resulted in a several degree increase in room temperature; however, these increases were drawn out over the full duration of the analyses. These experiments were intended to assess large, slow changes in air, and therefore electronics, temperature versus smaller, rapid changes in temperature as mediated by the aircon units.

Experiment F – δ -value Concentration Dependence and Data Correction

TILDAS, and other optical isotope spectrometers, determine δ -values by measuring and comparing the mole fractions of several isotopologues simultaneously (Griffith et al., 2012). Optically derived δ -values are calculated based on isotopologue mole fractions (χ), which are in turn related to the isotopologue measured concentration (C) in the optical cell (of volume, V). Adopting IUPAC notation, in which isotopologues are identified by the second digits of the atomic mass of their atoms (e.g., 627 = $^{16}\text{O}^{12}\text{C}^{17}\text{O}$ = $^{12}\text{C}^{17}\text{O}^{16}\text{O}$; Cohen et al., 2007; Coplen, 2008), the mole fraction of the $^{12}\text{C}^{17}\text{O}^{16}\text{O}$ isotopologue is calculated as: $\chi_{627} = C_{627}V/n$, where n is moles of gas. Similar equations, both here and throughout this section, can be derived for other isotopologues (e.g., $^{12}\text{C}^{18}\text{O}^{16}\text{O}$ and $^{13}\text{C}^{16}\text{O}^{16}\text{O}$; IUPAC identification 628 and 636, respectively). Spectroscopic delta values are then calculated using the measured mole fractions of the sample and reference gas by the equation:

$$\delta(627) = \left(\frac{\chi_{627}/\chi_{626}}{X_{627}/X_{626}} - 1 \right) \times 1000 \quad (2.3)$$

where c is the measured mole fraction of the sample (mol mol^{-1}) and X is the isotopologue abundance ratio (mol mol^{-1}) as specified by the high-resolution transmission molecular absorption database (HITRAN) (Rothman et al., 2005; Gordon et al., 2022). In this equation, c_{627} is analogous to the atomic isotope ratio of the sample ($^{17}\text{O}/^{16}\text{O}_{\text{sample}}$) as measured by IRMS, and X_{627} is analogous to the isotope ratio of the standard, ($^{17}\text{O}/^{16}\text{O}_{\text{std}}$).

Because spectroscopic δ -values are measured relative to HITRAN, they are not directly comparable to more commonly used isotope reference scales such as VSMOW2-SLAP2 or VPDB. Consequently, raw spectroscopic δ -values ($\delta(627)$, etc.) must first be converted to spectroscopic δ -values relative to the working reference gas ($\delta^{17}\text{O}$, etc.) before final calibration to a more widely used scale; in this project the VSMOW2-SLAP2 scale. The relationship between spectroscopic raw $\delta(627)$ and $\delta^{17}\text{O}$ values is defined as:

$$\delta^{17}\text{O}_{\text{meas}} = \left(\frac{\delta(627)_{\text{sam}}/1000 + 1}{\delta(627)_{\text{ref}}/1000 + 1} - 1 \right) \times 1000 \quad (2.4)$$

where the subscripts “sam” and “ref” denote sample and reference gas from which individual $\delta(627)$ values are determined by sequentially measured aliquots of the respective gases, and “meas” indicates a measured, uncorrected value. It must be noted that the spectroscopic δ -values

measured in this project are technically molecular abundance ratios and are inherently different than traditional IRMS δ -values, which measure atomic isotope abundance. However, the difference between the two is assumed to be negligible (Kerstel, 2004). As such, this project presents data using atomic isotopic composition notation to promote easy comparison to the existing literature.

Spectroscopic δ -values calculated relative to the working reference gas then require calibration and scaling corrections to be interpreted alongside existing atomic δ and $\Delta^{17}\text{O}$ values derived via IRMS. A scheme for such scaling corrections was first laid out by Griffith et al. (2012) for correcting spectroscopic $\delta^{13}\text{C}$ values to the VPDB scale. This project, and related publication Hare et al. (2022), have expanded the procedure initially put forth to be adaptable to oxygen isotopes in the triple oxygen isotope system. The scaling correction procedure employed here employs the notation $\chi'_{627} = \chi_{627}/X_{627}$, and modifies eq. (2.4) such that:

$$\delta^{17}\text{O}_{\text{meas}} = \left(\frac{a_{627}\chi'_{627} + b_{627}}{a_{626}\chi'_{626} + b_{626}} - 1 \right) \times 1000 \quad (2.5)$$

where a_{626} , a_{627} , b_{626} , and b_{627} are scaling factors relating HITRAN mole fractions to their equivalents on the VSMOW2-SLAP2 scale. For cleanliness, the notation $A_{627} = a_{627}/a_{626}$ is adopted and the factor of 1000 dropped, before modifying eq. (2.5) further to show that:

$$\delta^{17}\text{O}_{\text{std}} = \frac{\chi'_{626}}{A_{627}(\chi'_{626} - b_{626})} \left[\delta^{17}\text{O}_{\text{meas}} + \frac{(A_{627}b_{626} - b_{627})}{\chi'_{626}} - A_{627} + 1 \right] \quad (2.6)$$

thereby providing a single equation for correcting TILDAS measured δ -values to the VSMOW2-SLAP2 scale. $\delta^{17}\text{O}_{\text{std}}$ is either assumed or measured directly by IRMS. The constants A_{627} , b_{627} , and b_{626} are then determined by non-linear least squares fitting to eq. (2.6) in MATLAB.

This equation shows that uncorrected TILDAS derived δ -values are subject to a concentration dependency based on the concentration of the most abundant isotopologue, $^{12}\text{C}^{16}\text{O}^{16}\text{O}$, via the term χ'_{626} . Termed a “*concentration dependence due to scale-offset error*” this effect is purely a mathematical consequence of the definition of a spectroscopic value and the offset between the HITRAN and VSMOW2-SLAP2 isotopologue abundance scales (Hare et al., 2022). The same correction procedure is then performed to correct $\delta^{18}\text{O}_{\text{meas}}$ values to $\delta^{18}\text{O}_{\text{std}}$ (with constants A_{628} , b_{628} , and b_{626}) for the same samples. Interlaboratory comparisons of international carbonate standards (e.g., NBS18, NBS19, and IAEA-603) measured by TILDAS and IRMS can thus be performed by correcting TILDAS measurements using eq. (2.6). After correction, $\Delta^{17}\text{O}$ values for the sample are then calculated using eq. (2.2).

Figure 2.10 shows a framework of measurement error due to *scale-offset*. For a theoretical sample that is diluted and mixed such that the χ'_{626} of every aliquot perfectly matches the χ'_{626} abundance of the reference gas, the constants in eq. (2.6) are $A_{627} = 1$ and $b_{627}, b_{626} = 0$, the equation becomes $\delta^{17}\text{O}_{\text{std}} = \delta^{17}\text{O}_{\text{meas}}$, (and similar for other isotopologues), and the two scales are equal. In realistic cases, sample gas aliquots have varying concentrations which are not equal to the reference gas (represented by stars). In this framework, sample aliquots that are under-concentrated relative to the reference gas will trend more positive in $\delta^{17}\text{O}_{\text{meas}}$ and $\delta^{18}\text{O}_{\text{meas}}$ values,

and over-concentrated aliquots more negative (Hare et al., 2022). As a result, under-concentrated aliquots are expected to record systemically more negative $\Delta^{17}\text{O}$ than their true value (i.e., $\epsilon(\text{scale})$ for these aliquots is negative), and vice-versa for over-concentrated aliquots.

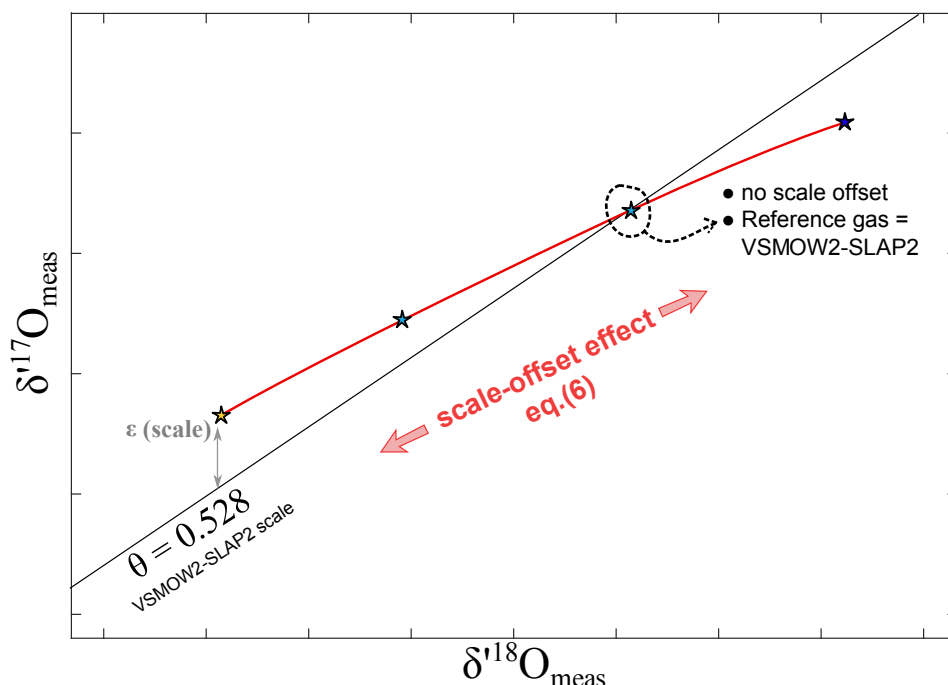


Figure 2.10: Figure adapted from Hare et al. (2022). Framework of TILDAS measurement error as a function of *scale-offset* ($\epsilon(\text{scale})$) between the HITRAN and VSMOW2-SLAP2 isotopologue abundance scales. Stars represent samples of different concentrations. When CO_2 concentration of the sample gas is equal to that of the reference gas, the HITRAN and VSMOW2-SLAP2 scales are equal, and no scale-offset is present.

In summary, all spectroscopically obtained δ -values, inherently relative to HITRAN, are influenced by the concentrations of each measured isotopologue when corrected to another isotopologue abundance scale (e.g., VSMOW2-SLAP2). This concentration dependence applies not only to the bulk sample but also to individual aliquots of the sample gas. The measured isotopologue mole fraction of each aliquot must be scaled relative to the respective reference gas aliquot they are compared against (eq. 2.4). Recently, Perdue et al. (2022) suggested there is no observable concentration effect when the mixing ratios of sample and reference gas are within 20 ppm ($\mu\text{mol mol}^{-1}$) of each other. However, the TILDAS instrument used in their study is a single laser instrument and avoids the complications of balancing absorption signals from two lasers. For the instrument used in this project data correction using eq. (2.6) is necessary for achieving <10 per meg $\Delta^{17}\text{O}$ precision when scaled to the VSMOW2-SLAP2 scale.

Chapter 3: Optimization of Automated Sample Preparation System and TILDAS Analyses[‡]

This chapter intends to describe the successes of both the sample preparation system introduced in this project and the TILDAS instrument. While the two halves of the greater system will be discussed separately, they are intimately connected. Ultimately, proper evaluation of the TILDAS analytical instrument and its various influences and limitations is purposeless without having a true understanding of the of the sample preparation system and the requirements thereof. As such, this chapter will follow the pattern of describing the evolution of the sample preparation system to meet the requirements of the TILDAS, followed by experimentally optimizing the TILDAS instrument and analytical method for high-precision measurements of $\Delta^{17}\text{O}$ in CO_2 . All mole fractions and concentrations reported in this chapter are as evaluated by the measured abundance of the $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue (χ_{626}) and reported in $\mu\text{mol mol}^{-1}$. Throughout this chapter, $\delta^{17}\text{O}_{\text{meas}}$ and $\delta^{18}\text{O}_{\text{meas}}$ values indicate uncorrected data as measured by the TILDAS, and $\delta^{17}\text{O}_{\text{corr}}$ and $\delta^{18}\text{O}_{\text{corr}}$ indicate data that has been corrected using eq. (2.6). Similarly, $\Delta^{17}\text{O}_{\text{meas}}$ values are calculated from $\delta^{17}\text{O}_{\text{meas}}$ and $\delta^{18}\text{O}_{\text{meas}}$ values, according to eq. (2.2), whereas $\Delta^{17}\text{O}_{\text{corr}}$ are calculated from $\delta^{17}\text{O}_{\text{corr}}$ and $\delta^{18}\text{O}_{\text{corr}}$ values.

Similar to the Chapter 2: Methods of this thesis, this chapter is divided into “Upstream” and “Downstream” sections, each with subsections correlating to the 6 “Experiments” previously described. As such, this chapter presents the results of the “Experiments” introduced in the Methods chapter and discusses their contributions to the successful optimization of the greater analytical system introduced in this project. The experiments in the “Upstream” section are largely discussed in the context of sample dilution accuracy and mixing extent, followed by a short discussion regarding uncorrected δ -values of standards as grouped by sample mixing strategy. The “Downstream” section is comparably less coherent in nature as several distinct aspects of the TILDAS instrument and analysis are optimized. However, this is not to say that they are disconnected. This section follows the pattern of optimizing measurement conditions, followed by identification of a precision-limiting electronics issue, and lastly discussion of the data correction scheme and presentation of international and internal carbonate standard data measured in this project to the VSMOW2-SLAP2 scale. Finally, this chapter concludes with a comparison between $\Delta^{17}\text{O}$ measurements made by IRMS and those made in this project.

3.1) Experimental

Upstream

Experiment A – Mixing via Passive Diffusion

Using diffusion as the driving force behind sample mixing comes with many limitations. Diffusion of CO_2 in N_2 is an exceptionally slow process. It is also difficult to model and predict,

[‡] Aspects of this research have been previously published in Hare et al. (2022). This thesis goes into more detail regarding the development of the sampling system and experimental identification of TILDAS operating conditions and limitations, conducted by the candidate. Said publication is cited several times throughout this chapter.

even more so in a non-steady state system such as the AutoCart, making the only real way to assess its efficacy simply by doing it and recording the results.

Results

Sample mixing by passive diffusion was performed on 2 samples of the internal laboratory standard Cavendish Marble (CM) in early February 2022 with allotted mixing times of 4.5 hours and 10 minutes, respectively. Other than different diffusion times, the samples were prepared identically on the AutoCart. Diffusion for both samples was limited to the flask volume to make full use of its intentional design. Resulting concentrations by measured aliquot for each of these samples are shown in Table 3.1.

	CM-1	CM-2
Aliquot	χ_{626}	χ_{626}
1	350.2	349.3
2	376.0	361.2
3	396.9	365.6
4	418.8	486.4
5	419.6	639.6
6	417.5	556.2
7	417.6	517.4
8	417.9	497.4
9	418.1	479.5
10	418.1	462.8
11	419.4	449.5
12		437.1
13		426.0
Average	406.4	463.7
1σ	23.1	81.6

Table 3.4: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) for samples mixed by passive diffusion only. CM is the internal laboratory standard Cavendish Marble.

CM-1 was prepared and diluted to sample pressure 582.4 mbar (99.53% accurate to the target pressure). The gas was allowed to passively diffuse in the flask for 4.5 hours before being introduced to the TILDAS. The sample pressure provided enough gas for 11 discrete aliquots to be expanded into and measured in the TILDAS optical cell. CM-1 sample concentration was $406.4 \pm 23.08 \mu\text{mol mol}^{-1}$ (1σ , $n = 11$). CM-2 was prepared and diluted to sample pressure 615.0 mbar (99.24% accurate) and allowed to diffuse in the flask for 10 minutes. The sample provided 13 aliquots of measured gas. CM-2 sample concentration was $463.7 \pm 81.56 \mu\text{mol mol}^{-1}$ (1σ , $n = 13$).

Discussion

CM-1 was allowed to diffuse within MV1 for 4.5 hours before expanding to the rest of the AutoCart's mixing volume. Despite this, the 11 measured aliquots of the sample had a χ_{626} range of 69.45 $\mu\text{mol mol}^{-1}$. This large range, $\sim 17\%$ of the overall average measured concentration, is indicative of diffusion being far from complete even on this timescale. Due to mass balance, it is expected that the remaining, unsampled gas will have an overall concentration less than 400 $\mu\text{mol mol}^{-1}$.

CM-2 was an experiment to observe near-immediate sample mixing extent after preparation on the AutoCart was completed. Given only 10 minutes to diffuse in MV1, the sample was then expanded throughout the full mixing volume of the AutoCart and TILDAS analysis began. Enough sample gas was prepared to measure 13 aliquots in the TILDAS optical cell. As can be seen in Figure 3.1, a slight upwards trend exists among the first 3 aliquots of measured sample. Sample CO_2 concentration then increases $\sim 120 \mu\text{mol mol}^{-1}$ between aliquots 3 and 4, and a further $\sim 150 \mu\text{mol mol}^{-1}$ from aliquot 4 to 5, before taking on a downward trending curve approaching the targeted 400 $\mu\text{mol mol}^{-1}$. Mass balance dictates that, because the volume of sample gas measured was overconcentrated in CO_2 , the sample remaining in the AutoCart mixing volume must be under concentrated.

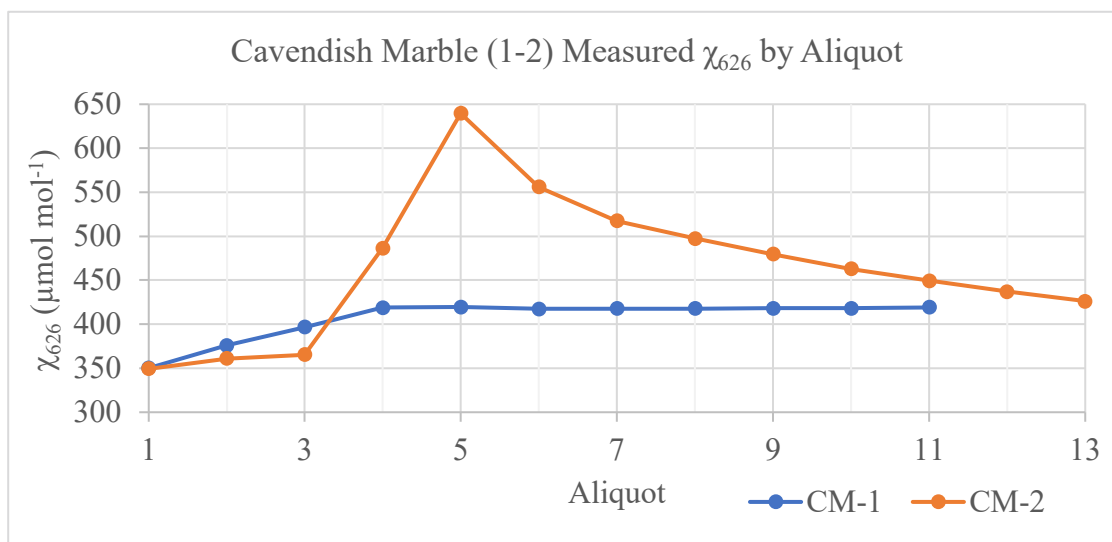


Figure 3.3: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) by aliquot for samples mixed by passive diffusion only. CM-1 and CM-2 were allowed to diffuse in the flask for 4.5 hours and 10 minutes, respectively.

Despite being only two data points, these samples offer significant insight to the limitations of mixing sample gas using only passive diffusion. First, it shows just how slow the diffusion process is. Even after 4.5 hours, CM-1 is still far from being well-mixed. The upward trend in concentration over the first several aliquots of both samples is thought to be connected to both the order of events in preparing the sample on the AutoCart and the geometry of the sampling flask (MV1). During sample preparation, the CO_2 is first expanded into MV1 before N_2 diluent is introduced. The positioning of the siphon suggests that dosed-in N_2 accumulates around the exit, creating a sort of N_2 “blanket” in the volume of the flask directly surrounding it. Sample CO_2 must diffuse evenly through this purely N_2 space to achieve complete mixing. Similarly, the

presence of the siphon causes a “plug” of N₂ being trapped within its volume which CO₂ must also diffuse up if perfectly even diffusion is to be achieved. Prior to TILDAS sampling, the bulk gas sample is expanded from MV1 to the rest of the mixing volume (i.e., evenly expanded between MV1,2,3). However, this expansion is nonturbulent and provides only ~17% more volume for the sample to mix in, and thus does not contribute significantly to sample mixing. During sample expansion from the AutoCart’s mixing volume into the TILDAS optical cell, the volume first sampled is that which first expanded out of MV1: the siphon and its surrounding egress volume. Effectively, the first several aliquots of sample gas are sourced from this “edge” of the mixing volume and give rise to the upward trend in concentration observed.

Experiment B – Mixing via Turbulent Advection

Diffusion as the mixing mechanism is clearly shown above to be ineffective on timescales relevant to laboratory practices and sample throughput. Consequently, a new mixing strategy was adopted in an attempt to increase sample mixing via repeated expansions of N₂ diluent into MV1. This technique consisted of pressurizing MV2,3 of the AutoCart to varying pressures of N₂ and rapidly expanding the gas into MV1 for sample mixing. Colloquially “sloshing”, this technique made better use of the flask’s design promoting turbulent advection and showed promise as a viable mixing method.

Results

The repeated expansion technique was initially trialed on several samples of Cavendish Marble (CM). These samples were all prepared by applying 5 expansions (“sloshes”) of N₂ at 1100 mbar, after which the remaining amount of N₂ required for accurate dilution was non-turbulently added. Mixing of these samples was concluded with a short diffusive mixing step, the duration of which varied between each of the samples. CM-3 was allowed 10 minutes of diffusion in the flask after initial mixing, CM-4 was given 40 minutes in the full MV1 volume (flask plus V16-V17 space) plus an additional 30 minutes in the entire mixing volume of the AutoCart, and CM-5 was 40 minutes of diffusion in the flask only. CM-3 measured CO₂ concentration was $466.58 \pm 64.97 \mu\text{mol mol}^{-1}$ (1σ , $n = 10$), CM-4 sample concentration was $461.17 \pm 98.12 \mu\text{mol mol}^{-1}$ (1σ , $n = 13$), and CM-5 sample concentration was $417.39 \pm 3.92 \mu\text{mol mol}^{-1}$ (1σ , $n = 12$), where n is the number of aliquots measured by the TILDAS. Overall average concentration repeatability for these three samples was $448.38 \pm 26.98 \mu\text{mol mol}^{-1}$ (1σ , $n = 3$).

Figure 3.2 shows the comparison between the demonstrated curve characterizing 1100 mbar expansions and observed resulting MV1,2,3 pressures during preparation of the sample CM-4. CM-4 was described by a slightly different curve than what was expected from the empirical function. Crucially, however, resulting MV1,2,3 pressures for the first 5 expansions are in near perfect agreement with the automated tests, as shown in the accompanying Table 3.2. Discrepancy between the projected curve and what is observed for CM-4 is due to a combination of imperfect prediction of MV1,2,3 pressures as pressure approaches 1100 mbar and inaccuracy in the WIKA S-20 pressure gauge. Imperfection as MV1,2,3 pressure approaches the expansion pressure is acceptable as samples are typically prepared to pressures in range of ~700-800 mbar, thus eliminating concerns of dilution accuracy at higher pressures. For context, CM-4 had a target dilution pressure of 736.4 mbar.

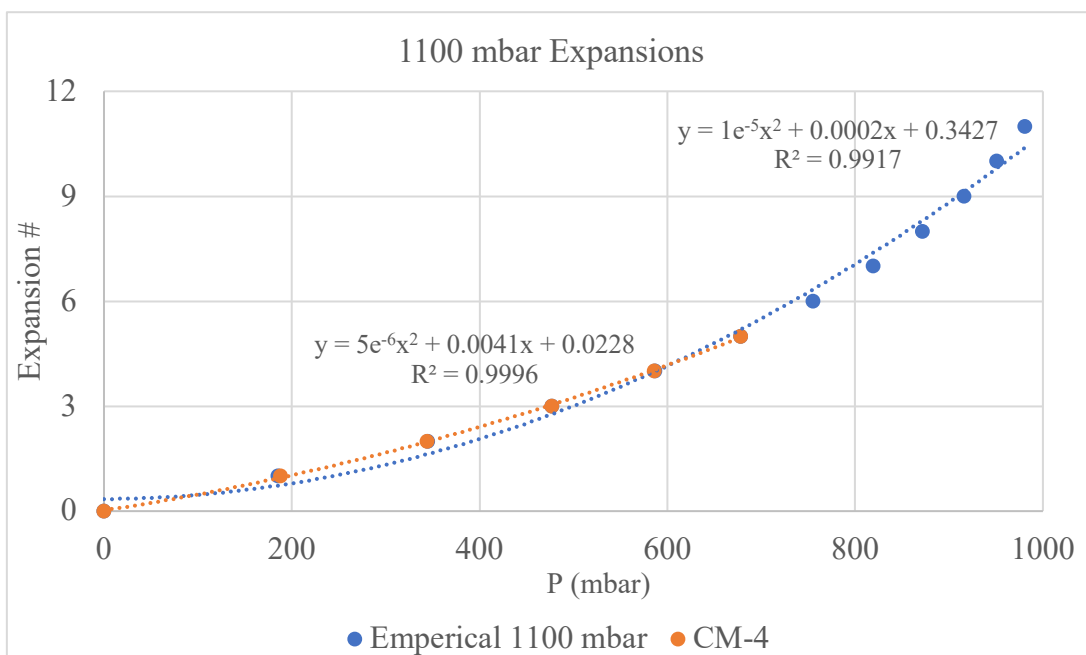


Figure 3.2: Comparison of resulting full Mixing Volume pressure between automated blank test of repeated 1100 mbar MV2,3 expansions and that observed during preparation of sample CM-4. Repeated expansions were performed filling MV2,3 to 1100 mbar before expansion into the full Mixing Volume (MV1,2,3). Close overlapping of data points for expansions 0-5 suggest high repeatability of repeated MV2,3 expansions.

	Automated test	CM-4
<i>n</i>	P (mbar)	P (mbar)
0	0	0
1	185	188
2	345	344
3	477	477
4	587	586
5	678	678
6	755	
7	819	
8	872	
9	916	
10	951	
11	981	

Table 3.5: Comparison of resulting full Mixing Volume pressure between automated blank test of repeated 1100 mbar MV2,3 expansions and that observed during preparation of sample CM-4. Repeated expansions were performed filling MV2,3 to 1100 mbar before expansion into the full Mixing Volume (MV1,2,3).

This mixing technique was then extended to the international carbonate standards NBS18 and NBS19. In total, 3 samples of NBS18 and 4 of NBS19 were prepared using the repeated

expansion mixing method and with varying times for diffusion afterwards. Of these, the first sample of each (NBS18-1 and NBS19-1) were mixed by 5 N₂ expansions to be consistent with the previous CM samples. NBS18-1 was combined with 40 minutes of diffusion and resulted in sample CO₂ concentration of $414.7 \pm 4.5 \text{ } \mu\text{mol mol}^{-1}$ (1σ , $n = 11$). NBS19-1 was allowed to diffuse in MV1 overnight and resulting CO₂ concentration was $424.4 \pm 0.1 \text{ } \mu\text{mol mol}^{-1}$ (1σ , $n = 16$).

The technique was then updated to determine the number of N₂ expansions best suited for mixing based on the recorded sample yield. This decreased the amount of N₂ added non-turbulently after expansions were completed and was expected to promote better mixing. This strategy was applied to 1 NBS18 (NBS18-2) and 3 NBS19 samples (NBS19-2, NBS19-3, NBS19-4) with varying allotted diffusion times following the initial advective mixing. NBS18-2 measured CO₂ concentration was $406.4 \pm 0.1 \text{ } \mu\text{mol mol}^{-1}$ (1σ , $n = 18$). NBS19-2 sample concentration was $397.9 \pm 3.9 \text{ } \mu\text{mol mol}^{-1}$ (1σ , $n = 21$), NBS19-3 sample concentration was $405.3 \pm 3.2 \text{ } \mu\text{mol mol}^{-1}$ (1σ , $n = 19$), and NBS19-4 sample concentration was $419.9 \pm 2.3 \text{ } \mu\text{mol mol}^{-1}$ (1σ , $n = 16$). Results of all NBS18 and NBS19 samples thus far prepared by repeated N₂ expansions are summarized in Table 3.3. Overall sample concentration repeatability for these 6 samples was $411.4 \pm 10.0 \text{ } \mu\text{mol mol}^{-1}$ (1σ , $n = 6$).

Sample	n	Expansion P (mbar)	# Expansions	t Diffusion	χ_{626} (1σ)
NBS18-1	11	1100	5	40 min	414.7 (4.5)
NBS19-1	16	1100	5	~15 hours	424.4 (0.1)
NBS18-2	18	1100	7	~14 hours	406.4 (0.1)
NBS19-2	21	1100	7	40 min	397.9 (3.9)
NBS19-3	19	1100	8	40 min	405.3 (3.2)
NBS19-4	16	1100	8	40 min	419.9 (2.3)

Table 3.6: ¹²C¹⁶O¹⁶O isotopologue abundance (χ_{626}) by aliquot for 2 NBS18 and 4 NBS19 samples mixed by repeated 1100 mbar MV2,3 expansions into MV1. n is the number of aliquots measured by TILDAS for each sample. After expansions, samples were allowed to diffuse in the flask for either 40 minutes or overnight. χ_{626} is $\mu\text{mol mol}^{-1}$ with 1σ in parenthesis.

The common NBS19-1 and NBS18-2 1σ aliquot concentration repeatability of $0.1 \text{ } \mu\text{mol mol}^{-1}$ given overnight diffusive mixing was encouraging for moving forward with more effortful samples. The first atmospheric sample, UCT-CPT-1, was collected on 14 February 2022 from Cape Point, Cape Peninsula, Western Cape, South Africa. This sample was initially mixed by 10 repeated 1100 mbar N₂ expansions into MV1 and allowed to mix by diffusion overnight. UCT-CPT-1 sample concentration was $374.2 \pm 0.5 \text{ } \mu\text{mol mol}^{-1}$ (1σ , $n = 16$). The sample was curiously over-diluted (target $400 \text{ } \mu\text{mol mol}^{-1}$), likely due to the contribution of residual atmospheric N₂ on measured sample yield which had condensed in the IN₂ trap during the CO₂ cryo-trapping step, thus impacting measured sample yield and offsetting the calculated dilution specifications. Figure 3.3 shows UCT-CPT-1 χ_{626} aliquot repeatability.

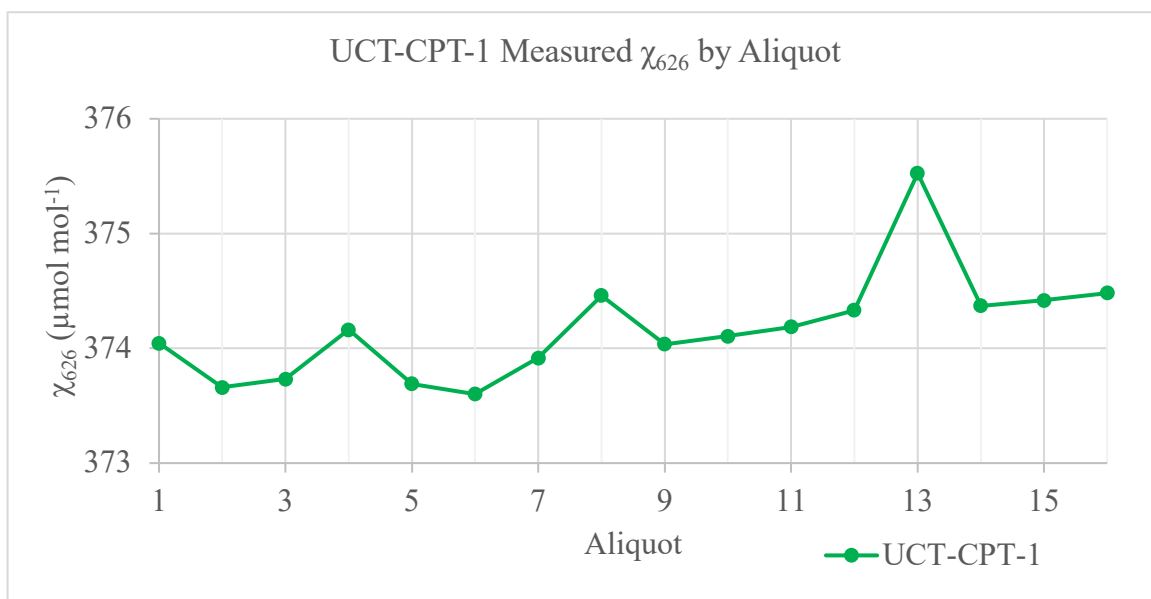


Figure 3.3: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) by aliquot for the atmospheric sample UCT-CPT-1. Sample was diluted by 10 1100 mbar MV2,3 expansions into MV1, then was allowed to diffuse in the flask overnight (~14 hours).

NBS18-5 followed up these samples by using N_2 expansions with a higher initial pressure than had been used thus far. For this sample, MV2,3 was pressurized to 1400 mbar, rather than 1100 mbar, to increase the pressure gradient between mixing volumes. Prepared by 5 1400 mbar N_2 expansions with 40 minutes of diffusion time added afterwards, NBS18-5 sample concentration was $419.9 \pm 1.3 \mu\text{mol mol}^{-1}$ (1σ , $n = 17$).

The improvement in sample mixing observed in NBS18-5 promoted further updates to the dilution technique to minimize the amount of N_2 diluent added after N_2 expansions were complete. This update introduced the use of “fractional sashes” which allowed the pressure of the final expansion to be unique to every sample, such that the amount of N_2 introduced via advection completely fulfills the amount of N_2 required for complete sample dilution to $400 \mu\text{mol mol}^{-1}$.

