# Movement and dietary preferences of migratory ungulates as estimated from stable isotope ratios in tail hair

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# Abstract

Globally, ungulate populations face considerable threats from human activities, which have resulted in the global decline (and local extinction) of major ungulate species including the African elephant (*Loxodonta Africana*), the black rhinoceros (*Diceros bicornis*) and most recently, the African giraffe (*Giraffa camelopardalis*) . However, the most common animal response is movement, and the most common human mitigation strategy has been expanding coverage of protected areas. Yet, a significant loss of ungulates' species and biodiversity has been reported across different protected areas, suggesting that expanding protected areas alone might not be the most effective solution. Restoring historical connectivity and migratory corridors might offer a more sustainable and manageable solution. However, to effectively apply conservation and restoration methods, historical information on the extent of animal movement patterns as well as their dietary preferences is of utmost importance. Yet, answering the question of where, how and why animals move has always been methodologically challenging, limiting our understanding of the extent of their movements as well their associated dietary preferences. The use of tracking devices and other traditional methods for studying animal movement has proven expensive and can pose serious concerns for animal welfare. Furthermore, studying movement retrospectively is impossible using the aforementioned techniques. Stable isotope ratio analysis techniques offer an alternative solution for exploring animal movement that is relatively cheap, easy to interpret and more importantly non-invasive to individual animals. Furthermore, the technique offers a possibility of understanding the historical range of animal movements retrospectively using archived materials such as specimens from museums. The primary contribution of this thesis is to demonstrate the applicability of isotopic analysis of tail hair to studies of movement and dietary interactions of migratory ungulates using the Serengeti ecosystem in East Africa as a case study.

The use of tail hair is promising because it represents a less-invasive way of recreating time-series information of animal's movement and feeding history. The Serengeti ecosystem presents a powerful model system to explore the use of isotopic methods applied to the tail hair of migratory ungulates because it harbors more than 27 species of ungulates including the migratory blue wildebeest (*Connochaetes taurinus*) and plain zebra (*Equus quagga*). Furthermore, the

ecosystem has a diversity of soil types as influenced by the underlying geological parent material, which gives the ecosystem a strong spatial variability in the isotope values. The study presented here seeks to: demonstrate the potential of utilizing a less-invasive technique of stable isotope ratios in tail hairs to investigate animal movement. Then, to examine the feasibility of using isotopic ratios in tail hair for geolocating migratory animals. Furthermore, the study aims to demonstrate how isotopic ratios in tail hair can help to distinguish between resident and migrant life history strategies in a mixed population. And lastly, to employ isotopic ratios in tail hair to gain insight into the periods of dietary convergence and partitioning during the annual cycle of co-migrating species.

Using spatial generalized additive model (GAM), this study establishes an interpolated map for the variation of sulfur stable isotope ratios ($\delta^{34}S$) in the grass across the Serengeti ecosystem (hereby referred to as the $\delta^{34}S$ isoscape). The isoscape was underpinned by a positive relationship between $\delta^{34}S$ and the local lithology. Using tail hairs sampled from cattle (*Bos taurus*), the created isoscape is used to test the hypothesis that $\delta^{34}S$ in tail hair reflects the $\delta^{34}S$ values of the diet (in this case, grass), and they can therefore be used for animal geolocation. Through a series of mathematical models and tail hair samples from GPS collared wildebeest, the $\delta^{34}S$ isoscape is used to identify the scenarios in which $\delta^{34}S$ in the tail hair are useful in the analysis of movement of migratory animals. Furthermore, I show using a series of state-space models of animal movement, how the $\delta^{34}S$ isoscape can be used in combination with variation of $\delta^{34}S$ in tail hair to differentiate resident versus migrant life-history forms of wildebeest. Using Stable Isotope Mixing Models (SIMMs), stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in the tail hair of wildebeest and zebra are used to understand seasonal periods of diet convergence and partitioning for the co-migrating animal species.

The results of this study suggest that the Serengeti ecosystem has strong spatial variability in $\delta^{34}S$ across its geological ranges. Furthermore, the results suggest that the $\delta^{34}S$ values in the tail hair strongly reflect the $\delta^{34}S$ values in the grass, suggesting that the variation of $\delta^{34}S$ in the tail hair can be used as a natural bio-logger for differentiating local versus non-local movement of animals. The output from the mathematical models suggests that the values of $\delta^{34}S$ take an average of 78 days from when the forage is ingested to isotope reflection in the tail hair of migratory wildebeest, suggesting that $\delta^{34}S$ might not be as effective for geolocating large mobile animals. On the other hand, the results from state-

space models of animal movement suggest that the variation of $\delta^{34}S$ in the tail hair is effective in differentiating resident from migrant life history strategies. This finding led to the creation of a classification key for identifying resident versus migrant life-histories. Results from SIMMs have demonstrated that, using time-series information generated from tail hair samples, we can detect the periods of diet convergence versus diet partitioning between co-migrating animals. For example, the highest dietary niche overlap between wildebeest and zebra has been observed in August and the lowest in May. This finding suggests that we can identify periods of strong competition for pasture and possible mitigations that species take to maintain their co-existence using stable isotope ratios technique.

The results from this thesis highlight the importance and application of isotopic methods to understand movement and dietary preferences of migratory ungulates. The created $\delta^{34}S$ isoscape for the Serengeti acts as a baseline from which other isotopic studies can be conducted. Furthermore, the study has developed a classification key which can be adopted, modified and applied to a diversity of taxa for identifying resident and migrant life-histories. The study has further helped an understanding of niche partitioning and coexistence of species especially in biodiverse regions where multiple species all focus on the same general resource.

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**“Nothing in the world is worth having or worth doing unless it means effort, pain or difficulty” Theodore Roosevelt.**

# Author’s declaration

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I affirm that I have not submitted this dissertation for any other degree at the University of Glasgow or any other institution, and that it does not exceed the prescribed word limit.

Signature

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# List of abbreviation

AIC - Akaike Information Criterion

AR 1 – First-order Autoregressive

ARC2 - African Rainfall and Climate version 2

CDT - Canyon Diablo Troilite

CEC – Cation Exchange Capacity

CIs - Credible Intervals

DDS - double distilled water

DICs - Deviance Information Criteria

GAM – Generalized Additive Model

IRMS -Isotope Ratio Mass Spectrometry

MAP – Mean Annual Precipitation

MCMC - Markov chain Monte Carlo

MRR - Mark Release Recapture

NDVI - Normalized Difference Vegetation Index

NM-AIST - Nelson Mandela African Institution of Science and Technology

NOAA - National Oceanic and Atmospheric Administration

PDB - Pee Dee Belemnite

SEAB – Bayesian Standard Ellipse Area

SIA - Stable Isotope Analysis

SIBER - Stable Isotope Bayesian Ellipses in R

SIMMR - Stable Isotope Mixing Models in R

SIMMs - Stable Isotope Mixing Models

SUERC - Scottish Universities Environmental Research Centre

UDSM – University of Dar es Salaam

VCDT - Vienna Canyon Diablo Troilite

VPDB - Vienna Pee Dee Belemnite

VSMOW - Vienna Standard Mean Ocean Water

# Chapter 1: Introduction

## 1.1 Global trend of ungulates' population and conservation

Worldwide, ungulate populations face considerable threats from climate change, zoonotic diseases and human activities (Buuveibaatar et al., 2016; Mduma, Hilborn, & Sinclair, 1998; Ogutu et al., 2009; Ogutu et al., 2016; Polfus & Krausman, 2012; Tucker et al., 2018). For example, over the past two decades, the world has experienced a global decline (and local extinction) of major ungulates' species including the African elephant (World Wildlife crime report, 2020), the black rhinoceros (World Wildlife crime report, 2020) and most recently the African giraffe (Muller et al., 2018). Many of these declines have been recorded across the African tropics (UNEP-WCMC, 2016). Therefore, understanding how to preserve the current population levels while ensuring a sustainable future of biodiversity conservation is of high scientific importance. However, addressing the threats to ungulates and biodiversity loss across developing countries has always been a challenge mainly due to both technological and financial constraints (UNEP-WCMC, 2016).

The most common response to ungulates and biodiversity loss has been an increased coverage of protected areas (Butchart et al., 2010; Jenkins & Joppa, 2009; Zimmerer et al., 2004). However, a significant loss of wildlife species and biodiversity is observed across different protected areas (Msuha et al., 2012; Ogutu et al., 2009; Seki et al., 2018; Western et al., 2009), suggesting that expanding protected areas is not a permanent solution to this problem. Restoring historical connectivity and migratory corridors might offer a more sustainable and manageable solution. To effectively apply conservation and restoration methods, historical information on the extent of animal movement patterns as well as their dietary preferences is of utmost importance (Borgmann & Conway, 2015). However, a thorough understanding of animal movement and their associated dietary preferences is always limited due to resource limitations and animal welfare concerns.

## 1.2 The concept of movement

Movement is the fundamental ecological and biological process that shapes the morphology, physiology, life-history, and behavior of an organism (Rubenstein & Hobson, 2004). Animal movement is a function of organism's health, experience, decision-making processes, navigation capacity as well as biotic factors (Nathan et al., 2008). Movement has important implications for an animal's reproductive capacity, survival, and population viability at larger-scales and its resultant influences on ecosystem processes.

Animal movement encompasses a broad range of an animal's behavior and activities. These have been categorized into four major groups based on their movement characteristics in space and time. These are; sedentarism, dispersal, nomadism, and migration. By being sedentary, an individual inhabits a relatively small area compared to the population distribution. An individual is required to have a stable home range or territory to occupy (Mueller & Fagan, 2008). Nomadism and migration differ only in the sense that nomadism does not result in a permanent settlement but an individual moving from place to place, seasonally and within a defined territory (Mueller & Fagan, 2008), but migration consists of seasonal movements between spatially disjointed and distinct areas. Dispersal refers to a move away from a birthplace to a place of reproduction. These categories vary in terms of pathways, distance, timing, shapes, and sizes of home ranges (Allen & Singh, 2016), all of which should be taken into account when planning, designing, and implementing different management actions.

## 1.3 Animal movement and species management

Animal movement is driven by both internal state (i.e. physiological and psychological conditions) and navigation capacity (Doherty & Driscoll, 2017; Nathan et al., 2008) and it plays a vital role in the conservation and management of wildlife species. The behavior and range of an animal's movement is influenced by their body size, human disturbances, diet availability and habitat quality (Tucker et al., 2018). For example, in the areas with high human footprint the extent of animal movement has been reported to be lower than in areas with low human footprint (Tucker et al., 2018). Therefore, the quality of the habitat plays a major role in determining the distribution and abundance of the organisms in the area (Boyce et al., 2016). If altered habitat results in insufficient resources or

unsustainable mortality levels, populations that lack the ability to move to alternative habitat may become extinct (Doherty & Driscoll, 2017; Fahrig & Merriam, 1985).

Animal movement provides links to core ecosystems processes such asthe redistribution of resources (i.e. nutrients in dung, seed dispersal, parasite dispersal) and genetic outbreeding (Allen & Singh, 2016), therefore, maintaining it is essential for the global ecosystem's health (Lundberg & Moberg, 2003; Massol et al., 2011). Although a lack of detailed information on where, when, how and why species move has always been a limiting factor for incorporating species movement into management objectives (Allen & Singh, 2016), a clear understanding of the movement attributes of species as well as their associated impacts on the ecosystem can help to identify the management actions that are relevant to the ecosystem's dynamics.

## 1.4 Studying movement

Over the last twenty years we have witnessed the rapid growth of animal movement research, and this has resulted in a multitude of articles being published in this field (e.g. Berger, 2004; Haché et al., 2014; Riggio & Caro, 2017; Rubenstein & Hobson, 2004; Sawyer et al., 2019; Shadrack et al., 2017; Trueman et al., 2012). Concurrently, this has led to the development of new methods and improvements on existing methods to analyze animal movement data (e.g. Bowen et al., 2005; Cagnacci et al., 2011; Hooten et al., 2018;Trueman et al., 2019). For example, the recent development of animal tracking technology has led to an exponential increase in movement data for wildlife globally (Joo et al., 2020). This has and continues to drive the development of new statistical methods to answer different animal movement research questions that are compatible with available data. This has helped to improve our understanding of how animals move from fine to coarser scale and its associated driving factors, behavioral changes under different environmental conditions, social interactions, and monitoring of wildlife in real time (Tucker et al., 2018), which is critical for wildlife managers and ecologists for sustainable management and conservation of declining wildlife species. Furthermore, the tracking technologies have helped to improve both

spatial and temporal [1]resolution of animal movement data (Bidder et al., 2015), such that, the time interval between consecutive locations of animal movement is shorter, making the movement data practically applicable (Wilmers et al., 2015).

Despite such advancement, animal movement is still a challenging field to study due to an inherent complexity of movement data that arises when it is collected from different sources (Table 1-1) such as mark release recapture (MRR) (Frame & Wagner, 1980; Magige & Senzota, 2006), different tracking devices like GPS collars and tags (Galanti et al., 2006; Sawyer et al., 2019; Tucker et al., 2018), photographic IDs (Araujo et al., 2019; Armstrong et al., 2019; Schofield et al., 2020) and intrinsic markers (Hobson, 2019; Hobson & Wassenaar, 2008;Trueman et al., 2012;Trueman & Glew, 2019). All these sources for collecting animal movement data have different strengths and limitations in making inferences when studying animal movement. This has and continues to stimulate debate around quantifying, improving, modelling statistical properties of animal movement, and making simulation-based inferences (e.g. Potts et al., 2018; Trueman et al., 2019).

Several methods exist for collecting and analyzing animal movement data. For example, our current understanding of the importance of animal movement was aided by the theory of Ideal Free Distribution (IFD) (Fretwell & Lucas, 1970). The theory states that animals will select prime habitats until the density is so great that it compromises their fitness, at which point animals will move to secondary habitats. The theory argues that individuals select habitats with low density to maximize their fitness. Several studies (e.g. Sutherland, 1996; Tregenza, 1995) have supported such assumptions including a theory of density dependent habitat selection (Rosenzweig, 1981). The theory has led to the development of increasingly sophisticated frameworks and models for dealing with movement data such as resource selection functions (Hooten et al., 2014; Johnson et al., 2013; Matthiopoulos et al., 2015), state-space models (Dorazio & Price, 2019; Patterson et al., 2008), hidden markov models (Adam et al., 2019; Bacheler et al., 2019; Goodall et al., 2019), and Brownian bridge movement models (Fischer et al., 2013; Kranstauber, 2019), to mention a few. These models provide insight

[1] Resolution in animal movement studies is the ability to collect details of movement information using a proposed technique, the details such as velocity, turning angles or stope over sites. Therefore, when a proposed technique can collect clear and relevant movement information based on either space that an animal is moving across (spatial) or the time scale of animal movement (temporal), then the resulting data will be of high resolution and of practical use.

into overall understanding of the mechanisms behind distribution and abundance of organisms, habitat usage, and how an animal moves and alters behaviors across different spatial and temporal scales (Cagnacci et al., 2010, 2011).

Table 1-1: Tools used to study movement for terrestrial animals, their strengths and weaknesses. Adapted from (Hobson, 2019)

| Technique | Strengths | Weaknesses |
|---|---|---|
| Mark release recapture | - Gives information at the start and the end of migration/journey<br>- Inexpensive<br>- Involves the use of leg bands, neck collars and dyes | - Small recovery rate<br>- Biased by animal size<br>- Labour intensive<br>- Requires handling the animal<br>- Mark may affect animal's behaviour |
| Radio transmitters and radar monitoring | - Small in size relative to GPS collars<br>- Not biased by animal size<br>- Gives relatively precise location and trajectories | - Moderately expensive<br>- Battery life is restricted<br>- Small sample sizes<br>- May affect behaviour of the tagged animal<br>- Requires capture and handling of animal |
| GPS tracking and satellite transmitters | - Small size<br>- High resolution<br>- Not species specific<br>- Provides precise location | - Expensive<br>- In some cases, individual must be recaptured to retrieve data<br>- May affect animal behaviour |
| Genetic markers | - Can be collected non-invasively without capturing an animal | - Low spatial resolution as a function of gentic map available<br>- Costly |

| | | |
|---|---|---|
| | - If capturing is necessary, only a single capture provides information on the origin of an animal | - Time intensive for data collection and analysis |
| Trace elements | - High spatial resolution<br>- Can use multiple elements | - Expensive<br>- Little is known about their variation across the globe<br>- Requires a continuous growing tissue |
| Stable isotopes | - Inexpensive<br>- Can use multiple elements to increase resolution<br>- Not species specific<br>- Can be collected in a less invasive way<br>- Stable across time | - Low spatial resolution<br>- Complication in interpretation due to animal's physiology and fractionation effects<br>- Limited knowledge about isotope variation in many landscapes<br>- Requires a continuous growing tissue |
| Pathogens and man-made contaminants | - High resolution given the clear understanding of the source of contaminat. | - Not very much is known about their geographical distribution<br>- Species specific especially pathogens<br>- Transport of contaminants might give defective geographical signals<br>- Invasive to animals |

## 1.5 Using diet to infer animal movement

An animal's diet has influence on both behavior and physiology (Han & Dingemanse, 2015). Diet is a fundamental environmental factor that determines animals' activity level including movement and mating (Han & Dingemanse, 2015). For instance, scientists have reported that an animal's diet is associated with different personalities, such as rule-based foraging decisions (Tremmel & Müller, 2013).

In recent years, scientists have been able to use GPS trackers to link an animals' diet and their movement patterns (e.g.Eikelboom et al., 2020; Frair et al., 2005). However, questions of why, when, how and what triggers animals to move have always been difficult to answer. A number of scientific approaches to movement ecology have been developed (e.g. Bacheler et al., 2019; Nathan et al., 2008), which have helped biologists to understand the complexities associated with animal movement. Recent advancements in technology have enabled scientists to use an animals' feeding history as a means to study their movement indirectly using intrinsic markers such as stable isotope ratios (Bowen et al., 2005; Hobson, 2019; Hobson & Wassenaar, 2008; Rubenstein & Hobson, 2004; Trueman et al., 2012), trace elements (Ethier et al., 2014), compound specific stable isotopes (Pilecky et al., 2022) or genetic markers (Clegg et al., 2003). Intrinsic markers present a promising future in the field of movement ecology because they help to disentangle difficult questions associated with animal movement. For example, through intrinsic markers, scientists have been able to shed some light onto the key questions of when (Hobson et al., 2012), how (Bowen et al., 2009; Garcia-Perez & Hobson, 2014), why (Haché et al., 2014; Hobson et al., 2014) and where (Garcia-Perez & Hobson, 2014) animals move.

## 1.6 Introduction to stable isotopes

Isotopes are elements with unique atomic masses. They have the same number of protons, but different numbers of neutrons. The most famous isotopes are the uranium isotopes which are used in nuclear reactors and atomic bombs (Fry, 2006) and they are examples of radioactive isotopes, which emit a variety of particles and decay or change into other elements. Stable isotopes are naturally occurring

stable forms of elements (Coiffait et al., 2009; Jianzhu et al., 2004) that do not decay after they are formed (Viljoen et al. 2016; West et al., 2006). However, their global concentration may change over time if they are formed by the radioactive decay of larger isotopes (i.e. they are radiogenic stable isotopes). In ecology, we are mostly interested in non-radiogenic stable isotopes whose global concentration is stable over geological times (Sharp, 2017).

The differences in nuclear masses of isotopes cause dissimilarity in their physical properties (Jianzhu et al., 2004; Sharp, 2017), leading to differences in their biogeochemical behaviors (Jianzhu et al., 2004; Rubenstein & Hobson, 2004). A good example of where these differences are evident is on the boiling point of pure water. If pure water is made of the rarer, heavy $^{18}O$ isotope it's boiling point is above 100°C (Chang, 2008), unlike the tap water which contains mostly the common, lighter isotope $^{16}O$. These differences cause the variation in the ratios of heavy to light isotopes in organic compounds (Ben-David & Flaherty, 2012). Usually, heavier stable isotopes of elements are rare and the lighter ones are abundant because of fractionation process (Hobson, 1999; Fry, 2006; Ben-David & Flaherty, 2012; Viljoen, et al. 2016). The abundance of lighter isotopes is influenced by both biological and biogeochemical processes and that of heavier isotopes is influenced by biogeochemical processes only (Fry, 2006; Rubenstein & Hobson, 2004; Sharp, 2017) causing lighter isotope to react more rapidly than the heavier isotope (Fry, 2006). In addition, chemical bonds formed by the heavier isotopes are more stable, bonds formed by lighter isotopes are broken more easily in chemical reactions.

Stable isotopes are safe to use unlike radioactive isotopes (Schellekens et al. 2011; Sharp, 2017), because they do not pose health impacts. Most elements consist of one or more stable isotopes (Ehleringer & Rundel 1989; Viljoen et al. 2016). For example, carbon has two stable forms; $^{12}C$ and $^{13}C$ and the ratio reported as ($\delta^{13}C$), oxygen has three stable forms; $^{16}O$, $^{17}O$ and $^{18}O$ and the ratio reported as ($\delta^{18}O$), nitrogen has two stable forms; $^{14}N$ and $^{15}N$ and the ratio reported as ($\delta^{15}N$), hydrogen has two stable forms; $^{1}H$ and $^{2}H$, formerly called deuterium (D) and the ratio reported as ($\delta^{2}H$) likewise sulfur has four stable forms; $^{32}S$, $^{33}S$, $^{34}S$ and $^{36}S$ and the ratio reported as ($\delta^{34}S$) (Camin et al., 2016; Ehleringer & Rundel, 1989; West et al., 2006). In all cases, the lighter isotopes (i.e. $^{12}C$, $^{1}H$, $^{14}N$, $^{32}S$ and $^{16}O$) are by far the most common, generally around 99% (Sharp, 2017).