In essence, N_2 was no longer added non-turbulently. This new technique was applied to 4 CM samples (CM-7, CM-8, CM-9, and CM-10) with varying expansion pressures. As a result of the updated expansion strategy the fraction of the final “sash” is simply the fraction of the final expansion pressure required to achieve sample dilution to $400 \mu\text{mol mol}^{-1}$ as determined by the sample yield (see Methods: Experiment B for detailed description and construction). Sample preparation parameters and results are reported in Table 3.4 and shown in accompanying Figure 3.4. CM-7 measured CO_2 concentration was $421.0 \pm 1.1 \mu\text{mol mol}^{-1}$ (1σ , $n = 17$), CM-8 sample concentration was $417.5 \pm 8.6 \mu\text{mol mol}^{-1}$ (1σ , $n = 13$), CM-9 sample concentration was $422.1 \pm 7.9 \mu\text{mol mol}^{-1}$ (1σ , $n = 14$), and CM-10 sample concentration was $429.9 \pm 0.8 \mu\text{mol mol}^{-1}$ (1σ , $n = 17$). Overall sample concentration repeatability of these samples was $422.6 \pm 5.2 \mu\text{mol mol}^{-1}$ (1σ , $n = 4$).

Sample	<i>n</i>	Expansion P (mbar)	# Expansions	t Diffusion	χ_{626} (1 σ)
CM-7	17	1400	4.868	40 min	421.0 (1.1)
CM-8	13	1500	4.643	10 min	417.5 (8.6)
CM-9	14	1450	4.951	10 min	422.1 (7.9)
CM-10	17	1450	4.870	40 min	429.9 (0.8)

Table 3.7: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) for 4 Cavendish Marble (CM) samples mixed by the “fractional sashes” method. *n* is the number aliquots measured by TILDAS for each sample. Expansion pressure varied slightly between samples from 1400 to 1500 mbar. Samples were allowed to diffuse in the flask for either 10 or 40 minutes. χ_{626} is $\mu\text{mol mol}^{-1}$ with 1 σ in parenthesis.

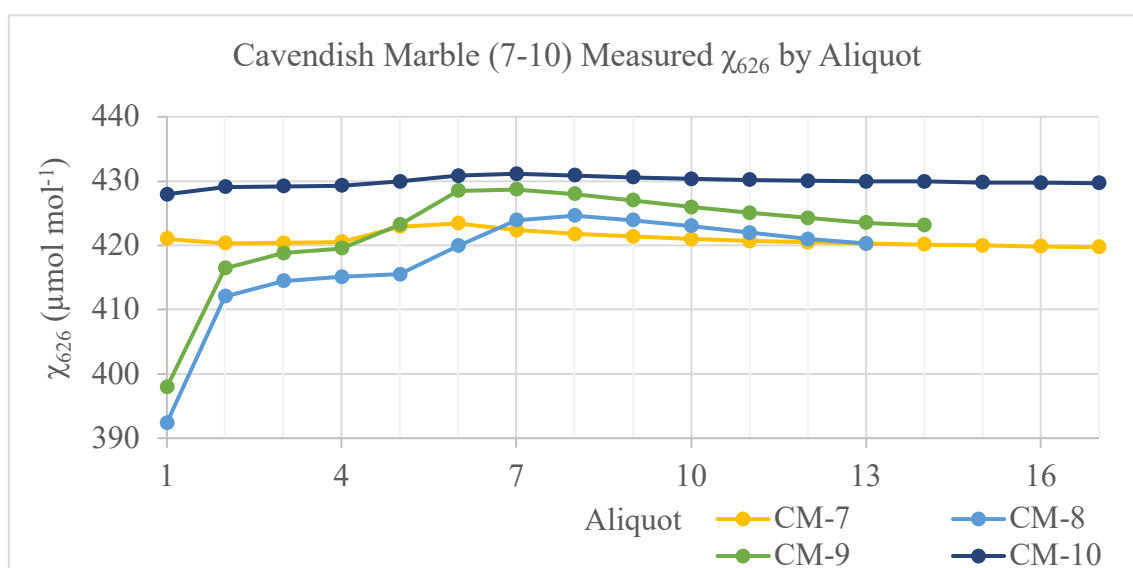


Figure 3.4: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) by aliquot for 4 Cavendish Marble (CM) samples mixed by the “fractional sashes” method. CM-7 and CM-10 were allowed to diffuse in MV1 for 40 minutes following mixing by expansions. CM-8 and CM-9 were allowed to diffuse in the flask for 10 minutes.

Discussion

Early attempts at mixing via repeated turbulent advection proved to still rely heavily on diffusion of the sample gas to achieve adequate mixing. Encouragingly, diluted sample CO_2 concentrations were observed to be impressively accurate to the targeted $400 \mu\text{mol mol}^{-1}$. Comparing CM-2 and CM-3 reveals little functional difference between the absence or presence of a few relatively low pressure N_2 expansions when identical times for diffusion are allotted. The concentration-by-aliquot profile of CM-4 stands out in that it constantly decreases throughout the duration of the analysis (Fig. 3.5). This is in contrast to most other samples in which diffusion played a significant role during the mixing process, where the first few aliquots are typically low concentration, followed by a sharp, sudden increase before tailing off for the remainder of the analysis. This is likely explained by the staggered diffusion time and volumes allowed for this sample. CM-4 was first given 40 minutes to diffuse in the entire MV1 volume, including the V16-V17 space above the flask, after which the sample was expanded into the full MV1,2,3 volume where it was allowed to diffuse for 30 minutes more. The inclusion of the V16-V17

volume in the initial mixing step may have trapped a plug of CO₂ which would not readily diffuse as constrained by the small diameter tubing making up that volume. Upon expansion into MV1,2,3, this plug would have been the first to rush to the new volume given its proximity to V17, thus creating an over-concentrated parcel of sample gas downstream, nearer the connection to the TILDAS. Analysis of this sample would have then pulled from this highly concentrated volume first, producing the concentration-by-aliquot profile observed.

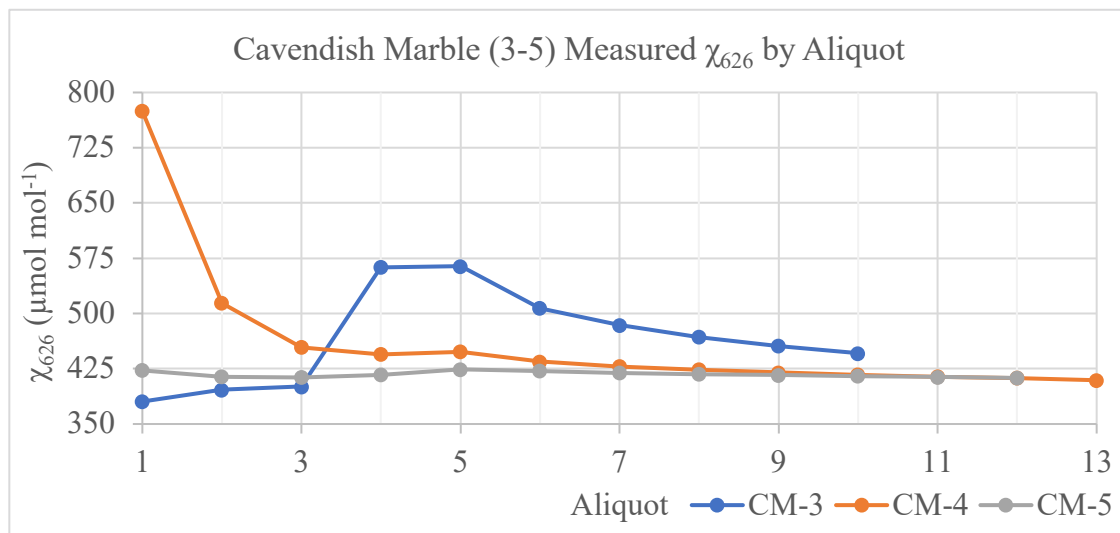


Figure 3.5: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) by aliquot for 3 Cavendish Marble (CM) samples mixed by 5 1100 mbar MV2,3 expansions into MV1. CM-3 was allowed to diffuse in the flask for 10 minutes, CM-4 for 40 minutes in the full MV1 volume (flask plus V16-V17 space) plus an additional 30 minutes in the full MV1,2,3 volume, and CM-5 40 minutes in the flask only.

With the exception of the samples allowed to diffuse overnight (NBS18-2, NBS19-1, UCT-CPT-1), TILDAS χ_{626} measurements of discrete aliquots of the samples presented here in Experiment B revealed improved, but yet insufficient, sample mixing. Consequently, the repeated expansion dilution and mixing technique was altered to simply allow for more expansions to occur, thereby reducing the amount of N₂ non-turbulently added. NBS19-2, NBS19-3, and NBS19-4 are all directly comparable to NBS18-1 where the only difference in sample preparation was the number of turbulent expansions used for dilution and initial mixing. NBS18-1 1 σ concentration of 4.5 $\mu\text{mol mol}^{-1}$ is similar to that of NBS19-2 where 1 σ concentration was 3.9 $\mu\text{mol mol}^{-1}$, suggesting little difference in mixing extent between samples prepared using either 5 or 7 N₂ expansions. The addition of an 8th expansion, however, directly improved 1 σ concentrations observed in both NBS19-3 and NBS19-4 which were 3.2 $\mu\text{mol mol}^{-1}$ and 2.3 $\mu\text{mol mol}^{-1}$, respectively. This improvement in mixing is presumably due to decreasing the amount of N₂ non-turbulently added after the sample-specific determined number of expansions were completed.

The persistent requirement of overnight diffusion times to achieve the desired extent of mixing offered little by means of sample throughput. The adjustment to expansions of N₂ at a greater pressures was key in improving sample mixing. 1 σ sample concentration improved by a factor of ~3.5 (4.5 $\mu\text{mol mol}^{-1}$ to 1.3 $\mu\text{mol mol}^{-1}$) between NBS18-1 and NBS18-5, the only preparation difference being the increase of repeated expansion pressure.

Developing and implementing the “fractional sashes” method was critical to mixing sample gas to the fullest extent the AutoCart would allow in its contemporaneous construction. Using this dilution and mixing technique, 1σ sample concentrations of $< 1 \mu\text{mol mol}^{-1}$ were achieved by allowing only a 40 minute diffusion afterwards. This technique was much more practical for consistent analysis and increased sample throughput. However, the objective of equating sample preparation time and TILDAS analysis time (~ 50 minutes) was still out of reach given a required wait time of 40 minutes every sample.

Experiment C – Mixing via Circulation

Experiment B revealed that diluting and mixing the sample gas by turbulent advection was not sufficient for achieving the extent of mixing required to eliminate concentration inconsistencies, and therefore correlated isotopic fractionation, caused by diffusion. In an effort to force further mixing a circulation loop was added to the AutoCart. The circulation loop allowed samples to be thoroughly mixed by forcing gas through the entire mixing volume of the system repeatedly.

Results

Mixing via circulation was first trialed on the internal laboratory standard Cavendish Marble (CM). Sample preparation parameters and mixing results are presented in Table 3.5. Resulting overall concentration repeatability of this suite of runs was $395.5 \pm 15.4 \mu\text{mol mol}^{-1}$ (1σ , $n = 5$). Heavily skewed by the first test of this new sequence (CM-13, $368.3 \pm 2.3 \mu\text{mol mol}^{-1}$), overall sample concentration repeatability of the 4 latter samples improves to $402.3 \pm 3.3 \mu\text{mol mol}^{-1}$ (1σ , $n = 4$). Within sample (aliquot) concentration repeatability for all of the samples ranged from 0.4 to $2.3 \mu\text{mol mol}^{-1}$.

Sample	<i>n</i>	Expansion P (mbar)	# Expansions	t Circulation	χ_{626} (1σ)
CM-13	18	1450	4.856	1 min	368.3 (2.3)
CM-14	18	1450	4.726	2 min	405.0 (1.1)
CM-15	17	1450	4.918	3 min	398.0 (0.4)
CM-16	16	1450	4	3 min	404.6 (0.6)
CM-17	18	1450	5	2 min	401.5 (0.8)

Table 3.8: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) for 5 Cavendish Marble (CM) samples mixed by varying circulation times. n is the number aliquots measured by TILDAS for each sample. All samples diluted and initially mixed by 1450 mbar MV2,3 expansions into MV1. χ_{626} is $\mu\text{mol mol}^{-1}$ with 1σ in parenthesis.

Following the tests on CM material, the international carbonate standards NBS18, NBS19, and IAEA-603 were analyzed to better assess the performance of the circulation loop and the degree of mixing. In total, 9 NBS18, 10 NBS19, and 11 IAEA-603 samples were prepared and measured. Samples of NBS18 had overall sample concentration repeatability of $400.9 \pm 11.8 \mu\text{mol mol}^{-1}$ (1σ , $n = 9$) and aliquot concentration repeatability ranged from 0.6 to $2.0 \mu\text{mol mol}^{-1}$ (1σ). Samples of NBS19 had overall sample concentration repeatability of $401.9 \pm 8.9 \mu\text{mol mol}^{-1}$ (1σ , $n = 10$) and aliquot concentration repeatability ranged from 0.6 to $1.2 \mu\text{mol mol}^{-1}$.

(1 σ). IAEA-603 samples had overall sample concentration repeatability of $401.3 \pm 11.0 \mu\text{mol mol}^{-1}$ (1 σ , $n = 11$) and aliquot concentration repeatability ranged from 0.5 to $1.2 \mu\text{mol mol}^{-1}$ (1 σ). Cumulatively, all international carbonate standards reported here had an overall concentration repeatability of $401.4 \pm 10.3 \mu\text{mol mol}^{-1}$ (1 σ , $n = 30$). Combined preparation parameters and results for all international carbonate samples are presented in Table 3.6.

Sample	<i>n</i>	Expansion P (mbar)	# Expansions	t Circulation	χ_{626} (1 σ)
NBS18-7	19	1450	5	2.5 min	402.0 (1.1)
NBS18-8	18	1450	4.998	2.5 min	398.1 (1.3)
NBS18-9	17	1450	5	2.5 min	376.4 (0.8)
NBS18-10	18	1450	5	2.5 min	393.5 (1.6)
NBS18-11	18	1450	4.801	2.5 min	401.1 (1.0)
NBS18-12	18	1450	4.586	2.5 min	409.7 (0.6)
NBS18-13	18	1450	4.879	2.5 min	405.3 (0.9)
NBS18-14	18	1450	5	2.5 min	402.1 (1.3)
NBS18-15	16	1450	4.742	2.5 min	419.9 (2.0)
				Average	400.9
				1σ	11.8
NBS19-5	19	1450	5	2 min	401.5 (0.8)
NBS19-6	21	1450	5.672	2 min	409.3 (1.2)
NBS19-7	18	1450	4.649	2 min	397.9 (1.1)
NBS19-8	18	1450	4.604	2.5 min	405.7 (0.6)
NBS19-9	16	1450	4	2.5 min	398.3 (0.8)
NBS19-10	18	1450	4.973	2.5 min	391.6 (1.0)
NBS19-11	17	1450	4	2.5 min	397.5 (1.0)
NBS19-12	18	1450	5	2.5 min	388.7 (1.1)
NBS19-13	16	1450	4	2.5 min	411.7 (1.1)
NBS19-14	18	1450	4.757	2.5 min	416.8 (1.0)
				Average	401.9
				1σ	8.9
IAEA-603-1	18	1450	5	2.5 min	407.3 (1.1)
IAEA-603-2	18	1450	4.907	2.5 min	389.1 (0.7)
IAEA-603-3	14	1450	3.855	2.5 min	379.7 (0.8)
IAEA-603-4	18	1450	4.978	2.5 min	393.5 (0.8)
IAEA-603-5	18	1450	4.684	2.5 min	404.9 (0.9)
IAEA-603-6	17	1450	4	2.5 min	412.8 (0.7)
IAEA-603-7	19	1450	5	2.5 min	394.0 (1.2)
IAEA-603-8	12	1450	4.860	2.5 min	402.1 (0.5)

IAEA-603-9	19	1450	5	2.5 min	405.5 (1.1)
IAEA-603-10	18	1450	4.769	2.5 min	415.7 (0.7)
IAEA-603-11	18	1450	4.617	2.5 min	410.1 (0.9)
				Average	401.3
				1σ	11.0
Overall				Average	401.4 (1.0)
				1σ	10.3

Table 3.9: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) for the international standards NBS18, NBS19, and IAEA-603. Most samples were mixed by 2.5 minutes of circulation, with the exception of NBS19-5, NBS19-6, and NBS19-7 which were mixed with 2 minutes of circulation. n is the number aliquots measured by TILDAS for each sample. All samples diluted and initially mixed by 1450 mbar expansions. χ_{626} is $\mu\text{mol mol}^{-1}$ with 1σ in parenthesis. The average concentration repeatability (1σ) of all 30 standards was $1.0 \mu\text{mol mol}^{-1}$.

In addition, two more internal standards, UCT-WC-1 and UCT-Et-1, both modern ostrich eggshell (OES), were also analyzed. The results of all of OES internal standard runs are reported in Table 3.7. Overall sample repeatability of all OES standards was $400.5 \pm 8.7 \mu\text{mol mol}^{-1}$ (1σ , $n = 17$). Within sample (aliquot) concentration repeatability ranged from 0.7 to $6.1 \mu\text{mol mol}^{-1}$ (1σ). When the last two UCT-WC-1 samples are excluded, average aliquot concentration repeatability (1σ) of the remaining 15 OES samples was improved to $1.0 \mu\text{mol mol}^{-1}$, equal to that of the international standards above, with a range of 0.7 to $1.3 \mu\text{mol mol}^{-1}$ (1σ).

Sample	n	Expansion P (mbar)	# Expansions	t Circulation	χ_{626} (1σ)
UCT-Et-1-1	18	1450	4.926	2.5 min	377.9 (0.7)
UCT-Et-1-2	18	1450	5	2.5 min	404.6 (1.2)
UCT-Et-1-3	18	1450	4.793	2.5 min	406.6 (1.3)
UCT-Et-1-4	18	1450	4.849	2.5 min	398.1 (0.8)
UCT-Et-1-5	22	1450	5	2.5 min	388.3 (1.3)
UCT-Et-1-6	19	1450	4.898	2.5 min	402.7 (0.8)
UCT-Et-1-7	18	1450	4.827	2.5 min	406.7 (1.2)
UCT-Et-1-8	17	1450	4.508	2.5 min	406.0 (1.2)
				Average	398.9
				1σ	10.5
UCT-WC-1-1	18	1450	4.938	2.5 min	388.3 (1.2)
UCT-WC-1-2	18	1450	4.853	2.5 min	403.9 (0.9)
UCT-WC-1-3	18	1450	4.994	2.5 min	396.5 (0.9)
UCT-WC-1-4	18	1450	4.967	2.5 min	402.3 (1.2)
UCT-WC-1-5	18	1450	5	2.5 min	396.8 (1.1)
UCT-WC-1-6	19	1450	5	2.5 min	411.7 (0.6)
UCT-WC-1-7	18	1450	5	2.5 min	405.0 (1.3)

UCT-WC-1-8	18	1450	5	2.5 min	404.0 (6.1)
UCT-WC-1-9	17	1450	5	2.5 min	408.7 (2.7)
				Average	401.9
				1σ	7.1
Overall				Average	400.5 (1.4)
				1σ	8.7

Table 3.10: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) for the internal OES standards UCT-Et-1 and UCT-WC-1. All samples were mixed by 2.5 minutes of circulation. n is the number aliquots measured by TILDAS for each sample. All samples diluted and initially mixed by 1450 mbar expansions. χ_{626} is $\mu\text{mol mol}^{-1}$ with 1σ in parenthesis. The average concentration repeatability (1σ) of all 17 samples was $1.4 \mu\text{mol mol}^{-1}$. Excluding UCT-WC-1-8 and UCT-WC-1-9, average concentration repeatability (1σ) of the remaining 15 samples was $1.0 \mu\text{mol mol}^{-1}$.

Discussion

Sample mixing extent was determined by TILDAS measurement of χ_{626} aliquot repeatability. It was quickly realized that circulating for 1 minute was not sufficient for achieving the required mixing extent for TILDAS $\Delta^{17}\text{O}$ analysis. Extension of circulation time to 2 and 3 minutes revealed diminishing mixing extent as the sample gas became more completely mixed. Ultimately, a circulation time of 2.5 minutes was decided upon as a compromise between sufficiently mixing the sample gas and limiting the amount of time for diaphragm pump leaking to contaminate the sample. Any atmosphere leaks into the system during this process would be easily observed in the measured δ -values of the standards/samples. Figure 3.6 shows the 1σ aliquot repeatability of concentration, χ_{626} , for a range of circulation times of CM, NBS18, NBS19, and IAEA-603 samples. All samples were prepared similarly with the fractional expansion dilution technique described above. The large spread in 1σ concentration precision at 2.5 minutes suggests that, by this time, sample mixing extent is not the limiting factor in achieving highly precise measured concentrations. This leads to the possibility that, even at complete mixing, instrument effects within the TILDAS (e.g., related to thermal instability, optical cell pressure variation, etc.) contribute to varied measured concentration precisions.

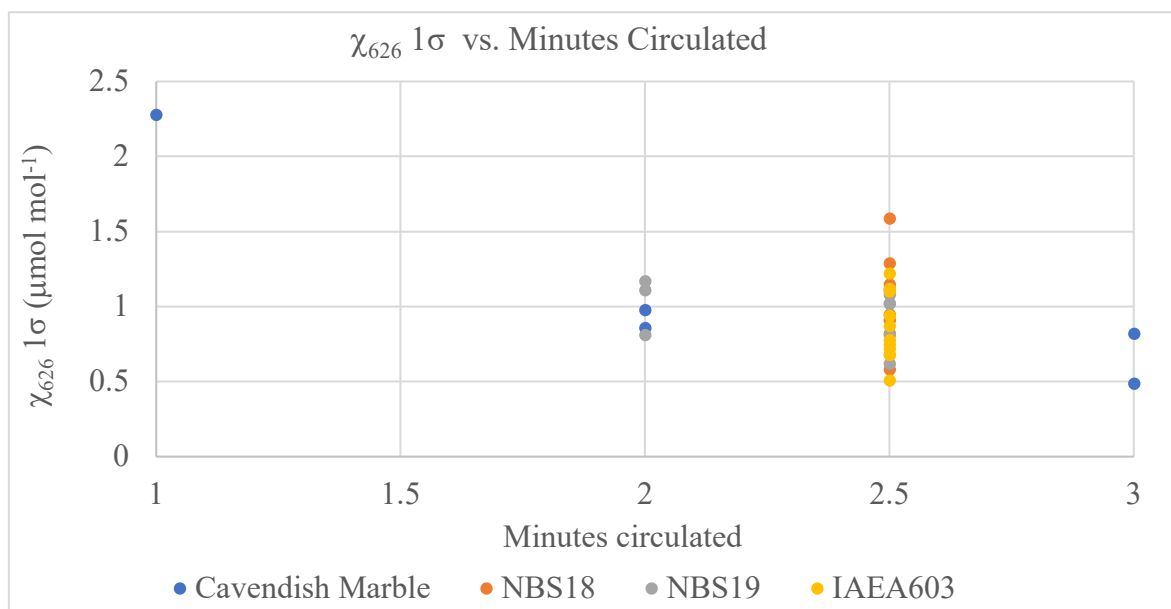


Figure 3.6: χ_{626} aliquot repeatability (1σ) with increasing circulation time. Efficacy of circulative mixing decreases after 2 minutes. Data is divided by standard materials: blue is Cavendish Marble, orange in NBS18, grey is NBS19, yellow is IAEA-603.

The mixing time necessary to achieve aliquot concentration repeatability $< 1 \mu\text{mol mol}^{-1}$ was reduced from 40 minutes by turbulent advection only to a few minutes when circulation was appended to the preparation process. This addition drastically improved sample throughput capabilities, as well as increased confidence in the extent of mixing in each sample. As the final evolution in forced bulk sample mixing, the implementation of the circulation loop allowed, for the first time, highly precise measurements of isotopologue abundances. As samples are well mixed, no assumptions must be made to account for the proportion of sample not analyzed by the TILDAS, a crucial aspect of the method presented here.

Focusing on the last two UCT-WC-1 samples, poor aliquot concentration repeatability is directly linked to inconsistent optical cell pressures during each of the analyses. The cause of this was determined to be the failing of the pneumatic valve separating the optical cell from the rest of the TILDAS sampling valve apparatus (V15). This valve failure caused poor evacuation of the optical cell between aliquots and, subsequently, irregular sample expansion into the optical cell due to the residual pressure within. Optical cell pressures over the course of these two analyses ranged from ~ 30 Torr to ~ 47 Torr, causing significant variation in the absolute amount of CO_2 in the cell between aliquots and, therefore, signal absorbance indicative of individual isotopologue concentrations.

Impact of Mixing via Circulation on Measured δ -values

Mixing sample gas has been shown above to drastically improve the extent of sample mixing in terms of aliquot-to-aliquot concentration repeatability. Mixing extent also impacts measured δ -values by minimizing mass-dependent fractionation caused by diffusion. Table 3.8 presents measured $\delta^{17}\text{O}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ values for samples of Cavendish Marble (CM), NBS19, and NBS18 prepared with and without mixing via circulation. Samples included in the “Mixed without circulation” subset were not all prepared equally but by a combination of the mixing

techniques presented in Experiments A and B above. Although no IAEA-603 samples were prepared prior to the incorporation of the circulation loop, data for 6 IAEA-603 samples prepared with circulation are also shown.

Not Mixed via circulation	<i>n</i>	$\delta^{17}\text{O}_{\text{meas}}$	1σ	$\delta^{18}\text{O}_{\text{meas}}$	1σ	$\Delta^{17}\text{O}_{\text{meas}}$	1σ
Cavendish Marble	9	10.597	0.284	20.123	0.707	-0.028	0.116
NBS19	4	14.068	0.121	27.072	0.319	-0.226	0.109
NBS18	3	3.566	0.317	6.960	0.609	-0.108	0.056
Mixed via circulation							
Cavendish Marble	4	10.471	0.206	20.274	0.385	-0.234	0.010
NBS19	7	14.336	0.117	27.550	0.237	-0.211	0.066
NBS18	4	3.755	0.105	7.300	0.175	-0.100	0.019
IAEA-603	6	14.283	0.037	27.455	0.090	-0.213	0.018

Table 3.8: Comparison of uncorrected TILDAS measured δ -values mixed with and without mixing via the circulation loop. All data are shown are in per mille (‰). 6 IAEA-603 standards mixed by circulation are also included.

There are several notable results from this experiment. $\delta^{17}\text{O}_{\text{meas}}$ and $\delta^{18}\text{O}_{\text{meas}}$ values for both the NBS19 and NBS18 standards shifted more positive after mixing via circulation was implemented. In contrast, CM $\delta^{17}\text{O}_{\text{meas}}$ decreased while $\delta^{18}\text{O}_{\text{meas}}$ increased with circulative mixing. While CM $\delta^{17}\text{O}_{\text{meas}}$ precision moderately improved (from 0.284‰ to 0.206‰, 1σ), $\delta^{18}\text{O}_{\text{meas}}$ precision was nearly halved (from 0.707‰ to 0.385‰, 1σ). Similarly, NBS19 $\delta^{17}\text{O}_{\text{meas}}$ precision was essentially unchanged while $\delta^{18}\text{O}_{\text{meas}}$ precision improved from 0.319‰ to 0.237‰ (1σ). The results of these asymmetric improvements in δ -value precision are most significantly observed in the -0.206‰ (-206 per meg) shift in CM $\Delta^{17}\text{O}_{\text{meas}}$ from -0.028‰ to -0.234‰. CM $\delta^{17}\text{O}_{\text{meas}}$ and $\delta^{18}\text{O}_{\text{meas}}$ precisions after circulation are noticeably worse than those for both NBS standards but are still on par with typical precisions reported by IRMS measurements. NBS18 1σ precisions for $\delta^{17}\text{O}_{\text{meas}}$ and $\delta^{18}\text{O}_{\text{meas}}$ were both significantly reduced when mixed via circulation (from 0.317‰ to 0.105‰ and 0.609‰ to 0.175‰, respectively, 1σ). Of most significance to this project, $\Delta^{17}\text{O}$ 1σ precision for all samples improved after circulation was applied. CM precision was improved by one order of magnitude (from 0.116‰ to 0.010‰, 1σ), and NBS18 improved by ~66% (from 0.056‰ to 0.019‰, 1σ). NBS19 $\Delta^{17}\text{O}$ precision, while improved by ~40%, stands out as having significantly worse $\Delta^{17}\text{O}$ precision than CM and NBS18 (and IAEA-603). This is likely due to imperfections in the standard material, as expanded upon in Experiment F below. IAEA-603 1σ precision for $\delta^{17}\text{O}_{\text{meas}}$ and $\delta^{18}\text{O}_{\text{meas}}$ are 0.037‰ and 0.090‰, respectively, both of which are exceptional, and $\Delta^{17}\text{O}$ precision is 0.018‰. As expected, all IAEA-603 isotope values are extremely similar to those of NBS19, aiding in confirming the accuracy of the measurement procedure employed in this project.

The nearly universal positive shift of $\delta^{17}\text{O}_{\text{meas}}$ and $\delta^{18}\text{O}_{\text{meas}}$ values is consistent with expectations of limiting the role of diffusion in sample mixing. This expectation comes from a combination of the increased diffusion rates of the lighter CO_2 isotopologues relative to heavier ones, the geometry of the mixing volumes of the AutoCart, and the observation that only ~40-

50% of prepared sample gas is actually measured by the TILDAS. Imagine a parcel of incompletely mixed sample gas contained within the MV1 volume. Given the geometry of MV1, initial aliquots of said sample are pulled from the “edge” of the flask volume near the bottom of the siphon. This same area around the siphon is also where the N₂ diluent is introduced. Given this, sample CO₂ must diffuse into this dominantly N₂ area as mixing progresses. Because the lighter CO₂ isotopologues diffuse more quickly than the heavier ones, sample gas pulled from this area will be depleted in both $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ relative to the same gas if it was completely mixed. As less than half of the prepared sample gets analyzed, incompletely mixed samples will thus be characterized as more depleted than what their true composition is. When samples are completely mixed (e.g., via circulation), this apparent depletion is eliminated, and resulting $\delta^{17}\text{O}_{\text{meas}}$ and $\delta^{18}\text{O}_{\text{meas}}$ values will be more positive.

Downstream

Experiment D – Gas Introduction and Spectroscopic Measurement

TILDAS isotopologue abundance measurements are subject to instrument drift over the course of an analysis. One tool for evaluating the stability of the instrument during the analysis is evaluation of the Allan variance and the associated plots (Werle et al., 1993). These plots show measurement precision of a selected variable (e.g., $\delta(628)$, $\Delta^{17}\text{O}$, χ_{626} , etc.) as a function of data integration time. These plots are used to determine optimal measurement parameters and quantify the amount of drift observed over the course of a measurement. Allan variance can be assessed on both short and long time scales (e.g., for a single measured aliquot or on the scale of tens of hours in the case of overnight stability tests). The point of highest measurement precision (lowest error) is called the Allan minimum (σ_{min}) and represents the turning point at which continued measurement begins to be dominated by drift and fails to improve measurement precision. The combination of these tools provides value when determining how long to measure discrete aliquots to achieve optimal measurement precision while maintaining an effective analytical routine.

Results

The measurement time applied to individual aliquots used in this project was determined based on observed Allan minima (σ_{min}) for the $\delta(627)$ and $\delta(628)$ isotopologues during a closed cell analysis of the reference gas. An 11-hour continuous analysis was performed on 24 February 2022 and assessed for Allan variance. This assessment revealed several distinct phases of long-term drift in measured $\delta(627)$ and $\delta(628)$ values. Much of the observed drift was correlated to changes in the local operational environment, most intensely aircon cycling frequency – which itself is a function of air temperature and thermal flux within the instrument room. Laboratory aircon influence on TILDAS precision is investigated and presented in detail in Experiment E below.

Focusing on a specific 15-minute interval, from 14:40-14:55, in which measurement conditions were optimal reveals exceptional analytical precision for all isotopologues. This period was identified based on its high stability of measured $\delta(627)$ and $\delta(628)$ values, as well as TILDAS electronics temperature – a significant constraint on analytical precision – also investigated at length in Experiment E alongside referenced laboratory aircon influence. Allan minima for both $\delta(627)$ and $\delta(628)$ were 0.008‰, obtained after 221s and 68s of data integration, respectively

(Fig. 3.7). For $\delta(628)$, Allan variance begins to trend upwards after 68 seconds of measurement, signifying that after this point the measurement is dominated by instrumental drift and continued measurement does not improve precision. In addition, the Allan minimum for $\delta(636)$, the $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue, was 0.002‰ after 221 seconds of integration. Although this isotopologue is not a focus of this project, the extremely high-precision of the measurement is worth noting.

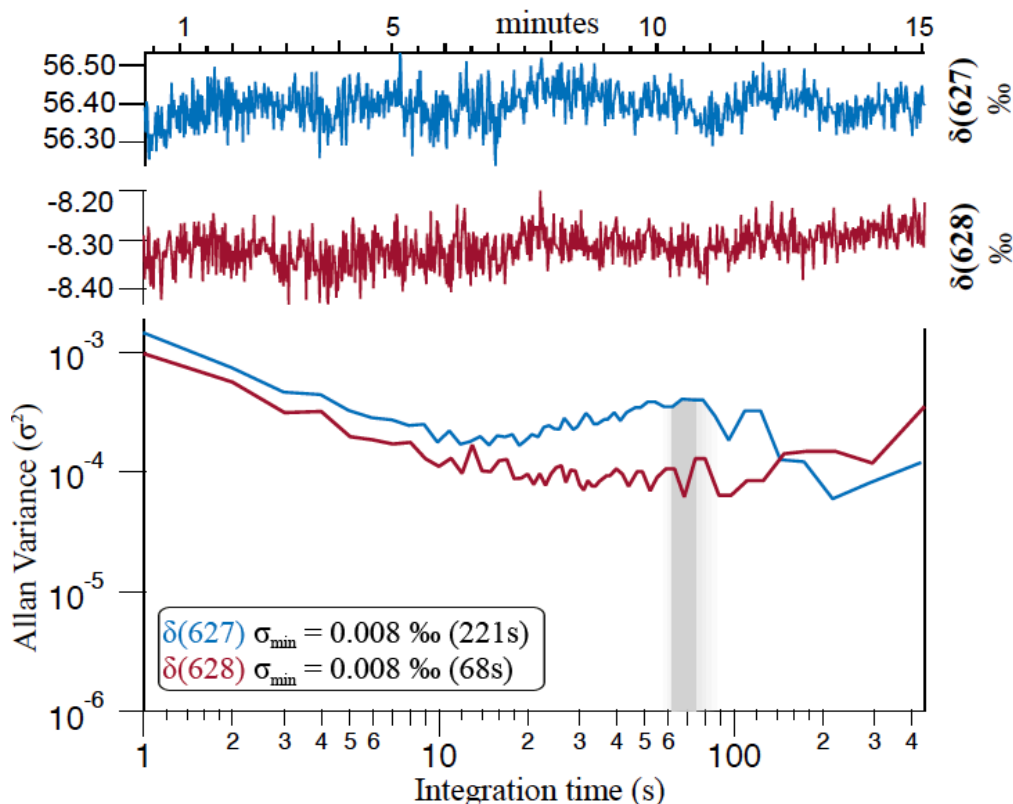


Figure 3.7: Figure adapted from Hare et al. (2022). Allan variance evaluation of a highly stable 15-minute interval of a closed cell analysis of the reference gas. The 15-minute period was identified based on its highly stable $\delta(627)$, $\delta(628)$, and TILDAS electronics temperature. Allan minima for $\delta(627)$ and $\delta(628)$ were both 0.008‰, occurring at 221s and 68s, respectively.

Comparatively, an overnight stability run was previously performed from the night of 30 August into the morning of 31 August 2021. This run is characterized by an initial period of significant drift, $\sim 4\text{‰}$, in measured $\delta(628)$ before reaching steady-state. This drift is similarly represented in $\delta(627)$ in direction, although occurring at a smaller scale ($\sim 1\text{‰}$). The drift is correlated to slight increases in optical cell and liquid coolant temperature, $\sim 0.6^\circ\text{C}$ and $\sim 0.05^\circ\text{C}$, respectively, as the instrument room became thermally stable. Assessing a relatively stable ~ 1 hour period of this measurement, from 2:30am to 3:30am, reveals the Allan minimum for $\delta(627)$ is 0.009‰, however this occurs after only 13 seconds of data integration, suggesting poor $\delta(627)$ measurement stability on longer timescales (Fig. 3.8). This is clearly seen in the cyclical drift in measured $\delta(627)$, on the order of 0.4‰, during this time period. The cyclical nature of the drift closely tracks laboratory aircon cycling, again an issue expanded upon in Experiment E below. The $\delta(628)$ Allan minimum for this same period was 0.007‰ after 107 seconds of integration.

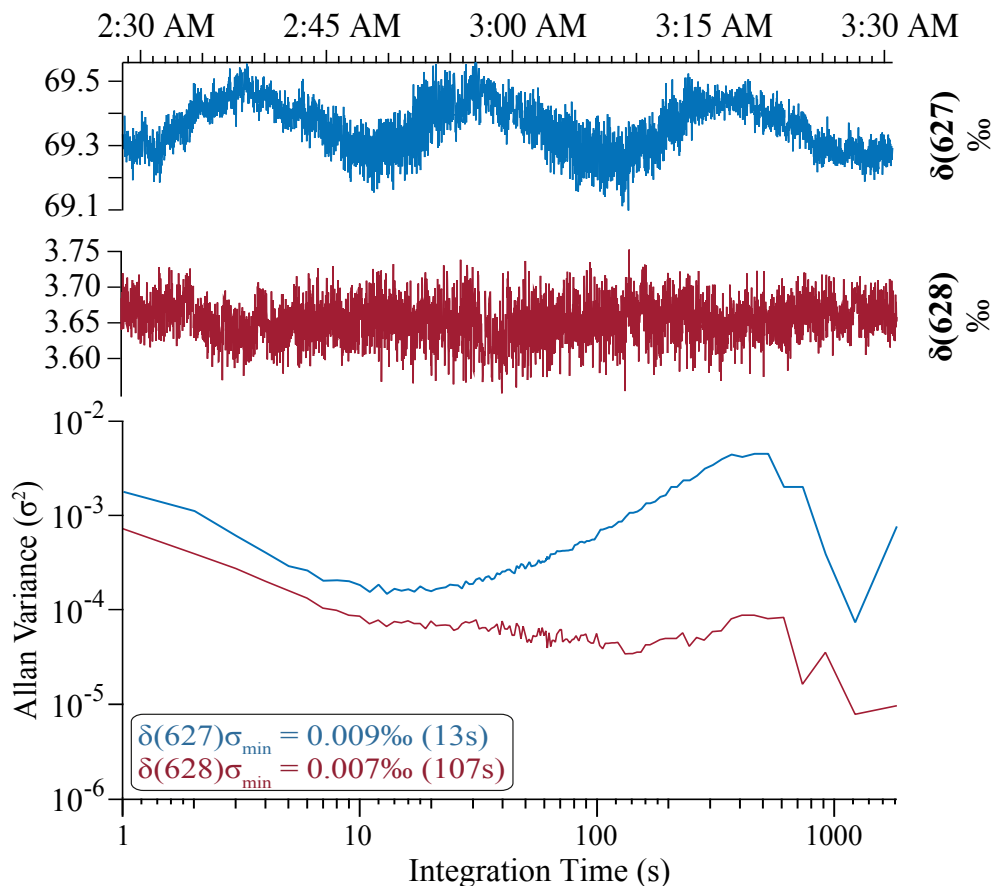


Figure 3.8: Allan variance of a 1-hour interval of an overnight closed cell analysis of the reference gas. Allan minimum for $\delta(627)$ was 0.009‰ after 13s of integration and for $\delta(628)$ was 0.007‰ after 107s. Variation in $\delta(627)$ closely tracks lab aircon cycling.

More recently, on 6 April 2022, a similar assessment of Allan variance was performed on a particularly high-precision analysis of the reference gas versus itself in discrete aliquots (Fig. 3.9). This analysis was intentionally designed to replicate the optimal measurement conditions observed in the identified 15-minute high-stability period assessed above. Evaluated over a 45 minute span of the analysis (52 minutes in total), Allan minimum for $\delta(627)$ was 0.024‰ with 59 seconds of integration time. Allan minimum for $\delta(628)$ was 0.015‰ after 78 seconds of integration (Fig. 3.9A). These Allan minima times closely agree with that previously obtained for $\delta(628)$ under optimal measurement conditions (68s, $\sigma_{\min} = 0.008‰$) shown above. In terms of absolute precision, the $\sigma_{\min}$ for $\delta(627)$ of 0.024‰ is unimpressive but not surprising given the absolute amount drift observed. Comparatively, Allan variance for $\delta(627)$ given 78 seconds of integration is 0.027‰, implying a period where continued measurement neither drastically improves nor worsens precision.

Highlighting a single aliquot (of which there are 27 in total), the Allan minimum was 0.007‰ for $\delta(627)$ and 0.004‰ for $\delta(628)$, occurring after 38 seconds of integration for both (Fig. 3.9B). As shown, Allan variance of both $\delta(627)$ and $\delta(628)$ continues to decrease as the measurement ends, suggesting continued measurement would have improved $\sigma_{\min}$ precision further. Referring back to Figure 3.7, Allan variance for both $\delta(627)$ and $\delta(628)$ under optimal conditions is not much

different at ~38 seconds as compared to 68 seconds – the Allan minimum of $\delta(628)$. This suggests that this specific aliquot, despite being limited by a shorter integration time, is not drastically compromised in terms of its absolute precision.

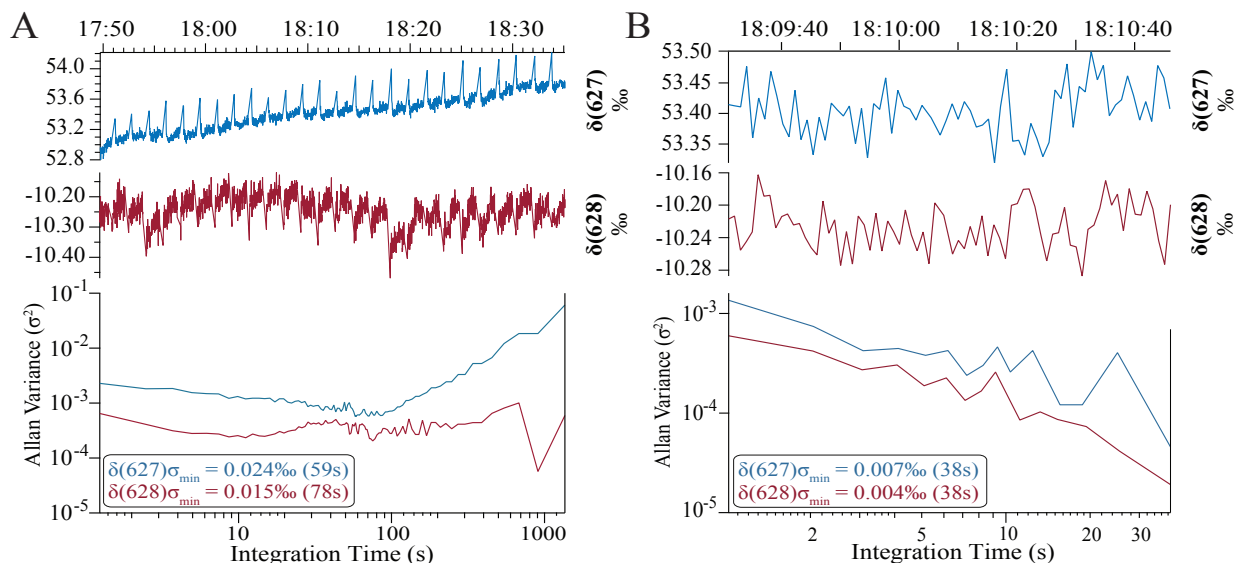


Figure 3.9: **A)** Allan variance for a ~45 minute period of the reference gas measured against itself in discrete aliquots. Allan minima for $\delta(627)$ and $\delta(628)$ were 0.024‰ and 0.015‰ and occurred after 59s and 78s of integration, respectively. **B)** Allan variance of single aliquot from the analysis shown in (A). Allan minima for $\delta(627)$ and $\delta(628)$ were 0.007‰ and 0.004‰, respectively, both occurring after 38s of integration.

Figure 3.10 shows $\Delta^{17}\text{O}_{\text{meas}}$ Allan minima at various integration times for a separate experiment in which the reference gas was again measured against itself in discrete aliquots. The data are an aggregation of several distinct analyses, all performed over the course of a single day, which were then assessed for Allan variance as a single, cohesive dataset. In theory, all $\Delta^{17}\text{O}_{\text{meas}}$ data points should equal exactly 0‰. As shown, the overall average $\Delta^{17}\text{O}_{\text{meas}}$ was $-0.004 \pm 0.080\text{‰}$ (1σ , $n = 93$), highlighting the high accuracy of the measurement procedure. Allan variance was evaluated for 3 well-separated time points: after 3 minutes, 21 minutes, and 2.4 hours of data integration. Allan minimum precision ($\sigma_{\min}$) at each of these points was 0.090‰, 0.026‰, and 0.004‰, respectively.

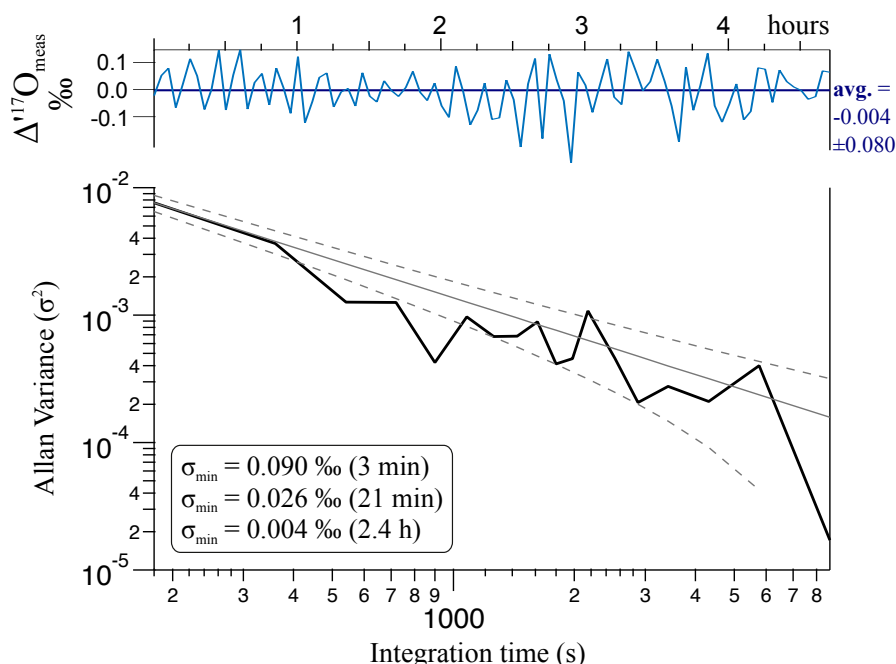


Figure 3.10: Figure from Hare et al. (2022). Allan variance of $\Delta^{17}\text{O}$ of the reference gas measured against itself in discrete aliquots. Data were collected from 5 distinct analyses and assessed as a single dataset. Measured $\Delta^{17}\text{O}$ of all data was $-0.004 \pm 0.080\text{‰}$ (1σ , $n = 93$). Allan minimum variation was assessed after 3 minutes, 21 minutes, and 2.4 hours of data integration with $\sigma_{\min}$ 0.090‰, 0.026‰, and 0.004‰, respectively.

Discussion

As a result of the observed Allan minimum of the optimal condition evaluation shown in Figure 3.7, all sample analyses presented in this project were accordingly performed such that discrete aliquots, of both sample and reference gas, were each measured in the optical cell for ~70-80 seconds. This time is a compromise between minimizing the contribution of drift in while also achieving good precision in both $\delta(627)$ and $\delta(628)$. Because spectroscopic $\Delta^{17}\text{O}$ values are ultimately calculated from measured $\delta(627)$ and $\delta(628)$, optimizing and balancing measurement of these two values is critical for reducing compounded error. The shortened 15-minute time period shown in Figure 3.7 represents a best case scenario for measurement stability as the gas within the optical cell would have reached thermal equilibrium, liquid coolant, electronics, and laser temperatures were all stable, and there was no external influence on the TILDAS instrument (e.g., laboratory aircon cycling, local environment heat sources are constant, etc.). The identical $\sigma_{\min}$ precision for both $\delta(627)$ and $\delta(628)$ is also encouraging, highlighting the ability of the TILDAS instrument to measure the several CO_2 isotopologues equally.

Noticeably, the cyclical drift in $\delta(627)$ in Figure 3.8 is not present in measured $\delta(628)$. This asymmetrical effect is a result of laboratory aircon exerting a disproportionately strong influence on measured $^{12}\text{C}^{17}\text{O}^{16}\text{O}$ isotopologue abundance determined by Laser 2, as compared to other isotopologue abundances as determined by Laser 1 (expanded upon in Experiment E below). The Allan minimum of $\delta(628)$ after 107 seconds of integration in this ~1 hour period is exceptional. Continued precision improvement with increasing data integration time is indicative of a highly

stable system yet to be limited by analytical environment conditions (e.g., fluctuations in optical cell and/or electronics temperature, laser span and current, etc.).

Allan variance evaluation on reference gas analyses performed by manner of repeated discrete aliquots suggests that the analytical parameters applied in this project are appropriate for the instrument used here. Similar $\sigma_{\min}$ times for both $\delta(627)$ and $\delta(628)$ to that of optimal condition closed-cell analyses yields confidence to the functioning of the TILDAS switching valve system – specifically the repeatability of reference gas dosing into the V1 volume. Poor repeatability in V1 filling would manifest in poor optical cell pressure repeatability, and by extension absolute CO₂ abundance. As spectroscopic δ -values are calculated from the measured raw abundances of the individual CO₂ isotopologues, relatively large variations in cell pressure and absolute CO₂ concentration are likely to cause poor δ -value precision. Slightly worse precision of such analyses is expected as individual aliquots are not perfectly equal in CO₂ abundance and optical cell pressure, among other slightly varying but important factors.

Allan minima times for $\delta(627)$ and $\delta(628)$ for the analysis shown in Figure 3.9 conveniently span that determined for the closed cell, optimal condition analysis ($\delta(628)$ $\sigma_{\min} = 0.008\text{‰}$, 68s). This lends confidence towards the applied measurement procedure, suggesting analyses performed under similar environmental conditions are not subject to drastic error caused by imperfect aliquot repeatability. The drift in $\delta(627)$ shown in Figure 3.9A is best attributed to an uncharacteristic optical cell temperature profile of the analysis as compared to other analyses run under similar conditions. Typically, cell temperature during such an analysis is stable to <0.1 K and only increases over its duration (a consequence of the absence of laboratory aircon) (see Methods: Fig. 2.10). However, this specific analysis displays a cell temperature profile that is U-shaped – decreasing by ~ 0.1 K over the first several aliquots before sharply rising for the remaining duration of the analysis. Optical cell temperature range is ~ 0.3 K, 3-4 times that of a typical analysis. Despite this drift, $\Delta^{17}\text{O}_{\text{meas}}$ precision from this analysis was exceptional (see Experiment E, sub-experiment 11 below).

Allan variance $\Delta^{17}\text{O}$ assessment of the reference gas versus itself presented in Figure 3.10 suggests that the reference gas has no measurable drift in isotopic composition, at least over the course of a full day of measurement. For this dataset, Allan variance steadily declines with increasing integration time over the cumulative ~ 4.5 hours of measurement, indicative of the system following noise-limited behavior. This continuous decline validates the stability of the reference tank and reinforces the viability of high-precision spectroscopic $\Delta^{17}\text{O}$ measurements in CO₂ directly. Seemingly patterned variation in $\Delta^{17}\text{O}_{\text{meas}}$ over the course of this combined dataset (e.g., extremely good repeatability between hours 1 and 2 versus lesser repeatability prior and following) is the result of slight differences in analytical conditions for each of the distinct analyses. All analyses represented in this dataset are a part of an investigation into the influence of TILDAS electronics temperature on $\Delta^{17}\text{O}$ precision, presented in Experiment E below.

Experiment E – Assessment of TILDAS Electronics Temperature on $\Delta^{17}\text{O}$ Precision

In the week from 31 March to 5 April 2022, laboratory aircon malfunctioning was noted and appeared to correlate with worsened measured $\Delta^{17}\text{O}$ precision. A closer look revealed that, specifically, variation in the temperature of the TILDAS electronics exhibited significant

influence on $\Delta^{17}\text{O}$ repeatability on an aliquot-by-aliquot basis. On 6 April 2022, a day long experiment was conducted to observe this relationship and attempt to quantify the applied influence. Sub-experiments 1 through 8 were conducted with laboratory aircon set to 23°C, matching the temperature of the TILDAS liquid chiller, sub-experiments 9 and 11 were run with laboratory aircon completely turned off, and sub-experiment 10 was run with aircon set to 24°C. All precisions presented here are calculated as 1σ and presented in terms of per mille (‰). In a theoretically perfect system, all measured $\Delta^{17}\text{O}$ values would equal exactly 0‰.

Results

To access the impacts of electronics temperature on measurement precision, the rate of change of the temperature (dT/dt ; $^{\circ}\text{K min}^{-1}$) of the electronics was mapped using a centered 100-second moving average. The curve produced from this moving average was plotted alongside calculated $\Delta^{17}\text{O}$ values for each reference gas vs. reference gas measurement cycle. A trend is observed correlating the amplitude (A) of the dT/dt moving average curve, hereafter $A(dT/dt)$, to measured $\Delta^{17}\text{O}$ standard deviation (1σ) (Fig. 3.11). Half of the data observed are explained by this relationship. The outlier in the trend is the direct result of an intentional disruption to the experimental run (Experiment 5) in which the cooling fan intake on the TILDAS computer was blocked with a sheet of paper for ~ 6 minutes during the run.

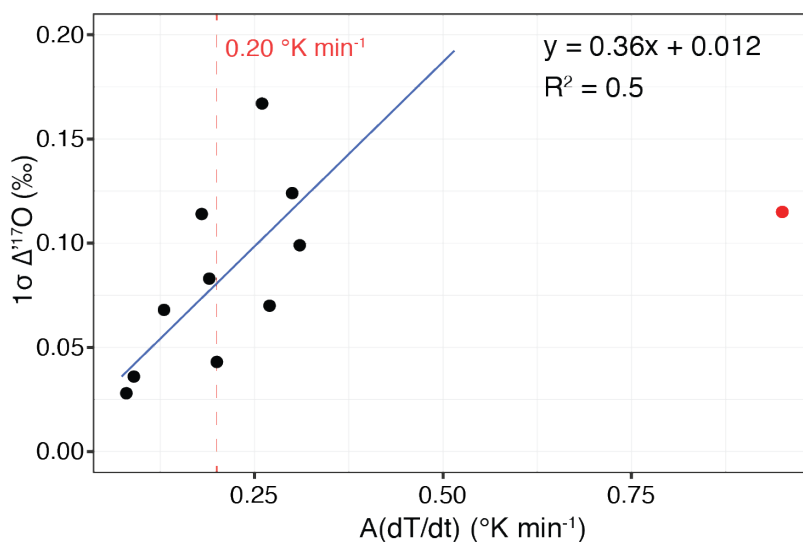


Figure 3.11: Figure from Hare et al. (2022). Amplitude of TILDAS electronics temperature curve (see Methods: Experiment E for details) versus $\Delta^{17}\text{O}$ 1σ precision. Red point marks Sub-Experiment E5 where TILDAS cooling fan intake was temporarily blocked during the analysis.

The entirety of the dT/dt sub-experiments is shown in Figure 3.12 and summarized in Table 3.9. Observably, measured $\Delta^{17}\text{O}$ repeatability is correlated not to the absolute temperature of the TILDAS electronic components, but rather their temperature's rate of change. Sub-experiment with a tightly fluctuating absolute electronics temperature, in the range of $\sim 1^{\circ}\text{K}$ (sub-experiments 1, 2, 3, 4, 6, 7, 8), are not necessarily associated with improved measured $\Delta^{17}\text{O}$ precisions. However, sub-experiments in which the electronics temperatures were not regulated (i.e., laboratory aircon was powered off; sub-experiments 9, 11) are described by having very good measurement precision while reflecting drifts in absolute electronics temperature on the scale of 3°K .

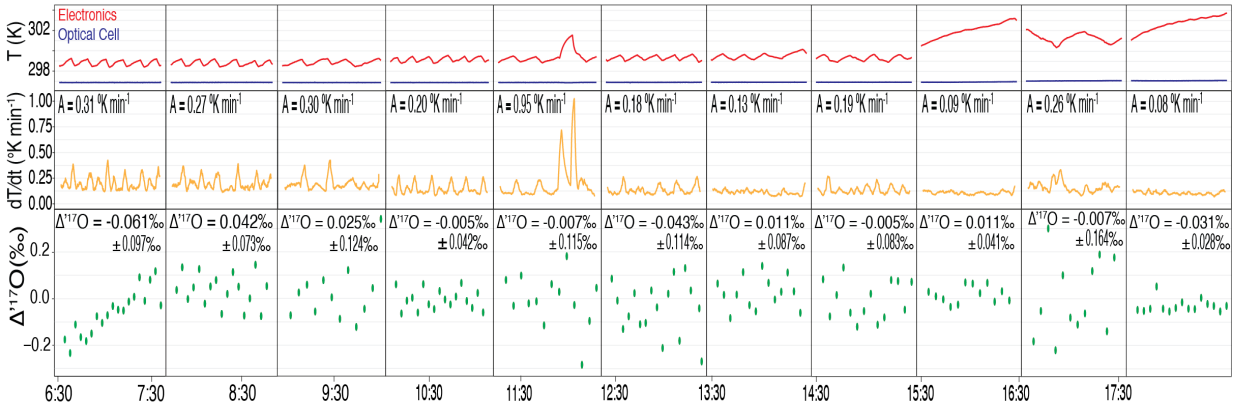


Figure 3.12: All sub-experiments presented in Experiment E. All analyses are the reference gas measured against itself in discrete aliquots. Top row shows TILDAS electronics (red) and optical cell (blue) temperature; middle row shows 100-second moving average of electronics rate of change (yellow); bottom row is measured $\Delta^{17}\text{O}$ for all aliquots (green data). $\Delta^{17}\text{O}$ precision is correlated to the rate of change TILDAS electronics temperature.

Sub-Experiment	A(dT/dt) (°K min ⁻¹)	$\Delta^{17}\text{O}$ (‰)	1 σ $\Delta^{17}\text{O}$ (‰)
E1	0.31	-0.061	0.097
E2	0.27	0.042	0.073
E3	0.30	0.025	0.124
E4	0.20	-0.005	0.042
E5	0.95	-0.007	0.115
E6	0.18	-0.043	0.114
E7	0.13	0.011	0.087
E8	0.19	-0.005	0.083
E9	0.09	0.011	0.041
E10	0.26	-0.007	0.164
E11	0.08	-0.031	0.028
Overall		-0.006	0.030

Table 3.9: Summary of results shown in Figure 3.12.

Focusing in on individual experiments from this day helps to clarify aspects of this newly discovered relationship. Sub-experiment 1 has a clear upward trend in measured $\Delta^{17}\text{O}$, likened to incomplete purging of TILDAS instrument internal housing. While N_2 purge rate of the instrument housing is taken to be inconsequential to measurement precision so long as it is sufficient to maintain a dry, N_2 dominated internal environment, incomplete purging implies the presence of atmospheric CO_2 and H_2O along the laser pathway prior to entering the optical cell. Absorption of light prior to entering the cell will have direct impacts on measured isotopologue abundance ratios and, therefore, δ -values. Given this, it is difficult to interpret the influence of the A(dT/dt) of $0.31^\circ\text{K min}^{-1}$ on its own. No matter the cause, the $\Delta^{17}\text{O}$ precision of 0.097‰ (1σ , $n = 19$) is poor and required improvement to be useful in future measurements.

Sub-experiment 2 plays the role of the initial baseline to which compare the following experiments performed throughout the day as measured $\Delta^{17}\text{O}$ values have no apparent trend. For this experiment, $A(dT/dt)$ equaled $0.27^\circ\text{K min}^{-1}$ and $\Delta^{17}\text{O}$ precision of 0.073‰ (1σ , $n = 18$). It is difficult to say whether the improvement was due to the different purge rate or simply a result of a more stable measurement environment.

Sub-experiment 3 displays a slightly different electronics temperature profile as compared to that of sub-experiment 2. In sub-experiment 3, it can be seen that the electronics temperature has a lengthened periodicity. The $A(dT/dt)$ profile also differs in that it is more characterized by broader peaks, suggesting periods of sustained high rates of temperature change, which could in turn lead to worsened $\Delta^{17}\text{O}$ precision. $\Delta^{17}\text{O}$ precision of 0.124‰ (1σ , $n = 12$) is heavily skewed by the final measurement in this run, where $\Delta^{17}\text{O} = 0.343\text{‰}$. Excluding this final measurement improves $\Delta^{17}\text{O}$ 1σ precision to 0.077‰ ($n = 11$), more consistent with expectations as based on the first two sub-experiments.

Sub-experiment 4 was setup identically to Experiment 2 and resulted in an $A(dT/dt)$ of $0.20^\circ\text{K min}^{-1}$ and $\Delta^{17}\text{O}$ precision of 0.042‰ (1σ , $n = 18$). This experiment stands out as a coincidental experiment regarding laboratory thermal stability in terms of thermal mass. During this experiment, 10 people spent notable amounts of time in the instrument room, as compared to 1-3 in previous and latter sub-experiments, contributing large amounts of heat energy and thermal mass, thus reducing the instantaneous effectiveness of the aircon units. This likely explains the decreased $A(dT/dt)$ as inefficiency in cooling via aircon directly results in slower rates of laboratory environment temperature change.

Sub-experiment 5 was a unique experiment performed regarding this relationship in that it was intentionally disrupted. By covering the fan intake on the TILDAS computer midway through the analysis, a rapidly heating internal environment was created. Clearly shown in both the absolute electronics temperature and dT/dt profile, this action had an immediate impact on measured $\Delta^{17}\text{O}$. The paper was removed after ~ 6 minutes, causing the electronics to rapidly cool. The drastic impact the electronics is observed as a double peak in the dT/dt profile of the experiment, the first of which is the rapid heating of the electronics when cooling air was cut off, and the second when the fan intake was uncovered, causing rapid cooling of the electronics. An extreme $A(dT/dt)$ of $0.95^\circ\text{K min}^{-1}$ expectedly corresponds to a poor $\Delta^{17}\text{O}$ precision, 0.115‰ (1σ , $n = 13$). Looking closer at the individual data points from the run, both the highest and lowest measured $\Delta^{17}\text{O}$ values fall within this 6 minute window, providing evidence supporting the direct responsibility of this action on the acquired values.

Sub-experiment 6 stands out as having equally poor $\Delta^{17}\text{O}$ precision, 0.114‰ (1σ , $n = 17$), as sub-experiment 5, with the distinction that no clear, singular cause can be attributed to it. Measured $\Delta^{17}\text{O}$ values appear to increase in range with time throughout the experiment, potentially related to worsening thermal stability of the electronics. Despite a modest $A(dT/dt)$ value of $0.18^\circ\text{K min}^{-1}$, the dT/dt profile of this run, sharp peaks interspersed by broad periods of moderate change, suggests inefficient temperature distribution of laboratory air, however this is difficult to verify.

Sub-experiment 7 $A(dT/dt)$ of $0.13^{\circ}\text{K min}^{-1}$ was expected to set the $\Delta^{17}\text{O}$ precision benchmark to which the previous experiments could be compared against. However, a $\Delta^{17}\text{O}$ precision of 0.087% (1σ , $n = 15$) is unremarkable despite the drastic improvement in electronics temperature stability. Comparing this experiment to sub-experiment 2, similar $\Delta^{17}\text{O}$ precisions are achieved despite a difference in $A(dT/dt)$ by a factor of ~ 2 . This experiment is difficult to interpret as there is no clear cause for either the improved temperature profile or the corresponding worse-than-expected $\Delta^{17}\text{O}$ precision. Although this experiment had no direct implication on measured $\Delta^{17}\text{O}$ values, improvement in the dT/dt profile was encouraging.

Sub-experiment 8 showed an $A(dT/dt)$ of $0.19^{\circ}\text{K min}^{-1}$, however the $\Delta^{17}\text{O}$ precision of 0.083% (1σ , $n = 14$) is worse than previous experiments with considerably worse temperature profiles. No singular cause has been linked to this. dT/dt individual peak shape is similar to that of sub-experiment 6 in which broadened intervals of moderate change exist alongside sharper, higher peaks, potentially linked to this poor precision. This is evidenced in the larger scattered of $\Delta^{17}\text{O}$ values early on in the experiment where the dT/dt profile is represented by these broad peaks as compared to more instantaneous changes in temperature later in the run.

Sub-experiment 9 was unique thus far in that it was the first experiment to be run with no laboratory aircon control. Rather, the room was allowed to heat over the course of the analysis to characterize the minimum rate of temperature change and the impact of absolute electronics temperature on measurement precision. This resulted in the lowest $A(dT/dt)$ recorded at the time, $0.09^{\circ}\text{K min}^{-1}$, and the best $\Delta^{17}\text{O}$ precision at 0.041% (1σ , $n = 13$). This combination of results was very encouraging as it showed that preventing instantaneous laboratory air temperature changes directly correlates to improvements in measured $\Delta^{17}\text{O}$. Although this physical characteristic was resulted differently, this follows similar logic to the good precision measured in sub-experiment 4, which achieved similar 1σ precision. The absolute temperature of the electronics increased by $\sim 3^{\circ}\text{K}$ and showed no correlation to measured $\Delta^{17}\text{O}$. This experiment provided the dT/dt baseline that could be expected when not exerting any control on the TILDAS operational environment.

Sub-experiment 10 acted as the antithesis of sub-experiment 9. For this experiment, laboratory aircon control was set to 24°C to observe the impacts of, effectively, lengthening the periodicity of electronics temperature change. In doing so, $\Delta^{17}\text{O}$ precision plummeted to 0.164% (1σ , $n = 13$), by far the worst precision yet recorded. Somewhat surprisingly, allowing laboratory air, and therefore electronics temperature, to drift upwards longer prior to cooling also resulted in a poor dT/dt profile, with a $A(dT/dt)$ of $0.26^{\circ}\text{K min}^{-1}$. This is likely related to worsened laser thermal control as the liquid chiller temperature setpoint, 23°C , was lower than the laboratory air temperature.

Sub-experiment 11 was the final experiment performed. Setup identically to sub-experiment 9, it was intended to verify the hypothesis that removing sources of rapid electronics temperature change leads to improved measured $\Delta^{17}\text{O}$ precision. With no laboratory aircon control influencing electronics temperature, the dT/dt profile of this experiment was very stable with a low $A(dT/dt)$ of $0.08^{\circ}\text{K min}^{-1}$, identical to that of sub-experiment 9. Measured $\Delta^{17}\text{O}$ precision improved further to 0.028% (1σ , $n = 15$). Although the average $\Delta^{17}\text{O}$ of the 15 measurement

cycles, -0.031‰ , is not within measurement error of the theoretical value of 0‰ , the drastic improvement in precision was extremely encouraging and laid the groundwork and minimum physical requirements for future analyses.

Taken together, the average $\Delta^{17}\text{O}$ of all 11 sub-experiment is $-0.006 \pm 0.030\text{‰}$ (1σ , $n = 167$). Excluding the first experiment, due to the clear trend in measured $\Delta^{17}\text{O}$, this improves to $-0.001 \pm 0.025\text{‰}$ (1σ , $n = 148$). This is an exceptional result and fully displays the long-term accuracy and stability the TILDAS instrument is capable of.