Stable isotope studies have been successfully applied to the resolution of fundamental problems in earth and planetary sciences, human sciences, biological sciences and several subdisciplines of chemistry (Schellekens et al. 2011; Sharp, 2017). Generally, the analysis of stable isotope ratios can provide powerful tracers and chronometers for geosciences (Stack & Rock, 2011), archaeology (Nehlich et al., 2011; Richards et al., 2001), anthropology (Thompson et al., 2009) and environmental studies (Bogaard et al., 2007; Heaton, 1986; Liu et al., 2016).

### 1.6.1 Isotope measurements

The analysis of stable isotopes in samples is conducted using isotope ratio mass spectrometry (IRMS) and, in the past 20 years, commonly in conjunction with an elemental analyzer (Figure 1-1). The samples are usually obtained as dry organic materials and ground into a fine powder, and then weighed into small tin capsules (normally, 8mm in height and 5mm in diameter). The elemental analyser functions by combusting the organic material at a high temperature (1100°C) to produce gas ($N_2$, $CO_2$, $H_2$, and $SO_2$). Next, the sample is purified, and the gases are separated and extracted into the mass spectrometer. Under vacuum, in the source, the gases are ionized, accelerated, and separated based on their mass-to-charge ratios using a magnetic field. The separated ions are collected in Faraday cups, where the ratios of the desired isotopes are measured. The output of the measurement is in the form of ratios, which can be converted internally to either an atom percentage or a delta value based on the standards used and the specific experimental requirements.

The current IRMS systems can accurately measure isotope ratios in samples as small as 10μg of carbon and 5μg of nitrogen, and the analysis typically takes between 5 and 10 minutes per sample, depending on the specific machine and its settings. For gas and liquid samples, laser and infrared techniques are used to determine stable isotope measurements quickly, even at very low concentrations (e.g. in picomoles). This presents a promising opportunity for the future expansion and affordability of isotope analysis.

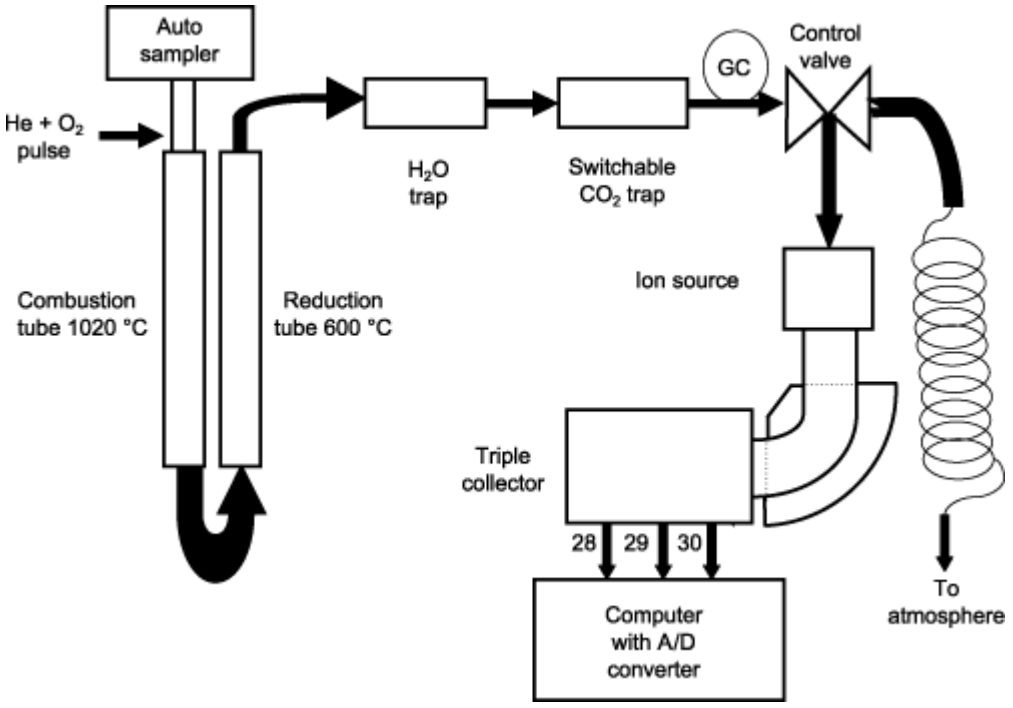


Figure 1-1: Graphic illustration elemental analyser linked to an isotope mass spectrometer. Adapted from (Hood & Blair, 2001).

### 1.6.2 Isotopes units and terminologies

To examine the isotopic composition of an element, the usual approach is to compare the ratio of its less common isotope to its more common isotope, and the resulting values are presented using the delta (δ) notation with reference to the global standard (Table 1-2), such that:

$$\delta^{H}X = \left[\frac{R_{sample}}{R_{standard}} - 1\right] * 1000 \qquad \text{Equation 1-1}$$

- Where X represents the element of interest (i.e. X = C,N,S,O or H),
- $\delta^{H}X$ is the heavy isotope mass of that element (i.e. $^{13}C$, $^{15}N$, $^{34}S$, $^{18}O$ or $^{2}H$) and
- R is the ratio of the heavy isotope to the light isotope for the element (i.e. $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$, $^{18}O/^{16}O$ or $^{2}H/^{1}H$).

The units for δ isotopes are represented by "‰" which is short for "permil" or "per mill" in Latin. This is because the difference between the samples and standards is quite small, so the δ values need to be amplified by multiplication with 1000 to make them more noticeable. For instance, a slight difference of 1 percent is multiplied by 1000 to become a 10 permil δ unit.

The reference point for measuring δ values is 0‰, meaning that any positive or negative values in the samples are relative to this standard. A δ value of 0‰ does not indicate the absence of isotopes, but rather means that the sample has no difference from the standard. For instance, a negative value does not imply that the sample contains a small amount of isotopes, but rather indicates that the sample is less heavy (i.e. has fewer heavy isotopes) compared to the standard. Conversely, a positive value means that the sample is relatively heavier (i.e. has more heavy isotopes) than the standard. The difference between heavy and lighter isotopes is caused by the fractionation process and more often the isotope values in the samples are depleted in their heavy isotopes (Fry, 2006).

A more detailed review of isotope abundances and their measurement is found in the isotope ecology book by Brian Fry (Fry, 2006).

Table 1-2: Isotope Compositions of International Reference Standards (*PDB and VPDB are considered equivalent and CDT and VCDT are considered equivalent). Adapted from (Gröning, 2004).

| Element | R isotopic ratio of the reference materials used for the delta scales | Reference materials used for the delta scales |
|---|---|---|
| Carbon $^{13}C/^{12}C$ | 0.0112372 | Pee Dee Belemnite (PDB) |
| *Carbon $^{13}C/^{12}C$ | 0.011224 | Vienna Pee Dee Belemnite (VPDB) |
| Nitrogen $^{15}N/^{14}N$ | 0.0036765 | Air (AIR) |
| Hydrogen $^{2}H/^{1}H$ | 0.00015576 | Vienna Standard Mean Ocean Water (VSMOW) |
| Oxygen $^{18}O/^{16}O$ | 0.0020052 | VSMOW |
| Sulfur $^{34}S/^{32}S$ | 0.0450045 | Canyon Diablo Troilite (CDT) |
| *Sulfur $^{34}S/^{32}S$ | 0.0441626 | Vienna Canyon Diablo Troilite (VCDT) |

### 1.6.3 Isotope fractionation

Isotope fractionation refers to relative partitioning of the heavier and lighter isotopes between two coexisting phases in a natural system (Fry, 2006; Gröning, 2004; Hood & Blair, 2001). This phenomenon is denoted by the Greek symbol Δ. The fractionation value that exists at the equilibrium of a system (i.e. the equilibrium constant) is known as the "fractionation factor" or "α," and it represents the isotopic partitioning between the two phases. This partitioning process is what creates the variations between the heavy and light isotopes within a given sample. The calculation of the isotope fractionation factor is as follows:

$$\Delta = (\alpha - 1) * 1000 \qquad \text{Equation 1-2}$$

Where Δ always gives the fractionation in (‰) as a positive value, because molecules of light isotopes react faster making this ratio always slightly greater than 1 (Fry, 2006; Sharp, 2017).

## 1.7 Application of stable isotopes to infer animal movement

Isotopic tracking of animal movements has been on-going since the 1990s, but was constrained by resource availability especially lab standards and facilities (Voigt & Lehnert, 2019). Only over the past two decades has the application of stable isotopes become popular in different movement studies such as (Hobson, 1999; Rubenstein & Hobson, 2004; Trueman et al., 2012; Trueman et al., 2017, 2019; Voigt & Lehnert, 2019). Intrinsic isotope markers allow the identification of the extent and patterns of isotope ratio variations in the environment to be traced through animal tissues (Bowen & West, 2019). However, their ability to reveal animal movement patterns depends on the assimilation and fixation of intrinsic stable isotope markers from environment to animal tissues (Bowen & West, 2019).

In the application of stable isotopes to study animal movement, there are several requirements to meet and principles to consider (Graham et al., 2010; Hobson, 2019). The first step is to have a tissue of interest from a consumer (e.g. Table 1-3). This is because stable isotopes reflect the dietary history of organisms through their tissues. Second is the time period of the tissue growth through which spatial isotopic signature is retained. This is used to estimate the amount of movement information that can be studied over the tissue growth period. For

example, a metabolically active tissue such as hair can provide a moving window of the dietary information throughout period of its growth while an inactive tissue e.g a feather, reflects a short period of its growth. Third is the construction of isotopic maps (isoscapes) across different spatial-temporal scales that are ecologically relevant to the movements of animal of interest. These maps represent the on-ground isotopic information which are related to isotopic information on animals' tissue (Bowen, 2010). Lastly is the mechanism related to diet transfer of isotopic signals. These mechanisms are important to understand isotope discrimination (fractionation) between consumer and diet.

Table 1-3: Different animal tissues for isotopic studies, their advantages, disadvantages and potential use.

| Tissue | Advantages | Disadvantages | uses |
|---|---|---|---|
| Blood | - Gives the most recent feeding information | - Informative of recent feeding history | - Diet elucidation<br>- Short term movements |
| Muscles | - Gives a detailed information of the most feeding history | - Differences in turnover, between and within species<br>- Can't be used to track long term movement | - Short-term movements |
| Nails and hooves | - Grow continuously<br>- Retain feeding history for long time | - Difficult sample preps and preservation<br>- Can grow moulds easily | In the studies of long movements and migration |
| Hairs | - Grow continuously | - Tissue turnover might pose a challenge<br>- Different species have different hair growth rate<br>- Difficult to estimate the actual growth rate | Useful in different dietary and medium-term movement studies |

| | | | |
|---|---|---|---|
| Tail hairs in ungulates / whiskers in carnivores | - Grow continuously<br>- Depending on the length of the hair, it can retain dietary information of up to three years<br>- Its sampling does not cause harm to animal | - Tissue turnover might pose a challenge<br>- Different species have different hair growth rate<br>- Different hairs of the same individual might have different growth rate<br>- Difficult to estimate the actual growth rate | Useful in different dietary and long-term movement studies |
| Tooth enamel and tusks | - Give a lifetime feeding history<br>- Excellent preservation of biogenic elements<br>- Its composition is a reliable indicator of childhood exposure | - Could be invasive to animals | - Useful in both archaeological and ecological studies<br>- Teeth of an individual therefore provide archive of both early and later childhood exposure because different teeth are formed at different stages of life |

| | | | |
|---|---|---|---|
| Bone and bone marrow | Provide a lifetime information of an animal | - Can cause harm to animal during sample collection | - Useful in life-time history of movement and forensic studies |
| Plasma | Provide information for the most recent diet history | Invasive to animal | - Short term dietary history |

### 1.7.1 Application of stable isotopes to study movement of terrestrial animals

Early studies that explored the ecology of migratory mammals using stable isotopes, established the $\delta^{13}C$ values to assess the diet of nectar feeding bat species in north America (Hobson, 1990). The $\delta^{13}C$ couldn't reveal the exact animal's movement pattern based on probability of origin approaches, but it highlighted the relevance of columnar cacti as a food source for migratory nectar-feeding bats. Normally, the suitability of $\delta^{13}C$ values for use in tracing spatial origins depends on whether $\delta^{13}C$ values vary in baseline food web samples, such as plant matter, across latitudinal, longitudinal, or elevational gradients.

#### 1.7.1.1 Hydrogen isotopes ($\delta^{2}H$)

When studying movement of animals using a stable isotope technique, the stable hydrogen ($\delta^{2}H$) or sulfur ($\delta^{34}S$) ratios are mainly used (Segers & Broders 2015). Hydrogen isotopes are used to make inferences of long-distance migrations because they vary across latitudinal gradients, reflecting local precipitation patterns (Hobson, 1999). They also vary seasonally and between marine and terrestrial sites (Rubenstein & Hobson 2004), enabling them to be used in tracing the origin of migrating species.

A major source of protein-based hydrogen in animal tissues is from food and water (Sharp et al., 2003). In early studies, hydrogen isotopes have been used as a tool for forensic and archaeological investigations (Sharp et al. 2003). The main approach that has been employed is to study sources of hydrogen isotopes from biologically inert tissue (Hobson, 2019), because hydrogen stable isotopes show distinct geographic patterns across the globe (Meehan et al., 2004).

#### 1.7.1.2 Oxygen isotopes

Oxygen isotopes vary as a function of climate and geography (Budd et al., 2004) and their main route of entering an animal's body is through drinking water (Longinelli, 1984). Oxygen isotopes undergo fractionation unlike strontium isotopes. Mammalian bodies have developed a mechanism to standardize fractionation between and within species through their constant body temperature maintenance (Bryant et al., 1996; Longinelli, 1984). Some species however, due to differences in body mass, diet and metabolism exhibit some variation but several developed calibrations have related oxygen isotopes ratios

to those of drinking water (Budd et al., 2004). This has enabled tracking of origin and movements using oxygen isotopes possible by using regional differences. For example, Budd et al. (2004) differentiated north and western Europe generations of human immigrants as well as their probable childhood residence using differences in the oxygen isotope ratios of human teeth.

#### 1.7.1.3 Sulfur isotopes ($\delta^{34}S$)

A major source of sulfur in terrestrial ecosystems is coal and crude oils (Ehleringer & Rundel, 1989). Plants and animals get their sulfur from soil sulfur which is affected by local lithology and rainfall (Nehlich & Richards, 2009; Peterson & Fry, 1987). Sulfur isotopes are mostly used to make inferences about marine and marsh food webs (Connolly et al., 2004; Krouse & Herbert, 1998), however they have been recently used in terrestrial ecosystems (Nehlich et al., 2011; Zazzo et al., 2011). Small fractionation factor of sulfur isotopes (Harrison et al., 2011; McCutchan et al., 2003; Richards et al., 2003; Zazzo et al., 2011) enables sulfur measurement of organisms to reflect the values of their local geology (Peterson & Fry 1987; Richards et al. 2003). Animals moving across distinct isotopic food webs retain information of their previous and current feeding locations (Connolly et al., 2004; Hobson et al., 2012; Hobson et al., 2014), enabling ecologists to predict their movement patterns. For instance, Krouse and Herbert, (1998) revealed $\delta^{34}S$ values of animals to be heavily dependent on locality. Also, Rossmann et al., (1998) identified the origin of milk because of similarities in $\delta^{34}S$ values between the milk and geology of the area from which the cow grazed.

#### 1.7.1.4 Strontium isotopes ($^{87}Sr/^{86}Sr$)

Strontium is a common trace element, forming part of the natural environment including rocks, soil, water and air. The amount of strontium and its isotopic composition in the environment varies depending on the local geology (Dasch, 1969; Graustein, 1989; Hurst & Davis, 1981). Some strontium bearing minerals are water soluble, bioavailable, and they move easily through the food chain and are incorporated into human and animal bodies through consumption of food and water. In animal and human bodies, strontium is incorporated in tissues by substitution for calcium in bones, hair, nails and enamel. There is no fractionation of the $^{87}Sr/^{86}Sr$ ratio which makes the Sr isotopic signatures of animal tissues mirror those of their food and water sources (Price et al., 2004). The strontium isotope ratios in different rocks are a direct function of the age and composition

of the material (Faure, 1986). Variation of strontium across the environment is less limited (Budd et al., 2004) making it a useful geolocator of animal movement.

## 1.8 Application of stable isotopes to study feeding ecology of animals

The primary use of stable isotopes in early 1970s was to study plant physiology (Smith & Epstein, 1971). In the late 1970s, ecologists explored the idea of applying stable isotope ratios to study feeding ecology of foraging animals (Deniro & Epstein, 1978, 1981). This was based on the fact that isotopic composition of the animal's body reflects the isotopic composition of its diet plus a few permille (Deniro & Epstein, 1978). Isotopes are powerful forensic recorders of different dietary sources, which can be spatially interpolated and clearly linked to the on-ground information (Hobson et al., 2012; Hobson & Wassenaar, 2008). Analysis of stable isotopes of animal tissues provides valuable information on diets and trophic levels of individuals and populations (Hobson et al., 1997; Hobson, 1990; Mizutani et al., 1990), because stable isotopes are directly incorporated into animal tissues with a varying degree of trophic enrichment from the consumed diet (Deniro & Epstein, 1978, 1981; Rubenstein & Hobson, 2004). Stable isotopes have been successfully applied in different animal diet studies to understand the importance of food sources by comparing distributions of isotope ratios in their tissues (Hopkins & Ferguson, 2012).

In natural ecosystems, isotopic ratios of carbon and nitrogen in animal tissues have been used to explain different eating behaviors and habitat usage by animals (Ambrose, 1987; Codron et al., 2011; Codron & Brink, 2007; Herrera et al., 2003; Newsome et al., 2007). This is because isotopic signatures of local food webs vary spatially due to different biogeochemical processes (Hobson, 1999; Rubenstein & Hobson, 2004), and such variations are very important in explaining ecosystem functioning (Fry, 2006). For instance, stable isotopes of nitrogen ($\delta^{15}N$) have proven to be an essential tool in assessing the dietary preferences of organisms in both extinct and extant ecosystems (Styring et al., 2010) as well as studying food web structures (Ambrose, 1987; Hesslein et al., 1991; Sponheimer et al., 2003; Porras-Peters et al., 2008). This is because the values of $\delta^{15}N$ of consumer tissues increases incrementally from food source to consumer, as a result they can be used to indicate trophic level position (Deniro & Epstein, 1981).

However, the applications of their $\delta^{15}N$ can be compromised with some confounding factors like aridity and rainfall (Handley et al., 1999; Heaton, 1986) which increase $\delta^{15}N$ values of plant and animals, making the interpretation of N isotopes complicated. Likewise with carbon isotope ($\delta^{13}C$), isotopic enrichment occurs between trophic levels, but generally much less significantly than $\delta^{15}N$ (Deniro & Epstein, 1978; Zanden & Rasmussen, 1999, 2001). Therefore, $\delta^{13}C$ has been mostly used to assess the influence of marine versus terrestrial food sources (Chisholm et al., 1982; Schoeninger & DeNiro, 1984; Walker & Deniro, 1986), because the values of $\delta^{13}C$ are always distinct between marine and terrestrial primary producers (Larsen et al., 2012). Stable isotope ratios of sulfur ($\delta^{34}S$) can be used to distinguish between marine, freshwater and terrestrial food consumptions (Britton et al., 2016; Nehlich et al., 2011; Weber et al., 2002; Zazzo et al., 2011), because the values of $\delta^{34}S$ vary widely between marine, freshwater and terrestrial ecosystems (Holser et al., 1989; Krouse et al., 1987; Peterson & Fry, 1987; Stack & Rock, 2011).

In general, stable isotope analysis technique is centered on the predictable relationship of the isotopic composition of the consumers and that of their prey, which allows the investigation of diet composition and energy flow (Fry, 2006; Herrera et al., 2003). The most recent advancements in mathematical modelling of ecological data have developed different Stable Isotope Mixing Models (SIMMs) such as MIxSIAR (Stock et al., 2018) and SIBER (Jackson et al., 2011) which are all very useful for understanding feeding ecology of animals using stable isotope analysis approach.

## 1.9 The concept of isoscape

An isoscape refers to spatially explicit prediction of isotopic values across a landscape. Isoscapes can be derived from interpolating geographically distributed observations or by process-level models that aim to capture and predict the observed heterogeneity from an understanding of isotope fractionation (Cheesman & Cernusak, 2016). It is the framework that enables researchers to understand different movement patterns and processes of the earth (J. B. West et al., 2010). The interpretation and utilization of the isoscape in different fields requires a detailed understanding of the studied ecosystem (Cheesman & Cernusak, 2016). Several isoscapes have been developed across the globe, and the most popular

one is that of hydrogen and oxygen (Terzer et al., 2013). Isoscapes are used to study retrospective animal movement by assigning an animal's location based on the isotope values in their tissue in relation to particular isotope values on the isoscape. However, the accuracy and precision of the technique depends on the accuracy and precision of the underlying isoscape (Meehan et al., 2004).

## 1.10 Application of hair in the isotopic studies

In a variety of big mammalian species, isotopically inert tissues have emerged as a potential tool for addressing individual seasonal variation in movement patterns and food composition. Hair particularly offers the largest potential especially in studies of isotope ecology (e.g. Cerling et al., 2006, 2009; Kaczensky et al., 2017; Zazzo et al., 2011), as it represents a unique feature of mammalian skin with similar properties for different species (Leblond & Walker, 1951; O'Connell & Hedges, 1999). For example, a hair consists of the persistent protein structure called keratin (Ebling, 1987), which can preserve isotopic information indefinitely making a hair an isotopic archive for conducting the studies of historical movements (Coutu et al., 2016) or dietary changes (Nehlich et al., 2011).