Discussion

TILDAS electronics temperature is measured by a thermistor located inside the electronics box, just behind the air intake. It is suspected that room air temperature impacts measurement precision by means of its effect on electronic components with a temperature coefficient (e.g., resistors). As the measured temperature inside the electronics box changes, those parts with temperature dependencies further influence their contingent components. In this manner, it is possible that changes in room air temperature effect laser parameters such as current offset and span. The extremely high sensitivity of the lasers implies that even tiny changes in applied current could induce increased measurement errors apparent even on the per meg scale.

For the system used in this project, it was realized that after optimizing and achieving all of the above, minimizing $A(dT/dt)$ is the most important factor in producing high-precision $\Delta^{17}\text{O}$ measurements. For the system used in this project maintaining a constant TILDAS electronics temperature rate of change near or below $\sim 0.20^\circ\text{K min}^{-1}$ for the duration of an analysis is the minimum requirement for achieving high-precision $\Delta^{17}\text{O}$ measurements (Fig. 3.12). To this end, it was best to perform analyses with no lab aircon control, allowing the room to slowly heat up over the course of a measurement (sub-experiments 9 and 11). While the absolute temperature of the electronics typically increases by several degrees when applying this strategy, the continuous but consistent heating minimizes drastic instantaneous changes in the electronics temperature, as represented by $A(dT/dt)$. Other, perhaps more practical long-term solutions to limit $A(dT/dt)$ could be the extension of the liquid cooling system to include more sensitive electronic components, adding a heat exchanger near the computer's cooling fan intake, or using a high quality aircon with PID control to continuously supply the lab space around the TILDAS with air of consistent temperature.

In summary, the results of the sub-experiments suggest the main control over measurement precision, after achieving sufficient sample preparation, to be dT/dt of the TILDAS electronics. The internal purge rate and absolute temperature of the electronics had little to no influence on the measured $\Delta^{17}\text{O}$ values, while the lab aircon temperature setting exerts its largest influence when set higher than the liquid chiller temperature. As the TILDAS system is constantly measuring and making corrections to adapt to its operational environment, it follows that rapid changes will exert a greater influence on instrument stability. Minimizing large instantaneous electronics temperature changes is key to achieving the necessary precision for relevant earth surface triple oxygen isotope studies using TILDAS. While changes in TILDAS electronics temperature do not perfectly explain all of the observed variation in measured $\Delta^{17}\text{O}$, it is clear that electronics dT/dt plays an increasing role as measurement 1σ precision approaches theoretical limitations (Fig. 3.11). This finding is relevant not only to this project but also to

future studies focusing on high-precision spectroscopic isotopologue abundance measurements as analytical conditions must be highly reproducible for widespread achievement of this common objective.

Experiment F – δ -value Concentration Dependence and Data Correction

The TILDAS instrument measures the relative abundances of several isotopologues in CO₂. As such, the δ -values measured in this project are technically molecular abundance ratios, as compared to atomic abundance ratios as traditionally measured by isotope ratio mass spectrometry (IRMS), although the difference between the two is assumed to be negligible (Kerstel, 2004). For this reason, all δ -values reported in this project adopt atomic δ -value notation to keep consistent with the current literature and promote effortless comparisons between analytical methods.

Data Correction and Results

Data correction to the VSMOW2-SLAP2 scale was performed using the equation:

$$\delta^{17}\text{O}_{\text{std}} = \frac{\chi'_{626}}{A_{627}(\chi'_{626} - b_{626})} \left[\delta^{17}\text{O}_{\text{meas}} + \frac{(A_{627}b_{626} - b_{627})}{\chi'_{626}} - A_{627} + 1 \right] \quad (3.1)$$

(identical to eq. (2.6); See Methods: Experiment F, eq. (2.3 – 2.6) for derivation and details). Calculation of the scaling factors in the applied correction was done using the carbonate standards NBS18 ($n = 4$) and IAEA-603 ($n = 6$). Assuming the VSMOW2-SLAP2 relative values for these standards reported by Wostbrock et al. (2020) for CO₂, measured $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values and χ'_{626} of all aliquots were fitted to eq. (3.1). In doing so, the fitted parameters for $\delta^{17}\text{O}$ were: $A_{627} = 0.674$, $b_{627} = -1974$, $b_{626} = -168$ ($R^2 = 0.999$); and for $\delta^{18}\text{O}$ were $A_{628} = 0.632$, $b_{628} = -3782$, $b_{626} = -207$ ($R^2 = 0.999$). The Wostbrock et al. (2020) data was chosen as the correction reference as they are relatively assumption free (Passey & Levin, 2021). All standards used in the correction were prepared identically on the AutoCart using the fully developed repeated advection and 2.5 minute circulation mixing technique described in Experiment C above. Note that not all standards presented above were used for developing the data correction scheme. Standards run between the dates of 31 March and 5 April 2022 have been excluded on the basis of poor thermal stability and lab aircon issues noted in Experiment E. Standards run during this time period include NBS18-9, NBS18-10, NBS18-11, NBS19-8, NBS19-9, NBS19-10, IAEA-603-1, IAEA-603-2, and IAEA-603-3. Results for both uncorrected ($\delta^{17}\text{O}_{\text{meas}}$, $\delta^{18}\text{O}_{\text{meas}}$) and corrected ($\delta^{17}\text{O}_{\text{corr}}$, $\delta^{18}\text{O}_{\text{corr}}$) and corrected ($\Delta^{17}\text{O}_{\text{corr}}$) values are presented in Table 3.10.

For all standards shown in Table10 overall sample concentration repeatability was 403.6 ± 8.2 $\mu\text{mol mol}^{-1}$ (1σ , $n = 17$). Within sample (aliquot) concentration repeatability ranged from 0.4 to 0.9 $\mu\text{mol mol}^{-1}$ (1σ). Both of these results show that the standards used for calibration are accurately diluted and well-mixed and offer target concentration guidelines to aim for when preparing non-standard samples. The range of concentrations (28.1 $\mu\text{mol mol}^{-1}$) also provides perspective on the applied correction scheme and its ability to accommodate varying CO₂ concentrations.

Sample	χ_{626}	$\delta^{17}\text{O}_{\text{meas}}^{\text{a}}$	$\delta^{18}\text{O}_{\text{meas}}^{\text{a}}$	$\delta^{17}\text{O}_{\text{corr}}^{\text{b}}$	$\delta^{18}\text{O}_{\text{corr}}^{\text{b}}$	$\Delta^{17}\text{O}_{\text{corr}}$
IAEA-603-4	393.3 (0.5)	14.311	27.560	20.036	38.250	-158
IAEA-603-5	404.8 (0.6)	14.290	27.435	20.045	38.220	-149
IAEA-603-6	412.8 (0.8)	14.288	27.471	20.097	38.374	-161
IAEA-603-7	393.8 (0.8)	14.287	27.493	20.012	38.181	-147
IAEA-603-9	405.3 (0.7)	14.277	27.435	20.034	38.224	-149
IAEA-603-10	415.6 (0.7)	14.216	27.300	20.000	38.172	-158
	Average	14.291	27.479	20.037	38.237	-151
	1σ	0.013	0.052	0.015	0.043	10
NBS18-8	397.7 (0.4)	3.605	6.925	8.941	17.148	-113
NBS18-12	409.6 (0.5)	3.791	7.374	9.075	17.389	-106
NBS18-13	405.1 (0.5)	3.840	7.409	9.150	17.463	-71
NBS18-14	401.8 (0.9)	3.763	7.313	9.112	17.461	-107
	Average	3.750	7.255	9.070	17.365	-99
	1σ	0.102	0.224	0.046	0.149	19
NBS19-5	401.4 (0.6)	14.164	27.295	19.895	38.052	-196
NBS19-6	409.0 (0.6)	14.269	27.435	20.053	38.266	-151
NBS19-7	397.6 (0.5)	14.244	27.500	19.978	38.222	-203
NBS19-11	397.3 (0.7)	14.406	27.796	20.146	38.524	-195
NBS19-12	388.5 (0.7)	14.492	27.868	20.208	38.515	-127
NBS19-13	411.3 (0.5)	14.443	27.678	20.224	38.530	-120
NBS19-14	416.6 (0.7)	14.418	27.798	20.184	38.582	-177
	Average	14.348	27.624	20.098	38.385	-150
	1σ	0.122	0.217	0.126	0.203	60

Table 3.10: Results from TILDAS analyses of CO_2 evolved from international carbonate standards IAEA-603, NBS18, NBS19. χ_{626} is measured abundance of the $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue, 1σ precision given in parentheses. All δ -values reported in ‰, $\Delta^{17}\text{O}$ reported in per meg ($\theta = 0.528$).

^a Molecular abundance ratios by optical spectrometry (e.g., $\delta(627)$) assumed equal to atomic abundance ratios (e.g., $\delta^{17}\text{O}$), and atomic notation is retained. ^b Corrected using eq. (3.1).

Results for the modern OES internal laboratory standards UCT-WC-1 and UCT-Et-1 are shown in Table 3.11. Excluded analyses among these internal standards are UCT-Et-1-5 and two UCT-WC-1 samples (UCT-WC-1-8 and UCT-WC-1-9). UCT-Et-1-5 is excluded due to severe Laser 2 instability during the analysis, and both UCT-WC-1 samples are excluded due to valve (V15) failures. Overall sample concentration repeatability of the non-excluded analyses was $400.3 \pm 8.7 \mu\text{mol mol}^{-1}$ (1σ , $n = 14$) and within sample (aliquot) concentration repeatability ranged from 0.4 to $0.9 \mu\text{mol mol}^{-1}$ (1σ).

Sample	χ_{626}	$\delta^{17}\text{O}_{\text{meas}}^{\text{a}}$	$\delta^{18}\text{O}_{\text{meas}}^{\text{a}}$	$\delta^{17}\text{O}_{\text{corr}}^{\text{b}}$	$\delta^{18}\text{O}_{\text{corr}}^{\text{b}}$	$\Delta^{17}\text{O}_{\text{corr}}$
UCT-Et-1-1	377.9 (0.5)	17.630	33.968	23.379	44.582	-160
UCT-Et-1-2	404.2 (0.5)	18.024	34.487	23.934	45.516	-98
UCT-Et-1-3	407.0 (0.6)	17.957	34.473	23.879	45.543	-167
UCT-Et-1-4	398.0 (0.4)	17.883	34.307	23.752	45.236	-132
UCT-Et-1-6	402.5 (0.4)	17.875	34.185	23.770	45.179	-84
UCT-Et-1-7	406.3 (0.5)	17.868	34.307	23.783	45.360	-167
UCT-Et-1-8	405.8 (0.7)	18.059	34.458	23.980	45.510	-49
	Average	17.862	34.325	23.736	45.266	-123
	1σ	0.167	0.178	0.226	0.314	46
UCT-WC-1-1	388.1 (0.8)	18.793	36.186	24.634	47.005	-185
UCT-WC-1-2	403.7 (0.4)	18.877	36.140	24.820	47.219	-111
UCT-WC-1-3	396.3 (0.5)	19.086	36.598	24.989	47.568	-127
UCT-WC-1-4	401.9 (0.3)	18.904	36.341	24.837	47.397	-189
UCT-WC-1-5	396.6 (0.7)	18.874	36.208	24.772	47.173	-135
UCT-WC-1-6	411.6 (0.5)	18.917	36.209	24.910	47.417	-127
UCT-WC-1-7	404.8 (0.9)	18.765	36.002	24.709	47.093	-156
	Average	18.888	36.241	24.810	47.267	-147
	1σ	0.104	0.187	0.119	0.200	30
Excluded						
UCT-Et-1-5	388.1 (1.2)	17.604	34.419	23.409	45.199	-456
UCT-WC-1-8	402.9 (7.6)	15.975	30.510	21.773	41.333	-51
UCT-WC-1-9	408.8 (2.6)	14.821	28.673	20.600	39.515	-263

Table 3.11: Results from TILDAS analyses of CO₂ evolved from internal OES standards. χ_{626} is measured abundance of the $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue, 1 σ precision given in parentheses. All δ -values reported in ‰, $\Delta^{17}\text{O}$ reported in per meg ($\theta = 0.528$). ^a Molecular abundance ratios by optical spectrometry (e.g., $\delta(627)$) assumed equal to atomic abundance ratios (e.g., $\delta^{17}\text{O}$), and atomic notation is retained. ^b Corrected using eq. (3.1).

Discussion

All analyses of NBS19 were excluded from correction fitting due to significantly worse $\Delta^{17}\text{O}_{\text{corr}}$ reproducibility (1 σ = 0.060‰, $n = 7$) as compared to NBS18 and IAEA-603. This is attributed to likely sample contamination as the vial of NBS19 material used here was exhausted by these analyses as this specific vial had been in regular use for ~30 years prior to these measurements. It is suspected that significant isotopic exchange with atmospheric moisture over this time period is the source of the large-scale measurement errors. This phenomenon has been brought up by other authors in the triple oxygen isotope community (Barkan et al., 2019). Alternatively, it has been suggested that significant variation may also be due to grain-scale heterogeneities within the standard material itself (Ishimura et al., 2008). Comparatively, a brand new vial of IAEA-603 was opened and used for all measurements presented in this project and resulted in extremely

high-precision measurements of $\delta^{17}\text{O}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ (0.015‰, 0.043‰, and 10 per meg, respectively).

Laser 1 is used to quantify the abundance of the $^{12}\text{C}^{18}\text{O}^{16}\text{O}$ isotopologue ($\delta^{18}\text{O}$), and Laser 2 the $^{12}\text{C}^{17}\text{O}^{16}\text{O}$ isotopologue ($\delta^{17}\text{O}$) (See Methods: Table 2.4). In the case of sample UCT-Et-1-5, instability in Laser 2 resulted in measured $\delta^{17}\text{O}$ values trending out of phase with $\delta^{18}\text{O}$ values, giving rise to the extremely negative $\Delta^{17}\text{O}$ (-456 per meg). Both of the UCT-WC-1 samples are excluded due to failure of the pneumatic valve separating the TILDAS optical cell from the valve switching system (V15), as discussed in Experiment C above. Extant $\Delta^{17}\text{O}_{\text{corr}}$ variability in the OES samples presented here is a subject worthy of future investigation. At present the observed variability is thought to be either due to deficiencies in the acid digestion process, or simply natural variation in the sample material. Deficiencies in sample acid digestion can be addressed in two ways; allowing longer reaction times prior to AutoCart sample preparation or using smaller grain sizes to promote complete digestion.

3.2) TILDAS vs. IRMS Comparison

In order to answer some of the most pressing terrestrial (paleo)climate questions being explored today, “high-precision” measurements of $\Delta^{17}\text{O}$, with analytical precisions in the realm of $\sim 0.010\text{‰}$ (10 per meg), are essential. The triple oxygen isotope composition of carbonates has typically been measured using isotope ratio mass spectrometry (e.g., Passey et al., 2014; Barkan et al., 2015; Wostbrock et al., 2020). Despite being the method of choice, IRMS has several drawbacks and limitations which make it unobtainable for some laboratories and difficult for those that can. A large laboratory footprint, the requirement of a dedicated power supply, and high-cost are all limiting factors for smaller, lesser equipped laboratories and institutions to perform high-precision $\Delta^{17}\text{O}$ measurements. Labs that are able to house IRMS equipment are then burdened with the complexities of sample preparation and the actual isotopic analysis. While these factors are not completely eliminated when using a TILDAS instrument, the reduced instrument cost, ability to plug it into a standard wall outlet, and newly developed methodology presented in this project increase accessibility into the triple oxygen isotope field.

To date, the various sample preparation methodologies for IRMS triple oxygen isotope analysis of carbonates can be grouped into two main categories: the “conversion” methods and the “exchange” methods (Passey & Levin, 2021). The former relies on quantitatively converting sample CO_2 evolved from carbonates into a more readily measured form, typically O_2 gas, by various means. The latter relies on controllably mediating isotopic exchange between sample CO_2 and another oxygen bearing species (e.g., H_2O , CuO , CeO) and measuring the CO_2 composition both before and after. The two categories of sample preparation do not always result in closely agreeing $\Delta^{17}\text{O}$ values, with exchange methods typically resulting in lower $\Delta^{17}\text{O}$. Each of these sample preparation techniques consumes $\sim 5\text{--}10$ mg of CaCO_3 per sample (as compared to the ~ 0.9 mg CaCO_3 needed for the technique introduced in this project).

The data used for development of the correction scheme presented in this project have been corrected to the VSMOW2-SLAP2 scale by assuming the previously reported standard values by Wostbrock et al. (2020), a group which uses a conversion method. As such the values reported in this project more closely match those given by conversion methods as compared to the exchange

methods. Figure 3.13 shows $\Delta^{17}\text{O}$ values of NBS18, NBS19, and IAEA-603 measured in this project (red triangles) compared to those reported by several IRMS based laboratories, and their associated errors. $\Delta^{17}\text{O}$ values of NBS18 and IAEA-603 determined in this project are internally consistent with Wostbrock et al. (2020) (blue circles) and are in good agreement with other studies using conversion methods (grey symbols). Due to its large uncertainty NBS19 agrees with most IRMS methods, however this is more likely to be an artifact of material contamination rather than indicative of shortcomings in the system presented in this project, as discussed above.

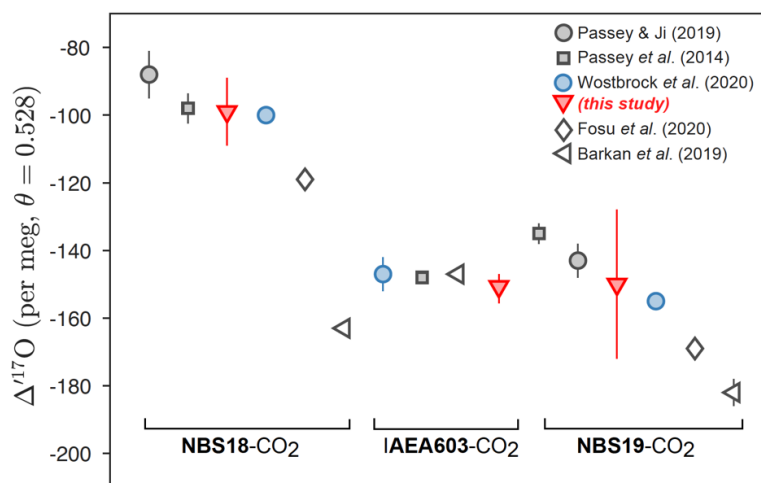


Figure 3.13: From Hare et al. (2022). Summary of reported $\Delta^{17}\text{O}$ of international standards NBS18, NBS19, and IAEA-603. Red triangles are this project. IRMS data organized by type of sample preparation method: conversion (grey), exchange (white). Blue data is Wostbrock et al. (2020), which uses a conversion method. Error bars are 1 SE.

Table 3.12, adapted from Passey & Levin (2021), shows reported $\Delta^{17}\text{O}$ values from several studies for these standards as grouped by analytical and sample preparation method. Encouragingly, despite the very large uncertainty of NBS19 reported here, the difference between the average $\Delta^{17}\text{O}$ values of NBS18 and NBS19 ($\Delta\Delta^{17}\text{O}$ NBS18-NBS19) in this project is in near perfect agreement with those of the conversion method IRMS studies. The extremely high variability of $\Delta^{17}\text{O}$ values of these standards reported by studies employing exchange methods highlights the decision to correct measured values in this project to reported values of a conversion method study.

Comparison of $\Delta^{17}\text{O}$ values measured in this project by TILDAS to those measured by several laboratories using IRMS displays the potential of viable spectroscopic $\Delta^{17}\text{O}$ measurements in unaltered CO_2 . The measurements presented here achieve the “high-precision” 10 per meg benchmark highly sought after by the triple oxygen isotope community. Future work expanding on these results includes performing CO_2 equilibrations with VSMOW2 and SLAP2 directly on the automated sample preparation system, in the MV1 volume. This effort will allow for direct calibration of spectroscopic measurements performed by the instrument used here to the VSMOW2-SLAP2 scale without the need for assuming IRMS obtained values. The advancement in spectroscopic isotopologue abundance measurements presented in this project has the potential to revolutionize the field of triple oxygen isotope studies. Creating and introducing the methodology and physical requirements to perform these measurements using an instrument that

is more easily accessible to a broader reach of researchers opens the door for future innovation and improvement in this young but rapidly growing field.

Study	NBS18		NBS19		IAEA-603		$\Delta\Delta^{17}\text{O}$
	$\Delta^{17}\text{O}$ CO ₂	$\Delta^{17}\text{O}$ CaCO ₃	$\Delta^{17}\text{O}$ CO ₂	$\Delta^{17}\text{O}$ CaCO ₃	$\Delta^{17}\text{O}$ CO ₂	$\Delta^{17}\text{O}$ CaCO ₃	NBS18- NBS19
<i>TILDAS</i>							
This project ^a	-99	-56	-150	-125	-151	-108	69
<i>IRMS – Conversion Methods</i>							
Passey et al. (2014) ^b	-98	-58	-135	-95	-	-	37
Passey & Ji (2019) ^b	-88	-48	-143	-103	-	-	55
Wostbrock et al. (2020) ^c	-100	-48	-155	-102	-147	-100	54
Michigan IsoPaleoLab ^b	-91	-51	-142	-102	-148	-108	51
Average	-94	-51	-144	-100	-147.5	-104	49
1σ	6	5	8	4	0.7	5	8
<i>IRMS – Exchange Methods</i>							
Barkan et al. (2015) ^d	3	54	-227	-176	-	-	230
Barkan et al. (2019) ^d	-163	-112	-182	-131	-187	-136	19
Sha et al. (2020) ^d	-225	-174	-	-	-267	-216	42 ^e
Fosu et al. (2020) ^d	-119	-67	-169	-118	-	-	50
Average	-126	-75	-193	-141	-227	-176	100
1σ	96	96	30	30	57	57	114

Table 3.12: Adapted from Passey & Levin (2021). $\Delta^{17}\text{O}$ values are reported in per meg relative to the VSMOW-SLAP scale, $\theta = 0.528$. CaCO₃ values are calculated based on CO₂ values using $\theta_{\text{acid}} = 0.5230$ (Wostbrock et al., 2020), $^{18}\alpha_{\text{acid}} = 1.0103$ (25 °C reactions), $^{18}\alpha_{\text{acid}} = 1.00871$ (70 °C reactions), and $^{18}\alpha_{\text{acid}} = 1.00812$ (90 °C reactions; Kim et al., 2007), except for the CaCO₃ values for Wostbrock et al. (2020), which are directly measured. Disagreement in $\Delta^{17}\text{O}_{(\text{CaCO}_3)}$ of NBS19 reported in this project compared to other studies shown is likely linked to the relatively poor $\Delta^{17}\text{O}$ precision obtained for this standard throughout this project. $\Delta\Delta^{17}\text{O}$ NBS18-NBS19 is the difference in $\Delta^{17}\text{O}$ between NBS18 and NBS19. ^a Calculated from molecular isotopologue abundance ratios of CO₂ produced by 70 °C acid digestion. Molecular abundance ratios are assumed equal to atomic abundance ratios. ^b Reduction of CO₂ produced by 90 °C acid digestion, and CoF₃ fluorination of the resulting water. ^c Direct BrF₅ fluorination of carbonate and of CO₂ produced by 25 °C acid digestion. ^d Pt-catalyzed exchange with O₂ of CO₂ produced by 25 °C acid digestion. ^e $\Delta^{17}\text{O}$ difference between NBS18 and IAEA-603.

Chapter 4: Results & Discussion – Triple Oxygen Isotope Composition of Ratite Eggshell

Throughout this chapter, all $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values are reported in per mille (‰) on the VSMOW scale. $\Delta^{17}\text{O}$ data are given in per meg (1‰ = 1000 per meg). $\Delta^{17}\text{O}$ is calculated as:

$$\Delta^{17}\text{O} = \delta^{17}\text{O} - \lambda_{\text{ref}} * \delta^{18}\text{O}$$

where $\lambda_{\text{ref}} = 0.528$. All standard deviations are reported as 1σ . All reported sample data are corrected via eq. (2.6) (identical to eq. (3.1)) as described in the Methods Chapter. Abbreviations used frequently in this chapter include RES (ratite eggshell), MCT (Miocene Climatic Transition), and MMCO (Mid-Miocene Climatic Optimum). Where applicable, consistent color schemes are used throughout this chapter to encourage easy interpretation.

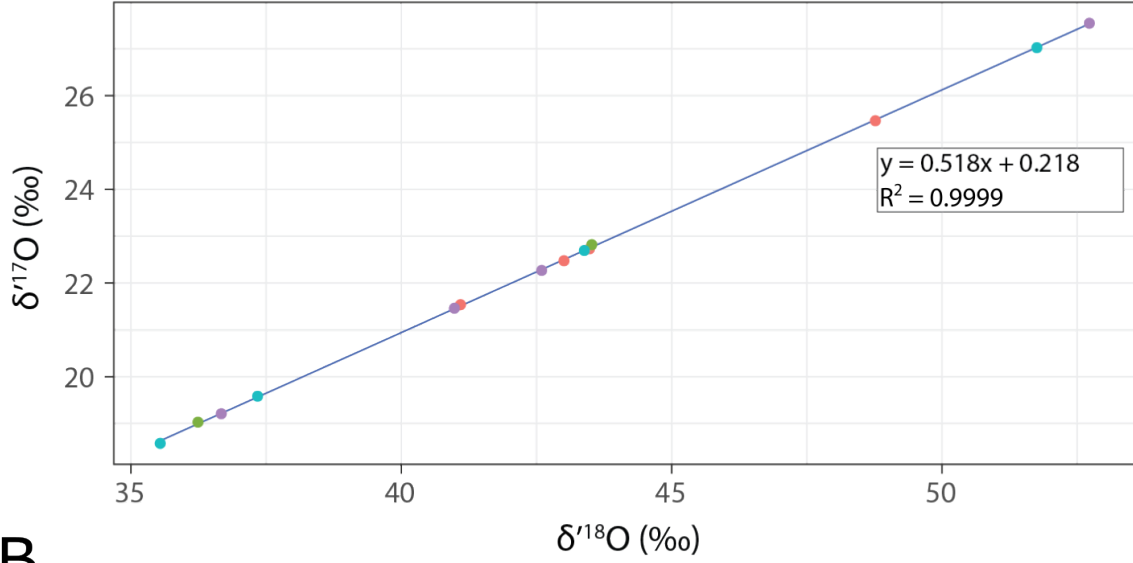
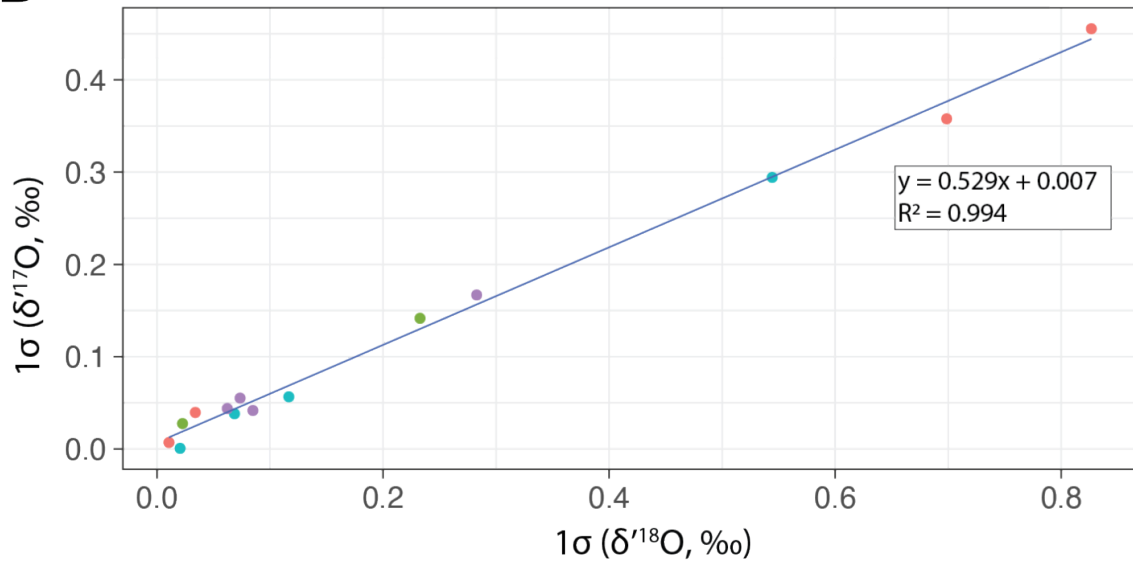
For all analyses of oxygen isotopes presented in this project, the first 3 measured aliquots of each analysis were ignored to allow the optical cell to reach thermal stability. After removal of these aliquots, outliers were identified and removed within an analysis (on a per aliquot basis) using the IQR method for outlier identification. Sample analyses were initially performed in duplicate. Some samples were analyzed in triplicate, and one in quadruplicate, when there was obvious disagreement between the first two/three analyses. Exclusion of replicate analyses (e.g., in the case a sample was run in triplicate) occurs where there were known issues during either the sample preparation or TILDAS analysis processes, or otherwise where sample results were clearly unrepresentative and/or unrealistic.

4.1) Results

Assessment of Measurement Performance & Accuracy

The sample data show near perfect correlation between $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$, as expected (Fig. 4.1A, $R^2 = 0.9999$). For all samples analyzed during this project, measurement errors between $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ were also highly correlated (Fig. 4.1B, $R^2 = 0.994$). The high degree of correlation suggests that the source of the error is instrumental and not reflective of real variation within the sample material. The strong correlation between δ' errors does not influence errors in $\Delta^{17}\text{O}$ (Table 4.1). Using samples UPMC-Ka-1-SC and UPMC-RO-1-NO as examples – it is clear that this effect works in practice, where a roughly 1 order of magnitude difference in δ' errors between samples results in nearly identical errors in $\Delta^{17}\text{O}$ (Table 4.1).

Sample replicate $\Delta^{17}\text{O}$ reproducibility ranged from 2 to 33 per meg – a range that agrees with that of the standards reported in Chapter 3. $\delta^{18}\text{O}$ reproducibility ranged from 0.011‰ to 0.827‰. This range in errors is large. However, given that 9 of the 14 samples analyzed showed $\delta^{18}\text{O}$ precision less than 0.2‰ (typically considered a very good level of reproducibility), overall $\delta^{18}\text{O}$ reproducibility was better represented by the lower end of the range (Table 4.1).

A**B**

● Quaternary ● Pliocene ● MCT ● MMCO

Figure 4.1: Correlation between **A)** measured $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ and **B)** 1σ $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ errors. Samples are colored by time period in both panels. MCT is Miocene Climatic Transition. MMCO is Mid-Miocene Climatic Optimum. Relative to the VSMOW2-SLAP2 scale.

In addition, the sample $\delta^{18}\text{O}$ data obtained in this project were compared with those of the original study from which the samples are sourced. $\delta^{18}\text{O}$ measured here show strong agreement with those determined by Ségalen et al. (2006) (Fig. 4.2, $R^2 = 0.9983$). Despite the strong agreement, there is a consistent positive offset of $\sim 2.5\text{‰}$ between the values obtained here and

those from the original study. This offset is best explained by the use of different acid digestion temperatures $\text{CO}_2\text{-CaCO}_3$ and/or acid fractionation factors. §

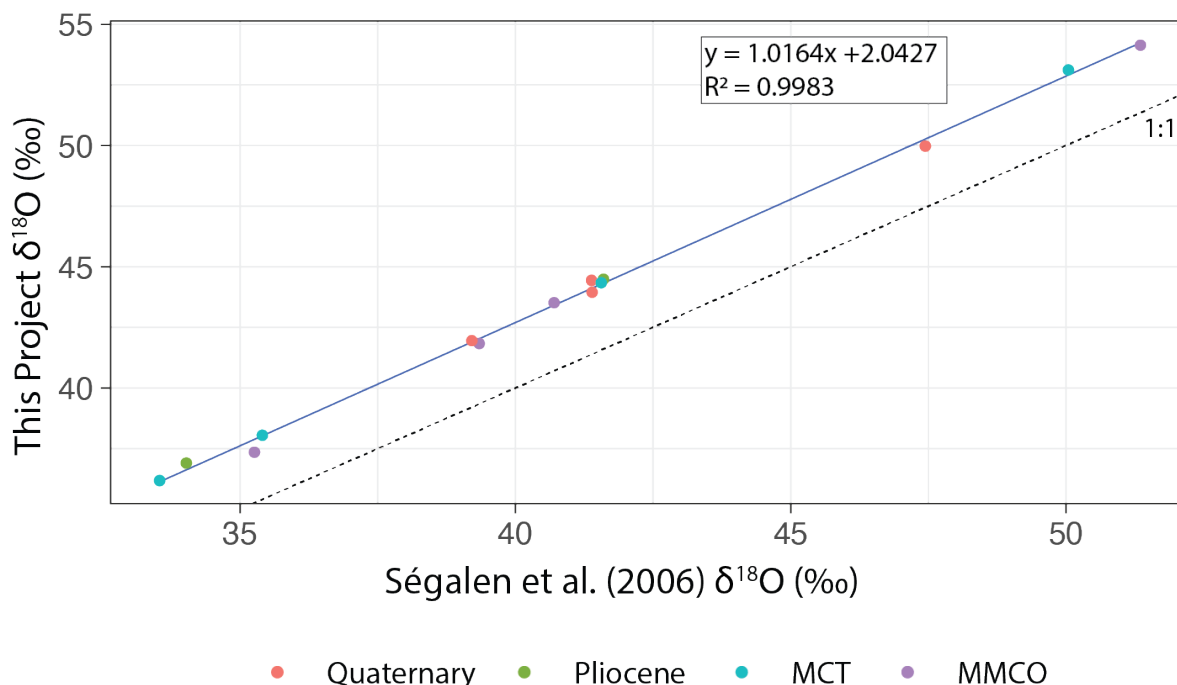


Figure 4.2: Agreement in measured $\delta^{18}\text{O}$ between values obtained by Ségalen et al. (2006) and this project. MCT is Miocene Climatic Transition. MMCO is Mid-Miocene Climatic Optimum. ~2.5‰ offset thought to be resultant lower temperature reactions and/or the use of different acid fractionation corrections, see footnote below. MCT is Miocene Climatic Transition. MMCO is Mid-Miocene Climatic Optimum. Relative to the VSMOW2-SLAP2 scale.

Sample Overview

The complete data for all samples analyzed in this project is given in Table 4.1. The slope (θ) of the line of best fit is 0.518, significantly less than the slope (λ_{ref}) of 0.528 applied to the global meteoric water line (GMWL), giving rise to the negative measured $\Delta^{17}\text{O}$ sample values (Fig. 4.1A).

Due to large disagreements between the first two replicates, samples UPMC-Ka-2-SC, UPMC-RO-1-SC, UPMC-GPP-2-SC, and UPMC-RO-1-NO were all analyzed in triplicate, each with one analysis of the 3 being excluded. These samples were excluded due to obviously high and not representative $\Delta^{17}\text{O}$ values. Excluded replicate analyses are UPMC-Ka-2-SC-1, UPMC-GPP-2-SC-2, UPMC-RO-1-SC-2, and UPMC-RO-1-NO-2. $\Delta^{17}\text{O}$ values and 1σ precision for all

§ The acid fractionation used in this project is from Kim et al. (2007). As Ségalen et al. (2006) predates the publication of this fraction correction, a different correction scheme for the sample acid digestion had to have been used. The specific correction used in the original study is not reported and has not been obtainable through personal communications. The original study also reports the use of reaction temperatures of both 70°C and 25°C , but fails to report which temperature, and associated fractionation correction, was used and applied for final data presentation. An offset of 2.5‰ is difficult to reckon, however it may be possible that the original study erred in their fractionation correction, using an equation for 25°C when the samples were actually reacted at 70°C .

replicates of these four samples are shown in Table S4.1 (Appendix). Samples UPMC-Ob-2-SD and UPMC-RO-4-DL were analyzed in triplicate and quadruplicate, respectively, with no individual analyses being excluded. Figure 4.3 shows all accepted analyses of each sample analyzed in this project.

Sample	<i>n</i>	Region	$\delta^{17}\text{O}_{\text{calcite}}$ (‰)	$\delta^{18}\text{O}_{\text{calcite}}$ (‰)	$\Delta^{17}\text{O}_{\text{calcite}}$ (per meg)
Quaternary (0-2 My)					
<i>Struthio camelus</i>					
UPMC-Ka-1-SC *	2	Central	25.464 (0.455)	48.768 (0.827)	-285 (18)
UPMC-Ka-2-SC *	2	Central	22.475 (0.040)	43.008 (0.034)	-233 (21)
UPMC-RO-1-SC *	2	Southern	22.728 (0.358)	43.479 (0.699)	-229 (11)
UPMC-GPP-2-SC *	2	Southern	21.537 (0.007)	41.096 (0.011)	-162 (2)
Pliocene (2-5 My)					
<i>Struthio daberasensis</i>					
UPMC-Ob-2-SD	3	Southern	22.818 (0.142)	43.523 (0.233)	-162 (22)
UPMC-K-3-SD *	2	Southern	19.030 (0.028)	36.239 (0.023)	-105 (15)
Miocene Climatic Transition (8-10 My)					
<i>Diamantornis laini</i>					
UPMC-AW-8-DL *	2	Central	27.024 (0.057)	51.754 (0.117)	-302 (6)
UPMC-Ts-2-DL	2	Central	18.576 (0.038)	35.542 (0.069)	-190 (3)
UPMC-RO-4-DL *	4	Southern	22.693 (0.294)	43.385 (0.544)	-214 (33)
UPMC-RO-1-DL *	2	Southern	19.584 (0.001)	37.343 (0.021)	-134 (12)
Mid-Miocene Climatic Optimum (15-16 My)					
<i>Namornis oshanai</i>					
UPMC-AW-7-NO *	2	Central	27.546 (0.044)	52.724 (0.062)	-293 (11)
UPMC-AW-9-NO *	2	Central	21.461 (0.042)	40.982 (0.085)	-178 (3)
UPMC-RO-1-NO *	2	Southern	22.268 (0.055)	42.596 (0.074)	-223 (16)
UPMC-K-1-NO *	2	Southern	19.209 (0.167)	36.670 (0.283)	-153 (17)

Table 4.1: RES sample isotopic composition. Species names are given in italics. Associated age ranges are as presented by Ségalen et al. (2006). Sample names marked with an asterisk (*) indicate samples originally analyzed for $\delta^{13}\text{C}$ by Ségalen et al. (2002). All samples were analyzed for $\delta^{18}\text{O}$ by Ségalen et al. (2006). 1σ standard deviations are given in parentheses. Relative to the VSMOW2-SLAP2 scale.

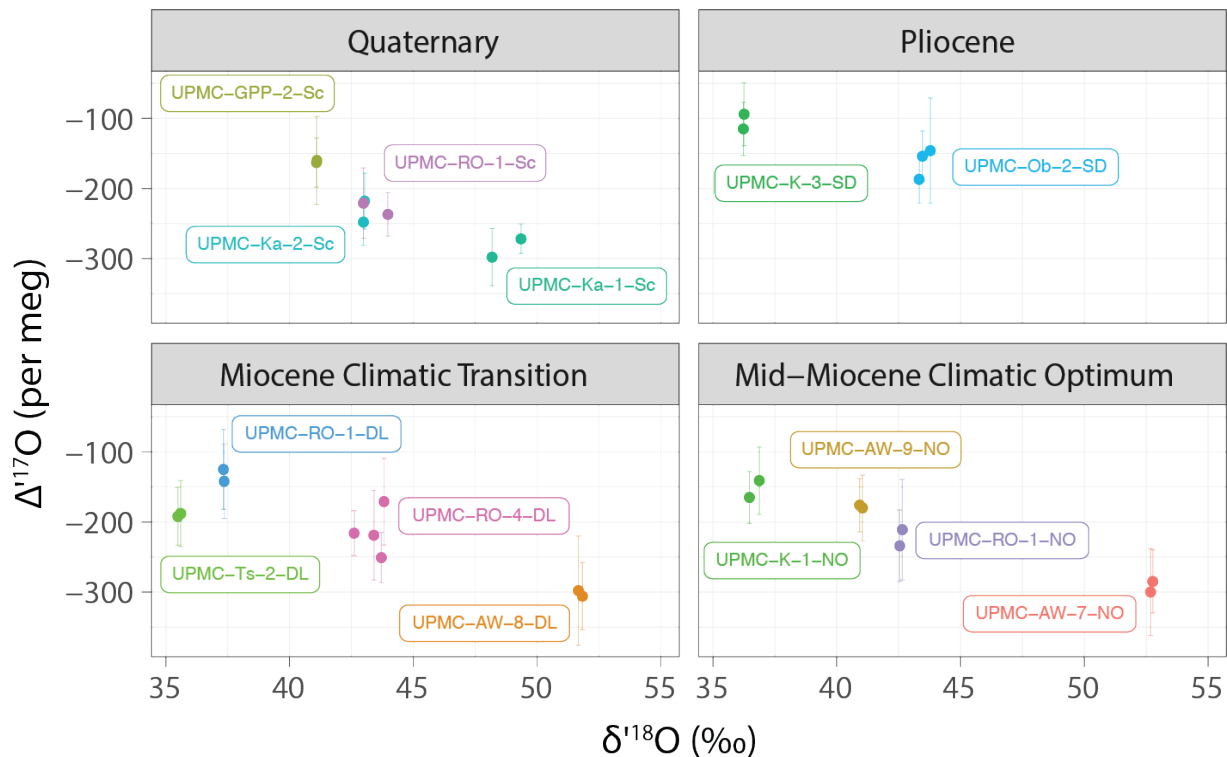


Figure 4.3: Accepted analyses of each sample. All error bars are 1σ . For all analyses, $\delta^{18}\text{O}$ 1σ error bars are smaller than plotted points. Relative to the VSMOW2-SLAP2 scale.

Because this project focuses on the extreme ends of available ratite eggshells (RES) through time, average δ' and $\Delta^{17}\text{O}$ values, and their associated standard deviations, are not necessarily representative of the local environment at the time of eggshell formation. Such averages are better used to constrain RES isotopic composition variability within a given time period and/or region. For this project, isotopic composition variations through time are interpreted by looking at the ranges between the sample groups, shown in Table 4.2. Overall, sample $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values are moderately correlated ($R^2 = 0.788$, Appendix Fig. S4.2). The Quaternary samples show the least amount of variation in both $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ for each time period (both on the whole and within each regional subgrouping) suggesting relative stability of the various influences over RES isotopic composition, which are expanded upon in depth below. Notably, the southern Namib samples show much less variability in any given time period than central Namib samples.

	Range $\delta^{17}\text{O}$ (‰)	Range $\delta^{18}\text{O}$ (‰)	Range $\Delta^{17}\text{O}$ (per meg)
Quaternary			
<i>Central</i>	2.989	5.760	52
<i>Southern</i>	1.191	2.384	67
Pliocene			
<i>Southern</i>	3.788	7.284	57
Miocene Climatic Transition			
<i>Central</i>	8.448	16.212	112
<i>Southern</i>	3.110	6.043	80
Mid-Miocene Climatic Optimum			
<i>Central</i>	6.086	11.742	115
<i>Southern</i>	3.059	5.926	70

Table 4.2: Range in calcite corrected $\delta^{17}\text{O}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ of each time period – region combination, where $n = 2$ for all. Ranges describe differences between final sample values obtained after replicate analyses.

Central & Southern Namib Overview

The data show two trends between the central and southern Namib. On the whole, ratite eggshell samples from the central Namib are more positive in $\delta^{18}\text{O}$ and more negative in $\Delta^{17}\text{O}$ than the southern Namib samples. This is consistent with the previous $\delta^{18}\text{O}$ study of these samples (Ségalen et al., 2006) and follows the expected $\delta^{18}\text{O}$ - $\Delta^{17}\text{O}$ relationship for arid environments. Altogether, the central Namib samples have an average $\delta^{18}\text{O}$ of $45.463 \pm 6.749\text{‰}$ and $\Delta^{17}\text{O}$ of -247 ± 54 per meg. Southern Namib samples have $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values of $40.541 \pm 3.248\text{‰}$ and -173 ± 45 per meg, respectively. The $\delta^{18}\text{O}$ range is 17.183‰ and 7.284‰ in the central and southern Namib, respectively. Data trends for the central and southern Namib samples are shown on common axes in Figure 4.4. This figure highlights the extent of both the reduced $\delta^{18}\text{O}$ range and general positive shift in $\Delta^{17}\text{O}$ of the southern Namib samples compared to the central.

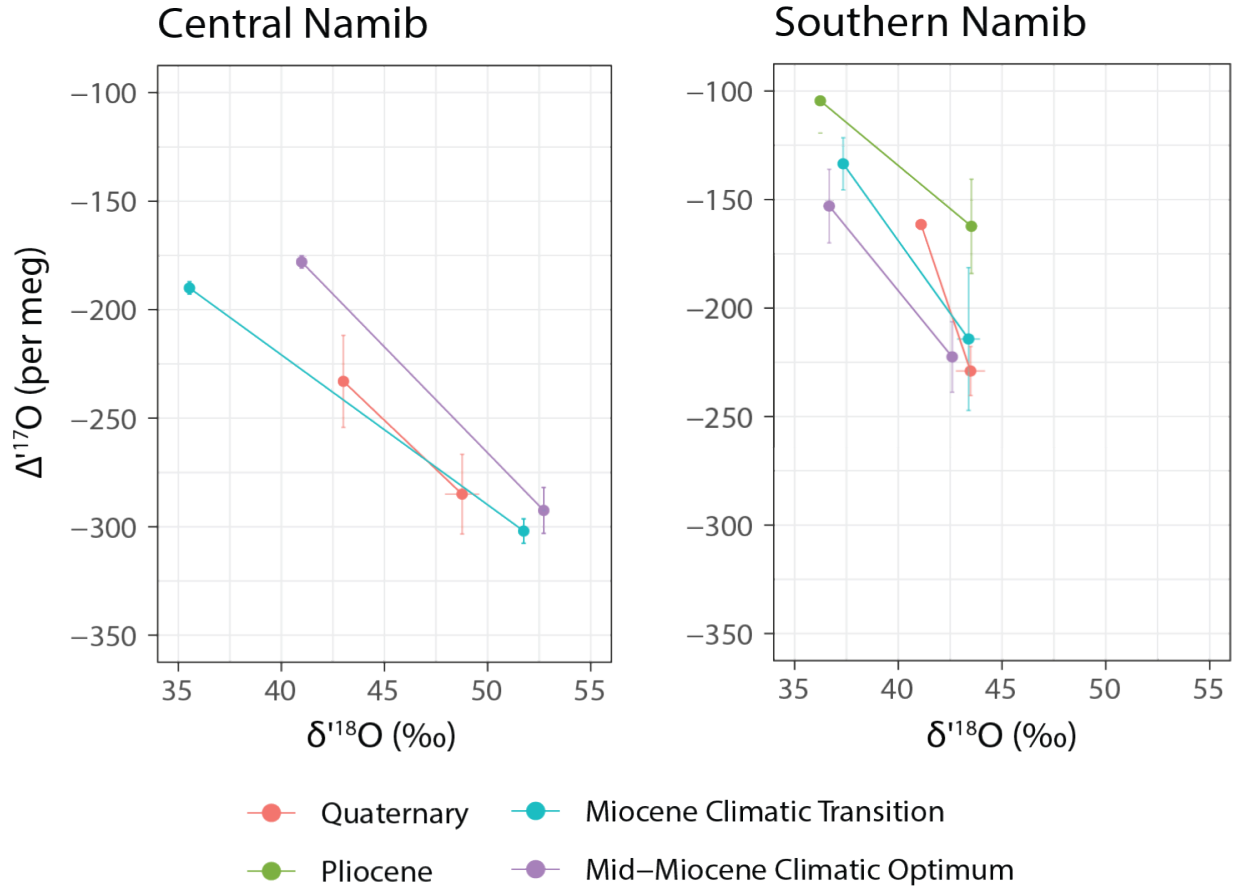


Figure 4.4: $\delta^{18}\text{O}$ – $\Delta^{17}\text{O}$ trend comparison between central and southern Namib samples. Data points are the composition of each RES sample as presented in Table 4.1. In general, central samples plot along a shallower slope, encompass a wider $\delta^{18}\text{O}$ range, and are more negative in $\Delta^{17}\text{O}$, than southern samples. Coloring between the two panels is consistent. Relative to the VSMOW2-SLAP2 scale.

Interestingly, this large range difference is not equally reflected in $\Delta^{17}\text{O}$ values, where ranges for the central and southern samples are 124 and 125 per meg, respectively. This is, however, at least partially due to sampling inequality. A significant part of the $\Delta^{17}\text{O}$ range found in the southern Namib samples comes from the very high $\Delta^{17}\text{O}$ of a Pliocene eggshell (UPMC-K-3-SD). Despite having very similar $\delta^{18}\text{O}$ as other low- $\delta^{18}\text{O}$ samples, the $\Delta^{17}\text{O}$ of -105 ± 15 per meg is by far the highest (least negative) value of all samples in this project and adds 29 per meg to the southern samples' $\Delta^{17}\text{O}$ range compared to the range calculated from the next least negative $\Delta^{17}\text{O}$ sample (UPMC-RO-1-DL) where $\Delta^{17}\text{O} = -134 \pm 12$ per meg. If both of the Pliocene samples are ignored, the range of $\Delta^{17}\text{O}$ of all other southern Namib samples becomes 96 per meg, and the $\delta^{18}\text{O}$ range is 6.809‰.

Within each applicable time period studied, central Namib samples have a wider range of $\delta^{18}\text{O}$ values than their southern Namib counterparts (Fig. 4.5). The range in $\delta^{18}\text{O}$ of southern Namib samples is also more consistent between time periods, compared to ranges in central samples that vary significantly through time, suggesting more stable local environmental conditions.

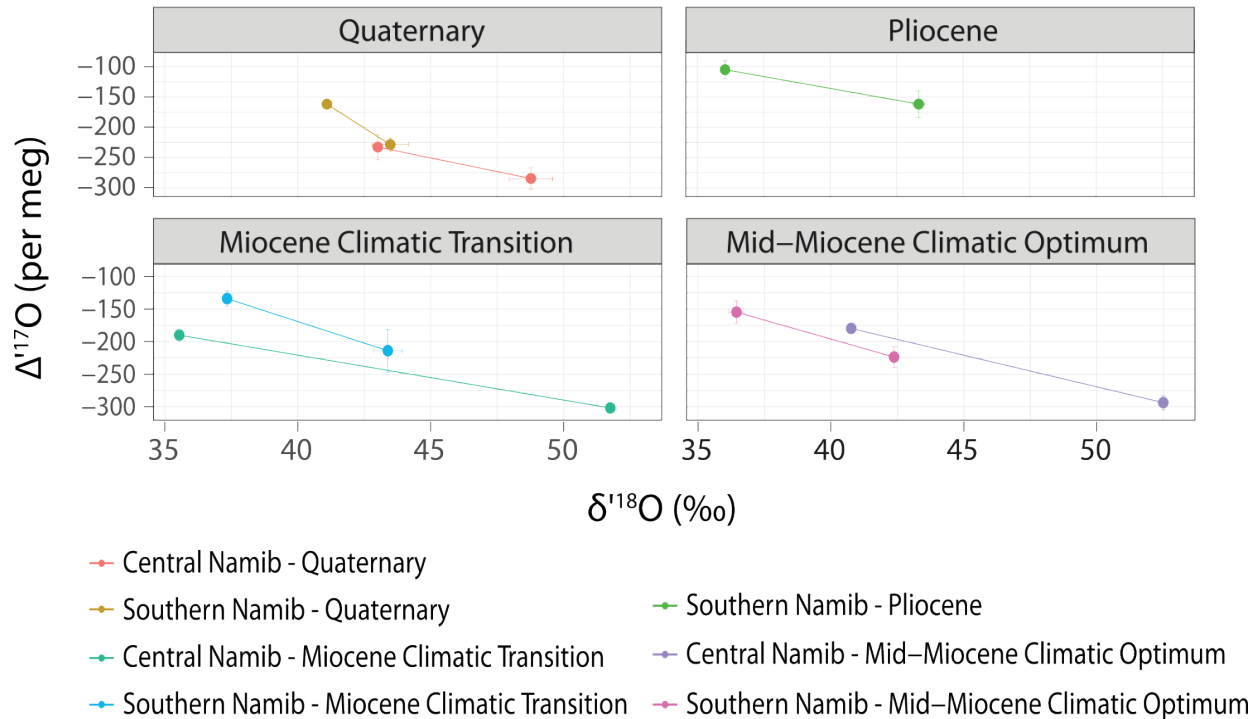


Figure 4.5: Sample ranges within each time period. Data points are the composition of each RES sample as presented in Table 4.1. Notably, southern Namib samples always lie along a steeper slope than central Namib samples within a given time period where comparison is possible. Common axes for all panels. Relative to the VSMOW2-SLAP2 scale.

Central Namib $\delta^{18}\text{O}$

The range of $\delta^{18}\text{O}$ values within a given time period changes through time. During the Mid-Miocene Climatic Optimum (MMCO), a difference of 11.742‰ is observed between the two samples analyzed. This range increases to 16.212‰ in the Miocene Climatic Transition (MCT) and is reduced to 5.760‰ in the Quaternary.

Maximum $\delta^{18}\text{O}$ values for each time period are $52.724 \pm 0.062\text{‰}$ during the MMCO, $51.754 \pm 0.117\text{‰}$ during the MCT, and $48.768 \pm 0.827\text{‰}$ during the Quaternary. The range of maximums across these time periods is 3.975‰. Minimum $\delta^{18}\text{O}$ values have a much larger range of 7.467‰. Varying from $40.982 \pm 0.085\text{‰}$ in the MMCO, to $35.542 \pm 0.069\text{‰}$ during the MCT, and $43.008 \pm 0.034\text{‰}$ during the Quaternary, the entirety of this range comes from the difference between the MCT and Quaternary low $\delta^{18}\text{O}$ samples.

Southern Namib $\delta^{18}\text{O}$

A much smaller and more stable range in $\delta^{18}\text{O}$ values is overserved through time in the southern Namib. During the MMCO, a $\delta^{18}\text{O}$ difference of 5.926‰ is observed between the two samples analyzed, a range which remains effectively unchanged through the MCT where a difference of 6.043‰ between maximum and minimum values are observed. This range then increases to 7.284‰ in the Pliocene before the range shrinks to 2.384‰ in the Quaternary.

The southern Namib records maximum $\delta^{18}\text{O}$ values that are nearly constant across all time periods sampled in this project. Maximum $\delta^{18}\text{O}$ is $42.596 \pm 0.074\text{‰}$ during the MMCO, 43.385

$\pm 0.544\text{‰}$ during the MCT, $43.523 \pm 0.233\text{‰}$ during the Pliocene, and $43.479 \pm 0.699\text{‰}$ during the Quaternary. The range of maximums of across these time periods is 0.927‰ , a fourth of that observed in the central Namib samples.

Minimum $\delta^{18}\text{O}$ varies from $36.670 \pm 0.283\text{‰}$ in the MMCO, to $37.343 \pm 0.021\text{‰}$ during the MCT, $36.239 \pm 0.023\text{‰}$ in the Pliocene, and $41.096 \pm 0.011\text{‰}$ during the Quaternary. Minimum $\delta^{18}\text{O}$ values have a much larger range of 4.857‰ , however most of this range stems from the higher $\delta^{18}\text{O}$ of the Quaternary samples, suggesting more constant environmental conditions from the MMCO through the Pliocene.

Central Namib $\Delta^{17}\text{O}$

The variable range of $\delta^{18}\text{O}$ values observed in the central Namib is not similarly represented in RES $\Delta^{17}\text{O}$. The MMCO samples record $\Delta^{17}\text{O}$ values of -293 ± 11 and -178 ± 3 per meg, a range of 115 per meg. A similar range, 112 per meg, is observed in the MCT samples, where $\Delta^{17}\text{O}$ values are -302 ± 6 and -190 ± 3 per meg. This range is halved in the Quaternary samples to 52 per meg, where sample $\Delta^{17}\text{O}$ values are -285 ± 18 per meg and -233 ± 21 per meg.

Notably, the lowest (most negative) $\Delta^{17}\text{O}$ values for each time period represented in the central Namib are all within error of each other, grouping together in a range of 17 per meg (from -302 to -285) suggesting little variation in the main controls over retained $\Delta^{17}\text{O}$ values (e.g., aridity). The most positive $\Delta^{17}\text{O}$ of each time period, however, do not overlap within error between any two periods. Such samples from the MMCO and MCT do, however, have similar $\Delta^{17}\text{O}$ values of 178 ± 3 and -190 ± 3 per meg, respectively, again suggestive of stable local environmental conditions across these time periods. The high $\Delta^{17}\text{O}$ sample from the Quaternary stands out as much more negative than these other samples, in agreement with expectations from its higher $\delta^{18}\text{O}$.

Southern Namib $\Delta^{17}\text{O}$

The decreased range of $\delta^{18}\text{O}$ values observed in the southern Namib is similarly represented in RES $\Delta^{17}\text{O}$ variability. The MMCO samples record $\Delta^{17}\text{O}$ values of -223 ± 16 and -153 ± 17 per meg, a range of 70 per meg. A range of 81 per meg is observed in the MCT samples, where $\Delta^{17}\text{O}$ values are -214 ± 33 and -134 ± 12 per meg. Samples from the Pliocene show $\Delta^{17}\text{O}$ range of southern samples of 58 per meg, where sample values are -162 ± 22 and -105 ± 15 per meg. The Quaternary samples range 68 per meg, where sample $\Delta^{17}\text{O}$ values are -229 ± 11 per meg and -162 ± 2 per meg.

Similar to the central Namib samples, the most negative $\Delta^{17}\text{O}$ values for the MMCO, MCT, and Quaternary are all within error of each other, clustering tightly together within a range of ~ 15 per meg in $\Delta^{17}\text{O}$ (and $\sim 1\text{‰}$ in $\delta^{18}\text{O}$). The most negative sample from the Pliocene (UPMC-Ob-2-SD), however, stands alone in $\Delta^{17}\text{O}$ (-162 ± 22 per meg) despite having an extremely similar $\delta^{18}\text{O}$ value ($43.534 \pm 0.233\text{‰}$) to the other high $\delta^{18}\text{O}$ samples from the southern Namib. The most positive $\Delta^{17}\text{O}$ samples from the MMCO and MCT (UPMC-K-1-NO and UPMC-RO-1-DL, respectively) share a slight potential overlap between -136 and -146 per meg. Due to its excellent precision, the high $\Delta^{17}\text{O}$ Quaternary sample (UPMC-GPP-2-SC) overlaps entirely within the range of UPMC-K-1-NO. The high $\Delta^{17}\text{O}$ Pliocene sample (UPMC-K-3-SD) stands out with

$\Delta^{17}\text{O}$ of -105 ± 15 per meg, not overlapping with any other sample irrespective of region or time period.

UPMC-RO-4-DL has a large $\Delta^{17}\text{O}$ error ($1\sigma = 33$ per meg) despite being run in quadruplicate. While the 4 separate analyses are not in particularly good agreement with each other, it is difficult to find reason to exclude any one analysis. Encouragingly, the agreement between analyses of this sample improved with subsequent analyses such that the last two analyses had near perfect agreement (-216 ± 32 and -219 ± 64 per meg, respectively), both of which agree well with the average value of all 4 analyses (-214 per meg), lending confidence to the final obtained value.

Ignoring the Pliocene samples (to provide a more direct comparison to the central Namib, where the Pliocene is not represented) results in significantly decreased ranges of each time period's maximum and minimum $\Delta^{17}\text{O}$ values (Table 4.3). In doing so, it is observed that, for all other time periods, the range of maximum $\Delta^{17}\text{O}$ values is nearly identical between the southern and central Namib. An opposite effect occurs for the range of minimum $\Delta^{17}\text{O}$ values, where the range is approximately halved. This is not to say that the central and southern Namib were environmentally similar during all other time periods studied in this project. It does, however, suggest that the Pliocene Namib may have been environmentally unique, or, perhaps, large scale changes in either global scale (e.g., oceanic circulation patterns) or local environmental (e.g., vegetation type) conditions were underway.

<i>Including Pliocene</i>	Range Max $\Delta^{17}\text{O}$	Range Min $\Delta^{17}\text{O}$	<i>Excluding Pliocene</i>	Range Max $\Delta^{17}\text{O}$	Range Min $\Delta^{17}\text{O}$
<i>Central</i>	17	55	<i>Central</i>	17	55
<i>Southern</i>	67	57	<i>Southern</i>	15	29

Table 4.3: Effect of Pliocene sampling inequality on ranges in $\Delta^{17}\text{O}$ between central and southern Namib samples, highlighting the importance of Pliocene central Namib samples to be acquired for future research.

4.2) Discussion

Comparison of Body Water Composition with Previously Published Studies

To allow for meaningful comparison between the RES data obtained in this project and previously published ratite eggshell data, correction from eggshell calcite to animal body water isotopic composition is necessary. Table 4.4 shows the RES data presented in this project corrected to body water $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values and includes the data of the 4 modern wild ostrich from Passey et al. (2014).

Sample	Region	$\delta^{18}\text{O}_{\text{bw}}$ (‰) ^a	$\Delta^{17}\text{O}_{\text{bw}}$ (‰) ^b	$\Delta^{17}\text{O}_{\text{bw}}$ (per meg)
Quaternary (0-2 My)				
<i>Struthio camelus</i>				
UPMC-Ka-1-SC	Central	11.472	-0.154	-154
UPMC-Ka-2-SC	Central	5.712	-0.102	-102
UPMC-RO-1-SC	Southern	6.183	-0.098	-98
UPMC-GPP-2-SC	Southern	3.800	-0.031	-31
Pliocene (2-5 My)				
<i>Struthio daberasensis</i>				
UPMC-Ob-2-SD	Southern	6.227	-0.032	-32
UPMC-K-3-SD	Southern	-1.057	0.026	26
Miocene Climatic Transition (8-10 My)				
<i>Diamantornis laini</i>				
UPMC-AW-8-DL	Central	14.458	-0.171	-171
UPMC-Ts-2-DL	Central	-1.754	-0.059	-59
UPMC-RO-4-DL	Southern	6.089	-0.084	-84
UPMC-RO-1-DL	Southern	0.047	-0.003	-3
Mid-Miocene Climatic Optimum (15-16 My)				
<i>Namornis oshanai</i>				
UPMC-AW-7-NO	Central	15.428	-0.162	-162
UPMC-AW-9-NO	Central	3.686	-0.047	-47
UPMC-RO-1-NO	Southern	5.300	-0.092	-92
UPMC-K-1-NO	Southern	-0.626	-0.022	-22
Passey et al. (2014) (Modern)^c				
<i>Struthio camelus</i>				
GON04-22-1.1	Gona, Ethiopia	7.696	-0.094	-94
ET05-AWSH-23	Awasy, Ethiopia	8.751	-0.113	-113
OLGO4M-11	Ologesaile, Kenya	6.838	-0.131	-131
SA13M-007	Elandsfontein, South Africa	9.880	-0.103	-103

Table 4.4: Sample data corrected to reflect animal body water composition in accordance with Passey et al. (2014, Table 3). ^a Calculated using $^{18}\alpha_{(\text{O}_2/\text{CaCO}_3)-(\text{O}_2/\text{H}_2\text{O})} = 1.0380$. ^b Calculated using $\Delta^{17}\text{O}_{(\text{H}_2\text{O})} = \Delta^{17}\text{O}_{(\text{O}_2/\text{CaCO}_3)} + 1000\ln\alpha_{(\text{O}_2/\text{CaCO}_3)-(\text{O}_2/\text{H}_2\text{O})} * [0.528 - \lambda_{(\text{O}_2/\text{CaCO}_3)-(\text{O}_2/\text{H}_2\text{O})}]$, where $\lambda_{(\text{O}_2/\text{CaCO}_3)-(\text{O}_2/\text{H}_2\text{O})} = 0.5245$. ^c Values as provided in Passey et al. (2014, Table 3). All relative to the VSMOW2-SLAP2 scale.

In Figure 4.6, the body water data are shown plotted overlaying Fig. 10B of Passey & Levin (2021), which shows the data of Passey et al. (2014) in the context of the predictions of a triple oxygen isotope enabled version of the Kohn (1996) body water model for three end member physiologies. Relevant to this project is the “max evap” (maximum evaporation) model which describes animals that get most of their water intake through leaf consumption. “Max evap” model animals are very water efficient, drinking little to no water, and include ostrich, deer, and giraffe (Passey & Levin, 2021). As shown, much of the data presented in this project fall outside the bounds provided by the limited number of Passey et al. (2014) samples. Encouragingly, 3 of the 4 Quaternary samples fall within or near this bounding, suggesting that the sampling and measurements performed in this project are accurate and behave as would be expected from near-modern samples.

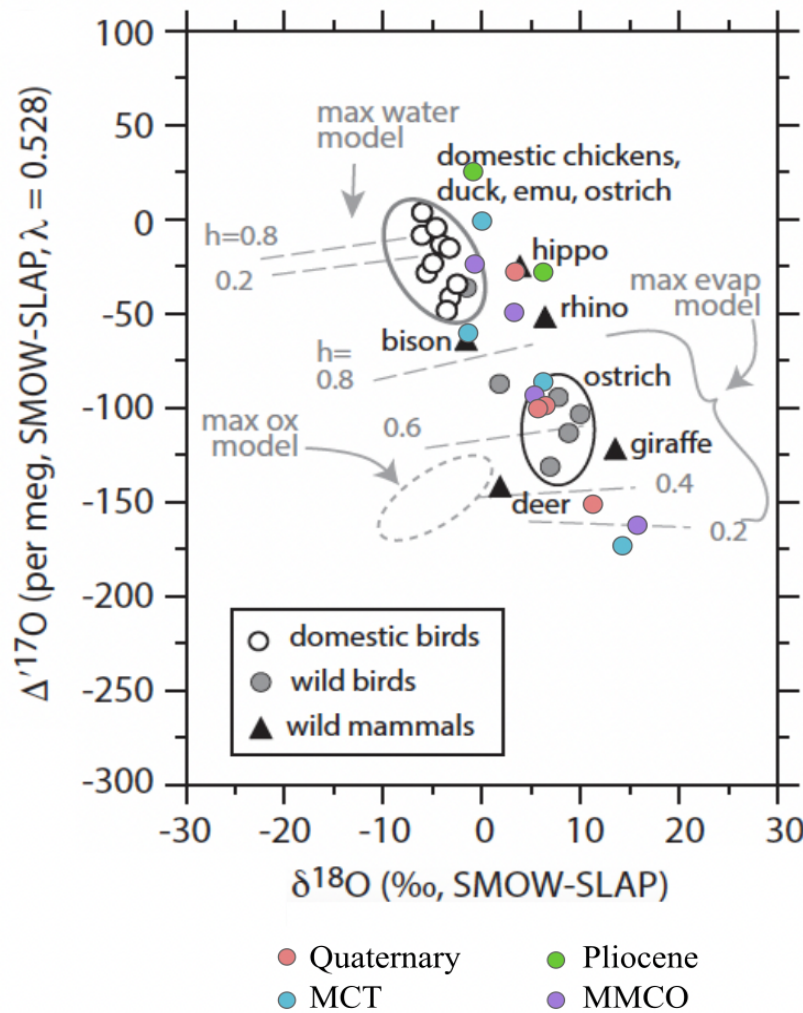


Figure 4.6: Adopted from Passey & Levin (2021, Figure 10B). All data shown has been corrected to body water composition. Colored data points are from this project. White, grey, and black data points are all modern samples from Passey et al. (2014) and are as labelled in the internal legend. Light grey lines and text represent the 3 model physiologies encompassed in the triple oxygen isotope enabled version of the Kohn (1996) body water model. Ostrich, and ratite in general, are examples of “max evap” animals. MCT is Miocene Climatic Transition. MMCO is Mid-Miocene Climatic Optimum. Relative to the VSMOW2-SLAP2 scale.