One advantage of using hair in mammalian research is that it can be acquired in a less invasive way and with minimal disturbance from the animal. Normally, a hair grows continuously from the base of the hair follicle and keratin is formed in the basal few millimeters beneath the skin (Ebling, 1987). Once the keratin is formed, a hair is metabolically inert (Ebling, 1987; Leblond & Walker, 1951), and therefore every section of the hair shaft keeps the isotopic information from the time when it was formed (Ebling, 1987; O'Connell & Hedges, 1999). A tail hair of a mammalian herbivore is even more interesting, because it can grow for a long period and therefore providing isotopic information over an extended period of time (Burnik Šturm et al., 2017; Kaczensky et al., 2017).

## 1.11 Stable isotope studies and facilities in Tanzania

Currently there are no active stable isotope facilities (i.e. a mass spectrometer for the analysis of isotope ratios) in Tanzania or nearby countries in East Africa. However, there are Tanzanian institutions such as Nelson Mandela African Institution of Science and Technology (NM-AIST) and the University of Dar es

Salaam (UDSM) which have facilities for analyzing elements which make up a target sample (i.e. an elemental analyzer). These machines operate in a similar way as the mass spectrometer, but the output is a total mass/concentration of elements in a sample and not the isotopes ratios. This type of work is different from stable isotopes because we cannot separate the isotopes (e.g. radioactive versus stable) or quantify different sources of these elements (e.g. organic versus non-organic).

The lack of stable isotope facilities has limited stable isotope related work for most Tanzanian scientists. Up to date, there is a very few published stable isotope related work that have been conducted by Tanzanian scientists in Tanzania. For example, works related to forest ecology (Mayengo et al., 2020; Muzuka & Shunula, 2006), ground water assessment (Bakari et al., 2012; Chacha et al., 2018), mosquito ecology (Opiyo et al., 2016), feeding ecology (Baino et al., 2022) and movement ecology (Kabalika et al., 2020). This is just a handful of works compared to a global rate of stable isotope related research publications and the great potential that stable isotope analysis technique offers in scientific research.

## 1.12 Objectives and research questions

The main objective of my PhD research was to apply stable isotope ratio techniques to understand movement patterns and dietary preferences of migratory animals using the Serengeti-Mara ecosystem in Tanzania as a case study. Specifically, I aimed to answer the following major questions:

(1) Are stable isotope ratios of sulfur ($\delta^{34}S$) a good geolocator of animal movement? The first aim was to assess the variation of $\delta^{34}S$ across the landscape (i.e. construct an isoscape from grass which is the animals' diet) and then test the idea that $\delta^{34}S$ values in the tail hair of an animal reflects the $\delta^{34}S$ values in the diet, and therefore the variation $\delta^{34}S$ in the animals' tissues can be used as a natural bio logger of animal movement.

(2) What are the prerequisites for applying $\delta^{34}S$ to geolocate migratory animals? My aim was to explore how long it takes between diet ingestion and isotope reflection in animal tissue, in this case, a tail hair. This was important because migratory animals encounter a wide range of habitat types along their migratory route, and to be able to geolocate them using $\delta^{34}S$, it is necessary to know the delay between ingestion at a location and the response seen in the hair.

(3) Can we use $\delta^{34}S$ to study resident versus migrant life history? My aim was to explore the idea that $\delta^{34}S$ can be used as a tool to differentiate a resident from a migrant individual in a mixed population. This was based on the idea that a migrant individual will have higher variability of $\delta^{34}S$ values across their tail hairs than a resident individual, because a migrant would encounter a wider range of habitat types over the migratory route and therefore their $\delta^{34}S$ values would reflect the isoscape across the migratory route.

(4) Can we detect periods of dietary convergence versus dietary partitioning in the annual cycle of co-migrating species? My aim was to explore the idea of diet partitioning and niche interaction between the co-migrating animal species of wildebeest and zebra. My major interest was to apply stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to understand the variation in isotopic niche and dietary preferences over the course migratory cycle.

The results presented here demonstrate an approach for studying animal movement as well as identifying resident and migrant life-history forms of migratory ungulates using variation of $\delta^{34}S$ in the tail hair. The technique presents a non-invasive and affordable way from which we can make population-wide inferences of animal movement patterns. Furthermore, the results help to inform the applicability of tail hair in providing continuous time series information of animal diet, which allows us to understand seasonal periods of diet convergence and partitioning between the co-habiting animal species. This approach is especially useful for understanding niche partitioning and coexistence of species, which is important for ecosystem functioning.

# Chapter 2: Tracking animal movements using biomarkers in tail hairs: a novel approach for geolocating from the Serengeti sulfur isoscape

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## 2.1 Abstract

Current animal tracking studies are most often based on the application of external geolocators such as GPS and radio transmitters. While these technologies provide detailed movement data, they are costly to acquire and maintain, which often restricts sample sizes. Furthermore, deploying external geolocators requires physically capturing animals, which poses an additional welfare concern. Natural biomarkers provide an alternative, non-invasive approach for addressing a range of geolocation questions and can, because of relatively low cost, be collected from many individuals thereby broadening the scope for population-wide inference.

We developed a low-cost, minimally invasive method for distinguishing between local versus non-local movements of cattle using sulfur isotope ratios ($\delta^{34}S$) in cattle tail hair collected in the Greater Serengeti Ecosystem, Tanzania. We used a Generalized Additive Model to generate a predicted $\delta^{34}S$ isoscape across the study area. This isoscape was constructed using spatial smoothers and underpinned by the positive relationship between $\delta^{34}S$ values and mean annual precipitation. We then established a strong correlation between $\delta^{34}S$ from recent sections of cattle tail hair and the $\delta^{34}S$ from grasses sampled in the immediate vicinity, suggesting $\delta^{34}S$ in the hair reflects the $\delta^{34}S$ in the environment. Finally, using a likelihood-based approach in which we assessed sequential sections of the hair over the entire length, we were able to differentiate local versus non-local movements of cattle over the preceding months. While the focus of our study was on cattle, our approach could be modified to understand movements in other

mobile organisms where the sulfur isoscape is sufficiently heterogeneous relative to the spatial scale of animal movements and where tracking with traditional methods is difficult.

## 2.2 Introduction

Movement is a fundamental characteristic of life (Nathan et al., 2008), yet its quantification across individuals and populations has remained a major methodological challenge in the field of movement ecology. Common animal tracking techniques such as GPS and radio transmitters (Coppolillo, 2000) are time-intensive to collect, require expensive equipment and pose welfare concerns (Bailey et al., 2017), and therefore may not be available in all settings nor necessary for addressing certain questions. For instance, characterizing population-level variation in movement patterns, such as the proportion of residents versus migrants, is likely to be inaccurate if one can only tag a handful of individuals over a relatively short period of time (Hurme et al., 2019). Forensically recreating movement paths from dead animals, or studying historical connectivity from archived specimens, or studying landscape connectivity, can provide useful insights into the drivers of population dynamics. There are few techniques curently available for retrospective animal movement tracking that allow the characterization of population-wide movements. The use of intrinsic markers to infer location of animals (Hénaux et al., 2011; Hobson et al., 2014; Rubenstein & Hobson, 2004) offers a relatively low-cost, noninvasive solution to study animal movement patterns.

Intrinsic markers are natural biological or biogeochemical tags that can be retrieved from animals' tissues (Rubenstein & Hobson, 2004). Biogeochemical markers are particularly promising for studying movements because they form links between seasons and across populations, and they give time-integrated information which can directly be linked to geographical regions (Rubenstein & Hobson, 2004). Stable isotopes of key elements, particularly sulfur ($\delta^{34}S$) and hydrogen ($\delta^{2}H$), are popular geolocators (Rubenstein & Hobson, 2004). Hydrogen isotopes are used to make inferences of long-distance migration because they vary over latitudinal gradients, reflecting local precipitation patterns (Hobson, 1999). Sulfur isotopes have been popular in different movement studies such as tracking distance to the sea (Zazzo et al., 2011) and in dietary studies for making

inferences about marine and marsh food webs (Krouse, 1988). The small fractionation factor of sulfur isotopes (Harrison et al., 2011) of -2‰ between soil and plants (Novák et al., 2001; Trust & Fry, 1992) and from -0.5‰ to +2‰ between animal diet and tissue (Krajcarz et al., 2019; McCutchan et al., 2003; Peterson & Fry, 1987; Richards et al., 2003), suggests that sulfur is a useful geolocator in animal tissue because of the way it reflects values of the local geology (Peterson & Howarth, 1987; Richards et al., 2003; Stack & Rock, 2011). The combination of inert biological material that acts as a natural biologger for geolocating animals and continuously growing tisssue that does not erode easily, can provide unique time series information about animal movement. This is because as individual animals move across distinct soils or between food webs, tissues that grow continuously (e.g. hair) retain the isotopic signatures of their present and previous feeding locations (Richards et al., 2001), potentially enabling ecologists to infer movement patterns from them.

This study demonstrates how variation in $\delta^{34}S$ along cattle tail hair can be used to study animal movement retrospectively. We first assess the variation of $\delta^{34}S$ across environmental space in the Serengeti landscape, and develop a sulfur isoscape for the ecosystem. We test the hypothesis that variation in $\delta^{34}S$ in grass samples is reflected in sections of the most recent growth of tail hair, which indicates whether tail hair has the potential to be used as a natural bio-logger of geolocation in cattle. Lastly, we test the hypothesis that the full tail hair sequence can be used determine the likelihood an individual animal undertook long-range movement in the time interval the sampled tail hair was generated. Agropastoral cattle provide an ideal system for developing these methods because 1) these animals often move long distances; 2) their movements play important roles in human-wildlife conflict and the epidemiology of livestock diseases; 3) cattle owners can help verify the animal's movement history; and 4) cattle are easier and cheaper to capture and sample than wild animals.

## 2.3 Materials & Methods

### 2.3.1 Study area

The study was conducted in the Serengeti ecosystem, Tanzania,(Figure 2-1a), a landscape that is approximately 25,000 $km^2$ in area (Emerton and Mfunda, 1999; Thirgood et al., 2004) and located between 34$^o$ and 36$^o$ E, and 1$^o$ and 3$^o$ (Sinclair

et al., 2015). The ecosystem is characterized by a subtropical climate, with a dry and relatively cool season from late May to August, and a warmer dry season from September to October. Rainfall is highly variable but normally peaks in December, and between March and May (Mwakatobe et al., 2013; Sinclair, 1995). The area's savanna vegetation is strongly influenced by soil type and rainfall (Sinclair, 1995) (Figure 2-1). The ecosystem is home to a diverse assemblage of both wild and domestic ungulates, including the largest mammal migration in the world (Hopcraft, 2015). The soils underlying the ecosystem are highly heterogeneous and largely volcanic in nature (Reed et al., 2009). The Eastern portion of the ecosystem, including the Serengeti plains, comprises alkaline soils derived from young volcanic ashes, tephra and calcareous tuffs deposited more than twenty thousand years ago from Ngorongoro rift region and more recently from Oldonyo Lengai volcano (Jager, 1982), the world's only active carbonatite volcano (Krafft and Keller, 1989; Sinclair et al., 2008). The Western portions of the ecosystem are dominated by older alluvial soils, formed from the erosion of Precambian volcanic rocks and banded-ironstones (Gerresheim, 1974; Jager, 1982; Sinclair et al., 2008). Other parts of the ecosystem inlcuding north of the Mara river and crater highlands are dominated by heavily leached soils from older parent materials including complex granite, igneous and volcanic rocks (Jager, 1982; Leger et al., 2015; Scoon, 2018) (Figure 2-1c). In addition to a number of protected areas, such as Serengeti National Park in Tanzania and the Masai Mara National Reserve in Kenya (Reid et al., 2015), the ecosystem supports a large human population consisting of pastoralists and agro-pastoralists living in close proximity to protected areas boundaries.

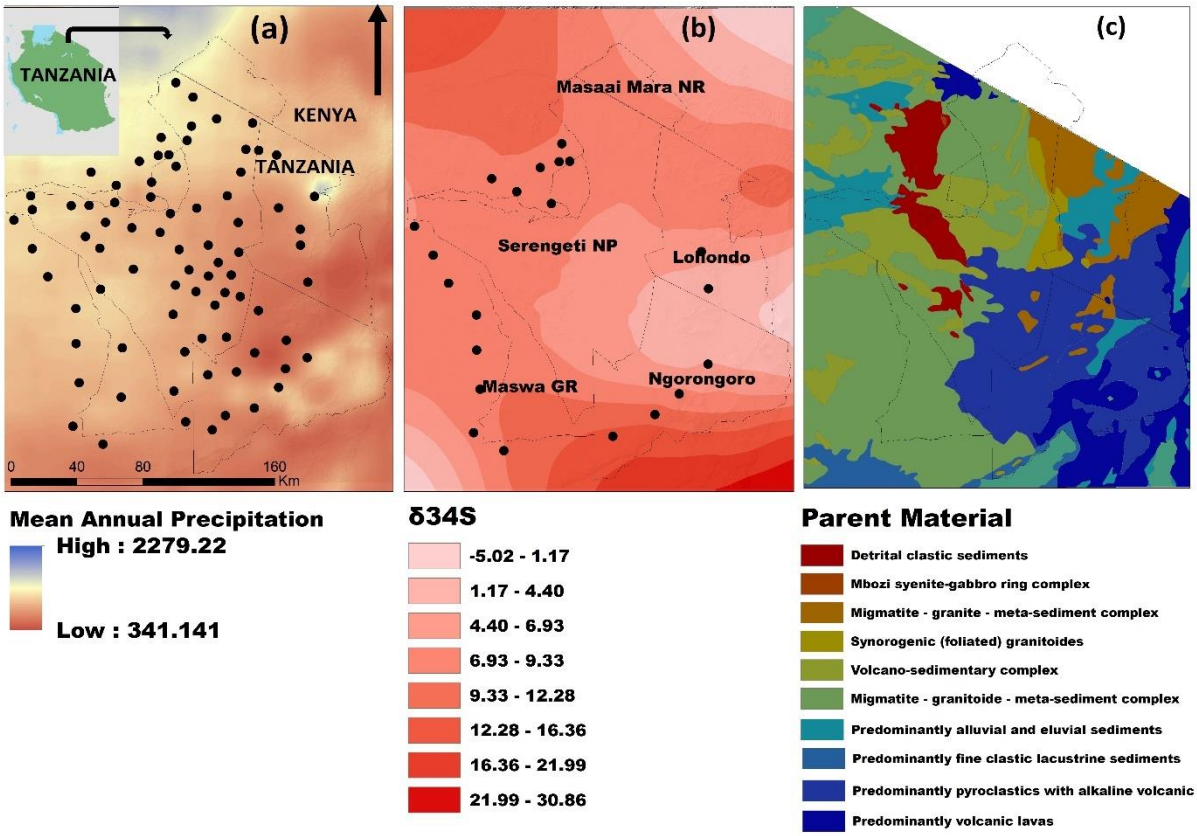


Figure 2-1. (a) The mean annual precipitation (b) the interpolated sulfur isoscape based on the output of a spatial GAM and (c) the underlying geology of the area. The boundaries of protected areas in the Serengeti ecosystem are illustrated with black lines. Sampling locations for (a) grass and (b) cattle are illustrated with black points

## 2.3.2 Data collection

All data collection including questionnaires, hair and grass samples was conducted from July 2017 to April 2018.

### 2.3.2.1 Environmental sampling

We collected grass samples from across the ecosystem to establish the spatial pattern of sulfur in the landscape. Grass samples were collected from 20 randomly selected villages bordering the Serengeti National Park but still within the Serengeti ecosystem (Figure 2-1a). A total of 116 grass samples were collected and analyzed for $\delta^{34}S$ to create a landscape-level isoscape. To collect grass samples, a 4 x 4 m plot was laid out and five sub samples (one from each corner and one at the middle) in a 25 x 25 cm quadrat were clipped to ground level and pooled together to make one single sample from each site (Hellmann et al., 2016). In addition, grass samples were collected (using the same sampling method) from

each village where cattle were sampled to compare local versus landscape level variation. At each village, grass samples were collected from three random points located in non-cultivated grazing fields and at least 100m away from roads and rivers. This minimized any potential sulfur contamination from industrial fertilizers, vehicle exhaust and road dust. Collected grass samples were kept in a paper envelope and stored in an open area at room temperature to prevent microbial activities and fungal development.

#### 2.3.2.2 Cattle hair sampling

In each of the 20 villages where grass samples were collected, between one and three cattle from random households were sampled for tail hairs, making a total of 46 tail hair samples. Tail hair samples were obtained by pulling hairs from the base of the tail of each animal. Pulling helped to remove the entire hair root, which represents the most recent feeding history of an animal. Cattle age, sex and color were recorded during sampling. After hair collection, all hairs from an individual were aligned by root to standardize time zero (i.e. the most recent time), tied together and stored at room temperature (Horacek et al., 2012; Rysava et al., 2016).

#### 2.3.2.3 Questionnaire

A set of eight close-ended questions (Appendix A-2) focused on exploring the movement history of cattle was posed to each cattle owner in relation to the sampled cattle. Questionnaires to cattle owners provided supplementary information on whether cattle had moved beyond the grazing area of the village or had been recently purchased from a neighboring village during the 5 months period prior to hair sampling. In addition to this binary response variable (moved versus not moved), these questionnaires also provided an estimate of distance travelled based on descriptions of the villages from which cattle had reportedly been moved

### 2.3.3 Sample preparation for stable isotope analysis

For grass samples, all non-grass species and debris were removed from samples prior to analysis. Grasses were thoroughly washed in double distilled water (DDS) to remove any soil. Grass samples were oven dried at $60^0$C for 48 hours, pulverized

into a fine powder and weighed (6.1 to 6.5 mg) into tin capsules (Hellmann et al., 2016), ready for isotopic analysis.

The range of hair bundles' length varied between 10 and 25 cm for adult cattle and between 5 and 7 cm for calves. The growth rate of cattle tail hair has been estimated to be 0.76 mm per day (Chen et al., 2017). In this study, tail hairs were sectioned into 10 x 8 mm segments representing 105 days of growth for adult cattle and 5-9 x 8 mm segments representing 52 to 95 days for calves. All samples were thoroughly washed in 2:1 chloroform: methanol to remove the impurities (Mekota et al., 2009), and then rinsed with DDS to remove the remnants of solvent. This process was repeated twice to ensure all possible contaminants had been removed from the samples. Samples were then oven dried, ground to powder and weighed (1.0 to 1.3 mg) into tin capsules as described above.

### 2.3.4 Stable isotope ratios analysis

All laboratory analyses for stable isotope ratios were performed at the NERC Life Sciences Mass Spectrometry Facility hosted by the Scottish Universities Environmental Research Centre (SUERC). All sample analyses were undertaken using a Pyrocube elemental analyser (Elementar nalysensysteme, Langenselbold, Germany) coupled to a VisION mass spectrometer (Elementar UK, Cheadle Hulme, Stockport, UK). Laboratory standards methanesulfonamide/Gelatine (MSAG2), methionine/alanine/glycine/gelatine (M2) and sulfanilamide/alanine/gelatine (SAAG2) were repeated after every 10 samples and were used to correct for linearity and instrument drift over a 72-hour analytical run. The isotope ratios for lab standards are determined relative to a range of international standards from IAEA (Vienna, Austria) and USGS (Reston, VA, USA). The analytical precision for sulfur isotopes was better than 0.7‰. The isotope ratios are expressed in the delta ($\delta$) notation in parts per million (‰): $\delta X = [(Rsample/Rstandard)-1]$ where $X= {}^{34}S$ and R = the ratio of $^{34}S/^{32}S$ isotopes in a given sample compared with V- CDT (Vienna - Canyon Diablo Troilite).

### 2.3.5 Sulfur isoscape

To develop a Serengeti grass sulfur isoscape, we fitted a spatial Generalized Additive Model (GAM) using the *mgcv* package (Wood, 2007, 2017). The fitted GAM of sulfur isotope ratios was modeled as a function of spatial and environmental

variables including Mean Annual Precipitation (MAP), Normalized Difference Vegetation Index (NDVI), Cation Exchange Capacity (CEC), the underlying geology (geology layer), elevation and the longitude and latitude. Longitude and latitude were modelled as a tensor product smoother to allow for construction of a single model matrix with multiple penalties (Wood, 2007, 2017). All environmental variables were prepared in R (R-Core-Team, 2017) using the *sp, raster, rdgal* and *rgeos* packages (Bivand, 2018; Bivand et al., 2013; Hijmans, 2017; Pebesma & Bivand, 2005; Roger et al., 2018). To characterize mean annual rainfall (MAP), we averaged African monthly data from The Climate Hazards Group Infrared Precipitation with station data (CHIRPS) 1981 and 2018 (Dinku et al., 2018; Funk et al., 2015). CHIRPS integrates 0.05°resolution satellite imagery with in-situ station data to create gridded rainfall time series for trend analysis and seasonal drought monitoring (Funk et al., 2015). The underlying geology and parent material for different soil types across the Serengeti ecosystem were characterized from the Minerogenic Map of Tanzania layer, from Geological Survey of Tanzania (Leger et al., 2015). The underlying geology was classified using lithology (Figure 2-1c) of the parent material. Model selection was based on Akaike Information Criterion (AIC, weight=0.99) (Appendix A-1) from the *stats* package (Sakamoto et al., 1986). Our final model included lithology as the main environmental predictor and longitude and latitude as a tensor product smoother. The model was analyzed for goodness of fit with the *gam.check* function from *mgcv* package (Wood, 2017) and was subsequently used to predict sulfur isotope values ($\delta 34S_{L,j}$) across the entire ecosystem (i.e. the 'isoscape') together with the standard deviation that captured the prediction uncertainty ($s_L$). The predicted $\delta^{34}$S isoscape was at the same spatial resolution as the geology layer (5 km$^2$ per pixel).