Also encouraging is the observation that, on the whole, the samples from this project plot along a steeper slope than the relationship that connects the “max water” model (i.e., much of body water comes from drinking water) with the “max evap” model. This implies that the $\delta^{18}\text{O} - \Delta^{17}\text{O}$ relationship behaves consistently with expectations through time and across a wide range of real world sample isotopic compositions. Clearly, much of the data do not fall within the Passey et al. (2014) sample bounding, or even within the predictions of the “max evap” model entirely, including one of the Quaternary samples. As a matter of fact, many data points land near or within the bounding of domestic birds described by the “max water” model. Of these 7 samples, 2 are from the central Namib and 5 the southern Namib. The near-“max water” values could be due to one or several different factors: (1) wetter environmental conditions at the time of eggshell formation; (2) changes in moisture source composition; (3) changes in atmospheric isotopic composition; and (4) slightly different behavior and/or water efficiencies between ratite species (e.g., varying degrees of nonobligate drinking, limited evolution of water conservation adaptations).

Environmental context

To place the samples into environmental context, a slightly modified version of the triple oxygen isotope animal body water model by Ben Passey and Huanting Hu (Hu, 2016) was used. The object of this exercise was to identify potential combinations of meteoric water composition, relative humidity, and $\Delta^{17}\text{O}$ of atmospheric O_2 ($\Delta^{17}\text{O}(\text{O}_2)$) which would allow for the RES isotopic compositions obtained in this project. Today, the Namib Desert experiences two, spatially separated, rainfall regimes. The central Namib is under the influence of a summer rainfall system characterized by hot, relatively humid summers and cool, lesser humid winters. In the summer rainfall regime, the available moisture originates in tropical systems to the east, with vapor sources reaching as far as the Indian Ocean (Ségalen et al., 2006; Wanke et al., 2018)). The opposite is true for the southern Namib; where the winter rainfall regime bringing cool, humid winters and hot, dry summers dominates. Southern Namib source waters derive from westerly winds off the Atlantic, which also drive the Benguela current from the Southern Ocean up the west coast of southern Africa.

Model Description & Inputs

$\delta^{18}\text{O}(\text{O}_2)$ and $\Delta^{17}\text{O}$ of meteoric water ($\Delta^{17}\text{O}_{\text{mw}}$) were kept constant at 23.881‰ and 0.020‰, respectively, throughout all model runs. Each of the model conditions were run across a range of relative humidity (from 10-90%) to capture a swath of potential environmental states.

The model variables that exerted the largest influence over resulting RES $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ value were $\delta^{18}\text{O}$ of meteoric water ($\delta^{18}\text{O}_{\text{mw}}$), $\Delta^{17}\text{O}(\text{O}_2)$, and the ratio of ingested leaves relative to the total vegetation ingested by the animal. To first order, the dominant downstream effects of each of these variables were: $\delta^{18}\text{O}$ of meteoric water determines the initial isotopic starting point from which subsequent fractionating processes exert their influence, thereby determining the possible general range of eggshell $\delta^{18}\text{O}$ values; $\Delta^{17}\text{O}(\text{O}_2)$ dominates the scaling of, and sets an effective minimum bound on, eggshell $\Delta^{17}\text{O}$; leaf ratio determines the influx of evaporatively enriched leaf water (~57% of body water oxygen contribution; Niespolo et al., 2020) and exerts a substantial “stretching” effect on eggshell $\delta^{18}\text{O}$, with relatively little (single-digit per meg) influence on $\Delta^{17}\text{O}$.

$\Delta^{17}\text{O}(\text{O}_2)$ is critically dependent on both pCO_2 and gross primary productivity (GPP; Young et al., 2014). Therefore, variation in $\Delta^{17}\text{O}(\text{O}_2)$ through time can arise due to changes in atmospheric pCO_2 , GPP, or some combination thereof. For example, a -70 per meg shift in $\Delta^{17}\text{O}(\text{O}_2)$ could be the result of an increase of ~ 150 ppm CO_2 or a decrease in GPP to $\sim 65\%$ of present-day levels (Fig. 4.7). Alternatively, the same shift could be caused by a ~ 50 ppm CO_2 increase and a decrease in GPP to $\sim 75\%$ present-day, although this combination of factors seems unlikely. Both pCO_2 and GPP are assumed to be global in scale, eliminating the possibility of localized variations in productivity and, consequentially, $\Delta^{17}\text{O}(\text{O}_2)$.

The results of these model runs are best interpreted alongside several existing models and $\Delta^{17}\text{O}(\text{O}_2) - \text{pCO}_2$ relationships. Specifically, the atmospheric $\Delta^{17}\text{O}$ model of Young et al. (2014), existing pCO_2 estimates for the Miocene (see review by Steinthorsdottir et al., 2021) and Plio-Pleistocene (Bartoli et al., 2011), and the $\Delta^{17}\text{O}(\text{O}_2) - \text{pCO}_2 - \Delta^{17}\text{O}_{\text{bw}}$ relationship described in Passey & Levin (2021, Fig. 4.11), itself an extension of the Cao & Bao (2013) $\text{pCO}_2 - \Delta^{17}\text{O}$ relationship.

We will first borrow Young et al. (2014) Fig. 4.7 which shows modelled $\Delta^{17}\text{O}(\text{O}_2)$ as a function of % present-day GPP and pCO_2 (Fig. 4.7). At present, due to the $\sim 1000\text{yr}$ residence time of atmospheric O_2 , $\Delta^{17}\text{O}(\text{O}_2)$ reflects a pre-industrial atmosphere (Passey & Levin, 2021). This is represented by the bold isopleth at 294 ppm CO_2 in Fig. 4.7.

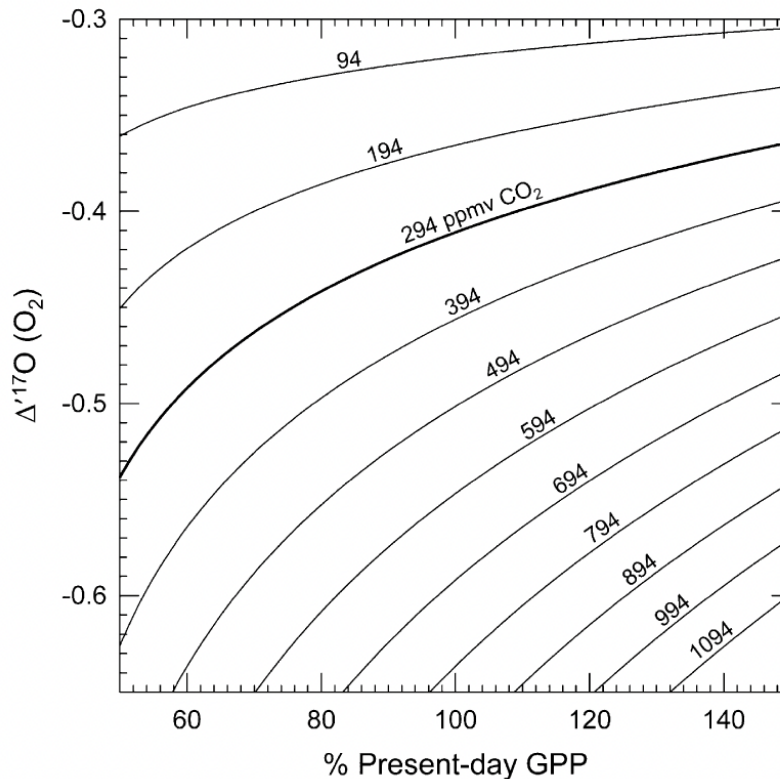


Figure 4.7: From Young et al. (2014). Calculated effects of atmospheric pCO_2 and % present-day GPP on $\Delta^{17}\text{O}(\text{O}_2)$. $\Delta^{17}\text{O}(\text{O}_2)$ on the y-axis is given in per mille ($-0.5\text{‰} = -500$ per meg). Net primary production and pO_2 are constant at present-day values.

For all model runs $\delta^{18}\text{O}_{\text{mw}}$ was held constant at -2‰, slightly more positive than the present-day rainy season values associated with the highly arid Etosha Pan in the far northern Namib where $\delta^{18}\text{O}_{\text{mw}}$ is typically ~ -4 to -8 ‰ (Wanke et al., 2018). $\Delta^{17}\text{O}(\text{O}_2)$ was assigned assuming GPP equal to present-day levels and pCO_2 representative of each time period in accordance with Fig. 4.7. Most recent reconstructions of early Miocene pCO_2 converge on values ranging between 400 and 600 ppm (Steinthorsdottir et al., 2021). As such, for assessment of the MMCO (15-16 My) samples the model was run at $\Delta^{17}\text{O}(\text{O}_2)$ representative of both pCO_2 of 400 and 600 ppm ($\Delta^{17}\text{O}(\text{O}_2) = -0.460$ ‰ and -0.550 ‰, respectively). pCO_2 for the MCT (8-10 My) samples is thought to be ~ 350 ppm (e.g., Franks et al., 2014; Kürschner et al., 2008, 1996; Wang et al., 2015), equating to $\Delta^{17}\text{O}(\text{O}_2)$ of -0.430 ‰. Pliocene pCO_2 is thought to have generally decreased while fluctuating between 250-400 ppm (Bartoli et al., 2011), thus a $\Delta^{17}\text{O}(\text{O}_2)$ of -0.410 ‰ reflective of $\text{pCO}_2 = 300$ ppm was chosen to assess the 2-5 My samples. Quaternary samples (0-2 My) are represented by pCO_2 of 280 ppm with equivalent $\Delta^{17}\text{O}(\text{O}_2)$ of -0.400 ‰. Assumed pCO_2 and corresponding $\Delta^{17}\text{O}(\text{O}_2)$ are summarized in Table 4.5, and sample values overlaying model predictions are shown in Fig. 4.8.

Time Period	pCO_2 (ppm)	$\Delta^{17}\text{O}(\text{O}_2)$ (‰)	$\Delta^{17}\text{O}(\text{O}_2)$ (per meg)
Quaternary			
<i>0-2 My</i>	280	-0.400	-400
Pliocene			
<i>2-5 My</i>	300	-0.410	-410
Miocene Climatic Transition			
<i>8-10 My</i>	350	-0.430	-430
Mid-Miocene Climatic Optimum			
<i>15-16 My</i>	400	-0.460	-460
	600	-0.550	-550

Table 4.5: Assumed pCO_2 and corresponding $\Delta^{17}\text{O}(\text{O}_2)$ (as estimated from Fig. 4.7) for each time period. $\Delta^{17}\text{O}(\text{O}_2)$ (‰) is the only value put directly into the model. For all model runs, $\delta^{18}\text{O}_{\text{mw}}$ was -2‰, relative to the VSMOW2-SLAP2 scale.

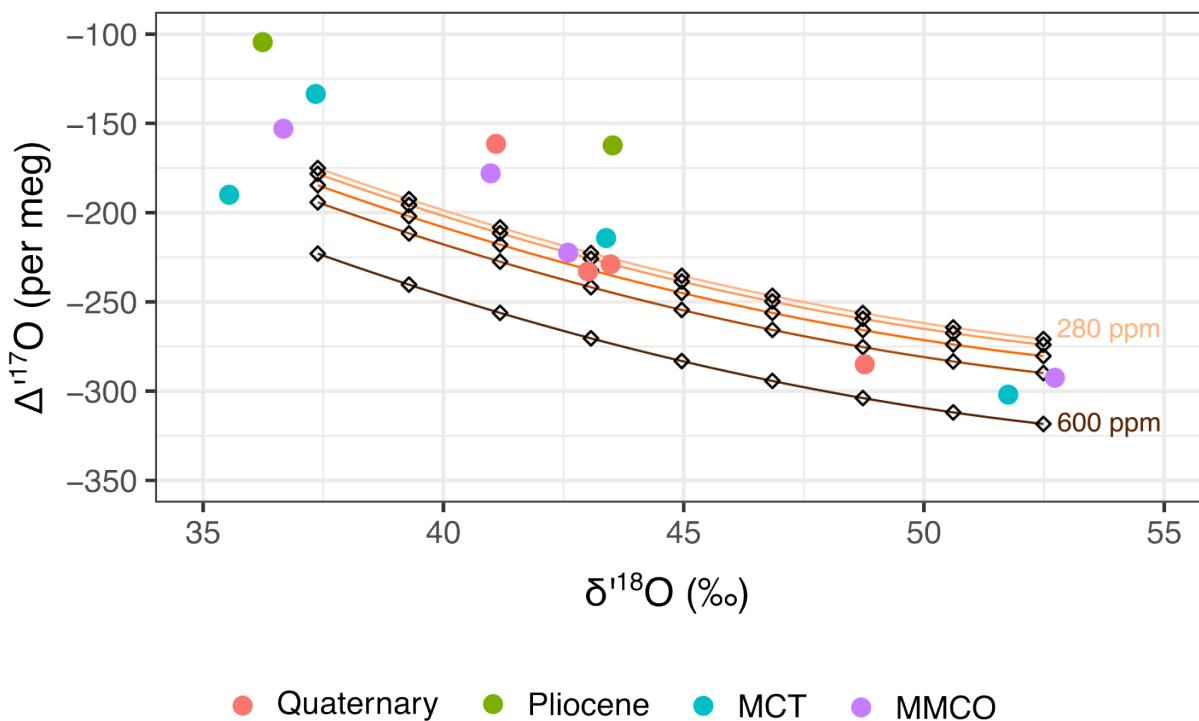


Figure 4.8: Sample data and modelled RES $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ with model inputs as described. The curves represent the various pCO_2 concentrations. Black diamonds mark relative humidity at 10% intervals, from 90% furthest left to 10% furthest right. In general, high- $\delta^{18}\text{O}$ – low- $\Delta^{17}\text{O}$ data are better recreated by the model than low- $\delta^{18}\text{O}$ – high- $\Delta^{17}\text{O}$ data. MCT is Miocene Climatic Transition. MMCO is Mid-Miocene Climatic Optimum. Relative to the VSMOW2-SLAP2 scale.

Comparison Between Sample and Modelled RES Values

Central Namib

The high- $\delta^{18}\text{O}$, low- $\Delta^{17}\text{O}$ samples from the central Namib suggest times of extreme aridity, with little evidence of deviation from such conditions through time. Immediately standing out is the extreme range of RES $\delta^{18}\text{O}$ against modelled conditions as described above. Using the leaf water model currently incorporated (combining aspects of Roden & Ehleringer, 1999 and Roden et al., 2000), it was difficult to recreate the full range of $\delta^{18}\text{O}$ observed for the central Namib samples. Because of this, the leaf ratio was kept constant throughout all models at 0.9 (i.e., 90% of ingested vegetation is leaves), a high but not unrealistic assumption. This shortcoming could potentially be alleviated by incorporating a more recent and detailed leaf water model (see Cernusak et al., 2016) which may better capture plant/leaf water fractionating processes. Central Namib samples are shown in relation to the modelled expectations in Fig. 4.9.

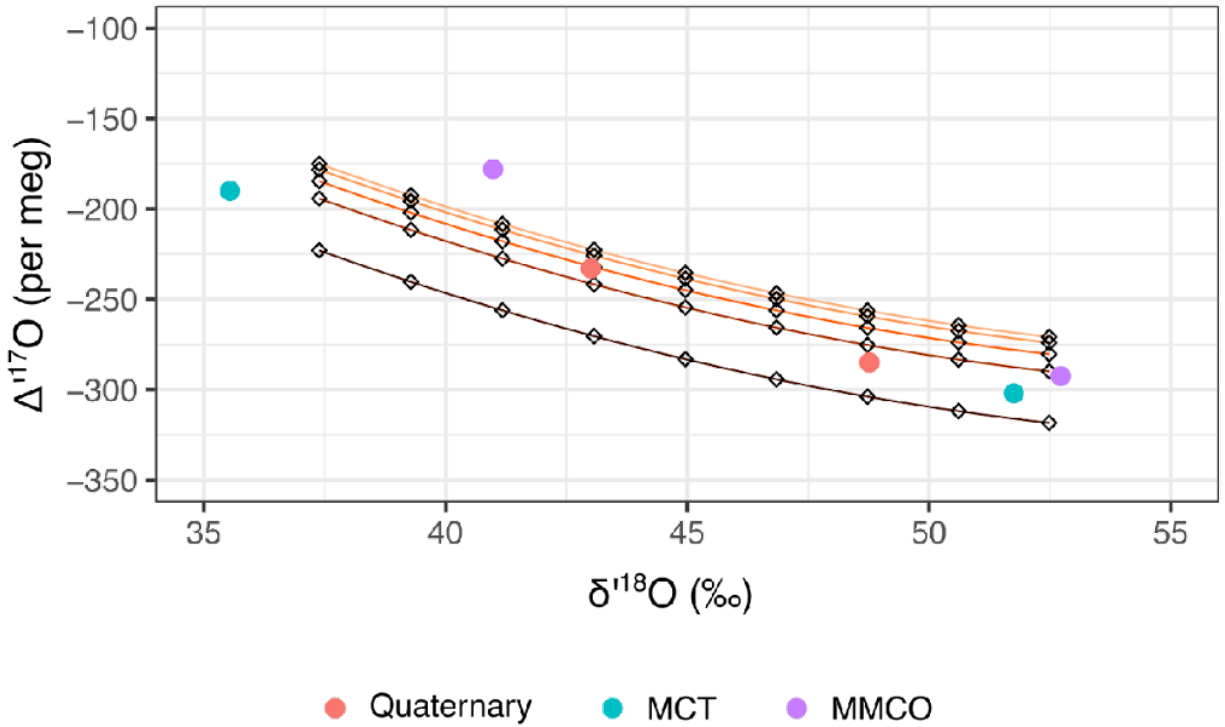


Figure 4.9: Same as Fig. 4.8 but showing only the central Namib samples. MCT is Miocene Climatic Transition. MMCO is Mid-Miocene Climatic Optimum. Relative to the VSMOW2-SLAP2 scale.

Mid-Miocene Climatic Optimum

The isotopic composition of central Namib RES samples observed in this project can be recreated using the current model with varying success. The MMCO samples do not fall cleanly into any of the trends created under the circumstances as described. The high $\delta^{18}\text{O}$ of UPMC-AW-7-NO plots well alongside the low (10%) relative humidity end member of all model runs. Concurrently, the predicted eggshell $\Delta^{17}\text{O}$ under an atmosphere of 400 ppm CO_2 is nearly identical to the measured value. The low $\delta^{18}\text{O}$ MMCO sample, UPMC-AW-9-NO, is more poorly represented, with a $\Delta^{17}\text{O}$ roughly 50 and 80 per meg higher than the modelled value under 400 and 600 ppm CO_2 , respectively. Neither sample reflects an atmosphere of 600 ppm CO_2 and present-day GPP. To produce measured RES $\Delta^{17}\text{O}$ under 600 ppm CO_2 and $\delta^{18}\text{O}_{\text{mw}}$ of -2‰, GPP on the order of ~150% or more present-day would be required.

A positive shift in $\delta^{18}\text{O}_{\text{mw}}$ on the scale of ~5‰ under the current model conditions is required to reproduce the measured range of RES $\delta^{18}\text{O}$. While a significant shift, such conditions are not impossible given the likely limited degree of (proto-)Namib evolution during the MMCO and early Miocene in general. Kaseke et al. (2018) report annual (hydrological year; Oct-Sept) precipitation isotopic composition from Windhoek, Namibia from 2012-2016. Their data show substantial shifts (up to ~7‰; VSMOW) in $\delta^{18}\text{O}$ from year-to-year between 2012 and 2015. In 2012-13, $\delta^{18}\text{O}$ was +0.94‰. In 2013-14 this decreased to -5.97‰ before rising again to -0.41‰ in 2014-15. While Windhoek is not located within the Namib, it does reside within the same summer rainfall regime, and thus is likely to experience similar precipitation changes. Shifts of similar scale could also alleviate the necessity of significantly higher GPP to explain the MMCO samples while maintaining pCO_2 around 400-450 ppm.

Miocene Climatic Transition

Miocene Climatic Transition RES samples are generally better represented by the model. Although the full extent of eggshell $\delta^{18}\text{O}$ is not easily reproduced, the discrepancy between the modelled and measured values on either end is within 1‰. The samples record eggshell $\Delta^{17}\text{O}$ more negative than predicted under modelled conditions ($p\text{CO}_2$ 350 ppm). Rather, the samples are more consistent with $p\text{CO}_2$ around 450 ppm, substantially higher than current predictions. Alternatively, and perhaps more likely, a decrease in GPP to ~80% of present-day levels causing a shift in $\Delta^{17}\text{O}(\text{O}_2)$ of ~ -40 per meg, would translate well to measured RES $\Delta^{17}\text{O}$.

Quaternary

The Quaternary samples are also fairly well captured by the model. The low $\delta^{18}\text{O}$ sample (UPMC-Ka-2-SC) plots nearly exactly where predicted under an atmosphere of around 280-300 ppm and present-day GPP. This sample aligns with a relative humidity of ~45%, reasonable for the rainy season. The high $\delta^{18}\text{O}$ sample, UPMC-Ka-1-SC, plots ~20 per meg more negative than predicted. Slight alterations to a few combinations of model input values (e.g., $\Delta^{17}\text{O}_{\text{mw}}$ and $\delta^{18}\text{O}_{\text{mw}}$ both = 0.000‰), could account for this discrepancy while maintaining accurate predictions of UPMC-Ka-2-SC. Likewise, a decrease in GPP to ~90% present-day describes a trend that reasonably captures $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ of both samples. However, 20 per meg is well within reasonably expected RES natural variation and should not be heavily scrutinized.

Southern Namib

To maintain consistency with interpretation of the central Namib samples, all southern Namib samples are also interpreted through model runs where model leaf ratio was 0.9. Compared to the central Namib, southern Namib RES samples are tightly constrained in $\delta^{18}\text{O}$ and consistently plot within the range expected for relative humidity between 50 – 90% (Fig. 4.10). This is likely reflective of less arid conditions associated with the winter rainfall regime as lower temperatures limit evaporative $\delta^{18}\text{O}$ enrichment. $\Delta^{17}\text{O}$ of all samples is also higher than their respectively comparable central Namib counterparts. This inconsistency suggests that another factor exerts a larger influence over RES $\Delta^{17}\text{O}$ in these samples. As a result, the samples are related by steeper $\delta^{18}\text{O} - \Delta^{17}\text{O}$ trends, consistent with expectations of less arid systems.

In addition, all southern Namib samples plot with $\Delta^{17}\text{O}$ substantially higher than predicted under their respective estimated $p\text{CO}_2$. A clear trend in $\Delta^{17}\text{O}$ exists through time in the southern Namib where, from the MMCO through the Pliocene, RES $\Delta^{17}\text{O}$ increases with each subsequent time period. This pattern is interrupted in the Quaternary samples resulting in a much steeper, and more tightly constrained, $\delta^{18}\text{O} - \Delta^{17}\text{O}$ relationship, interpreted here as a change in source water dynamics.

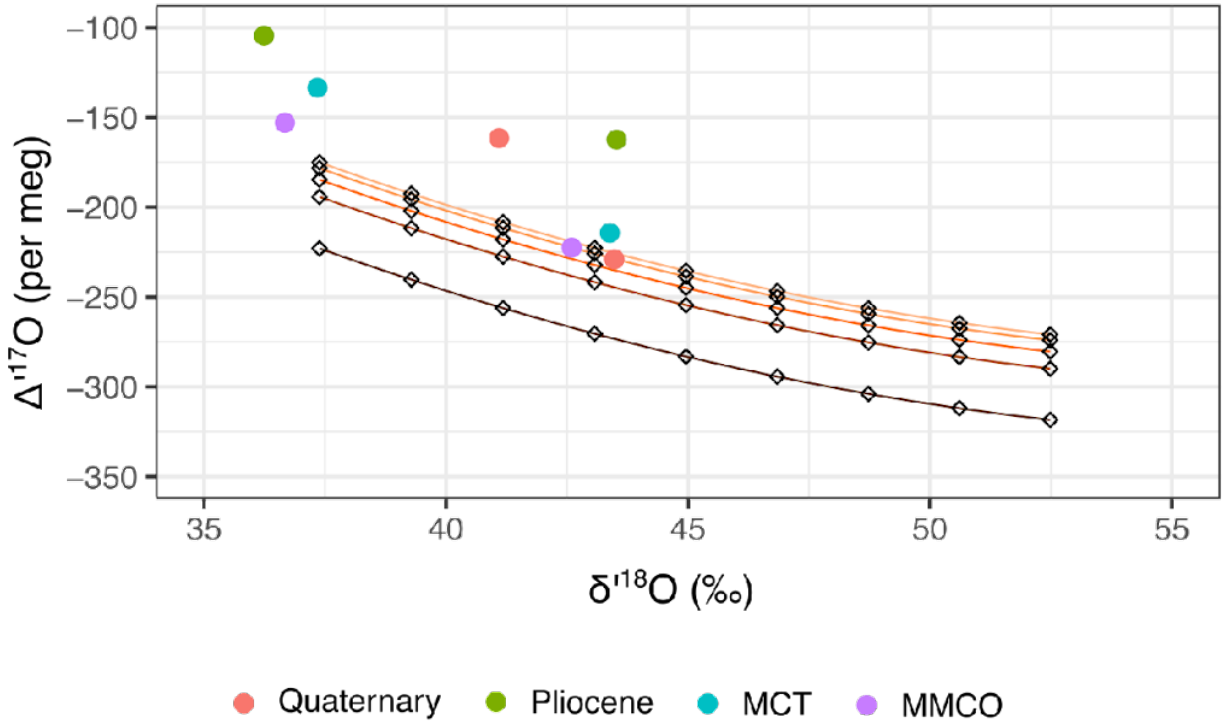


Figure 4.10: Same as Fig. 4.9 but showing only southern Namib samples. MCT is Miocene Climatic Transition. MMCO is Mid-Miocene Climatic Optimum. Relative to the VSMOW2-SLAP2 scale.

Mid-Miocene Climatic Optimum

Both MMCO samples record $\Delta^{17}\text{O}$ between $\sim 20 - 40$ per meg higher than predicted by the model under $p\text{CO}_2$ 400 ppm, and $\sim 50 - 70$ per meg too high under 600 ppm CO_2 . Under the above model conditions and present-day GPP, the samples are best characterized when $\Delta^{17}\text{O}(\text{O}_2)$ is ~ -370 per meg – equivalent to $p\text{CO}_2 \sim 220$ ppm. This unrealistic requirement suggests that $p\text{CO}_2$ is not the dominant influence over RES $\Delta^{17}\text{O}$ here. Likewise, under 400 ppm CO_2 , $\text{GPP} > 150\%$ present-day would be required to produce the measured RES $\Delta^{17}\text{O}$. As such conditions would not allow for the measured RES $\Delta^{17}\text{O}$ of the central Namib MMCO samples, significant increase in GPP is likely not the main control over RES $\Delta^{17}\text{O}$ here. Estimation of required GPP under 600 ppm CO_2 cannot be meaningfully done using Fig. 4.7 as the x-axis does not extend far enough and truncates the representative isopleth when $\Delta^{17}\text{O}(\text{O}_2)$ is ~ -460 per meg.

Alternatively, the measured sample values can be recreated, under 400 ppm $p\text{CO}_2$, with a positive shift in $\Delta^{17}\text{O}_{\text{mw}}$ of 50 per meg to 0.070‰. As the main water source for the southern Namib is oceanic, this may be unlikely but not impossible. Under 600 ppm $p\text{CO}_2$, this shift would need to be 80 per meg, making $\Delta^{17}\text{O}_{\text{mw}}$ 0.100‰. The most likely scenario from which such shifts could result would be increased evaporative flux from the ocean surface, causing increased $\Delta^{17}\text{O}$ of the resultant water vapor. This scenario is explored in greater depth later in this chapter.

Miocene Climatic Transition

The MCT samples plot similarly too positive in $\Delta^{17}\text{O}$ compared to the model predictions under representative $p\text{CO}_2$ and present-day GPP. Again, either unrealistically low $p\text{CO}_2$ or high GPP are required to recreate measured RES $\Delta^{17}\text{O}$, and the most accurate estimation results from increased $\Delta^{17}\text{O}_{\text{mw}}$. For these samples, $\Delta^{17}\text{O}_{\text{mw}}$ of 0.080‰, an increase of 60 per meg compared to the initial model conditions provides the best reconstruction.

Pliocene

Continuing with the trend to more positive RES $\Delta^{17}\text{O}$, the Pliocene samples are the most positive of all throughout the dataset presented in this project. The samples are not able to be described by the above model conditions and plot ~ 70 per meg more positive than predicted. Rather, the sample data is best recreated with a positive shift in $\Delta^{17}\text{O}_{\text{mw}}$ of ~ 100 per meg from 0.020‰ to 0.120‰. These samples also have the shallowest $\delta^{18}\text{O} - \Delta^{17}\text{O}$ slope of all sample groups presented here. It is difficult to infer the cause(s) of this shallow relationship. At its simplest, it could be due to a relatively dry rainy season at the time of egg laying for the low- $\delta^{18}\text{O}$ sample and/or wet rainy season for the high- $\delta^{18}\text{O}$ sample.

Quaternary

The southern Namib Quaternary samples have the steepest $\delta^{18}\text{O} - \Delta^{17}\text{O}$ relationship of all sample groupings presented in this dataset. While the high- $\delta^{18}\text{O}$ sample is indistinguishable from those of all other southern Namib sample sets, the low- $\delta^{18}\text{O}$ sample is shifted ~ 5 ‰ higher than the low- $\delta^{18}\text{O}$ samples of the other time periods. This is likely due to increased aridity more ressemblant of present-day. Ségalen et al. (2006) suggest higher present aridity may be linked to strengthening of the Benguela upwelling system during the Plio-Pleistocene.

Main Controls of RES $\Delta^{17}\text{O}$

Global vs. Local Scale Influences

The major controls over recorded RES $\Delta^{17}\text{O}$ are evaporation/aridity, atmospheric $p\text{CO}_2$, and GPP. One way to explain the discrepancies between the central and southern Namib samples and their model predictions is the possibility that aridity and larger scale atmospheric conditions play leveraging roles against each other. The interpretation put forth here is that each of these factors influences RES $\Delta^{17}\text{O}$, and that each exerts dominant influence under varying environmental and atmospheric circumstances. It may be that when aridity is relatively low, the comparatively large water pool drowns out potential signals expected from $p\text{CO}_2$ and/or GPP. Similarly, when aridity is high, it may be easier to infer variations in RES $\Delta^{17}\text{O}$ caused by atmospheric/climatic conditions as the high-aridity end member scenario is approached. In such a case, the central Namib samples would be better suited for making global scale inferences, while the southern Namib samples would provide more direct local environmental insights.

Alternatively, vegetation changes (e.g., expansion of C3 grasses, late expansion of C4 plants) may play a large role in RES isotopic composition. Ségalen et al. (2006) note significant and correlated positive shifts in both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ from ~ 6 My to ~ 3 My, and again from ~ 3 My to 0 My in both the central and southern Namib. They go on to suggest an increasing presence of C4 and CAM plants in the central Namib beginning in the Pliocene. Helliker & Ehleringer (2000) showed that, in growth chamber experiments, evaporative leaf water $\delta^{18}\text{O}$ enrichment is highest in C4 grasses (14.7 ± 3.0 ‰; VSMOW), followed by C3 grasses (7.2 ± 2.0 ‰), then C3

dicots ($3.9 \pm 1.0\text{‰}$). It would be expected that these significant differences would be reflected in animal body water and, in this case, ratite eggshell. However, to my knowledge, similar enrichment differences have not been described in terms of $\Delta^{17}\text{O}$. If vegetation type exerts a large influence over leaf water $\Delta^{17}\text{O}$, vegetation changes through time will, perhaps substantially, influence RES $\Delta^{17}\text{O}$.

Effects of Changes in Evaporative Flux & Relative Humidity at the Moisture Source

Another, potentially crucial, variable with downstream influence on RES $\Delta^{17}\text{O}$ is relative humidity at the moisture source. $\Delta^{17}\text{O}$ of waters decreases with increasing evaporation and aridity. By mass balance, $\Delta^{17}\text{O}$ of the resultant water vapor, and the precipitation from subsequent condensation (represented by $\Delta^{17}\text{O}_{\text{mw}}$ in the current model), increases. As such, changes in relative humidity and/or evaporative flux at the moisture source, and therefore source vapor composition, will produce downstream isotopic composition effects in any moisture dependent material. Aron et al. (2021) note the emergence of a mid-latitude pattern of $\Delta^{17}\text{O}$ seasonality in hydrological samples with lower values in the summer and higher in the winter. This pattern may be linked to such variations in relative humidity and/or evaporative fluxes at or above distant moisture sources (Tian et al., 2018; Uechi & Uemura, 2019), and fits nicely with the observed discrepancies between central and southern Namib RES data in this project.

One possibility for producing the increased $\Delta^{17}\text{O}_{\text{mw}}$ required to reproduce the southern Namib RES data is an increase in evaporation from the Atlantic Ocean surface. It is known from ODP data that the African plate moved northwards $\sim 5^\circ$ through the Miocene and Pliocene (Ségalen et al., 2006). This northward traverse would have increased solar energy absorption in and around southern Africa, possibly resulting in the increased evaporation suggested. Consequently, this locally increased vapor content may have manifested as fog being the dominant precipitation in the southern Namib. As diffusion and condensation of water vapor both increase $\Delta^{17}\text{O}$ of the vapor and resultant precipitation respectively, this scenario may be the best explanation of the high RES $\Delta^{17}\text{O}$ observed in this project for the southern Namib.

Increased solar insolation and evaporation at the moisture source of the central Namib likely would not be reflected as strongly in RES $\Delta^{17}\text{O}$ due to the continental east-to-west traverse of this moisture through tropical settings. The large water pool of the tropics would continuously have a “resetting” effect on moisture $\Delta^{17}\text{O}$ to values closer to the local meteoric water. Therefore, by the time this moisture makes fall in the central Namib, the extent of isotopic alteration we observe should be more representative of regionally averaged conditions.