## 2.3.6 Validation of $\delta^{34}$S methodology

To understand whether $\delta^{34}$S in the i$^{th}$ tail hair sample from the j$^{th}$ individual ($\delta^{34}S_{T,i,j}$) linearly reflects the local $\delta^{34}$S signature in vegetation ($\delta^{34}S_{L,j}$), and to account for multiple observations of tail hair from the same individual, and that the true values of explanatory variable ($M_{L,j}$) are latent, and only observed with error ($s_M$) we constructed a latent 'error in variables' model wherein:

$$d34S_{T,i,j} \sim \alpha_j + \beta\, M_{L,j} + \epsilon_i \qquad \text{Equation 2-1}$$

$$\delta 34S_{L,j} \sim N(M_{L,j}, \sigma_M) \qquad \text{Equation 2-2}$$

The model was fitted in Stan (Stan version 2.23, (Carpenter et al., 2017) using the R interface RStan version 2.19), using 3 chains for 10,000 iterations after 5,000 as warmup, thinning to generate 3000 posterior samples per parameter. We used weakly informative priors (Gelman, 2013) for all parameters: for regression coefficients we used *t* distributions with 3 degrees of freedom and for standard deviations half t with 3 degrees of freedom (except for $s_M$ where the prior was modelled as $N(s_L, 0.25)$. We used the fitted model to generate predictions and associated uncertainty in these predictions for each observation from the most recent segment of tail hair.

### 2.3.7 Studying movements using $\delta^{34}S$ across the Serengeti sulfur isoscape

Relocation data from 49 GPS collared cattle in Western Serengeti (Ekwem, 2020) suggested that cattle rarely moved farther than 5km from their home bomas (i.e. only 1.7% of relocations). Therefore, we expected longer distance movement to be relatively rare. To determine how far an animal would need to travel in order to robustly detect movement from $\delta^{34}S$ tail hair signatures, we identified 50,000 random pairs of points across the isoscape, predicted $\delta^{34}S_L$ for each point, and estimated the mean distance an animal needed to move in order to detect statistically significant differences in isotope values in its tail hair, given the propagated uncertainty in predicting values of $\delta^{34}S_L$ from the landscape data, and $\delta^{34}S_T$ from $\delta^{34}S_L$. We then compared the output with our actual distances travelled by cattle established from the questionnaire (above).

## 2.4 Results

### 2.4.1 Variation of sulfur stable isotope ratios across the Serengeti ecosystem

$\delta^{34}S$ values of grass range between +2.82 ‰ and +13.04 ‰ (Appendix A-3), consistent with the terrestrial nature of the ecosystem (Coplen et al., 2002). Our final predicted model of $\delta^{34}S$ values in Serengeti included lithology as the main environmental predictor, and the latitudes and longitudes and their interaction as

spatial smoothing parameters (AIC weight = 0.99: Appendix A-1). From the GAM, we identified the following statistically significant relationships; a) a positive relationship between sulfur isotope ratios and pyroclastic-alkaline volcanic lavas (ß = 0.079 ± 0.029, t = 2.699, p = 0.008) and b) a negative relationship between sulfur isotope ratios and volcanic ash/tephra (ß = 0.05 ± 0.023, t = 2.175, p = 0.032). Other relationships that were not statistically significant from the model included: a negative relationship between sulfur isotopes ratios and granitoids (ß = 0.021 ± 0.015, t = 1.345, p = 0.181) and between sulfur isotope ratios and volcanic lava (ß = 0.016 ± 0.017, t = 0.975, p = 0.332), as well as a positive relationship between sulfur isotope ratios and mafic volcanic meta-basalts (ß = 0.032 ± 0.021, t = 1.533, p = 0.128). The mean standard deviation of predictions estimated directly from the GAM was $s_L$ = 1.00, and inferred from the full model, $s_M$ = 1.810.

### 2.4.2 Relationship between cattle locations and tail hair isotope values

The 'error in variables' model showed good convergence and effective sample sizes for all posteriors (Rhat < 1.01, neff > 1400). The $\delta^{34}S$ values of grasses from locations where tail hair was sampled, and the most recent tail hair section were strongly and positively related (Figure 2-2) with slope ß = 1.736 (95% credible interval (CIs) 1.466 – 2.058) confirming that fractionation of $\delta^{34}S$ in the hair reflects the $\delta^{34}S$ in the surrounding grass (Richards et al., 2003; Zazzo et al., 2011). Furthermore, the intercept (i.e. the baseline fractionation rate between grass to herbivore tissue) was -4.670 (95% CIs -7.563 - -2.279) coincident with the estimated range of fractionation rates from previous studies (Richards et al., 2003; Webb et al., 2017). With segment-level standard deviation of $\sigma_\varepsilon$ = 0.64, and individual level standard deviation of $\sigma_\alpha$ = 0.292, segment level variation in $\delta^{34}S_T$ accounted for 83% of overall variation in recorded $\delta^{34}S_T$ values.

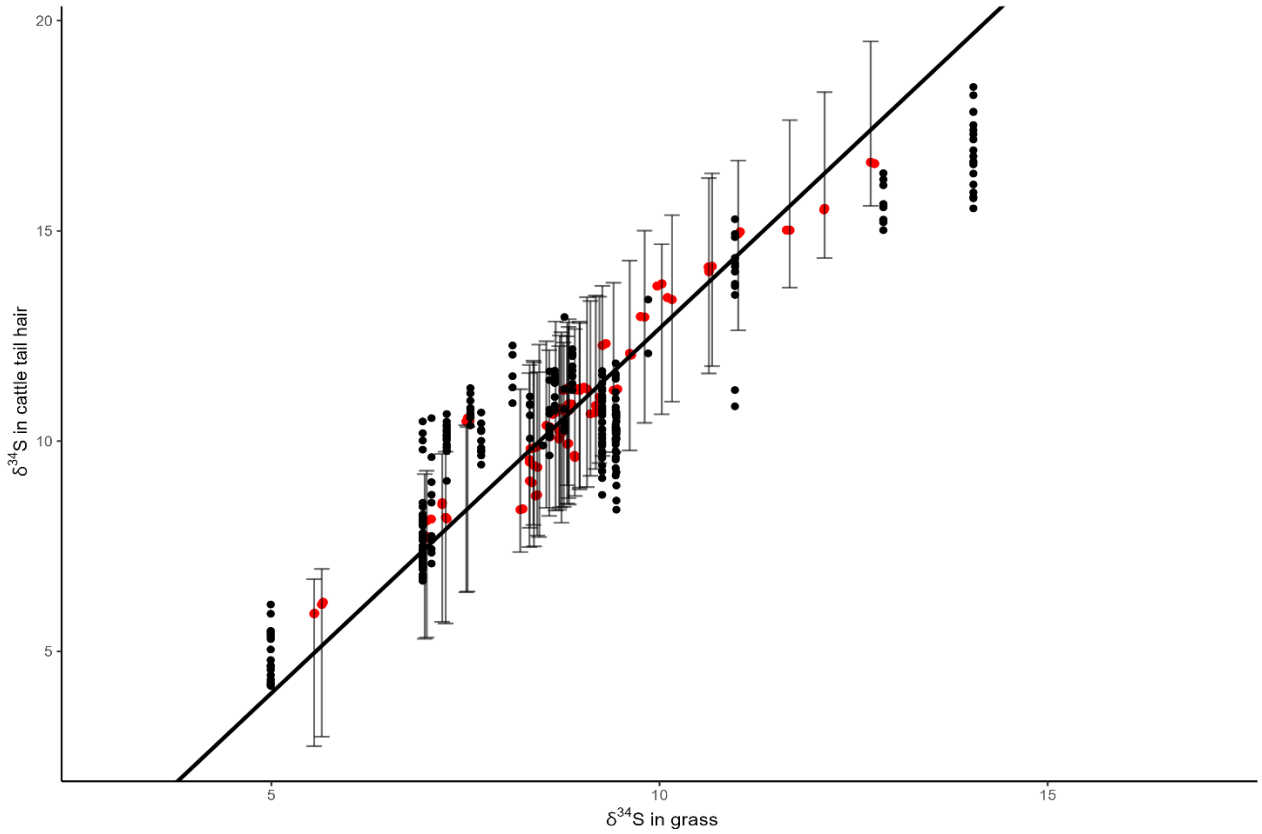


Figure 2-2. The observed relationship between $\delta^{34}S$ in grass and the most recent segment of the cattle tail hair, suggesting that $\delta^{34}S$ in the hair reflects the $\delta^{34}S$ in the landscape and can be used as a reliable biomarker of location. Red points (slightly 'jittered' for clearer visualization), show the most recent segment of a tail hair (i.e. the root), and black points show the rest of the segments in the tail hair. Error bars represent the credible intervals of $\delta^{34}S$ values for the most recent segments predicted from the isoscape.

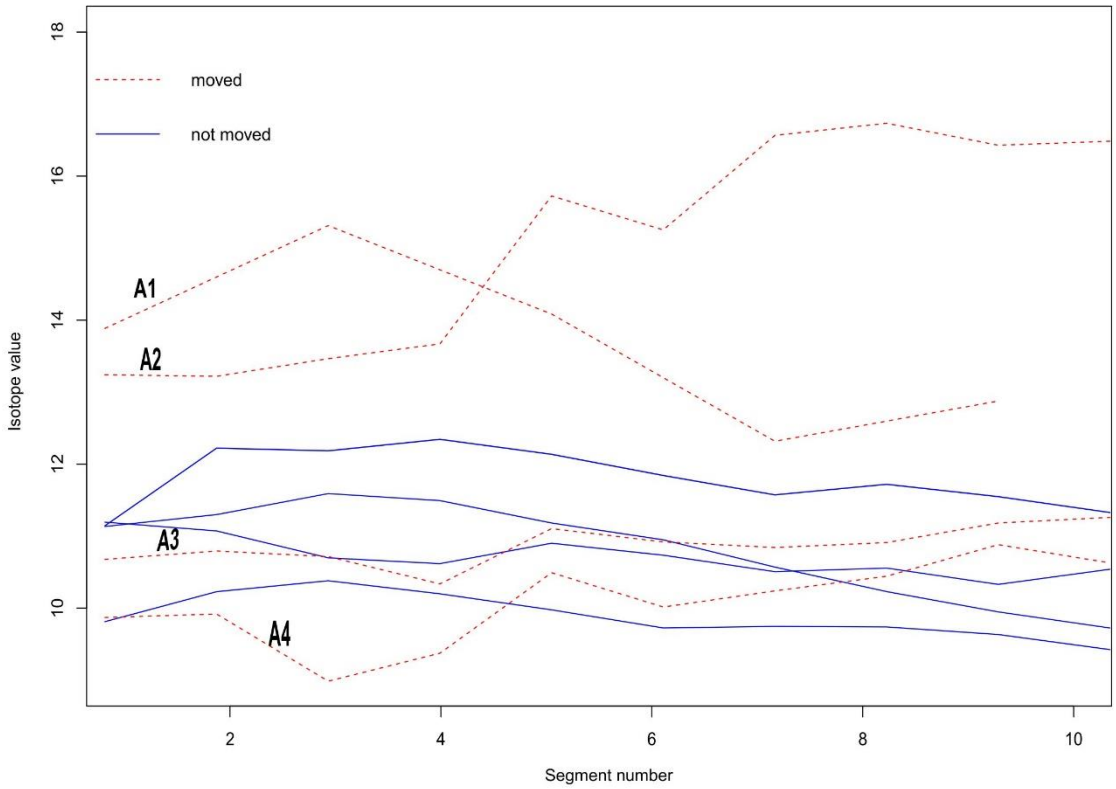


Figure 2-3: Variation of $\delta^{34}S$ across length of tail hairs for representative individual cattle in Serengeti, showing sulfur isotopes profiles for reportedly moved (with their associated distance moved. A1 = 10.607 Kms, A2 = 13.636 Kms, A3 = 3.79 Kms and A4 = 9.318 Kms) and animals that did not move

### 2.4.3 Power analysis result

The standard deviation on predicting tail hair $d^{34}S$ values from the isoscape was typically very close to 1(range 0.987 – 1.184). Our power analysis results suggested that an animal needed to move typically about 100 km (Figure 2-4). in order to generate a difference in tail hair $d^{34}S$ values likely to be judged significant. However, the nature of our sulfur isoclines across the Serengeti isoscape suggests effective distance is not equal in all cardinal directions. For instance, animals moving in a north-east to south-west pattern would in general have to move less than animals moving due north or south or east-west for the movement to be reliably detectable using $\delta^{34}S$ (Figure 2-4). Additionally, a questionnaire report suggested that our sampled cattle had not moved enough distance to establish their movement pattern using sulfur stable isotopes ratios, because the highest distance travelled by the cows was 52 Km with the majority having travelled less than 10 Km (Appendix A-4).

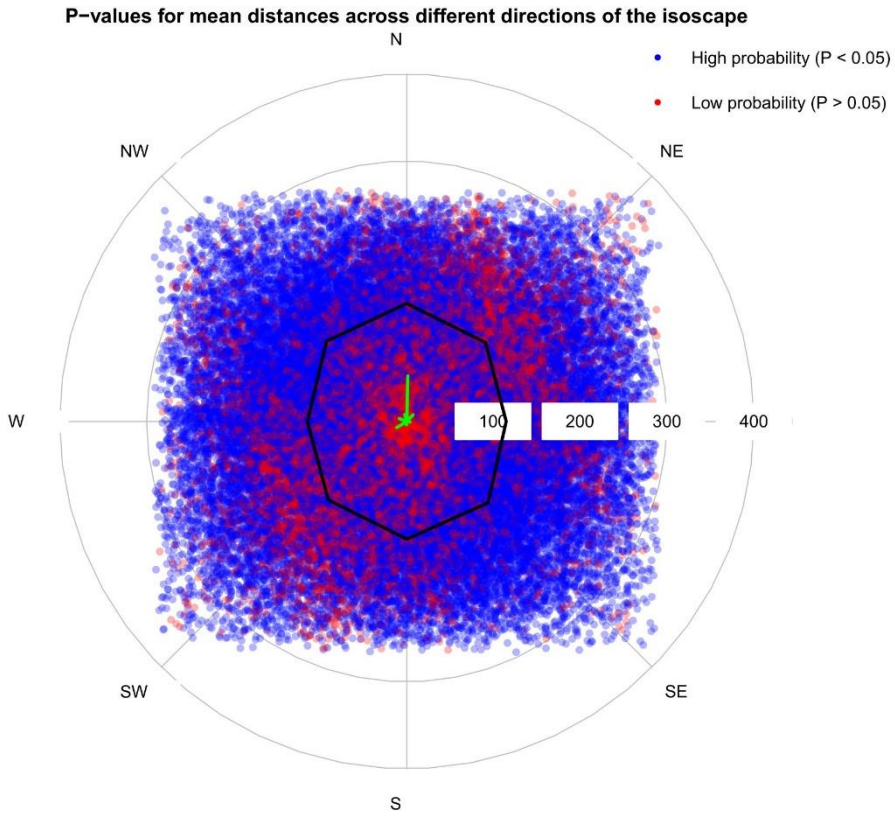


Figure 2-4. Black polygon shows mean distance required to move to detect movement across the Serengeti isoscape in different directions; green lines show the actual distance travelled by our cattle; red points correspond to distances and directions in which sulfur values in tail hair are not predicted to change significantly (based on comparing two segments, $P > 0.05$), while blue points correspond to distances and directions in which movement is predicted to result in a statistically significant change in sulfur tail values ($P < 0.05$).

## 2.5 Discussion

The application of sulfur stable isotope ratios to quantify the movement of animals holds considerable benefits. The major findings from our study suggest that: 1) sulfur stable isotope ratios in the vegetation across our study area vary across local lithology; 2) sulfur stable isotopes ratios in the vegetation are reflected in cattle tail hairs (Figure 2-2), and therefore 3) variation  tail hair could be used to potentially identify non-local movements of animals.

Feeding studies for grazing animals have derived fractionation factors of -0.5‰ to 2‰ (Krajcarz et al., 2019; Richards et al., 2003; Zazzo et al., 2011) between

fodder and keratin. This is a relatively small change when compared with the range of values we have from grass samples across the Serengeti ecosystem and indicates the potential of sulfur isotope values as a dietary tracer within this system. The mean distance required to detect movement is about 100 km and modest compared to how far cattle or other animal species can move across this system and beyond. For example, pastoralist cattle can be transported by lorry many hundreds of kilometers across the country, and wildebeest which undertake a routinely cyclic migratory pattern every year can move about 250 km from southern to northern part of the ecosystem. However, the effectiveness of $\delta^{34}S$ in studying movements for animals moving less than this distance in the Serengeti ecosystem would be dependent on local isotopic gradients.

### 2.5.1 Variation of sulfur stable isotope ratios across the Serengeti ecosystem

The major source of sulfur in terrestrial plants is from soil, which is mostly derived from bedrock weathering in the form of sulphate (Krouse, 1991). In addition, the $\delta^{34}S$ of soil is influenced by local lithology and rainfall (Coplen et al., 2002; Peterson & Fry, 1987), aerobic and anaerobic growing conditions (Rubenstein & Hobson, 2004), microbial processes(Peterson & Fry, 1987; Peterson & Howarth, 1987), fertilization procedures, as well as atmospheric deposition including the sea-spray effect (Krouse & Mayer, 2000). Normally, the $\delta^{34}S$ value for bedrock varies with rock type and age (Peterson & Howarth, 1987; Thode, 1991), and terrestrial plants exhibit a wide range of $\delta^{34}S$ values between ~ − 10 and + 35 ‰ (Krouse & Mayer, 2000; Webb et al., 2017). In the Serengeti ecosystem, grass samples have $\delta^{34}S$ values of 2.82‰ to + 13.04‰ (Appendix A-3), midway in the global range. The lowest $\delta^{34}S$ values in the Serengeti were observed on the eastern side of the ecosystem (Figure 2-1b), which is dominated by volcanic ash tephra. The highest $\delta^{34}S$ values in the isoscape come from the extreme south-eastern side of the Serengeti ecosystem beyond the Ngorongoro rain shadow (Figure 2-1b) which is characterized by soils from pyroclastic-alkaline and volcanic lavas. The areas with the strongest gradients in the isoscape are south-eastern and north-western parts of the ecosystem (Figure 2-1b), suggesting that animal movement across these isoclines could be detected over relatively short distances. In addition, the areas exhibit intermediate levels of mean annual precipitation of

800-1000 mm (Prins and Loth, 1988) (Figure 2-1a), providing plants growing in the area with an important nutrient found in rain water (e.g. nitrate) for their growth.

GAM results suggest the high values of grass sulfur in this region are mainly correlated with lithology. However, microbial processes and volcanic activity in the Rift Valley (Ueda & Sakai, 1984) likely contribute to these high $\delta^{34}S$ values. For example, the southern Serengeti plains are composed of tephra from volcanic eruptions from Ngorongoro highlands and other adjacent volcanoes including Lemagurut and Olmoti (McHenry et al., 2008). Volcanic gases and rocks have a wider range of $\delta^{34}S$ values caused by inorganic chemical reactions, and this can change the $\delta^{34}S$ signature of the soil (Puchelt et al., 1971; Thode, 1991). Aerosols and dust from volcanic fumaroles and eruptions can be dispersed by both prevailing winds and rainfall. The potential to include environmental variables in predicting sulfur variation, offers a useful predictive power to the isoscape, suggesting the method could be applied to other landscapes, particularly where there is soil heterogeneity and geological gradients. The nature of the Serengeti sulfur isoscape suggests that sulfur isotopes ratios are relatively invariant across the center and more heterogeneous across North East (NE) and South West (SW) gradients (Figure 2-1b). Such a pattern indicates that an animal needs to travel longer distances along the East-West direction than NE-SW direction to detect movement (Figure 2-4).

### 2.5.2 Variation of sulfur stable isotopes in nature and environment

Sulfur is an essential element for both plant and animal growth (Saito, 2004). It is a component of important biochemical compounds like cysteine, methionine, glutathione (Stipanuk, 1986). Geologically, sulfur is present as a minor constituent in most igneous and metamorphic rocks, and as a major component of organic substances like coal and crude oils (Coplen et al., 2002; Thode, 1970; Worden et al., 1997). Plants and animals obtain their sulfur from soil whose composition varies as a function of both local lithology (Stack & Rock, 2011) and rainfall (Peterson & Fry 1987; Nehlich & Richards 2009), as well as atmospheric deposition including the sea-spray effect (Krouse & Mayer 2000). The variation of sulfur stable isotope ratios across the Serengeti ecosystem we report in this work is determined

largely by local lithology and historic volcanic ash deposition, and therefore, the values are expected to be stable and seasonally invariant over 'biological' time.

### 2.5.3 Sulfur stable isotopes in ecological studies

This study has demonstrated the applicability of sulfur stable isotopes ratios in studying the movement of animals. Historically, sulfur stable isotopes have been difficult and expensive to analyze compared to carbon and nitrogen (Connolly & Schlacher, 2013; Hesslein et al., 1991; Richards et al., 2001), which limited their applications to different studies (Connolly et al., 2004). The recent advances in continuous-flow isotope-ratio mass spectrometry (CF-IRMS) have allowed sulfur isotopes to be measured and analyzed from both organic and inorganic materials in relatively small amounts (Richards et al., 2003; Richardson, 2011; Sayle et al., 2013). This has increased their applicability in different fields of study including archaeology and ecology (Nehlich et al., 2011; Richards et al., 2003).

Low variation in $\delta^{34}S$ values of our cattle tail hairs (Figure 2-3) suggests that cattle either foraged in a single location or foraged within an isotopically similar region of the isoscape (i.e. along the same isocline). Conversely, high variation in $\delta^{34}S$ values in the tail hair (Figure 2-3) should be taken as a primary indicator of movement or supplementary feeding using forage grown in a different isotopic setting (Zazzo et al., 2011). This finding agrees with other recent works (e.g. Zazzo et al., 2011), which revealed changes in $\delta^{34}S$ in hair following the movement of sheep relative to the proximity to the sea.

### 2.5.4 Estimating movement and wildlife home ranges of domestic and wild grazers

This study provides a template for understanding animal movement in and outside the Serengeti ecosystem. The question of where, how and when livestock move remains critical for informing policies aimed at maximizing livestock productivity, minimizing disease spread while also maintaining traditional pastoralist livelihoods. Quantifying livestock movements, such as those occurring in remote agro-pastoralist settings, or through illegal transboundary trade or theft, is challenging to study using other methods. Our approach provides a non-invasive way to infer whether an animal has moved long-distances across sulfur gradients

in the previous six months. The same principle could potentially be applied in other areas with sufficient variation in $\delta^{34}S$ values where tail hair can be readily sampled (e.g. from carcasses) to study wide-ranging wildlife species (e.g. wildebeest and zebra). For example, this method could distinguish between migratory and non-migratory individuals and establish the frequency of different movement strategies in a partially migratory system such as Serengeti.

## 2.6 Conclusion and recommendations

This study has shown the potential for using sulfur stable isotopes ratios in studying movement ecology of herbivores. For example, the $\delta^{34}S$ isoscape of the Serengeti provides baseline information on how $\delta^{34}S$ can be applied to understand spatial and movement ecology of livestock across the landscape. However, the use of single element, such as $\delta^{34}S$, does not capture all the isotopic variation across the landscape and limits our ability to recreate detailed movement patterns of individual animals. The inclusion of additional elements such as Strontium ($^{37}Sr$), which tends to have a well-defined geological distribution (Dasch, 1969; Graustein, 1989), or $\delta^{2}H$ which tends to differ by watershed (Bowen et al., 2005), could improve the technique by adding more isotopic axes from which the location can be more accurately triangulated. Future studies could incorporate data from other tracking techniques, such as GPS telemetry or genetic markers, to further calibrate these isotopic techniques which would ultimately improve our ability to forensically determine the movement history of unmarked animals.

# Chapter 3: Using sulfur stable isotope ratios ($\delta^{34}S$) for animal geolocation: estimating the delay mechanisms between diet ingestion and isotope incorporation in tail hair.

This chapter has been submitted and is currently under review in Rapid Communications for Mass Spectrometry journal as: Kabalika. Z., Haydon. D.T., McGill. R.A.R., Morales. J.M., Morrison. T. A., Newton. J. & Hopcraft, G.J.C. (2023). Using sulfur stable isotope ratios ($\delta^{34}S$) for animal geolocation: Estimating the delay mechanisms between diet ingestion and isotope incorporation in tail hair. *RCM-22-0189.*

## 3.1 Abstract

Metabolism and diet quality play an important role in determining delay mechanisms between an animal ingesting an element and depositing the associated isotope signal in tissue. While many isotope mixing models assume instantaneous reflection of diet in an animals' tissue, this is rarely the case. Here we use data from wildebeest to measure the lag time between ingestion of $^{34}S$ and its detection in tail hair. We use time-lagged regression analysis of $\delta^{34}S$ data from GPS collared blue wildebeest from the Serengeti ecosystem in combination with $\delta^{34}S$ isoscape data to estimate the lag time between an animal ingesting and depositing $^{34}S$ in tail hair. The best fitting regression model of $\delta^{34}S$ in tail hair and an individual's position on the $\delta^{34}S$ isoscape is generated assuming an average time delay of 78 days between ingestion and detection in tail hair. This suggests that sulfur may undergo multiple metabolic transitions before being deposited in tissue. Our findings help to unravel the underlying complexities associated with sulfur metabolism and are broadly consistent with results from other species. These findings will help to inform research aiming to apply the variation of $\delta^{34}S$ in inert biological material for geolocation or understanding dietary changes, especially for fast moving migratory ungulates such as wildebeest.

Key words: Lag time, wildebeest tail hair, Serengeti ecosystem, turnover rate, isotope delay mechanisms.

## 3.2 Introduction

While the application of sulfur stable isotope ratios ($\delta^{34}S$) in ecological studies is not new (e.g. Peterson et al., 1985), it has increased over the past two decades (Richards et al., 2003), mainly due to technological advances in mass spectrometry (Fourel et al., 2014; Sayle et al., 2013). Specifically, their applicability to reconstructing animal movement trajectories and diet shows promise for ecologists. For example, $\delta^{34}S$ has been used in dietary studies about marine and marsh food webs (Connolly et al., 2004), and $\delta^{34}S$ in hair has been applied to study movement of animals in terrestrial (Kabalika et al., 2020) and marine habitats (Zazzo et al., 2011). However, the delay between an animal ingesting and depositing sulfur in inert biological materials such as hair has rarely been explored or quantified, which limits the applicability of using $\delta^{34}S$ for geolocation particularly for migratory animals.

Sulfur stable isotope ratios are generally considered to have small fractionation factor (i.e. diet-tissue difference in $\delta^{34}S$) during incorporation into both plant and animal tissues (Krouse & Herbert, 1998; McCutchan et al., 2003; Trust & Fry, 1992), and their values tend to vary with local geology (Richards et al., 2003; Stack & Rock, 2011). The reported fractionation values for $\delta^{34}S$ isotopes between diet and animal tissues are between -3‰ and +4‰ (Harrison et al., 2011; Krajcarz et al., 2019; McCutchan et al., 2003; Richards et al., 2001, 2003; Webb et al., 2017) and between -8‰ and +4‰ between soil and plants (Trust & Fry, 1992). Although there are reported outlying values as high as +7‰ (McCutchan et al., 2003). $\delta^{34}S$ has the potential to be a good tracer of animal movement and diet in tissues because it stably reflects the $\delta^{34}S$ in their local environment.

A hair root is metabolically active as it grows, but dead when the hair fiber leaves the skin (Ebling, 1987). One of the advantages of using biological inert materials such as hair and feathers (Hobson et al., 2014) is that they provide unique time-series $\delta^{34}S$ data from which animal movement and diet can be inferred (Burnik Šturm et al., 2017; Kaczensky et al., 2017). This is because as animals move between different habitats, the information of past and present feeding is recorded and retained in these continuously growing tissues, enabling

scientists to infer movement history or diet change over time (Haché et al., 2014; Hobson et al., 2014). Furthermore, biologically inert material is stable over long periods of time following synthesis, making them a useful archive of diet (Schwertl et al., 2003). Tail hairs are particularly interesting as longer lengths of hair can provide information over an extended period of time (Burnik Šturm et al., 2015; Kaczensky et al., 2017; Rysava et al., 2016).

However, the use of stable isotopes in animal tissues to infer movement requires the consideration of two important aspects: First, establishing the diet-tissue discrimination factor which accounts for how the isotope value differs between tissue and diet (Rubenstein & Hobson, 2004; J. B. West et al., 2006). And second, estimating the temporal lag between ingestion and the appearance of an isotopic change in animal tissue. Animal tissue does not immediately reflect the isotopic composition of diet, given that metabolism has an important role to play in determining the time before isotopic changes in diet and corresponding changes in tissues (Zanden et al., 2015). For example, sulfur is metabolized differently from carbon and nitrogen and across different tissues and species, because it occurs at low concentration in animals' tissues, and it is mostly bound within amino acids (Zanden et al., 2015). Since different elements are metabolized differently and different tissues have different turnover and growth rates, therefore, there will be different delay effects (Zanden et al., 2015). However, many isotope mixing models (e.g. Parnell et al., 2013; Stock et al., 2018)) assume that the isotope composition of animal tissue is in short-term equilibrium with its diet (i.e., there is instantaneous reflection of diet in the animals' tissue). This is unlikely to be the case and could mislead interpretation of isotopic results (O'Reilly et al., 2002). Therefore, understanding the delay mechanisms associated with sulfur utilization in inert biological materials is an important prerequisite to using the variation of $\delta^{34}S$ to study different ecological processes.

In this study, we use $\delta^{34}S$ data from GPS-collared migratory wildebeest from the Serengeti ecosystem to demonstrate the delay mechanisms involving the $\delta^{34}S$ isotope incorporation in tail hairs. We use GPS collared wildebeest to obtain the exact georeferenced location of animals in the landscape and compare the corresponding $\delta^{34}S$ isoscape values against those values observed in the tail hair during the time of growth. We compare regression models between the landscape and the tail hair lagged over a period of 8 months. Our study provides insights into

the processes behind $\delta^{34}S$ signal delays in tail hair and helps to improve interpretation of $\delta^{34}S$ results when making ecological inferences.

## 3.3 Materials & Methods

### 3.3.1 Study area

Wildebeest tail hair samples were collected from the Serengeti-Mara ecosystem in East Africa (Figure 3-1), between $34^0$ and $36^0$ E, and $1^0$ and $3^0$ N covering northern part of Tanzania and southern part of Kenya. The area is characterized by wet and dry seasons with rainfall of between 500 and 1200 mm per year (Figure 3-1a). Normally the dry season lasts for 5 months (June-October) and the wet season for 5 months (December – April) with November and May being transition months from dry to wet and vice versa, respectively (Fryxell et al., 2015). The ecosystem has a high gradient of soil fertility caused by heterogeneity of the underlying geology from young mineral-rich pyroclastic material to ancient leached and eroded granite material (Hopcraft, 2015) (Figure 3-1c). These different soil types provide the ecosystem with a strong gradient of sulfur stable isotope ratios as reflected in the grass isoscape (Figure 3-1b). The interpolated Serengeti grass sulfur isoscape ranges in $\delta^{34}S$ values between -5 ‰ and 30‰ with the laboratory measured $\delta^{34}S$ values in grass ranging between +2.82‰ and +13.04‰ (Kabalika et al., 2020).

The ecosystem is home to 27 species of African ungulates including wildebeest which is the largest population of ungulates in the system (~1.3 million) (Fryxell et al., 2015; Hopcraft, 2015). The wildebeest population is comprised of a mixture of migratory (~1.2 million) and resident individuals. Migrants move along a north-south trajectory which enables animals to capitalize on the grazing resources associated with the rainfall and soil fertility gradients (Hopcraft, 2015).

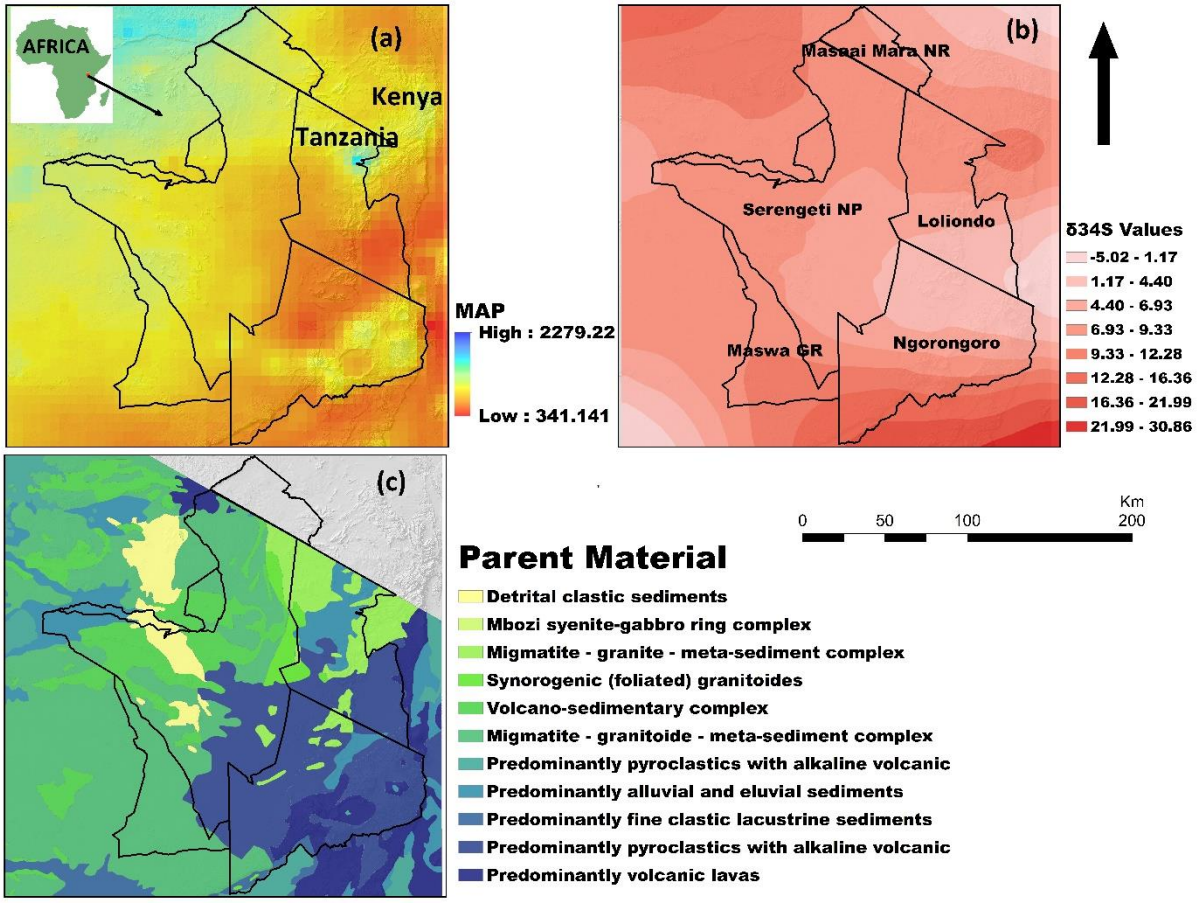


Figure 3-1: A map of Serengeti ecosystem showing a) mean annual precipitation (MAP; data from CHIRPS repository: https://www.chc.ucsb.edu/data/chirps); b) the variation of $\delta^{34}S$ isotopes measured in the grass (data from Kabalika et al. (2020); and c) the underlying parent material (data from Tanzania geological survey: https://www.gmis-tanzania.com/). Protected area boundaries are shown in black solid lines.

### 3.3.2 Collection and processing of biological materials

We collected tail hair samples from 11 GPS collared individual wildebeest (6 residents and 5 migratory individuals). At the time of first capture, wildebeest were equipped with a GPS collar and the right side of their tail was shaved to skin level. The date, age, sex and reproductive status (i.e., whether pregnant or lactating) was recorded for each animal before it was released. After approximately a year, the collared animals were recaptured, and the regrown tail hair was collected along with the ancillary data as described above. The regrown tail hair from each animal was aligned and packed in paper envelopes pending laboratory analysis. The start and end dates of the sample allowed us to estimate

tail hair growth rate, and the GPS data provided daily locations of the animal for the entire period of regrowth.

A small bundle of approximately 25 tail hairs from each individual were tied together so the proximal ends were aligned. Each bundle was prepared by washing in 2:1 chloroform: methanol and rinsed with double distilled water to remove the remnants of solvent (Kabalika et al., 2020; Rysava et al., 2016). Samples were dried for 48 hours at room temperature. After drying, the total length of the tail hair was measured. This was to be used to calculate the tail hair growth rate. Tail hair samples were sectioned into 8mm segments which correspond to approximately two weeks growth. The sectioning proceeded from the most recent part of the hair (proximal end) to the oldest (distal end). The segments were then powdered in a Retch MM400 (Germany) ball grinder using metal grinding tubes. The metal grinding tubes were immersed in liquid nitrogen ($LN_2$) for 60 seconds to embrittle the hair for easy powdering. The samples were ground for 90 seconds at 600 rpm speed. The powdered samples were weighed using a min-balance from a Mettler Toledo, Model MX5 calibrated to three digits. Samples were weighed between 1.0 - 1.3mg.

Samples were analyzed for $\delta^{34}S$ using a Pyrocube elemental analyser (Elementar Analysensysteme, Langenselbold, Germany) coupled to a VisION mass spectrometer (Elementar UK, Cheadle Hulme, Stockport, UK). Laboratory standards-methanesulfonamide/Gelatine(MSAG2), methionine/ alanine/ glycine/ gelatine (M2) and sulfanilamide/alanine/gelatine (SAAG2) were repeated with every 10 samples and were used to correct for linearity and instrument drift over a 72-hour analytical run. The analytical precision (i.e., the reproducibility of our lab standard) for $\delta^{34}S$ was better than 0.7‰. The isotope ratios are expressed in the delta (δ) notation in parts per million (‰): $\delta X = [(Rsample/Rstandard)-1]$ where $X= {}^{34}S$ and R = the ratio of $^{34}S/^{32}S$ isotopes in a given sample compared with V-CDT (Vienna - Canyon Diablo Troilite).

### 3.3.3 Estimating tail hair growth rate

We calculated tail hair growth rate in order to estimate the location of each individual wildebeest at the time the tail hair sample was growing. To calculate growth rate of a tail hair, we divided total length of a tail hair for each individual by the number of days that a hair grew (i.e., the difference between collaring and

recapture dates). To assess whether older hair might fragment at a faster rate than younger hair, we tested if the growth rate was different between individuals whose hair grew for longer than 13 months (i.e., 395 days) against the ones whose hair grew for shorter than this time using a generalized linear model. We also calculated what proportion of tail hair growth period occurs during wet and dry seasons as well as during the lactation period (note wildebeest reproduction is highly synchronous with calving in February and weaning in September, which enables us to estimate the lactation period for each animal). We included this information in the generalized linear model to test if tail hair growth rate differed by season and reproductive status. To test the statistical power of detecting the true effect of season and lactation on the growth rate of wildebeest tail hair using our model and our current sample size (i.e. 11 GPS collared wildebeest), we conducted power analysis by resampling our growth rate data from a normal distribution with the observed (mean ± SD), refitting the statistical model and examining the frequency of significant results in 1000 replicates.

### 3.3.4 Establishing the lag time (turnover rate) for $\delta^{34}S$ absorption in a tail hair for the migratory wildebeest

To establish lag time for $\delta^{34}S$ absorption between ingestion and deposition in tail hair of the migratory wildebeest (*N=5*), we georeferenced each segment of the tail hair (*N=118*) assuming a value for the tail hair growth rate for each wildebeest, conditional on an assumed wildebeest specific growth rate, and extracted the corresponding mean $\delta^{34}S$ isotope value from the Serengeti sulfur isoscape (Kabalika et al., 2020). We extracted the $\delta^{34}S$ values for every GPS point during the period of growth of each section of tail hair to get the mean value for the 8mm section as a whole. The growth rate for each wildebeest was taken from a normal distribution parameterized by the mean and standard deviation of the observed net growth rate. We extracted the $\delta^{34}S$ values for every GPS point during the period of growth of each section of tail hair to estimate the mean value for the 8mm section as a whole. We then fitted a linear regression in which the slope was fixed to be one between the isotope reading of the tail hair segment against the corresponding mean isotope value from the isoscape at lags ranging from 0 to 160 days at 10-day intervals. We used the r-squared metric from each regression to determine the lag that generated the best fitting regression model. We

repeated this process 5000 times (bootstrapping the individual wildebeest tail hair growth rates) to generate the mean and 95th percentile intervals (PIs) on the estimated lag time.

## 3.4 Results

### 3.4.1 Tail hair growth rate

Individual net rate of growth of tail hair from GPS collared wildebeest varied between 0.40mm and 0.63mm per day (mean = 0.511, SD= 0.062) (Table 3-1). We did not observe any significant effect of either season (coefficient= -0.178, t= 0.819, p = 0.439) or reproductive status (coefficient = -0.077, t=0.544, p = 0.603) on the wildebeest net tail hair growth rate. Our power analysis results suggested that, season had an overall effect size of 31.8% on the growth rate of wildebeest tail hair, but our sample size gave us only 18.08% power to detect it. Likewise, lactation had an overall effect size of 10%, but our power to detect it was only 6.6%. Furthermore, there was no evidence that net growth rate of hair that grew for more than 13 months was different than hair that grew for less than 13 months (coefficient = 0.022, t = 0.049, p=0.662), suggesting that net growth rate remains relatively constant. However, we note that if the distal end of the tail hair is fragmenting (i.e., eroding, Appendix B-3), independently of the age of the hair, then our estimated net growth rate is likely to be lower than the true growth rate.