4.3) Future Research

While this project has nearly quadrupled the number of published ratite eggshell $\Delta^{17}\text{O}$ data, there are yet several areas of requiring further research to better flush out the intricacies of (paleo-)RES $\Delta^{17}\text{O}$. As a consequence of the sample selection strategy (i.e., capturing maximum $\delta^{18}\text{O}$ ranges) in this project it is impossible to identify or rule out the influence of any singular or otherwise infrequent local weather events or environmental conditions at the formation of each sample eggshell. Actually, due to this project’s intentional selection of samples with extreme high and low δ -values, it may be that this project preferentially reflects such conditions.

Ultimately, better overall approximation of the relevant environments and avoidance of this sort of sampling bias will only be able to be evaluated with further research and larger sample pools.

In particular, modern samples will provide the most value in furthering our understanding of RES triple oxygen composition. Modern samples will allow us to improve our understanding of the leveraging global climatic and local environmental influences over RES $\Delta^{17}\text{O}$ under known conditions. For (paleo-)environmental and climatic interpretations, increased sampling of materials of moderate isotopic composition, as opposed to the intentional end-member composition sample selection of this project, will enable improved estimates of “typical” conditions. Quaternary focused research could, in particular, benefit greatly from increased sampling as a means to better sequence and interpret the highly variable climate states representative of the period.

Perhaps the most consequential area of further research for paleo studies is better refining the $\text{pCO}_2 - \Delta^{17}\text{O}(\text{O}_2) - \text{GPP}$ relationship. In this project, the relationship as modelled by Young et al. (2014) was used for consideration of model inputs (Fig. 4.7, Table 4.5), as described above. However, other models exist and are referred to in the general environmental triple oxygen isotope literature, most commonly that of Cao & Bao (2013). At lower pCO_2 , these two models calculate similar $\Delta^{17}\text{O}(\text{O}_2)$. However, they diverge significantly with increasing pCO_2 (e.g., at $\text{pCO}_2 = 600$ ppm: $\Delta^{17}\text{O}(\text{O}_2) = -550$ per meg and -750 per meg by Young et al. (2014) and Cao & Bao (2013), respectively). Running the RES model with $\Delta^{17}\text{O}(\text{O}_2) = -550$ and -750 per meg, and all other variables the same, results in a discrepancy in predicted RES $\Delta^{17}\text{O}$ of ~ 60 per meg, a significant difference which would invoke alternative RES data interpretations (e.g., significantly lower GPP through time).

Also necessary for improving our understanding and predictions are enhanced leaf water isotopic composition models and detailed studies of leaf water $\Delta^{17}\text{O}$ variation across plant types (e.g., C3 vs. C4). Development of a model which takes into an animal’s proportional plant type intake, using yet to be described $\Delta^{17}\text{O}$ enrichment differences, would almost certainly improve RES $\Delta^{17}\text{O}$ model predictions.

Chapter 5: Conclusions

This project was designed to be two-fold. First, the goal was to design, implement, and optimize a new methodological technique for preparing and analyzing triple oxygen isotopes directly on CaCO_3 sample-derived CO_2 . The second phase of this project was the application of these developments towards measuring the triple oxygen composition of ratite eggshell (RES) and assessing their usefulness for making inferences about (paleo-)environmental and global (paleo-) climatic conditions at the time of eggshell formation. For this, a framework for interpreting the various influences of atmospheric $p\text{CO}_2$, global gross primary production (GPP), and relative humidity (Rh) was suggested (Chapter 1) and later used to interpret the RES data obtained in this project (Chapter 4).

The automated sample preparation system introduced in Chapter 2 was the foundation for this project. The system, designed to mix sample CO_2 in N_2 to very precise concentrations (single sample concentration $1\sigma \sim 1$ ppm), was required to meet the operating specifications of the TILDAS instrument used for making the isotopic composition measurements. Critical to the optimal analysis of samples was the condition that the sample gas was well mixed before being introduced to the TILDAS, as even slight concentration gradients impact measurement accuracy and precision (Chapter 2). After a series of iterations, a system capable of reliably preparing and mixing sample gas was achieved (Chapter 3), where project-long ($n = 141$) sample concentration reproducibility, relative to the targeted concentration, was $99.8 \pm 1.2\%$ (corresponding to 399.1 ± 4.8 ppm CO_2 in N_2).

After construction and optimization of the sample preparation system, a series of experiments were carried out on the TILDAS instrument aimed at identifying optimal measurement conditions and analytical limitations (Chapter 3). These experiments revealed the major factors which limit the isotopologue spectroscopic measurement precision of isotopologues, which include single aliquot measurement duration, previously unexplored temperature dependencies of instrument electronic components, and a strong, within sample concentration dependence. In depth exploration of each of these major factors ultimately enabled the achievement of TILDAS measurement precisions meeting those typically stated as necessary for meaningful $\Delta^{17}\text{O}$ data interpretation ($1\sigma \sim 10$ per meg). This achievement also showed that TILDAS instruments are capable of achieving similar $\Delta^{17}\text{O}$ precision compared to the traditionally used IRMS techniques for triple oxygen isotope analyses.

The realized successes of the sample preparation system and measurement optimization enabled its application to fossil ratite eggshell samples (Chapter 4). These samples, collected from the central and southern Namib Desert, span ~ 16 My and represent distinct environments characterized by the summer and winter rainfall regimes, respectively. At present only 5 ratite eggshell triple oxygen data are published, all of which are modern samples of *Struthio camelus*. Thus, the analyses of the 14 RES samples introduced in this project have nearly quadrupled the amount of RES triple oxygen data available and are the first not-modern samples to be published or otherwise made openly available. The data can be interpreted in terms of distinct differences between the central and southern Namib through time and suggest variable leveraging of the dominant factors controlling $\Delta^{17}\text{O}$ signal retention. These factors are both global ($p\text{CO}_2$, GPP) and local (e.g., aridity, moisture source, vegetation type) in scale. Changes in $p\text{CO}_2$ over the last

~16 My alone are not sufficient to reproduce the $\Delta^{17}\text{O}$ values obtained in this project, thus revealing a lack in our understanding of the potential paleo- pCO_2 barometer proposed to be possible in RES triple oxygen isotope records. Likewise, reasonable changes in total GPP are also unable to account for the $\Delta^{17}\text{O}$ variation observed. Rather, the data obtained in this project suggest that local environmental factors may contribute largely to the retained $\Delta^{17}\text{O}$ signal (e.g., in the southern Namib). The specific local conditions which result in either constructive or destructive global scale influence recording in RES are an area of future research that will be necessary to meaningfully interpret $\Delta^{17}\text{O}$ data when targeting global scale conditions.

Overall, this project yielded advancements to both the methodological and real-world sample application aspects of $\Delta^{17}\text{O}$ measurements. Aspects of the automated sample preparation system and instrumental advances and optimizations have been published with all crucial information and code available to encourage future replication, and hopeful verification, of this work. Interpretation of the RES data obtained in project has revealed areas necessary future research to allow for more accurate and meaningful interpretation of $\Delta^{17}\text{O}$ data, particularly important for fossil samples. In concluding this project, I suggest the following points be addressed as top priority by future research: (1) the development and adoption of international and/or interlaboratory standard materials that better represent the ranges in isotopic composition observed in RES; (2) further research into the major influences of leaf water $\Delta^{17}\text{O}$, prioritizing the quantification of the variation in leaf water $\Delta^{17}\text{O}$ between plant types (e.g., C3 vs. C4, C3 grasses vs. C3 dicots); and (3) a significant increase in the number of published modern RES samples analyzed for $\Delta^{17}\text{O}$. The combination of progress in these 3 directed areas will allow for improvements in our understanding of modern global and local influences over RES $\Delta^{17}\text{O}$ and will therefore improve our ability to reconstruct the paleo-environments from which fossil RES was created.

Works Cited

- Aron, P. G., Levin, N. E., Beverly, E. J., Huth, T. E., Passey, B. H., Pelletier, E. M., Poulsen, C. J., Winkelstern, I. Z., & Yarian, D. A. (2021). Triple oxygen isotopes in the water cycle. *Chemical Geology*, 565, 1-23. Elsevier B.V. <https://doi.org/10.1016/j.chemgeo.2020.120026>
- Assonov, S. S., & Brenninkmeijer, C. A. M. (2001). A new method to determine the ^{17}O isotopic abundance in CO_2 using oxygen isotope exchange with a solid oxide. *Rapid Communications in Mass Spectrometry*, 15, 2426–2437. <https://doi.org/10.1002/rcm.529>
- Baker, L., Franchi, I. A., Maynard, J., Wright, I. P., & Pillinger, C. T. (2002). A Technique for the Determination of $^{18}\text{O}/^{16}\text{O}$ and $^{17}\text{O}/^{16}\text{O}$ Isotopic Ratios in Water from Small Liquid and Solid Samples. *Analytical Chemistry*, 74(7), 1665–1673. <https://doi.org/10.1021/ac010509s>
- Bao, H., & Gu, B. (2004). Natural Perchlorate Has a Unique Oxygen Isotope Signature. *Environ. Sci. Technol.*, 38(19), 5073–5077. <https://doi.org/10.1021/es049516z>
- Bao, H., Cao, X., & Hayles, J. A. (2016). Triple Oxygen Isotopes: Fundamental Relationships and Applications. *Annual Review of Earth and Planetary Sciences*, 44, 463–492. <https://doi.org/10.1146/annurev-earth-060115-012340>
- Bao, H., Lyons, J. R., & Zhou, C. (2008). Triple oxygen isotope evidence for elevated CO_2 levels after a Neoproterozoic glaciation. *Nature*, 453(7194), 504–506. <https://doi.org/10.1038/nature06959>
- Barkan, E., & Luz, B. (2005). High precision measurements of $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ ratios in H_2O . *Rapid Communications in Mass Spectrometry*, 19(24), 3737–3742. <https://doi.org/10.1002/rcm.2250>
- Barkan, E., & Luz, B. (2007). Diffusivity fractionations of $\text{H}_2^{16}\text{O}/\text{H}_2^{17}\text{O}$ and $\text{H}_2^{16}\text{O}/\text{H}_2^{18}\text{O}$ in air and their implications for isotope hydrology. *Rapid Communications in Mass Spectrometry*, 21, 2999–3005. <https://doi.org/10.1002/rcm.3180>
- Barkan, E., & Luz, B. (2012). High-precision measurements of $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ ratios in CO_2 . *Rapid Communications in Mass Spectrometry*, 26, 2733–2738. <https://doi.org/10.1002/rcm.6400>
- Barkan, E., Affek, H. P., Luz, B., Bergel, S. J., Voarintsoa, N. R. G., & Musan, I. (2019). Calibration of $\delta^{17}\text{O}$ and ^{17}O excess values of three international standards: IAEA-603, NBS19 and NBS18. *Rapid Communications in Mass Spectrometry*, 33, 737–740. <https://doi.org/10.1002/rcm.8391>
- Barkan, E., Musan, I., & Luz, B. (2015). High-precision measurements of $\delta^{17}\text{O}$ and ^{17}O excess of NBS19 and NBS18. *Rapid Communications in Mass Spectrometry*, 29(23), 2219–2224. <https://doi.org/10.1002/rcm.7378>
- Bartoli, G., Hönisch, B., & Zeebe, R. E. (2011). Atmospheric CO_2 decline during the Pliocene intensification of Northern Hemisphere glaciations. *Paleoceanography*, 26(4). <https://doi.org/10.1029/2010PA002055>
- Bergel, S. J., Barkan, E., Stein, M., & Affek, H. P. (2020). Carbonate ^{17}O excess as a paleo-hydrology proxy: Triple oxygen isotope fractionation between H_2O and biogenic aragonite, derived from freshwater mollusks. *Geochimica et Cosmochimica Acta*, 275, 36–47. <https://doi.org/10.1016/j.gca.2020.02.005>
- Beverly, E. J., Levin, N. E., Passey, B. H., Aron, P. G., Yarian, D. A., Page, M., & Pelletier, E. M. (2021). Triple oxygen and clumped isotopes in modern soil carbonate along an aridity gradient in the Serengeti, Tanzania. *Earth and Planetary Science Letters*, 567. <https://doi.org/10.1016/j.epsl.2021.116952>

- Blunier, T., Barnett, B., Bender, M. L., & Hendricks, M. B. (2002). Biological oxygen productivity during the last 60,000 years from triple oxygen isotope measurements. *Global Biogeochemical Cycles*, 16(3), 1029. <https://doi.org/10.1029/2001GB001460>
- Cao, X., & Bao, H. (2013). Dynamic model constraints on oxygen-17 depletion in atmospheric O₂ after a snowball Earth. *Proceedings of the National Academy of Sciences of the United States of America*, 110(36), 14546–14550. <https://doi.org/10.1073/pnas.1302972110>
- Cao, X., & Liu, Y. (2011). Equilibrium mass-dependent fractionation relationships for triple oxygen isotopes. *Geochimica et Cosmochimica Acta*, 75(23), 7435–7445. <https://doi.org/10.1016/j.gca.2011.09.048>
- Cernusak, L. A., Barbour, M. M., Arndt, S. K., Cheesman, A. W., English, N. B., Feild, T. S., Helliker, B. R., Holloway-Phillips, M. M., Holtum, J. A. M., Kahmen, A., Mcinerney, F. A., Munksgaard, N. C., Simonin, K. A., Song, X., Stuart-Williams, H., West, J. B., & Farquhar, G. D. (2016). Stable isotopes in leaf water of terrestrial plants. *Plant Cell and Environment*, 39(5), 1087–1102. <https://doi.org/10.1111/PCE.12703>
- Cohen, E. R.; Cvitaš, T.; Frey, J. G.; Holmström, B.; Kuchitsu, K.; Marquardt, R.; Mills, I.; Pavese, F.; Quack, M.; Stohner, J.; et al. *Quantities, Units and Symbols in Physical Chemistry*; IUPAC, 2007.
- Coplen, T. B. *Explanatory Glossary of Terms Used in Expression of Relative Isotope Ratios and Gas Ratios*; IUPAC, 2008
- Cowie, B. R., & Johnston, D. T. (2016). High-precision measurement and standard calibration of triple oxygen isotopic compositions ($\delta^{18}\text{O}$, $\Delta^{17}\text{O}$) of sulfate by F₂ laser fluorination. *Chemical Geology*, 440(15), 50–59. <https://doi.org/10.1016/J.CHEMGEO.2016.07.003>
- Craig, H. (1961). Isotopic Variations in Meteoric Waters. *Science (New York, N.Y.)*, 133(3465), 1702–1703. <https://doi.org/10.1126/SCIENCE.133.3465.1702>
- Dansgaard, W. (1964). Stable isotopes in precipitation. *Tellus*, 16(4), 436–468.
- Evans, N. P., Bauska, T. K., Gázquez-Sánchez, F., Brenner, M., Curtis, J. H., & Hodell, D. A. (2018). Quantification of drought during the collapse of the classic Maya civilization. *Science*, 361, 498–501. <https://www.science.org>
- Ferhi, A., & Letolle, R. (1977). Transpiration and evaporation as the principal factors in oxygen isotope variations of organic matter in land plants. *Physiologie végétale.*, 15, 363–370.
- Flanagan, L. B., Bain, J. F., & Ehleringer, J. R. (1991). Stable oxygen and hydrogen isotope composition of leaf water in C3 and C4 plant species under field conditions. *Oecologia*, 88(3), 394–400. <https://doi.org/10.1007/BF00317584>
- Flores, E., Le Viallon, J., Moussay, P., Griffith, D. W. T., & Wielgosz, R. I. (2017). Calibration Strategies for FT-IR and Other Isotope Ratio Infrared Spectrometer Instruments for Accurate $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ Measurements of CO₂ in Air. *Analytical Chemistry*, 89(6), 3648–3655. <https://doi.org/10.1021/acs.analchem.6b05063>
- Förstel, H. (1978). Radiation and Environmental Biophysics The Enrichment of ¹⁸O in Leaf Water Under Natural Conditions. *Radiation and Environmental Biophysics*, 15, 323–344.
- Fosu, B. R., Ghosh, P., Weisenberger, T. B., Spürgin, S., & Viladkar, S. G. (2021). A triple oxygen isotope perspective on the origin, evolution, and diagenetic alteration of carbonatites. *Geochimica et Cosmochimica Acta*, 299, 52–68. <https://doi.org/10.1016/j.gca.2021.01.037>
- Fosu, B. R., Subba, R., Peethambaran, R., Bhattacharya, S. K., & Ghosh, P. (2020). Technical Note: Developments and Applications in Triple Oxygen Isotope Analysis of Carbonates.

- ACS Earth and Space Chemistry*, 4(5), 702–710.
<https://doi.org/10.1021/acsearthspacechem.9b00330>
- Franks, P. J., Royer, D. L., Beerling, D. J., Van De Water, P. K., Cantrill, D. J., Barbour, M. M., & Berry, J. A. (2014). New constraints on atmospheric CO₂ concentration for the Phanerozoic. *Geophysical Research Letters*, 41(13), 4685–4694.
<https://doi.org/10.1002/2014GL060457>
- Gehler, A., Gingerich, P. D., & Pack, A. (2016). Temperature and atmospheric CO₂ concentration estimates through the PETM using triple oxygen isotope analysis of mammalian bioapatite. *Proceedings of the National Academy of Sciences of the United States of America*, 113(28), 7739–7744. <https://doi.org/10.1073/pnas.1518116113>
- Gonfiantini, R., Gratziu, S., & Tongiorgi, E. (1965). Oxygen isotope composition of water in leaves. *Isotopes and Radiation in Soil-Plant Nutrition Studies*. IAEA.
- Gordon, I. E., Rothman, L. S., Hargreaves, R. J., Hashemi, R., Karlovets, E. v., Skinner, F. M., Conway, E. K., Hill, C., Kochanov, R. v., Tan, Y., Weisło, P., Finenko, A. A., Nelson, K., Bernath, P. F., Birk, M., Boudon, V., Campargue, A., Chance, K. v., Coustenis, A., ... Yurchenko, S. N. (2022). The HITRAN2020 molecular spectroscopic database. *Journal of Quantitative Spectroscopy and Radiative Transfer*, 277.
<https://doi.org/10.1016/J.JQSRT.2021.107949>
- Griffith, D. W. T. (2018). Calibration of isotopologue-specific optical trace gas analysers: A practical guide. *Atmospheric Measurement Techniques*, 11(11), 6189–6201.
<https://doi.org/10.5194/amt-11-6189-2018>
- Griffith, D. W. T., Deutscher, N. M., Caldow, C., Kettlewell, G., Riggenbach, M., & Hammer, S. (2012). A Fourier transform infrared trace gas and isotope analyser for atmospheric applications. *Atmospheric Measurement Techniques*, 5(10), 2481–2498.
<https://doi.org/10.5194/amt-5-2481-2012>
- Hanekom, N., Randall, R. M., Nel, P., & Kruger, & N. (2009). *WEST COAST NATIONAL PARK STATE OF KNOWLEDGE REPORT*.
- Hare, V. J., Dyroff, C., Nelson, D. D., & Yarian, D. A. (2022). High-Precision Triple Oxygen Isotope Analysis of Carbon Dioxide by Tunable Infrared Laser Absorption Spectroscopy. *Analytical Chemistry* 94(46), 16023–16032.
<https://doi.org/10.1021/ACS.ANALCHEM.2C03005>
- Helliker, B. R., & Ehleringer, J. R. (2000). Establishing a grassland signature in veins: ¹⁸O in the leaf water of C3 and C4 grasses. *Proceedings of the National Academy of Sciences of the United States of America*, 97(14), 7894–7898.
<https://doi.org/10.1073/PNAS.97.14.7894>
- Hipondoka, Martin & Kempf, Juergen & Jousse, Helene. (2013). Palaeo and Present Ecological Value of the Etosha Pan, Namibia: An Integrative Review. 61. 61-2013.
<https://www.researchgate.net/publication/274959124>
- Hofmann, M. E. G., & Pack, A. (2010). Technique for high-precision analysis of triple oxygen isotope ratios in carbon dioxide. *Analytical Chemistry*, 82(11), 4357–4361.
<https://doi.org/10.1021/ac902731m>
- Horváth, B., Hofmann, M. E. G., & Pack, A. (2012). On the triple oxygen isotope composition of carbon dioxide from some combustion processes. *Geochimica et Cosmochimica Acta*, 95, 160–168. <https://doi.org/10.1016/J.GCA.2012.07.021>

- Hu, H. (2016). *TRIPLE OXYGEN ISOTOPES OF BIOMINERALS: A NEW PROXY FOR RECONSTRUCTING PALEOARIDITY, PALEOECOPHYSIOLOGY AND PALEO-CARBON-CYCLING*. PhD dissertation, Johns Hopkins University (143p)
- Ishimura, T., Tsunogai, U., & Nakagawa, F. (2008). Grain-scale heterogeneities in the stable carbon and oxygen isotopic compositions of the international standard calcite materials (NBS19, NBS18, IAEA-CO-1, and IAEA-CO-8). *Rapid Communications in Mass Spectrometry*, 22(12), 1925–1932. <https://doi.org/10.1002/RCM.3571>
- Johnson, B. J., Fogel, M. L., & Miller, G. H. (1993). Paleoecological reconstructions in southern Egypt based on the stable carbon and nitrogen isotopes in the organic fraction and stable carbon isotopes in individual amino acids of fossil ostrich eggshell. *Chemical Geology*, 107(3–4), 493–497. [https://doi.org/10.1016/0009-2541\(93\)90238-E](https://doi.org/10.1016/0009-2541(93)90238-E)
- Johnson, B. J., Fogel, M. L., & Miller, G. H. (1998). Stable isotopes in modern ostrich eggshell: A calibration for paleoenvironmental applications in semi-arid regions of southern Africa. *Geochimica et Cosmochimica Acta*, 62(14), 2451–2461. [https://doi.org/10.1016/S0016-7037\(98\)00175-6](https://doi.org/10.1016/S0016-7037(98)00175-6)
- Kaseke, K. F., Wang, L., Wanke, H., Tian, C., Lanning, M., & Jiao, W. (2018). Precipitation Origins and Key Drivers of Precipitation Isotope (^{18}O , ^2H , and ^{17}O) Compositions Over Windhoek. *Journal of Geophysical Research: Atmospheres*, 123(14), 7311–7330. <https://doi.org/10.1029/2018JD028470>
- Kawagucci, S., Tsunogai, U., Kudo, S., Nakagawa, F., Honda, H., Aoki, S., Nakazawa, T., & Gamo, T. (2005). An Analytical System for Determining $\delta^{17}\text{O}$ in CO_2 Using Continuous Flow-Isotope Ratio MS. *Analytical Chemistry*, 77(14), 4509–4514. <https://doi.org/10.1021/AC050266U>
- Kerstel, E. (2004). Isotope Ratio Infrared Spectrometry. *Handbook of Stable Isotope Analytical Techniques*, 759–787. <https://doi.org/10.1016/B978-044451114-0/50036-3>
- Kim, S. T., Mucci, A., & Taylor, B. E. (2007). Phosphoric acid fractionation factors for calcite and aragonite between 25 and 75°C: Revisited. *Chemical Geology*, 246(3–4), 135–146. <https://doi.org/10.1016/j.chemgeo.2007.08.005>
- Kohn, M. J. (1996). Predicting animal $\delta^{18}\text{O}$: Accounting for diet and physiological adaptation. *Geochimica et Cosmochimica Acta* 60(23), 4811–4829.
- Kürschner, W. M., Kvaček, Z., & Dilcher, D. L. (2008). The impact of Miocene atmospheric carbon dioxide fluctuations on climate and the evolution of terrestrial ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, 105(2), 449–453. <https://doi.org/10.1073/PNAS.0708588105>
- Kürschner, W. M., Van Der Burgh, J., Visscher, H., & Dilcher, D. L. (1996). Oak leaves as biosensors of late Neogene and early Pleistocene paleoatmospheric CO_2 concentrations. *Marine Micropaleontology*, 27, 299–312.
- Kuyper, B., Wingrove, H., Lesch, T., Labuschagne, C., Say, D., Martin, D., Young, D., Khan, M. A. H., O'Doherty, S., Davies-Coleman, M. T., & Shallcross, D. E. (2020). Atmospheric Toluene and Benzene Mole Fractions at Cape Town and Cape Point and an Estimation of the Hydroxyl Radical Concentrations in the Air above the Cape Peninsula, South Africa. *ACS Earth and Space Chemistry*, 4(1), 24–34. <https://doi.org/10.1021/acsearthspacechem.9b00207>
- Landais, A., Barkan, E., Yakir, D., & Luz, B. (2006). The triple isotopic composition of oxygen in leaf water. *Geochimica et Cosmochimica Acta*, 70(16), 4105–4115. <https://doi.org/10.1016/j.gca.2006.06.1545>

- le Roux, C. J. G., Grunow, J. O., Bredenkamp, G. J., Morris, J. W., & Scheepers, J. C. (1988). A classification of the vegetation of the Etosha National Park. *South African Journal of Botany*, 54(1), 1–10. [https://doi.org/10.1016/S0254-6299\(16\)31355-2](https://doi.org/10.1016/S0254-6299(16)31355-2)
- Lee-Thorp, J. A., & Ecker, M. (2015). Holocene Environmental Change at Wonderwerk Cave, South Africa: Insights from Stable Light Isotopes in Ostrich Eggshell. *African Archaeological Review*, 32(4), 793–811. <https://doi.org/10.1007/s10437-015-9202-y>
- Lehmann, B., Wahlen, M., Zumbunn, R., Oeschger, H., & Schnell, W. (1977). Isotope Analysis by Infrared Laser Absorption Spectroscopy. *Applied Physics*, 13, 53–158.
- Leuthold, W. (1977). Notes on the breeding biology of the ostrich *Struthio camelus* in Tsavo East National Park. *Ibis*, 119(4), 541–544.
- Levin, N. E., Cerling, T. E., Passey, B. H., Harris, J. M., & Ehleringer, J. R. (2006). A stable isotope aridity index for terrestrial environments. *Proceedings of the National Academy of Sciences of the United States of America*, 103(30), 11201–11205. www.pnas.org/cgi/doi/10.1073/pnas.0604719103
- Li, S., Levin, N. E., Soderberg, K., Dennis, K. J., & Caylor, K. K. (2017). Triple oxygen isotope composition of leaf waters in Mpala, central Kenya. *Earth and Planetary Science Letters*, 468, 38–50. <https://doi.org/10.1016/j.epsl.2017.02.015>
- Luz, B., & Barkan, E. (2010). Variations of $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ in meteoric waters. *Geochimica et Cosmochimica Acta*, 74(22), 6276–6286. <https://doi.org/10.1016/j.gca.2010.08.016>
- Luz, B., Barkan, E., Bender, M. L., Thiemens, M. H., & Boering, K. A. (1999). Triple-isotope composition of atmospheric oxygen as a tracer of biosphere productivity. *Nature*, 400, 547–550. <https://doi.org/https://doi.org/10.1038/22987>
- Ma, Q., Wu, S., & Tang, Y. (2008). Formation and abundance of doubly-substituted methane isotopologues ($^{13}\text{CH}_3\text{D}$) in natural gas systems. *Geochimica et Cosmochimica Acta*, 72(22), 5446–5456. <https://doi.org/10.1016/J.GCA.2008.08.014>
- Mahata, S., Bhattacharya, S. K., Wang, C. H., & Liang, M. C. (2013). Oxygen isotope exchange between O_2 and CO_2 over hot platinum: An innovative technique for measuring $\Delta^{17}\text{O}$ in CO_2 . *Analytical Chemistry*, 85(14), 6894–6901. https://doi.org/10.1021/AC4011777/ASSET/IMAGES/AC4011777.SOCIAL.JPEG_V03
- Matsuhisa, Y., Goldsmith, J. R., & Clayton, R. N. (1978). Mechanisms of hydrothermal crystallization of quartz at 250°C and 15 kbar. *Geochimica et Cosmochimica Acta*, 42(2), 173–182. [https://doi.org/10.1016/0016-7037\(78\)90130-8](https://doi.org/10.1016/0016-7037(78)90130-8)
- McManus, J. B., Nelson, D. D., & Zahniser, M. S. (2015). Design and performance of a dual-laser instrument for multiple isotopologues of carbon dioxide and water. *Optics Express*, 23(5), 6569. <https://doi.org/10.1364/oe.23.006569>
- Meijer, H. A. J., & Li, W. J. (1998). The Use of Electrolysis for Accurate $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ Isotope Measurements in Water. *Isotopes in Environmental and Health Studies*, 34(4), 349–369. <https://doi.org/10.1080/10256019808234072>
- Milatz, J. M. W., Kluyver, J. C., & Hardebol, J. (2004). Determination of Isotope Ratios, e.g., in Tracer Work, by an Infrared Absorption Method. *The Journal of Chemical Physics*, 119(7), 887. <https://doi.org/10.1063/1.1748402>
- Miller, M. F. (2002). Isotopic fractionation and the quantification of ^{17}O anomalies in the oxygen three-isotope system: an appraisal and geochemical significance. *Geochimica et Cosmochimica Acta*, 66(11), 1881–1889. [https://doi.org/10.1016/S0016-7037\(02\)00832-3](https://doi.org/10.1016/S0016-7037(02)00832-3)

- Miller, M. F., Franchi, I. A., Thiemens, M. H., Jackson, T. L., Brack, A., Kurat, G., & Pillinger, C. T. (2002). Mass-independent fractionation of oxygen isotopes during thermal decomposition of carbonates. *Proceedings of the National Academy of Sciences of the United States of America*, 99(17), 10988–10993.
<https://doi.org/10.1073/PNAS.172378499/ASSET/9E343966-FE14-4521-917F-88EA42DB1893/ASSETS/GRAPHIC/PQ1723784005.JPEG>
- Milton, S. J., Dean, W. R. J., & Siegfried, W. R. (1994). Food Selection by Ostrich in Southern Africa. *The Journal of Wildlife Management*, 58(2), 234. <https://doi.org/10.2307/3809386>
- Mrozek, D. J., Van Der Veen, C., Kliphuis, M., Kaiser, J., Wiegel, A. A., & Röckmann, T. (2015). Continuous-flow IRMS technique for determining the ^{17}O excess of CO_2 using complete oxygen isotope exchange with cerium oxide. *Atmospheric Measurement Techniques*, 8(2), 811–822. <https://doi.org/10.5194/amt-8-811-2015>
- Niespolo, E. M., Sharp, W. D., Tryon, C. A., Faith, J. T., Lewis, J., Ranhorn, K., Mambelli, S., Miller, M. J., & Dawson, T. E. (2020). Carbon, nitrogen, and oxygen isotopes of ostrich eggshells provide site-scale Pleistocene-Holocene paleoenvironmental records for eastern African archaeological sites. *Quaternary Science Reviews*, 230.
<https://doi.org/10.1016/j.quascirev.2019.106142>
- Nys, Y., Gautron, J., Garcia-Ruiz, J. M., & Hincke, M. T. (2004). Avian eggshell mineralization: Biochemical and functional characterization of matrix proteins. *Comptes Rendus - Palevol*, 3(6-7 SPEC.ISS.), 549–562.
<https://doi.org/10.1016/J.CRPV.2004.08.002>
- Ono, S., Wang, D. T., Gruen, D. S., Lollar, B. S., Zahniser, M. S., Mcmanus, B. J., & Nelson, D. D. (2014). Measurement of a Doubly Substituted Methane Isotopologue, $^{13}\text{CH}_3\text{D}$, by Tunable Infrared Laser Direct Absorption Spectroscopy. *Analytical Chemistry*, 86(13), 6487–6494. <https://doi.org/10.1021/ac5010579>
- Pack, A., & Herwartz, D. (2014). The triple oxygen isotope composition of the Earth mantle and understanding $\Delta^{17}\text{O}$ variations in terrestrial rocks and minerals. *Earth and Planetary Science Letters*, 390, 138–145. <https://doi.org/10.1016/j.epsl.2014.01.017>
- Pack, A., Gehler, A., & Süssenger, A. (2013). Exploring the usability of isotopically anomalous oxygen in bones and teeth as paleo- CO_2 -barometer. *Geochimica et Cosmochimica Acta*, 102, 306–317. <https://doi.org/10.1016/j.gca.2012.10.017>
- Passey, B. H., & Ji, H. (2019). Triple oxygen isotope signatures of evaporation in lake waters and carbonates: A case study from the western United States. *Earth and Planetary Science Letters*, 518, 1–12. <https://doi.org/10.1016/j.epsl.2019.04.026>
- Passey, B. H., & Levin, N. E. (2021). Triple Oxygen Isotopes in Meteoric Waters, Carbonates, and Biological Apatites: Implications for Continental Paleoclimate Reconstruction. *Reviews in Mineralogy and Geochemistry*, 86(1), 429–462.
<https://doi.org/10.2138/rmg.2021.86.13>
- Passey, B. H., Hu, H., Ji, H., Montanari, S., Li, S., Henkes, G. A., & Levin, N. E. (2014). Triple oxygen isotopes in biogenic and sedimentary carbonates. *Geochimica et Cosmochimica Acta*, 141, 1–125. <https://doi.org/10.1016/j.gca.2014.06.006>
- Perdue, N., Sharp, Z., Nelson, D., Wehr, R., & Dyroff, C. (2022). A rapid high-precision analytical method for triple oxygen isotope analysis of CO_2 gas using tunable infrared laser direct absorption spectroscopy. *Rapid Communications in Mass Spectrometry*, 36(21), e9391. <https://doi.org/10.1002/rcm.9391>

- Prokhorov, I., Kluge, T., & Janssen, C. (2019). Laser Absorption Spectroscopy of Rare and Doubly Substituted Carbon Dioxide Isotopologues. *Analytical Chemistry*, 91(24), 15491–15499. <https://doi.org/10.1021/acs.analchem.9b03316>
- Quade, J., Cerling, T. E., & Bowman, J. R. (1989). Systematic variations in the carbon and oxygen isotopic composition of pedogenic carbonate along elevation transects in the southern Great Basin, United States. *GSA Bulletin* 1989; 101 (4): 464–475. doi: [https://doi.org/10.1130/0016-7606\(1989\)101<0464:SVITCA>2.3.CO;2](https://doi.org/10.1130/0016-7606(1989)101<0464:SVITCA>2.3.CO;2)
- Riddell, E. S., Kilian, W., Versfeld, W., & Kosoana, M. (2016). Groundwater stable isotope profile of the Etosha National Park, Namibia. *Koedoe*, 58(1), 1329. <https://doi.org/10.4102/koedoe>
- Roden, J. S., & Ehleringer, J. R. (1999). Observations of Hydrogen and Oxygen Isotopes in Leaf Water Confirm the Craig-Gordon Model under Wide-Ranging Environmental Conditions 1. *Plant Physiology*, 120, 1165–1173. <https://academic.oup.com/plphys/article/120/4/1165/6085541>
- Roden, J. S., Lin, G., & Ehleringer, J. R. (2000). A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochimica et Cosmochimica Acta*, 64(1), 21–35. [https://doi.org/10.1016/S0016-7037\(99\)00195-7](https://doi.org/10.1016/S0016-7037(99)00195-7)
- Rothman, L. S., Jacquemart, D., Barbe, A., Benner, D. C., Birk, M., Brown, L. R., Carleer, M. R., Chackerian, C., Chance, K., Coudert, L. H., Dana, V., Devi, V. M., Flaud, J. M., Gamache, R. R., Goldman, A., Hartmann, J. M., Jucks, K. W., Maki, A. G., Mandin, J. Y., ... Wagner, G. (2005). The HITRAN 2004 molecular spectroscopic database. *Journal of Quantitative Spectroscopy and Radiative Transfer*, 96(2), 139–204. <https://doi.org/10.1016/J.JQSRT.2004.10.008>
- Sakai, S., Matsuda, S., Hikida, T., Shimono, A., Mcmanus, J. B., Zahniser, M., Nelson, D., Dettman, D. L., Yang, D., & Ohkouchi, N. (2017). High-Precision Simultaneous $^{18}\text{O}/^{16}\text{O}$, $^{13}\text{C}/^{12}\text{C}$, and $^{17}\text{O}/^{16}\text{O}$ Analyses for Microgram Quantities of CaCO_3 by Tunable Infrared Laser Absorption Spectroscopy. *Analytical Chemistry*, 89(21), 11846–11852. <https://doi.org/10.1021/ACS.ANALCHEM.7B03582>
- Sarma, V. V. S. S., Abe, O., & Saino, T. (2003). Chromatographic Separation of Nitrogen, Argon, and Oxygen in Dissolved Air for Determination of Triple Oxygen Isotopes by Dual-Inlet Mass Spectrometry. *Analytical Chemistry*, 75(18), 4913–4917. <https://doi.org/10.1029/2001gb001460>
- Ségalen, L., & Lee-Thorp, J. A. (2009). Palaeoecology of late Early Miocene fauna in the Namib based on $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios of tooth enamel and ratite eggshell carbonate. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 277(3–4), 191–198. <https://doi.org/10.1016/j.palaeo.2009.03.018>
- Ségalen, L., Renard, M., Lee-Thorp, J. A., Emmanuel, L., Le Callonnec, L., de Rafélis, M., Senut, B., Pickford, M., & Melice, J. L. (2006). Neogene climate change and emergence of C4 grasses in the Namib, southwestern Africa, as reflected in ratite ^{13}C and ^{18}O . *Earth and Planetary Science Letters*, 244(3–4), 725–734. <https://doi.org/10.1016/J.EPSL.2005.12.012>
- Ségalen, L., Renard, M., Pickford, M., Senut, B., Cojan, I., Le Callonnec, L., & Rognon, P. (2002). Environmental and climatic evolution of the Namib Desert since the Middle Miocene: the contribution of carbon isotope ratios in ratite eggshells. *Comptes Rendus Geoscience*, 334(12), 917–924.
- Senut, B., Pickford, M., & Ségalen, L. (2009). Neogene desertification of Africa. *Comptes Rendus Geoscience*, 341(8–9), 591–602. <https://doi.org/10.1016/j.crte.2009.03.008>

- Sha, L., Mahata, S., Duan, P., Luz, B., Zhang, P., Baker, J., Zong, B., Ning, Y., Brahim, Y. A., Zhang, H., Edwards, R. L., & Cheng, H. (2020). A novel application of triple oxygen isotope ratios of speleothems. *Geochimica et Cosmochimica Acta*, 270, 360–378. <https://doi.org/10.1016/j.gca.2019.12.003>
- Sharp, Z. D., Wostbrock, J. A. G., & Pack, A. (2018). Mass-dependent triple oxygen isotope variations in terrestrial materials. *Geochemical Perspectives Letters*, 7, 27–31. <https://doi.org/10.7185/geochemlet.1815>
- Sinclair, A. R. E. (1978). Factors effecting the food supply and breeding season of resident birds and movements of Palaeoarctic migrants in tropical African savannah. *Ibis*, 120(4), 480–497.
- Steinthorsdottir, M., Coxall, H. K., de Boer, A. M., Huber, M., Barbolini, N., Bradshaw, C. D., Burls, N. J., Feakins, S. J., Gasson, E., Henderiks, J., Holbourn, A. E., Kiel, S., Kohn, M. J., Knorr, G., Kürschner, W. M., Lear, C. H., Liebrand, D., Lunt, D. J., Mörs, T., ... Strömberg, C. A. E. (2021). The Miocene: The Future of the Past. *Paleoceanography and Paleoclimatology*, 36(4). <https://doi.org/10.1029/2020PA004037>
- Steur, P. M., Scheeren, H. A., Nelson, D. D., McManus, J. B., and Meijer, H. A. J. (2021) Simultaneous measurement of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ of atmospheric CO_2 – performance assessment of a dual-laser absorption spectrometer, *Atmos. Meas. Tech.*, 14, 4279–4304, <https://doi.org/10.5194/amt-14-4279-2021>
- Stoltmann, T., Casado, M., Daëron, M., Landais, A., & Kass, S. (2017). Direct, Precise Measurements of Isotopologue Abundance Ratios in CO_2 Using Molecular Absorption Spectroscopy: Application to $\Delta^{17}\text{O}$. *Analytical Chemistry*, 89(19), 10129–10132. https://doi.org/10.1021/ACS.ANALCHEM.7B02853/ASSET/IMAGES/ACS.ANALCHEM.7B02853.SOCIAL.JPEG_V03
- Surma, J., Assonov, S., Bolourchi, M. J., & Staubwasser, M. (2015). Triple oxygen isotope signatures in evaporated water bodies from the Sistan Oasis, Iran. *Geophysical Research Letters*, 42(20), 8456–8462. <https://doi.org/10.1002/2015GL066475>
- Surma, J., Assonov, S., Herwartz, D., Voigt, C., & Staubwasser, M. (2018). The evolution of ^{17}O -excess in surface water of the arid environment during recharge and evaporation. *Scientific Reports*, 8(1). <https://doi.org/10.1038/s41598-018-23151-6>
- Tian, C., Wang, L., Kaseke, K. F., & Bird, B. W. (2018). Stable isotope compositions ($\delta^2\text{H}$, $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$) of rainfall and snowfall in the central United States. *Scientific Reports*, 8(1), 6712. <https://doi.org/10.1038/s41598-018-25102-7>
- Tittel, F. K., Richter, D., & Fried, A. (2003). Mid-Infrared Laser Applications in Spectroscopy. *Topics in Applied. Physics*, 89, 445–516. https://doi.org/10.1007/3-540-36491-9_11
- Uechi, Y., & Uemura, R. (2019). Dominant influence of the humidity in the moisture source region on the ^{17}O -excess in precipitation on a subtropical island. *Earth and Planetary Science Letters*, 513, 20–28. <https://doi.org/10.1016/J.EPSL.2019.02.012>
- Voigt, C., Herwartz, D., Dorador, C., & Staubwasser, M. (2021). Triple oxygen isotope systematics of evaporation and mixing processes in a dynamic desert lake system. *Hydrology and Earth System Sciences*, 25, 1211–1228. <https://doi.org/10.5194/hess-25-1211-2021>
- von Schirnding, Y., Van der Merwe, N. J., & Vogel, J. C. (1982). Influence of diet and age on carbon isotope ratios in ostrich eggshell. *Archaeometry*, 24(1), 3–20. <https://doi.org/10.1111/J.1475-4754.1982.TB00643.X>

- Wang, Y., Momohara, A., Wang, L., Lebreton-Anberrée, J., & Zhou, Z. (2015). Evolutionary History of Atmospheric CO₂ during the Late Cenozoic from Fossilized Metasequoia Needles. *PLOS ONE*, 10(7), e0130941. <https://doi.org/10.1371/JOURNAL.PONE.0130941>
- Wang, Z., Nelson, D. D., Dettman, D. L., McManus, J. B., Quade, J., Huntington, K. W., Schauer, A. J., & Sakai, S. (2020). Rapid and Precise Analysis of Carbon Dioxide Clumped Isotopic Composition by Tunable Infrared Laser Differential Spectroscopy. *Analytical Chemistry*, 92(2), 2034–2042. <https://doi.org/10.1021/acs.analchem.9b04466>
- Wanke, H., Gaj, M., Beyer, M., Koeniger, P., & Hamutoko, J. T. (2018). Stable isotope signatures of meteoric water in the Cuvelai-Etosha Basin, Namibia: Seasonal characteristics, trends and relations to southern African patterns. *Isotopes in Environmental and Health Studies*, 54(6), 588–607. <https://doi.org/10.1080/10256016.2018.1505724>
- Wen, X. F., Meng, Y., Zhang, X. Y., Sun, X. M., & Lee, X. (2013). Evaluating calibration strategies for isotope ratio infrared spectroscopy for atmospheric ¹³CO₂/¹²CO₂ measurement. *Atmospheric Measurement Techniques*, 6(6), 1491–1501. <https://doi.org/10.5194/AMT-6-1491-2013>
- Werle, P., Mücke, R., & Slemr, F. (1993). The limits of signal averaging in atmospheric trace-gas monitoring by tunable diode-laser absorption spectroscopy (TDLAS). *Applied Physics B Photophysics and Laser Chemistry*, 57(2), 131–139. <https://doi.org/10.1007/BF00425997>
- West, A. G., February, E. C., & Bowen, G. J. (2014). Spatial analysis of hydrogen and oxygen stable isotopes (“isoscapes”) in ground water and tap water across South Africa. *Journal of Geochemical Exploration*, 145, 213–222. <https://doi.org/10.1016/J.GEXPLO.2014.06.009>
- Whiteman, J. P., Sharp, Z. D., Gerson, A. R., & Newsome, S. D. (2019). Relating Δ¹⁷O values of animal body water to exogenous water inputs and metabolism. *BioScience*, 69(8), 658–668. <https://doi.org/10.1093/biosci/biz055>
- Williams, J. B., Siegfried, W. R., Milton, S. J., Adams, N. J., Dean, W. R. J., Du Plessis, M. A., Jackson, S., Roy, W., Jackson, S., & Nagy, K. A. (1993). Field metabolism, water requirements, and foraging behavior of wild ostriches in the Namib. *Ecology*, 74(2), 390–404.
- Wostbrock, J. A. G., Cano, E. J., & Sharp, Z. D. (2020). An internally consistent triple oxygen isotope calibration of standards for silicates, carbonates and air relative to VSMOW2 and SLAP2. *Chemical Geology*, 533(5), 1–9. <https://doi.org/10.1016/j.chemgeo.2019.119432>
- Wostbrock, J. A. G., Sharp, Z. D., Sanchez-Yanez, C., Reich, M., van den Heuvel, D. B., & Benning, L. G. (2018). Calibration and application of silica-water triple oxygen isotope thermometry to geothermal systems in Iceland and Chile. *Geochimica et Cosmochimica Acta*, 234, 84–97. <https://doi.org/10.1016/J.GCA.2018.05.007>
- Young, E. D., Galy, A., & Nagahara, H. (2002). Kinetic and equilibrium mass-dependent isotope fractionation laws in nature and their geochemical and cosmochemical significance. *Geochimica et Cosmochimica Acta*, 66(6), 1095–1104.
- Young, E. D., Yeung, L. Y., & Kohl, I. E. (2014). On the δ¹⁷O budget of atmospheric O₂. *Geochimica et Cosmochimica Acta*, 135, 102–125. <https://doi.org/10.1016/j.gca.2014.03.026>

Appendix

Chapter 3 Supplementary:

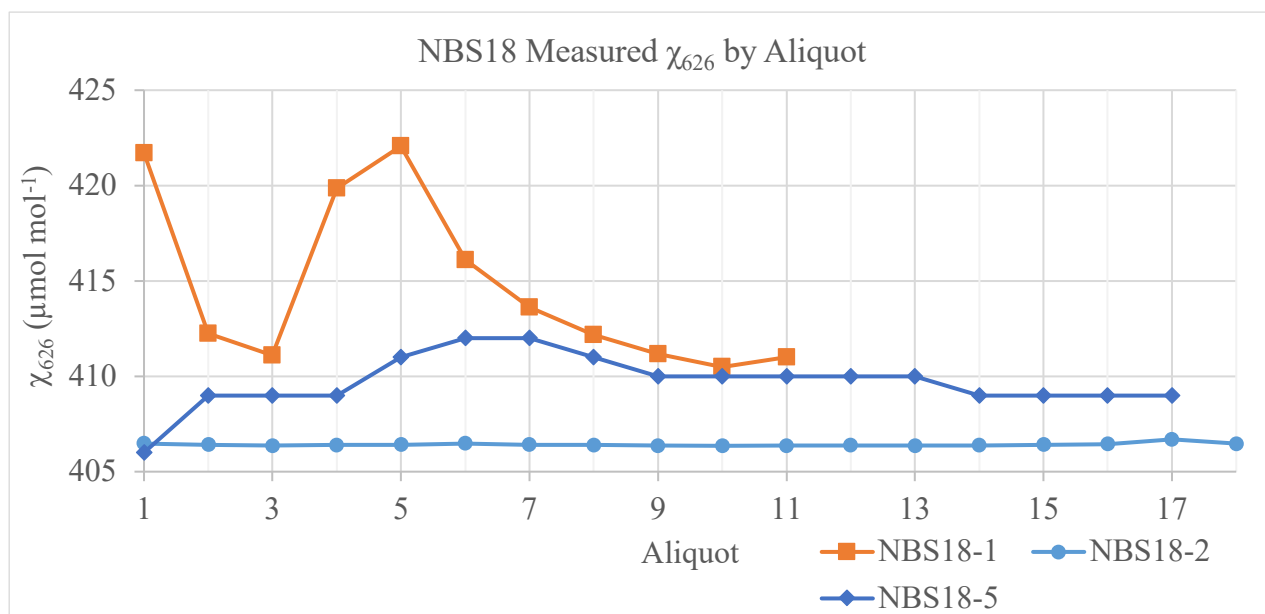


Figure S3.1: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) by aliquot for 3 NBS-18 samples mixed by the “Turbulent Advection” method. Data given in Experiment B.

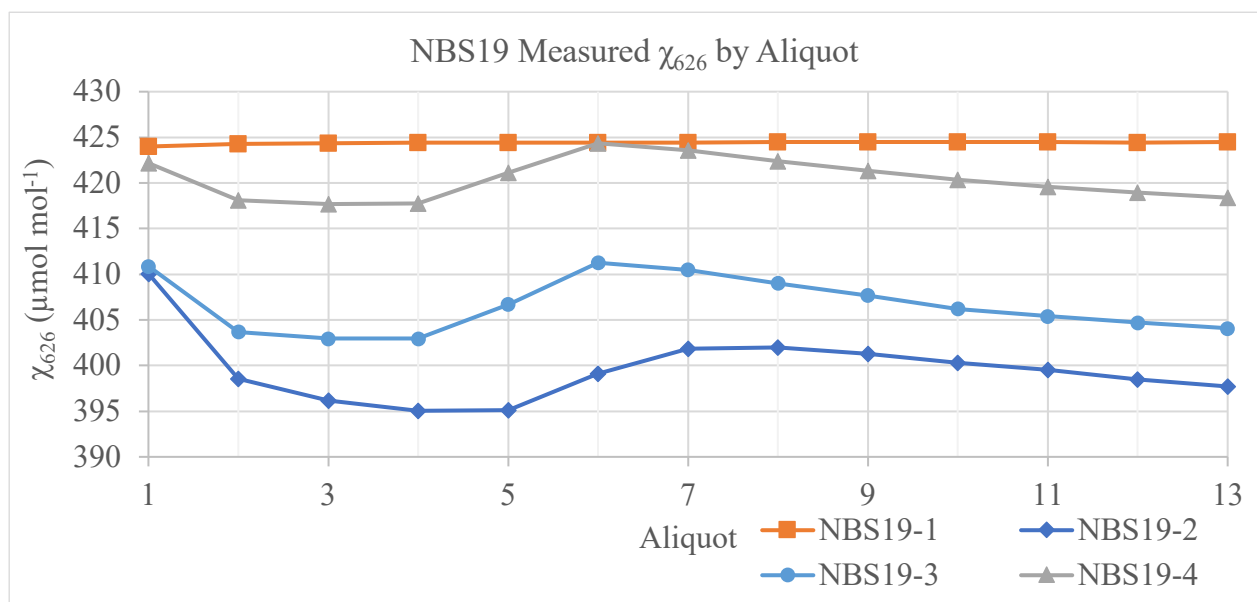


Figure S3.2: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) by aliquot for 4 NBS-19 samples mixed by the “Turbulent Advection” method. Data given in Experiment B.

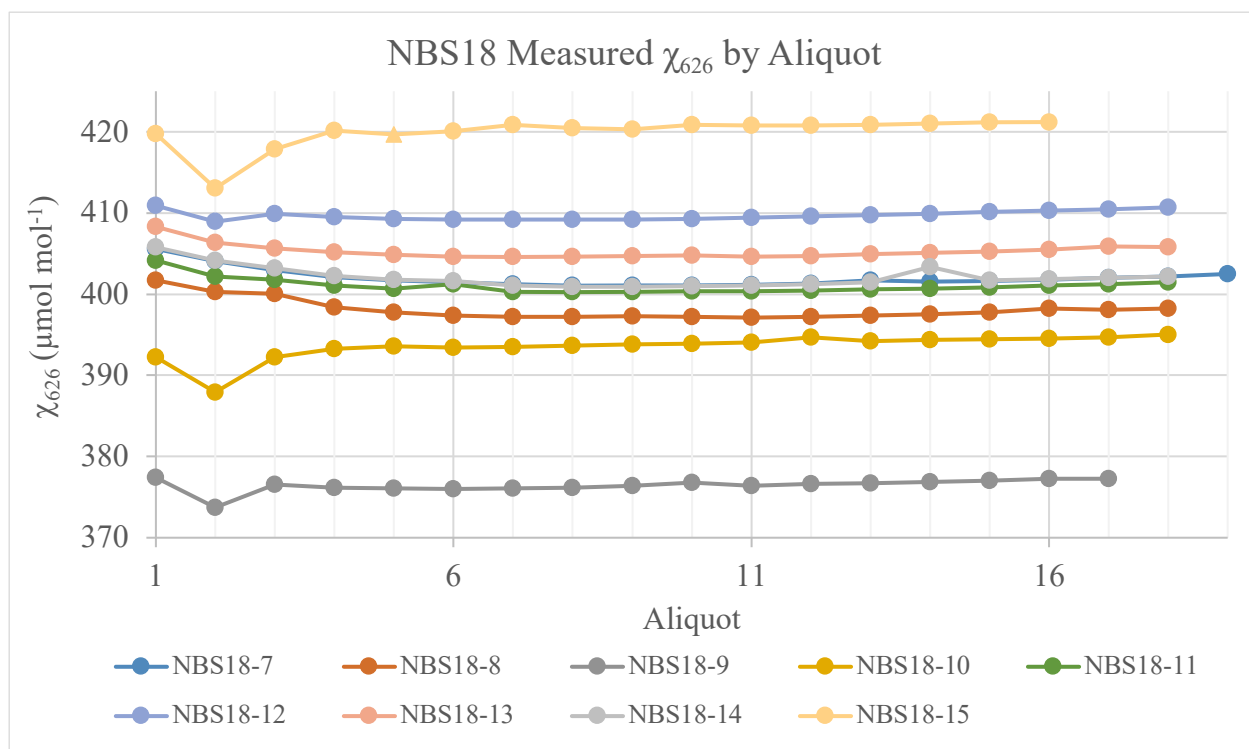


Figure S3.3: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) by aliquot for 9 NBS-18 samples mixed by the “Circulation” method. Data given in Experiment C.

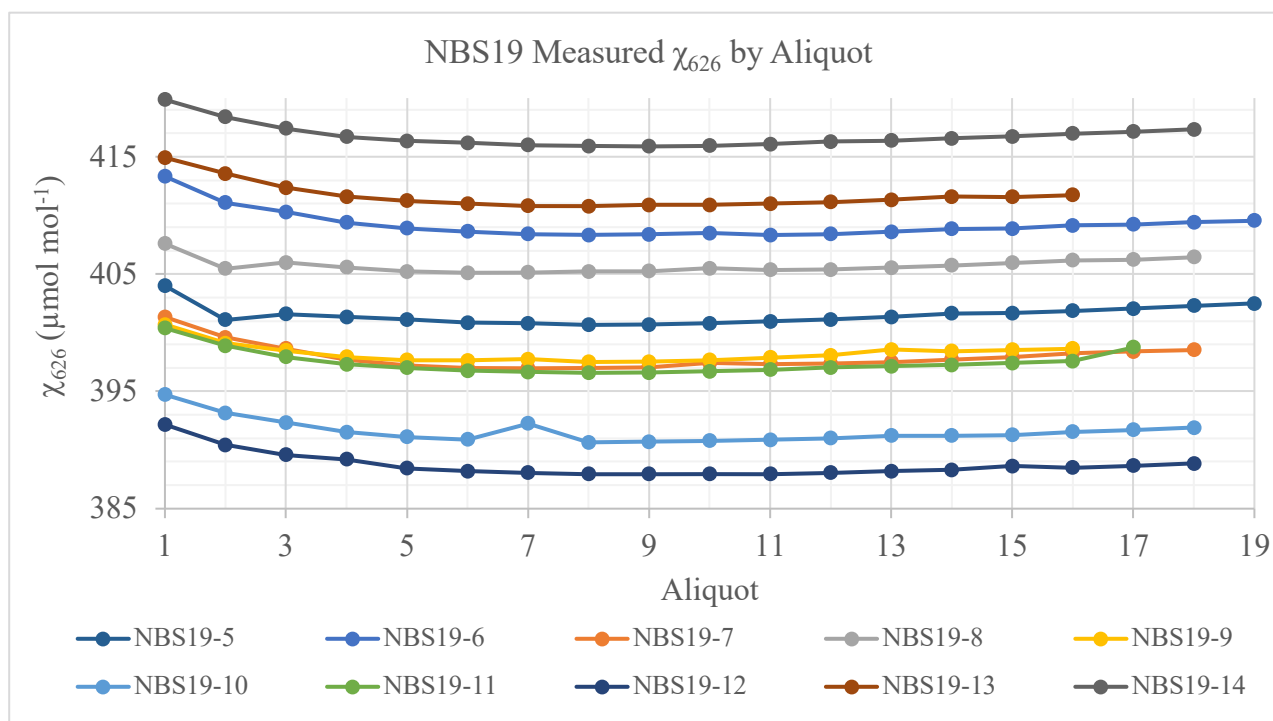


Figure S3.4: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) by aliquot for 10 NBS-19 samples mixed by the “Circulation” method. Data given in Experiment C.

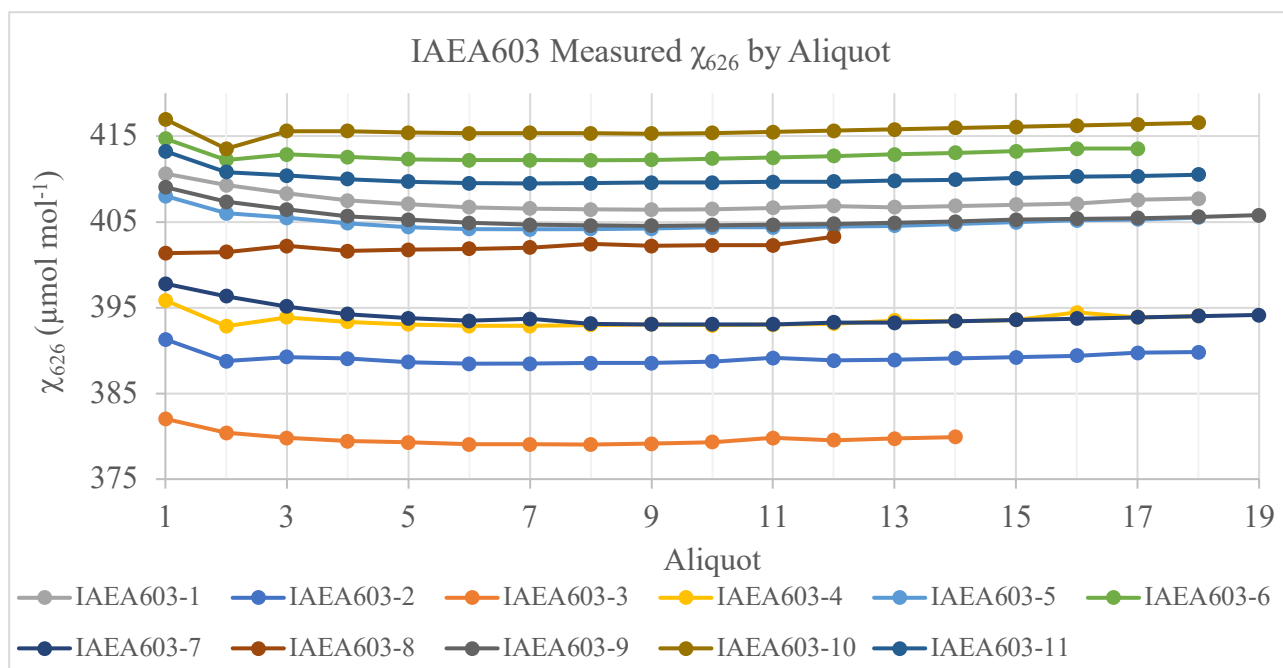


Figure S3.5: $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ isotopologue abundance (χ_{626}) by aliquot for 11 IAEA603 samples mixed by the “Circulation” method. Data given in Experiment C.

Chapter 4 Supplementary:

Sample	$\Delta'^{17}\text{O}$ (per meg)	1σ
UPMC-Ka-2-Sc-1	-143	38
UPMC-Ka-2-Sc-2	-218	40
UPMC-Ka-2-Sc-3	-248	33
UPMC-GPP-2-Sc-1	-163	35
UPMC-GPP-2-Sc-2	-84	35
UPMC-GPP-2-Sc-3	-160	63
UPMC-RO-1-Sc-1	-237	31
UPMC-RO-1-Sc-2	48	22
UPMC-RO-1-Sc-3	-221	50
UPMC-RO-1-NO-1	-234	51
UPMC-RO-1-NO-2	-147	63
UPMC-RO-1-NO-3	-211	72

Table S4.1: Sample analyses excluded from final data results highlighted in red.

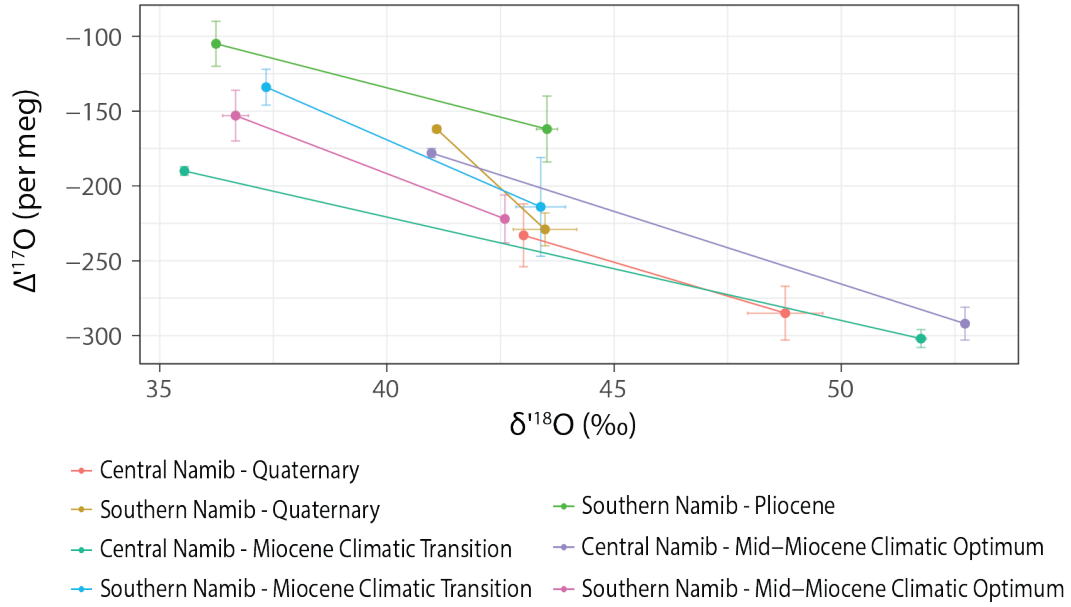


Figure S4.1: Sample trends from each region – time period combination. All error bars are 1σ . Relative to the VSMOW2-SLAP2 scale.

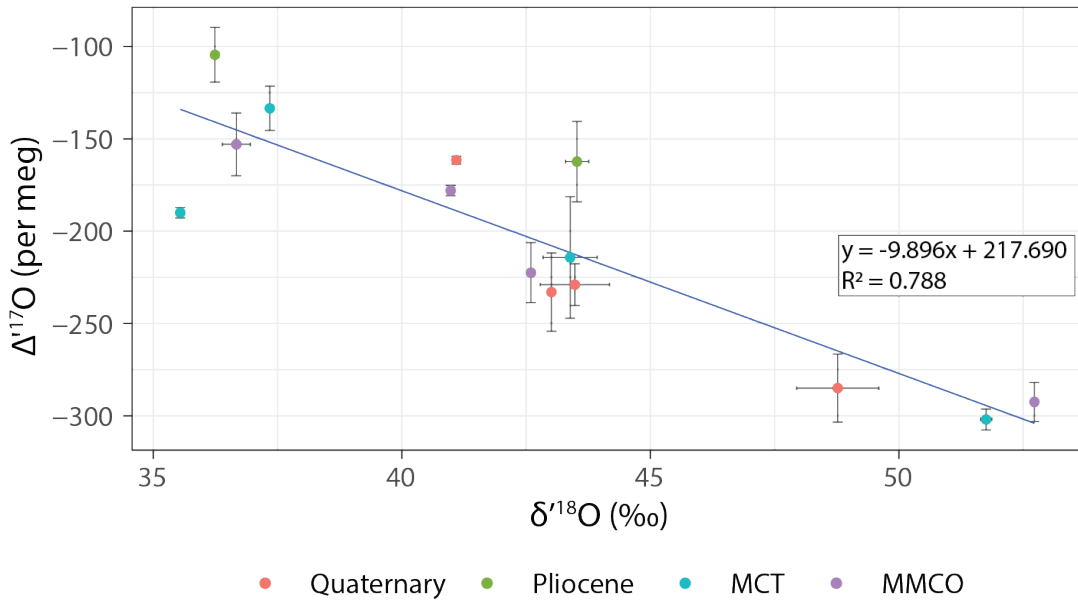


Figure S4.2: Sample $\delta^{18}\text{O}$ - $\Delta^{17}\text{O}$ isotopic composition, corrected for calcite. All error bars are 1σ . MCT is Miocene Climatic Transition. MMCO is Mid-Miocene Climatic Optimum. Relative to the VSMOW2-SLAP2 scale.

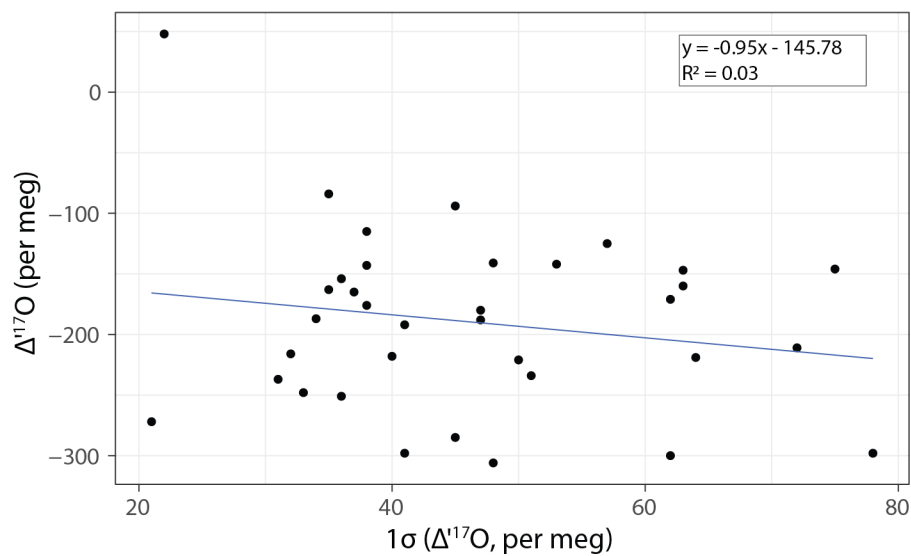


Figure S4.3: Comparison between sample $\Delta^{17}\text{O}$ value (y-axis) and $\Delta^{17}\text{O}$ 1σ precision (x-axis) shows no correlation between absolute $\Delta^{17}\text{O}$ value and size of 1σ error. All sample replicate analyses, including those excluded from final data analysis as given in Table S4.1, are plotted. Relative to the VSMOW2-SLAP2 scale.