Table 3-1: Tail hair growth rate per day for each individual wildebeest and a mean growth rate for all eleven GPS collared wildebeest.

| ID | Start date | End date | No segments | Tail length (mm) | Growth days | Days dry | Days wet | Days lactating | Growth rate (mm/day) |
|---|---|---|---|---|---|---|---|---|---|
| W417 | 08/06/2013 | 02/07/2014 | 25 | 200 | 389 | 135 | 254 | 120 | 0.514 |
| W418 | 09/06/2013 | 03/07/2014 | 23 | 184 | 389 | 150 | 239 | 0 | 0.473 |
| W419 | 08/06/2013 | 05/07/2014 | 24 | 192 | 392 | 144 | 248 | 120 | 0.489 |
| W420 | 10/06/2013 | 06/07/2014 | 26 | 208 | 391 | 165 | 226 | 120 | 0.531 |
| W422 | 10/06/2013 | 06/07/2014 | 21 | 168 | 391 | 180 | 211 | 120 | 0.429 |
| W551 | 26/05/2016 | 30/11/2017 | 37 | 296 | 553 | 345 | 208 | 240 | 0.535 |
| W552 | 26/05/2016 | 30/11/2017 | 39 | 312 | 553 | 345 | 208 | 0 | 0.564 |
| W553 | 26/05/2016 | 29/11/2017 | 33 | 264 | 552 | 345 | 207 | 240 | 0.478 |
| W578 | 24/03/2018 | 23/06/2019 | 36 | 288 | 456 | 210 | 246 | 120 | 0.631 |
| W580 | 27/04/2017 | 24/06/2019 | 40 | 320 | 788 | 210 | 578 | 120 | 0.406 |
| W582 | 24/03/2018 | 25/06/2019 | 33 | 264 | 458 | 195 | 263 | 0 | 0.576 |
| **GROWTH RATE (mm/day)** | | | | | | | **mean:** | | **0.511** |
| | | | | | | | **SD:** | | **0.062** |

### 3.4.2 $\delta^{34}S$ turnover rate in a tail hair for the migratory wildebeest

The best fitting regression model between $\delta^{34}S$ in the tail hair and $\delta^{34}S$ on the isoscape was found assuming a lag time of 78 days (95th PI 60-110 days, Figure 3-2a), and a baseline fractionation factor of 2.118‰ (95th PI 2.000 – 2.156‰, Figure *3*-2b). The lag estimate is insensitive to lower tail hair growth rates, but increases by about 10 days for every unit standard deviation (0.062) tail hair growth rate is increased by. Slopes of more or less than one can be imposed on the analysis, generating fits with equivalently well-fitting models, and slightly different lag-times (for example a slope of 0.75 generates lower lags of around 60 days, and 1.25 generates higher lags of around 90 days). Fitting both the intercept and slope results in very marginally better fitting model and a lower lag estimate of closer to 40 days but unrealistically high intercept of about 5.

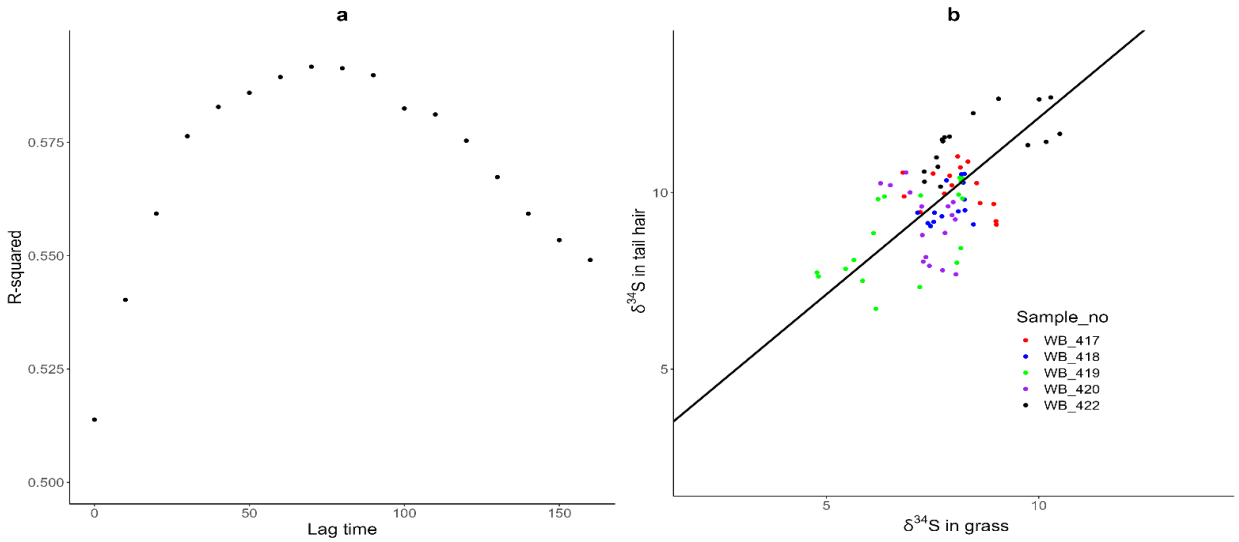


Figure 3-2. a) r-squared values from regression models relating $\delta^{34}S$ in tail with georeferenced $\delta^{34}S$ isoscape values at different time-lags; and b) the regression plot of our optimum 78 day lagged model.

## 3.5 Discussion

The primary result from this study suggests the delay between ingestion and deposition of $\delta^{34}S$ isotopes in the tail hair is substantial and here estimated to be about 78 days. This suggests that sulfur in the animal's body passes through a number of different metabolic processes before being deposited in the tail hair.

These findings are important because understanding how metabolic delays and processing speed influence the variation of $\delta^{34}S$ in biological material such as hair has important implications for making inferences about animal movements or dietary changes. Secondary results indicate that wildebeest tail hair grows at a constant rate invariant of season and pregnancy status, and there is no evidence of differential distal fraying or disintegration, and thus that tail hair length maps straightforwardly onto time.

There are several metabolic processes in the body that use sulfur and which may account for the long lags we observed between ingestion and deposition of $^{34}S$ in tail hair as indicated by our results. For example, Fry and Arnold, (1982), suggested isotopic turnover to be a result of two processes: tissue growth and catabolic turnover. The slow $\delta^{34}S$ turnover rate we observe could represent the fact that S is not directly involved in the process of obtaining metabolic energy, unlike C and N (Bahar et al., 2009). For example, sulfur in the hair is primarily derived from cysteine and methionine (Bin et al., 2017; Zanden et al., 2015). Methionine is a nutritionally indispensable amino acid that is acquired entirely from dietary sources (Elango, 2020). Because animals do not have the assimilatory mechanisms for inorganic sulfur (Saito, 2004); they therefore require methionine as a precursor for cysteine (Elango, 2020) and a source of their sulfur nutrients (Saito, 2004). However, the transformation of methionine to cysteine in animal's body involves a series of metabolic processes, such that; first, the formation of a high-energy sulfonium compound called AdoMet (i.e. S-adenosyl-L-methionine), which is used for both transmethylation reactions of methyl and as a precursor for the polyamines (Stipanuk, 1986). Then, the AdoMet passing through a series of multiple isozymes before being hydrolyzed to form cystathionine (for more detailed explanation of methionine metabolism see: (Stipanuk, 1986; Elango, 2020). Furthermore, the isotope turnover rate in the tail hair is not linear, it is controlled by multiple isotope pools (Ayliffe et al., 2004). Therefore, the $\delta^{34}S$ we observe in the tail hair is likely required to pass through multiple series of metabolic processes as well as multiple isotope pools before being laid down in the tail hair sequentially in the order the materials are consumed as suggested by Ayliffe et al., (2004).

The isotopic turnover rate is not only a function of the metabolic processes and tissues, but also scales with body mass (Thomas & Crowther, 2014; Zanden et al., 2015) which may compromise the utility of S isotopes for geolocating large

animals. Small animals such as mice (Arneson et al., 2006) integrate isotope signals over a short period of time compared to large animals (Carleton & Del Rio, 2005). For instance, Bahar et al., (2009) reported the turnover rate of $\delta^{34}S$ in the longissimus muscle of beef cattle is in excess of 219 days (approximate weight; 500 kgs), while our study estimates 78 days for $\delta^{34}S$ in wildebeest tail hair (approximate weight: 160kgs) suggesting the $\delta^{34}S$ metabolism in small ungulates may be faster than in large ungulates. Therefore, using $\delta^{34}S$ in tail hair to study animal movement may only be useful in small to medium sized animals with relatively long hair but may not be applicable for animals with short hair or very large herbivores such as elephant.

The rate at which $\delta^{34}S$ is processed in herbivores may also be a function of diet quality. For instance, forage with high protein content such as $C_3$ forbs are processed differently from the forage with low protein such as $C_4$ grass (Lee, 2018). Variations in dietary protein also alter the isotopic discrimination (Harrison et al., 2011; Richards et al., 2003) between different tissues within the body such as muscle, blood or skeletal tissue (Robbins et al., 2005). For example, Richards et al., 2003, switched the diet of two horses from their long-term $^{34}S$-rich diet ($\delta^{34}S$ = 10.8‰) to a $^{34}S$-poor diet ($\delta^{34}S$ = -1.9‰) for a period of 21 weeks, before switching back to a $^{34}S$-rich diet ($\delta^{34}S$ = 10.5‰) for a further 19 weeks. Tail hair was collected from each individual, subsampled into 1.5cm sections and analyzed for $\delta^{34}S$. They noted the $C_3$ and $C_4$ diets they supplied the horses with, were isonitrogenous but had different protein content with the $C_3$ having higher protein content than the $C_4$ based feed. The authors reported a larger diet-hair fractionation when horses were fed the protein poor $C_4$ based feed but lower fractionation levels when they are on the $C_3$ hays. The lower digestible protein in the $C_4$ feed could be associated with increased recycling of body proteins constructed while on the $C_3$ feed. Perhaps the time-lag we report for wildebeest could also be controlled by diet type. Wildebeest have been reported to be mostly grazers, feeding on $C_4$ grass (Codron et al., 2011; Codron & Brink, 2007; Owaga, 1975), but have also been reported to supplement their diet with $C_3$ plants (Zyl, 1965; *Personal Observation*), therefore the 78-day lag might be an averaged time delay of both diets.

On the other hand, the constant tail hair growth rate we report in wildebeest could be due to the fact that hair grows continuously regardless of the season or reproductive status (i.e. due to recycling taking place in the body) as

previously reported from some controlled studies such as (Codron et al., 2013; Dunnett & Lees, 2003; Dunnett, 2012; West et al., 2004), or our limited sample size (i.e. 11 individuals) has restricted our ability to detect some temporal variation in the growth rate caused by either the changes in season (affecting food quality and quantity) or lactation (affecting physiology). Therefore, care should be exercised when interpreting the resulting tail hair growth rate.

The spatial variation of forage quality is a function of the soil properties such as parent material or cation exchange capacity (Lee, 2018), however, parent material also determines $\delta^{34}S$ (Richards et al., 2003; Thode, 1970, 1991). Therefore, in areas with diverse parent material such as Serengeti, the protein content of the forage may be correlated with $\delta^{34}S$ isotopes (Nehlich et al., 2011)(Appendix B-2). In these instances, using $\delta^{34}S$ to make ecological inferences about animal movement may be complicated by the quality of the diet as well as collinearities between forage protein and $\delta^{34}S$.

## 3.6 Caveats

Application of $\delta^{34}S$ for studying the origin and movement of animals has been demonstrated successfully for a number of species (e.g. Hobson et al., 2012; Hobson & Wassenaar, 2008; Nehlich et al., 2011; Sayle et al., 2013; Weber et al., 2002). However, its utility to accurately geolocate animals has not been explored. In this study, we have tested the applicability of $\delta^{34}S$ for geolocating migratory animals. The major challenge that we have observed are the long lags in the continuous growing tissues (in this case, tail hair). The long lags suggest that S as an element is challenging to use for animal geolocation. We didn't detect seasonal differences in $\delta^{34}S$ (e.g. Coplen et al., 2002; Stack & Rock, 2011; Valenzuela et al., 2011). However, there could be patterns of seasonal variability in the growth rate of a tail hair caused by for example, changes in metabolic rates (Tieszen et al., 1983) which have not been established in this study. Therefore, the major caveats of this work in establishing the lag estimates of $\delta^{34}S$ in the tail hair are; a) small sample size probably limiting our ability to detect the effect of season or lactation in the growth rate of wildebeest tail hair, (b) error in some assumptions (e.g. the constant tail hair growth rate across seasons and lactation or limited information on S metabolism in the wildebeest tail hair), and c) single point sampling (i.e. one tissue, one location and one isotope), which might have limited

observation of other patterns such as seasonality in the created isoscape. Therefore, sampling repeatedly at different times can help to establish temporal variability of $\delta^{34}S$ across our study area and improve the accuracy of our lag estimates. Furthermore, application of compound specific isotope such as amino acids analysis (McMahon & Newsome, 2019) can also be helpful in explaining the context of S metabolism in the wildebeest tail hair.

## 3.7 Conclusion and future studies

Our analysis demonstrates the underlying complexities when using $\delta^{34}S$ to estimate animal movement. These complexities are likely caused by a mixture of animal's physiology (metabolism) and diet quality. Since wildebeest are eating a mixture of both low and high protein diets seasonally as they migrate between areas of high and low $\delta^{34}S$, the $\delta^{34}S$ deposited in the hair likely represents an averaged value. Therefore, $\delta^{34}S$ in the tail is a challenging approach for geolocation of wildebeest because of the long-time-lags between $\delta^{34}S$ ingestion and deposition, lags that may also depend on forage quality. However, if animals were moving across an S isoscape but were on a single diet with stable protein concentrations then these challenges may be overcome. A possible avenue for future studies might be to explore $\delta^{34}S$ as incorporated in only the essential amino acids (i.e., amino acids that are only available from the diet and which cannot be manufactured within the body, such as methionine). The $\delta^{34}S$ of essential amino acids may be a truer reflection of $\delta^{34}S$ in the landscape. Currently the $\delta^{34}S$ we observe in the tail hair of wildebeest is composed of both essential and non-essential amino acids that are embedded within forage of different protein concentrations which complicates the applicability of using $\delta^{34}S$ as a geolocator for fast moving migratory animals. This calls for more controlled diet studies in which we can establish the timing of dietary shifts for both wild and domesticated ungulates to ascertain this information. Furthermore, an understanding of a timing of dietary shift in wildebeest might also help to improve the estimates of our lag time.

# Chapter 4: Application of isotopic methods to study life histories: a novel approach for differentiating a resident and a migrant individual in mixed populations using sulfur isotopes

This chapter is based on the manuscript to be submitted in Methods in Ecology & Evolution Journal as: Kabalika. Z., Morales. J.M., Haydon. D.T., McGill. R.A.R., Morrison. T. A., Newton. J. & Hopcraft, G.J.C. (2023): Application of isotopic methods to study life histories: a novel approach for differentiating a resident and a migrant individual in mixed populations using sulfur isotopes.

## 4.1 Abstract

Differentiating resident versus migrant life history strategies in populations where both forms exist in sympatry is crucial for identifying the underlying drivers of large-scale animal movement. For instance, an understanding of movement of animals in response to climate anomalies, or the reproductive advantage conferred by moving are important ecological questions, which can help to predict future trends of population dynamics. However, differentiating resident from migrant individuals in a mixed population has always remained a methodological challenge due to limited resources and animal welfare issues. Here we demonstrate how the variation of sulfur stable isotope ratios measured in a tail hair of an animal can be used as a tool to differentiate a resident from a migrant individual, using a case study of GPS collared wildebeest of the Serengeti ecosystem, Tanzania. We employ three state-space models of animal movement, namely, stationary model, linear model and cyclic model. In the models, we compare amplitudes of the cyclic model, credible intervals of the amplitudes, slopes of the linear model and use the DIC values to identify the most parsimonious model(s) for the GPS collared individuals that are known to be resident (n=5) and the ones that are known to be migrant (n=5). We use the models' outputs to create a classification key and use it to classify samples of unknown trajectories collected from carcasses (n=7). We then use the sampling location and date of sampling to

estimate the most likely life history strategy and compare the results with our models' predictions. Our results suggest that migrant wildebeest have higher amplitudes of their cyclic model, wider credible intervals of their amplitudes as well as greater slopes of their linear model than the residents. The most parsimonious models for migrant wildebeest are linear and cyclic, and for the residents are stationary and linear. We assigned five out of seven wildebeest of unknown trajectories to be migrants and two to be residents, which matched with the suggested life histories based on the time and sampling location of the tail hair. Our study adds an important component in the field of movement ecology by providing an alternative way of making a population-wide inference of animal movement that is non-invasive to animals, relatively cheaper to analyze and easy to interpret. The approach can be widely adopted and applied to a diversity of taxa to identify their life histories, provided there is enough variation of $\delta^{34}S$ across the landscape, and time series of $\delta^{34}S$ can be re-created from animal's biological inert tissues.

Key words: wildebeest migration, the Serengeti ecosystem, Tanzania, state-space models, tail hair, East Africa.

## 4.2 Introduction

A variety of animal taxa undertake round trip movements between separate areas that are not used in other times of the year called seasonal migrations (Berger, 2004). These migrations help to reduce food limitation and facilitate compensatory grass growth (Holdo et al., 2009; Mcnaughton et al., 1997). Migration is considered to be an adaptive and spatially extensive strategy for utilization of resources that enhances individual fitness (Mariani et al., 2016). However, migration is not of unitary nature, it varies across populations, sex, age and sometimes between years within individuals (Bairlein & Coppack, 2006). For example, partial migration, where one part of a population migrates and the other does not is a common phenomenon in nature (Chapman et al., 2011). It has been reported in a diversity of taxa including large ungulates such as deer (Cagnacci et al., 2011; Sawyer et al., 2019), elk (Eggeman et al., 2016) and wildebeest (Hopcraft, 2015). Therefore, a detailed knowledge of variation of migratory patterns in a population and its underlying causes is important for understanding

the role of migration in the life cycle of migrating species (Bairlein & Coppack, 2006). For instance, quantifying a proportion of resident and migrant individuals in a population can help to elucidate aspects of disease transmission in disease ecology or predict future trends of predator-prey interactions (Dingle, 2006).

However, differentiating resident from migrant individuals in a partially migratory population has always remained methodologically challenging. This is because most of the evolutionary adaptations to migration are physiological and behavioral which are difficult to observe phenotypically (Lennox et al., 2016). Although the use of GPS trackers, accelerometers and other telemetry approaches have proven valuable in this context (Cagnacci et al., 2011; Sawyer et al., 2019), the costs associated with running, maintaining and interpreting the resulting data is high, thus limiting its utility for many ecologists, especially from developing countries. The use of stable isotope techniques offers a potential solution to identifying life history strategies of individual animals that is relatively cheap, easy to interpret and non-invasive to the animals.

Sulfur isotope ratios ($\delta^{34}S$) are stable across seasons (Nehlich et al., 2011; Newton & Bottrell, 2007) but vary across local geology (Kabalika et al., 2020; Krouse, 1988), making them a useful proxy in different fields of studies such as animal movement in both terrestrial (Kabalika et al., 2020) and marine (Zazzo et al., 2011) habitats, food web analysis (Connolly et al., 2004) or paleodietary studies (Nehlich et al., 2011; Richards et al., 2003; Sayle et al., 2013). Because the values of $\delta^{34}S$ in animal tissues reflects those of their local surface geology (Harrison et al., 2011; Krajcarz et al., 2019; McCutchan et al., 2003) sulfur isotopes can be particularly useful to infer movement between discrete areas. However, the potential for using variation of $\delta^{34}S$ in animals' tissues to differentiate resident and migrant individuals in partially migratory populations has not been explored.

Keratinized tissues such as hairs and feathers, are metabolically inert and yet they can preserve their isotopic records for the entire growth period (Burnik Šturm et al., 2017; Hobson & Koehler, 2015; Kabalika et al., 2020; Rysava et al., 2016), which means past diet and habitat selection can be traced for relatively long periods of time (Kaczensky et al., 2017). Tail hairs are particularly interesting because they grow continuously, providing unique time-series information on dietary or habitat changes over the period of growth (Burnik Šturm et al., 2017; Kabalika et al., 2020; Kaczensky et al., 2017). Exploring the variation of $\delta^{34}S$ in a

tail hair can provide ecologists with a means to understand the migratory history of individual animals.

In this study, we test if the variation of $\delta^{34}S$ in a tail hair can be used as an alternative approach to differentiate resident and migrant life histories in ungulates. We use tail hair data from GPS collared Serengeti wildebeest of known migratory status to model a time series of $\delta^{34}S$ isotope across the length of the tail hair using state-space models. We then use the parameters of these models to differentiate resident and migrant wildebeest, and to assign the most likely migratory strategy for unknown wildebeest sampled from carcasses. We hypothesize that a resident individual will have stationary (i.e. stable) values of $\delta^{34}S$ across the length of a tail hair and a migrant individual will have variable $\delta^{34}S$ values as it passes over a landscape of variable geology. The Serengeti wildebeest population provides an ideal system for testing these hypotheses because: 1) we know that the wildebeest population is comprised of both resident and migrant animals (Hopcraft, 2015), 2) the ecosystem has a strong gradient of $\delta^{34}S$ across its different geologies (Kabalika et al., 2020) and 3) we have GPS collared migrant and resident wildebeest from which we can track their movement for the entire growth period of tail hairs.

## 4.3 Materials & Methods

### 4.3.1 Study area and study species

The study was located in the Serengeti ecosystem which is in East Africa covering the northern part of Tanzania and the southern part of Kenya (Figure 4-1). The ecosystem has a diversity of soil types. The southeast is composed of nutrient rich pyroclastic soils resulting from deposits of volcanic ash. The north and central regions are composed of nutrient poor sandy soils from eroded granite. The west is composed of silts and clays which are the product of alluvial processes associated with the rivers flowing to Lake Victoria (Sinclair, 1995). Different parent material and soil types give rise to a steep gradient of sulfur stable isotope ratios ($\delta^{34}S$) with the values of between -5‰ and +30‰ (Figure 4-1; Kabalika et al., 2020). The ecosystem has a strong rainfall gradient ranging between 500mm per year in the southeast to 1200mm in the northwest (Holdo et al., 2009).

The area harbors a diverse assemblage of 27 species of ungulates including wildebeest (Fryxell et al., 2015; Hopcraft, 2015). Wildebeest are the most

abundant ungulate in the system and outnumber all other species combined (Hopcraft, 2015). There are two migratory strategies of wildebeest in the Serengeti; migrants and residents (Hopcraft, 2015). The migratory wildebeest (approximately 1.2 million animals) move in a circular pattern from south to west to north following a counter gradient of rainfall and soil quality (Holdo et al., 2009). The migrants range over an area of approximately 25,000 $km^2$, consuming grasses with different $\delta^{34}S$ values across the gradient as they move.

There are three separate populations of resident wildebeest in the ecosystem located in the Western Corridor (n= ~ 10,000) (Hopcraft, 2015), the Ngorongoro crater (n = ~ 14,700) (Estes & Small, 1981) and Maasai-Mara National Reserve in Kenya, which harbors the largest population of resident wildebeest (n= ~ 20,000) (Ottichilo et al., 2001). Western Corridor residents have a restricted home range which is limited to the western arm of the ecosystem covering areas of Kirawira, Ndabaka, Ndoha, Musabi and Sibora plains (Figure 4-1)(Hopcraft, 2015). Home range size of Ngorongoro crater residents is limited to within the crater, with occasional movements outside the crater during the wet season (Hopcraft, 2015; Watson, 1967) (Figure 4-1). Maasai-Mara residents are primarily restricted to the northern part of the ecosystem with occasional short movements to the Loita plains during the wet season; the Mara residents are the remnants of the collapsed Loita-Mara migration (Figure 4-1) (Hopcraft, 2015; Watson, 1967). Approximately 35% of the resident wildebeest from Ngorongoro crater spend up to six months of time outside the crater in the Olbalbal depression between February and April where they mix with the Serengeti wildebeest migration onto the short grass plains and at the base of crater highlands (Watson, 1967; Serneels and Lambin, 2002; Thirgood et al., 2004; Msoffe et al., 2019) (Figure 4-1). The resident population from the Western Corridor mix with the migratory population between May and July in Ndabaka and Musabi plains as the migration passes through to complete its annual cycle (Thirgood et al., 2004), (Figure 4-1). The abundance of resident populations has remained unchanged for nearly five decades (Hopcraft, 2015), with the exception of the Mara herd which has collapsed by 70% from ~120,000 (Ogutu et al., 2016; Ottichilo et al., 2001; Serneels & Lambin, 2002). Resident wildebeest population generally has smaller home range sizes than the migratory population. They tend to move up and down the hill slope by season (Bell, 1971), and their home range size typically does not exceed 2500 $km^2$.

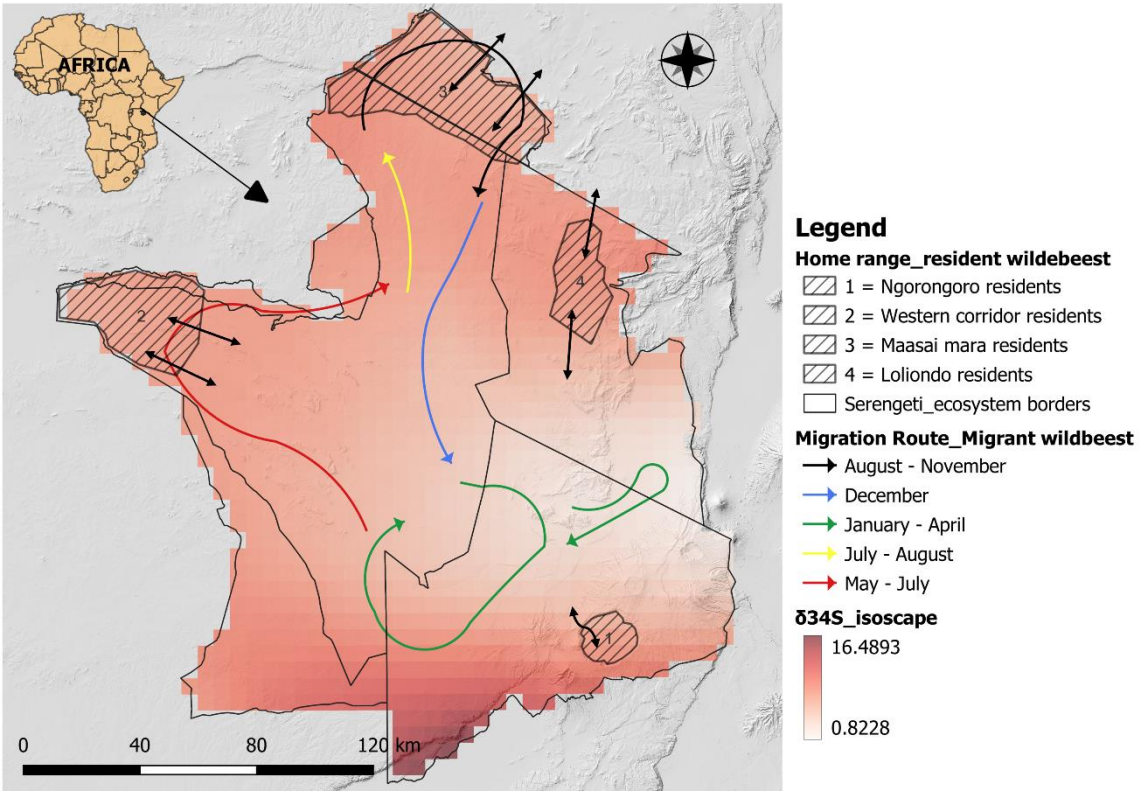


Figure 4-1:The Serengeti ecosystem (black solid lines) showing the gradient of $\delta^{34}S$ across the system (Kabalika et al., 2020), home range of resident wildebeest (shaded blocks) together with their movement patterns (black arrows) and migratory route across different months of the year for migrant wildebeest (Torney et al., 2018).

### 4.3.2 Tail hair sample collection

We collected a total of 17 wildebeest tail hair samples of which 10 were from GPS collared individuals with known movement trajectories (5 resident and 5 migrant), and 7 were from carcasses with unknown movement trajectories. GPS collars were deployed on reproductively active female wildebeest. At the time of immobilization the right side of a tail was shaven to skin level before the animal was released. Collared animals were then recaptured approximately 1 year later and the regrown tail hair was collected. All corresponding GPS locations during the period of hair growth (i.e. between collaring and recapture) were downloaded and the movement trajectories plotted, allowing differentiation between resident and migrant animals. Additional samples from carcasses were collected by pulling the hair by the root. For every sample the following ancillary data were collected; date, animal ID, collar ID (for live animals), age, reproductive status, and a

possible cause of death (for carcass). Samples were tied together at the proximal end to standardize time zero and stored in paper envelopes for laboratory analysis.

### 4.3.3 Sample preparation for stable isotope analysis

Tail hair samples were washed in 2:1 chloroform methanol solution to remove all the impurities and rinsed in double distilled water to remove the remnants of a solution. Samples were dried for 48 hours at room temperature and sectioned in 8mm segments which corresponded to approximately 16 days of growth (based on a wildebeest growth rate of 0.511 mm per day estimated by (Kabalika et al., under review). Hairs were sectioned in order from the proximal to distal end (i.e. from the most recent to the oldest sections). Samples were powdered using Retch MM400 Ball mill grinder using metal grinding tubes. Before powdering, grinding tubes were dipped in liquid nitrogen ($LN_2$ ) for 60 seconds to freeze the hair making it fragile for grinding. Samples were ground for 90 seconds at 600 rpm. Powdered samples were weighed using mini-balance from Mettler Toledo, Model MX5 calibrated to three digits. The samples weighed between 1-1.3mg.

### 4.3.4 Sample analysis for stable isotope ratios

$\delta^{34}S$ was measured at the Scottish Universities Environmental Research Centre (SUERC) laboratory using a Pyrocube elemental analyser (Elementar Analysensysteme, Langenselbold, Germany) coupled to a VisION mass spectrometer (Elementar UK, Cheadle Hulme, Stockport, UK). Laboratory standards(methanesulfonamide/ gelatine(MSAG2), methionine/ alanine/ glycine/ gelatine (M2) and sulfanilamide/alanine/gelatine (SAAG2)) were repeated with every 10 samples and were used to correct for linearity and instrument drift over a 72-hour analytical run. The analytical precision for sulfur isotopes was better than 0.7‰. The isotope ratios are expressed in the delta (δ) notation in parts per million (‰): $\delta X = [(Rsample/Rstandard)-1]$ where X= $^{34}S$ and R = the ratio of $^{34}S/^{32}S$ isotopes in a given sample compared with V- CDT (Vienna - Canyon Diablo Troilite).

### 4.3.5 State-space models for $\delta^{34}S$ in wildebeest tail hair

To model the time series of $\delta^{34}S$ across the length of a tail hair, we used three different state-space models reflecting different resident and migrant life history strategies. The stationary model represents a relatively stable isotopic signature in the tail hair over time, as would be expected for a resident animal that occupies a small and stable home range for a long period of time. The linear model represents a changing isotopic signature in a tail hair over time (i.e. a linear function with either a positive or negative slope). This case would be expected for a dispersing animal, which moves between distinct isotopic regions and does not return. The cyclic model represents a fluctuating isotopic signature in a tail hair that cycles between high and low $\delta^{34}S$ values with a periodic pattern over time. This case would be expected from a migratory animal that performs a full cycle across a wide range of isotopic distinct regions. Due to the sequential nature of data, we defined all our models as first order autoregressive (AR 1). The state-space models consisted of an observation model accounting for sampling noise in the estimates of isotope ratios, and a process model describing how the system changes through time. The observation model was the same for all three models and assumed that the isotope ratio recorded at time $\mathrm{y_t}$ (corresponding to 16 days of tail hair growth) is sampled from a normal distribution with a mean equal to the true isotope value ($\mathrm{x_t}$ ) and standard deviation ($\mathrm{sd_{obs}}$) representing uncertainty due to measurement error.

$$y_t \sim N(x_t, sd_{obs}) \qquad \text{Equation 4-1}$$

The process models defined the three different ways in which the isotopic signature could change over time as a function of animal movement (i.e. stationary, linear or cyclic). To model process variability, we used a normal distribution, with the modelled expected isotopic value ($\mathrm{m_t}$) and standard deviation ($\mathrm{sd_{proc}}$) so that the realized isotope value at time $t$, is defined by:

$$x_t \sim N(m_t,\ sd_{proc}) \qquad \text{Equation 4-2}$$

$$m_t = \mu_t + x_{t-1} \times \rho \qquad \text{Equation 4-3}$$

where $\mu_t$ is modelled differently for the 3 models, and $\rho$ is an autocorrelation parameter.

Our state-space models for different life history strategies were defined as follows: For the stationary model, the value of $\mu_t$ is a constant that needs to be estimated. For the linear model, $\mu_t$ is a linear function of time with intercept, $a$ and slope, $b$ parameters (Equation 4-4)

$$\mu_t = a + b \times \tau_t \quad \text{Equation 4-4}$$

where $\tau_t$ is the corresponding time period.

For the cyclic model, $\mu_t$ is modelled as a periodic function of time with parameters α, ß and $\gamma$ (Equation 4-5)

$$\mu_t = \alpha + \beta \times cos\left(\gamma + 2\pi \frac{16}{365} \times \tau_t\right) \quad \text{Equation 4-5}$$

where α is a scale parameter, ß controls the amplitude of the cycle and $\gamma$ controls the phase shift of the annual cycle. Given that the tails segments measure sulfur content for 16 days (Kabalika et al., under review), the $\frac{16}{365}$ was set to obtain an annual cycle.

We fitted the models to data for each tail hair sample using a Bayesian approach and obtained posterior samples from all parameters using MCMC implemented in JAGS using R software (R-Core-Team, 2017). To improve the efficiency of MCMC samples, the isotope values for each animal were centered on their mean. We used an informative prior for the measurement error $(\mathrm{sd_{obs}})$ based on the estimated instrument error of 0.7‰ as calculated from SUERC lab standards. This was sampled from a normal distribution with mean 0.7 and precision of 500 (i.e. 0.002 variance) to account for the high accuracy of the mass spectrometer. The prior for process variability $(\mathrm{sd_{proc}})$ that accounts for variability in isotope values in the tail hair, was uniform between 0 and 10. This ensured the deviation values were non-negative and within a reasonable range. To avoid estimates greater than 1 for the autocorrelation parameter ρ, we used a vague Beta prior (with parameters 1.1, 1.1). For the stationary model (resident), we set the prior for μ as a normal distribution with mean 0 and standard deviation of 1. For the linear model (disperser), we used a normal distribution with mean 0 and standard deviation of 1 for both intercept and the slope. For the cyclic model (migrant), we used a normal distribution with mean of 0 and a standard deviation of 1 for α and ß, and a truncated normal with mean 0 and a standard deviation of

1 for $\gamma$. We ran three MCMC chains for 50,000 iterations with half of them as burn-in. We checked for convergence using R-hat <= 1.1 for all parameters.

### 4.3.6 Differentiating resident and migrant wildebeest.

To understand the differences in variability in $\delta^{34}S$ over the length of the tail hair between resident and migrant wildebeest, we compared the slopes and amplitudes for both the linear and cyclic state-space models respectively for all GPS collared wildebeest. We use Deviance Information Criteria (DICs) to determine the most parsimonious model of $\delta^{34}S$ over time in the tail hair. The models with a DIC difference of less than two units were considered not to be different (Berg et al., 2004). We report the Credible Intervals (CIs) for slopes and amplitudes for the animals that are known to be residents separately from those that are known to be migrants based on their GPS trajectories. We then used this information (i.e. amplitudes and slopes including their credible intervals), as well as the DIC for the most parsimonious model to create the simplest classification key that most consistently differentiates samples collected from resident versus migrant individuals.

To assign a life history strategy (i.e. resident or migrant) to wildebeest carcasses with unknown trajectories we used the classification key created from the information of the GPS collared wildebeest. We tested the accuracy of the classification key by comparing the outcome from our models' classification (i.e. resident or migrant) with the date and location of the sampled carcass. Because the general timing and movement patterns of wildebeest migration in the Serengeti are known, we can assign a probable life-history strategy to each carcass. For instance, a carcass sampled in the western corridor (*Figure 4-1*) during the peak of the wet season (i.e. between February-April) or the peak of the dry season (August-October), is likely to be from a resident individual because the migrants are typically in the extreme south or north during these periods. Conversely, a carcass sampled in the southern plains during the wet season is likely to be a migrant because resident animals do not leave venture far from their home ranges (see methods section for explanations about the timing of movement and mixing between resident and migrant wildebeest).

# 4.4 Results

## 4.4.1 Model parameters for GPS collared wildebeest

Amplitudes for the cyclic model and slopes for the linear model for the migrant wildebeest were larger than those of the residents (Figure 4-2). The absolute values for the amplitudes of resident individuals were less than 0.184 (0.095 - 0.461, 97.5% CI), and those of migrants were greater than 0.337 (0.177 – 0.802, 97.5% CI) (Figure 4-2 & Figure 4-3; Table 4-1). The absolute values for slopes of residents were less than 0.017 (0.003-0.032, 97.5% CI), while those of migrant wildebeest were greater than 0.026 (0.019 - 0.075, 97.5% CI), (Figure 4-2 & Figure 4-3; Table 4-1). Results from the stationary model did not show any specific pattern across the isotope time series of a tail hair between resident and migrant wildebeest (Figure 4-2a). Resident wildebeest had the smallest DICs values for either stationary or linear models (Table 4-1), but not for the cyclic model. Migrant wildebeest had the smallest DICs values for either linear or cyclic models (Table 4-1), but not for the stationary model. This information was used to generate the classification key in Figure 4-4.

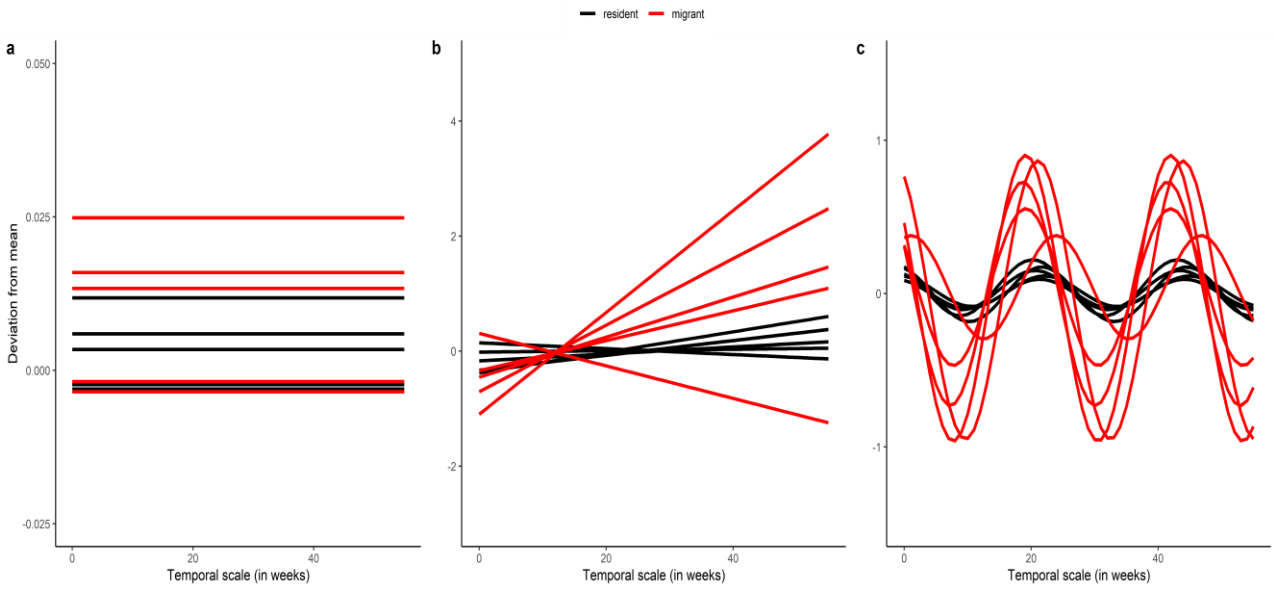


Figure 4-2: $\delta^{34}S$ isotope time series in a tail hair for resident and migrant wildebeest when tested against a) stationary model b) linear model and c) cyclic model.

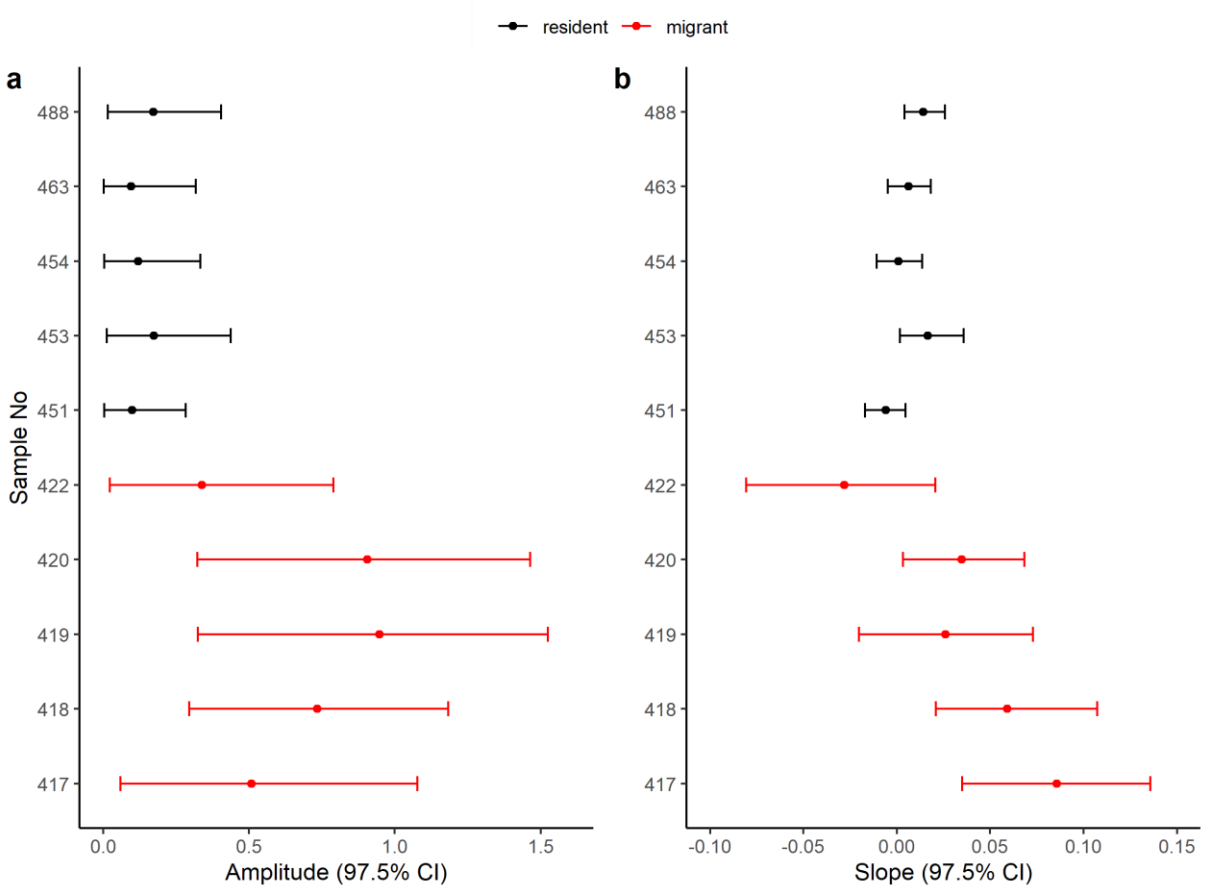


Figure 4-3: Credible intervals for a) slope of linear model and b) amplitude of the curve for cyclic model for resident and migrant wildebeest.

Table 4-1: Showing numerical values for amplitudes and slopes (together with their associated credible intervals) as well as the DICs for the most parsimonious model(s) for resident and migrant GPS collared wildebeest.

| Sample No | amplitude (97.5% CI) | slope (97.5% CI) | Δ DIC (stationary model) | Δ DIC (linear model) | Δ DIC (cyclic model) | Most parsimonious model | Life history strategy |
|---|---|---|---|---|---|---|---|
| 488 | 0.173 (0.016 - 0.404) | 0.014 (0.004 - 0.025) | 10.41 | 0.00 | 6.27 | Linear model | Resident |
| 463 | 0.096 (0.002 - 0.318) | 0.006 (-0.004 - 0.018) | 0.00 | 2.82 | 0.86 | Stationary model | Resident |
| 454 | 0.12 (0.004 - 0.333) | 0.001 (-0.01 - 0.013) | 0.00 | 5.47 | 1.53 | Stationary model | Resident |
| 453 | 0.174 (0.012 - 0.438) | 0.017 (0.001 - 0.013) | 12.38 | 0.00 | 7.60 | Linear model | Resident |
| 451 | 0.1 (0.004 - 0.282) | 0.006 (-0.017 - 0.004) | 0.00 | 1.53 | 1.63 | Stationary model | Resident |
| 422 | 0.339 (0.023 - 0.790) | 0.028 (-0.08 - 0.020) | 0.61 | 1.43 | 0.00 | Cyclic model | Migrant |
| 420 | 0.906 (0.323 - 1.463) | 0.035 (0.003 - 0.068) | 4.75 | 7.77 | 0.00 | Cyclic model | Migrant |
| 419 | 0.948 (0.324 - 1.524) | 0.026 (0.02 - 0.072) | 2.05 | 5.73 | 0.00 | Cyclic model | Migrant |
| 418 | 0.734 (0.295 - 1.182) | 0.059 (0.021 - 0.107) | 10.41 | 3.22 | 0.00 | Cyclic model | Migrant |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 417 | 0.509 (0.058 - 1.078) | 0.086 (0.035 - 0.135) | 7.84 | 0.00 | 14.09 | Linear model | Migrant |

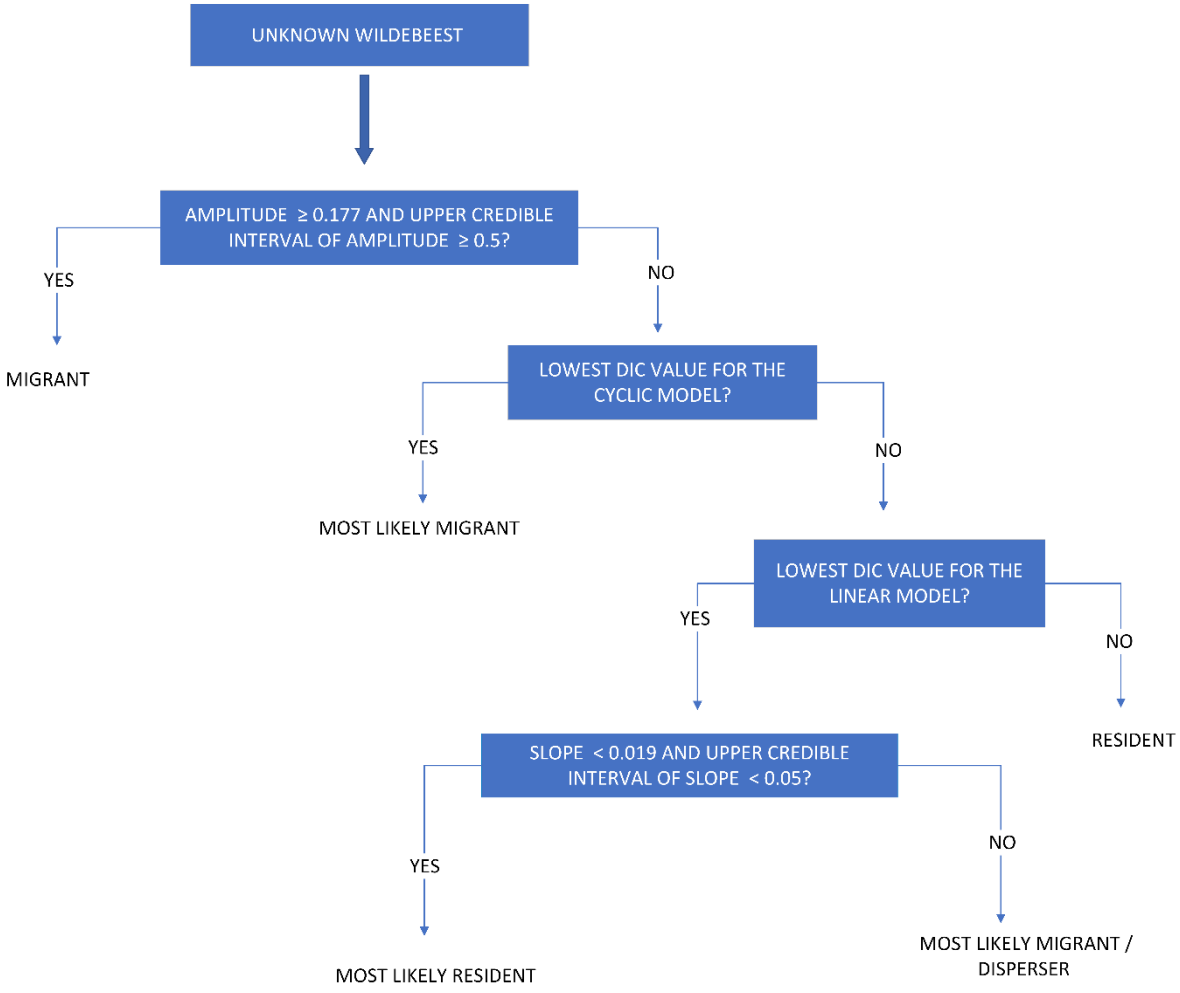


Figure 4-4: A classification key for identifying life histories of unknown wildebeest from the Serengeti ecosystem.

### 4.4.2 Classifying the 'unknown' individuals

Slopes of the linear models and amplitudes of the cyclic models for the unknown individuals included both large and small values suggesting a mixture of both resident and migrant animals (Figure 4-5 & Figure 4-6). The following individuals had amplitudes of greater than or equal to 0.177 (i.e. the lower 97.5% CI for migrant wildebeest) and subsequently upper credible intervals of amplitude of

greater than or equal to 0.5: 224 (green), 238 (black), 259 (purple), 266 (yellow) and 274 (brown). Therefore, these animals are most likely migrants (Figure 4-5; Table 4-2). The following individuals had amplitudes of less than 0.095 (i.e. the lower 97.5% CI for resident wildebeest) and small upper credible intervals of amplitude of less than 0.5: 50 (blue) and 108 (red). Therefore, these animals are most likely residents (Table 4-2). Furthermore, the DIC value for individual 50 (blue) supported a linear model and the slope was less than or equal to 0.019 which is the lower 97.5% CI for resident wildebeest (Figure 4-6; Table 4-2), suggesting this animal is most likely a resident. The DIC value for individual 108 (red) supported a stationary model (Table 4-2).

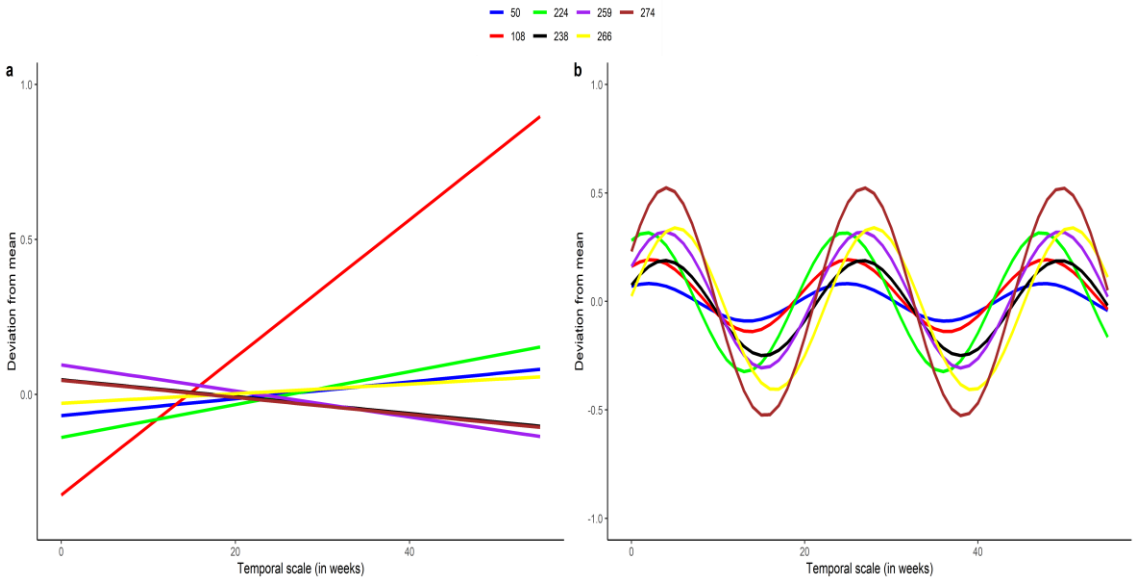


Figure 4-5: a) slopes for linear model and b) amplitudes for the cyclic model for the 'unknown' individuals, suggest a mixture of both resident and migrant wildebeest.

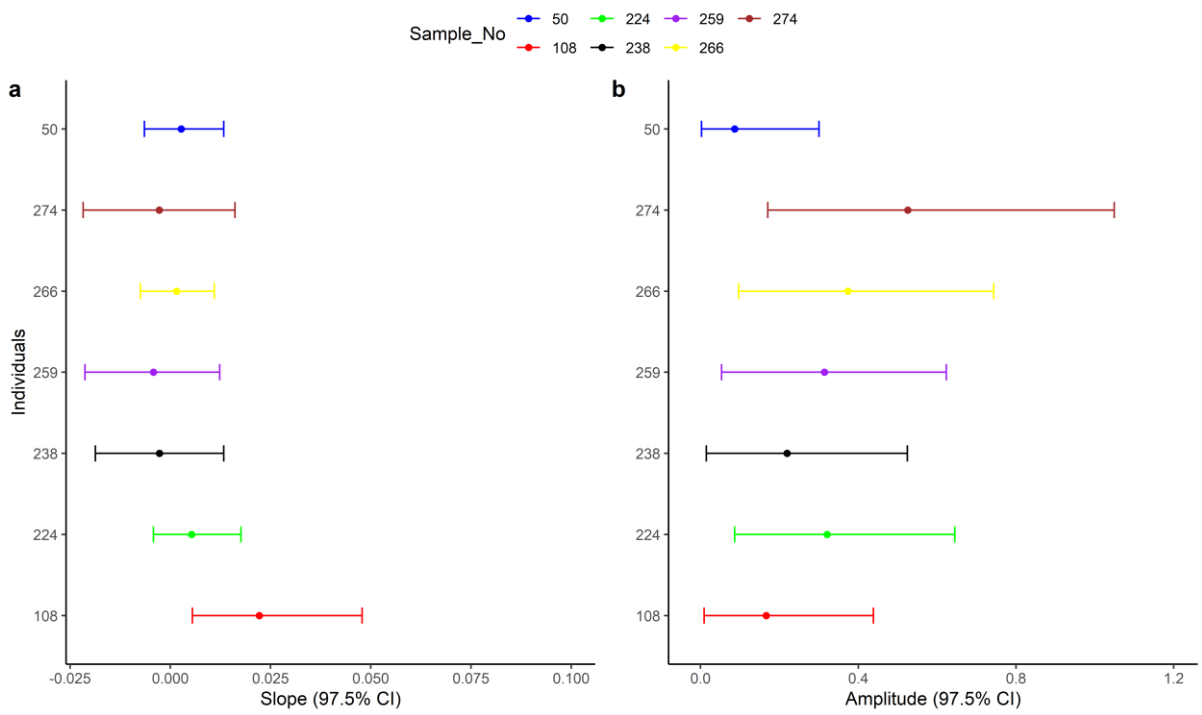


Figure 4-6: Credible intervals for unknown individuals showing a) slope of linear mean model and b) amplitude of the curve of the cyclic mean model

Table 4-2: The DICs for the most parsimonious model(s) supporting different life history strategies for the unknown individuals, and possible life histories based on the date and location of sampling. Please note that individuals 259, 266 and 274 have depicted their most parsimonious models as stationary model, but they have been classified as migrants based on their amplitudes and slopes according to the classification key

| Sample No | Amplitude (97.5% CI) | slope (97.5% CI) | Δ DIC - stationary model | Δ DIC - linear model | Δ DIC - cyclic model | Most parsimonious model | Proposed life history strategy by our key | Possible life history strategy by sampling location |
|---|---|---|---|---|---|---|---|---|
| 50 | 0.082 (0.002-0.3) | 0.003 (-0.006 - 0.015) | 1.87 | 0.00 | 1.28 | Linear model | Resident | Resident |
| 108 | 0.166 (0.008 - 0.438) | 0.021 (0.005 - 0.048) | 0.00 | 1.91 | 2.14 | Stationary model | Resident | Mara-resident |
| 224 | 0.32(0.086 - 0.644) | 0.005 (-0.004 - 0.018) | 20.73 | 14.40 | 0.00 | Cyclic model | Migrant | Migrant |
| 238 | 0.219 (0.014 - 0.524) | 0.002 (-0.019 - 0.013) | 3.21 | 3.69 | 0.00 | Cyclic model | Migrant | Migrant |
| 259 | 0.314 (0.053 - 0.623) | 0.004 (-0.021 - 0.012) | 0.00 | 1.70 | 1.90 | Stationary model | Migrant | Migrant |
| 266 | 0.373 (0.096 - 0.743) | 0.001 (-0.007-0.01) | 0.00 | 3.04 | 8.72 | Stationary model | Migrant | Migrant |
| 274 | 0.525 (0.17-1.048) | 0.002 (-0.02-0.016) | 0.00 | 0.74 | 14.29 | stationary model | Migrant | Migrant |

## 4.5 Discussion

The major finding from this study suggests that variation of $\delta^{34}S$ across length of the tail hair has potential to be used as a tool to differentiate resident and migrant life history strategies in ungulates. Our results suggest that migratory wildebeest have higher slopes and amplitudes (together with their associated credible intervals) for their linear and cyclic models than the residents. Our results further suggest that the most parsimonious model for the variation of $\delta^{34}S$ across length of a tail hair for migratory wildebeest is either linear or cyclic, but never stationary. The most parsimonious model for resident wildebeest is either stationary or linear, but never cyclic. This suggests that state-space models of variation of $\delta^{34}S$ across length of the tail hair can be used to identify the migratory strategy of a randomly sampled wildebeest, and suggesting similar approaches could be used for other species in other ecosystems.

The large slopes and high amplitudes observed in migrant wildebeest are most likely caused by the fluctuations of $\delta^{34}S$ they encounter over the course of migration. Migratory animals such as the Serengeti wildebeest move across a wide range of habitat types in a periodic pattern in search of high-quality food and water (Haché et al., 2014; Holdo et al., 2009). The variation of $\delta^{34}S$ observed in tail hair as it grows reflects the variation of $\delta^{34}S$ in the vegetation over the migratory route (Kabalika et al., 2020; Kaczensky et al., 2017). This is because animal's inert biological materials such as hairs can grow for a long period of time, and the isotopic composition of the new tissues reflects the diet of an animal during the time of synthesis (Burnik Šturm et al., 2017; Horacek et al., 2012).

The cyclic model is favored by migratory wildebeest likely because it is designed to capture the patterns of large-scale movements through fluctuating periodic patterns of $\delta^{34}S$. Migratory wildebeest move across a large spatial scale of $\delta^{34}S$ values across the Serengeti in a predictable seasonal pattern. As a result, annual values of their $\delta^{34}S$ across tail hair are highly variable.

The scenario of the linear model is favored by both resident and migrant wildebeest likely because the model is designed to capture a trend related to range shift, such as a resident animal moving up and down the hill (Bell, 1971), or a migrant animal moving over a short period of time without completing a full migratory cycle. For example, the home range size of resident wildebeest is restricted to between top and bottom of the hills, and the wildebeest move between these areas by season, such that the hill tops in the wet and the hill

bottoms in the dry season (Bell, 1971; see section 4.3.1). The variation of S in the tail hair of residents is influenced by the soil on which the grass is growing. Therefore, the linear patterns of $\delta^{34}S$ we see in residents may also be due to season movement up and down the low-lying hill. Migrant animals do not all start their journey at the same time and the speed of movement varies between individuals (Hopcraft et al., 2014). As a result, the linear model may support migrants that move rapidly across the landscape because they do not linger and consume a large amount of vegetation at each stage as one would expect from a slow moving migrant. In addition, the route of the migration varies between years based on rainfall, therefore the amplitude of $\delta^{34}S$ in the tail hair may vary annually. An alternative behavior that would support a linear model is that of a dispersing animal that moves across a large geographic area that differs in vegetative $\delta^{34}S$ and without returning. Dispersal has never been observed in Serengeti wildebeest, however this could be a mechanism for other species, especially for territorial animals moving away from their natal range.

The stationary model did not show any patterns between resident and migrant wildebeest. This is most likely because the stationary model is designed to capture the patterns one would expect from animals that move across areas with a very small $\delta^{34}S$ gradient either because the home range is very constrained, or because the animal moves along $\delta^{34}S$ isocline (Kabalika et al., 2020). Both resident and migrant wildebeest of the Serengeti move across different spatial ranges of $\delta^{34}S$, therefore the model was unable to consistently identify patterns that could differentiate between these life histories.

The classification key for life histories of unknown individuals capitalized on the differences in amplitudes, slopes, and credible intervals between resident and migrant wildebeest. However, the most parsimonious models across some individuals were ambiguous in such a way that the DICs difference was less than 2 for all three models (e.g. individual 259, Table 4-2), providing an avenue of further exploration for improving our models. For example, an additional secondary isotope like $\delta^{13}C$, which tends to vary with photosynthetic pathways between $C_3$ and $C_4$ plants (Spasojevic & Weber, 2021; Yan et al., 2020) can help to differentiate $C_3$ versus $C_4$ regions (Janzen et al., 2020; Spasojevic & Weber, 2021) and improve the accuracy of our models. The Serengeti $\delta^{34}S$ isoscape has strong variability of $\delta^{34}S$ across its geological ranges (Kabalika et al., 2020), suggesting the movement of other species such as zebra, eland, Thomson's gazelle, Grant's

gazelle and giraffe should be detectable using similar approaches. However, some species may not move across the entire $\delta^{34}S$ range. Therefore, the accuracy of using a state-space model to identify migratory strategy depends on the extent and direction of movement relative to bearing of the isoclines as well as the strength of gradient of the isoscape. The approach would likely be more effective in areas with higher variability of $\delta^{34}S$ than the Serengeti but impractical in areas with less or no $\delta^{34}S$ variability. Assuming these conditions are met, the technique of using state-space models of $\delta^{34}S$ variation in a tail hair (or other biologically inert tissues such as horn, hoof or teeth) to study life histories can be widely applied to a variety of species.

## 4.6 Caveats

This work has demonstrated the applicability of using $\delta^{34}S$ in the tail hair of a grazer to differentiate resident and migrant life history strategies. However, its major limitation is the fact that it cannot be used to accurately geolocate animals. It can only be used to detect the patterns of movements, such that whether an animal is a resident or a migrant. Furthermore, our inability to establish patterns of seasonality in resident using our approach could be a limiting factor when adopting the technique across other species or ecosystems. This is because home range sizes of resident individuals from other species or systems might be broader than those of the Serengeti wildebeest, causing high fluctuations in their $\delta^{34}S$ values. Therefore, it is important to have a clear understanding of home range sizes for the resident individuals before adopting and employing this technique. Characterizing migration patterns using hair samples offers a useful approach for understanding both current and historic characteristics of populations, which is useful for planning and implementing different species' management strategies.

## 4.7 Conclusions and future studies

The classification key created from state-space models' for identifying migratory strategies of ungulates has demonstrated a promising degree of accuracy in identifying the life history status of unknown samples. The classification key identified all seven individuals as either resident or migrant in a way that matches the strategies suggested by date and location of the sampled carcass (Table 4-2).

This suggests the analysis of $\delta^{34}S$ in a tail hair using state-space models could be an effective approach for making a population-wide inference of migratory strategy, but not as a tool for animal geolocation. Further cross-validation using more tail hair samples from animals with known GPS trajectories from other species in different ecosystems would improve this approach and facilitate the creation of a more robust classification key.

# Chapter 5: Seasonal dietary convergence and partitioning of co-migrating ungulates: the case of Serengeti wildebeest and zebra

This chapter is based on the manuscript to be submitted in the Journal of Animal Ecology as: Kabalika. Z., McGill. R.A.R., Newton. J., Morales. J.M., Morrison. T. A., Haydon. D.T., & Hopcraft, G.J.C. (2023): Seasonal dietary convergence and partitioning of co-migrating ungulates: the case of Serengeti wildebeest and zebra.

## 5.1 Abstract

Quantifying dietary preferences and niche partitioning between co-migrating species provides insights on resource use and interaction patterns, such as periods of diet convergence versus diet partitioning. Using wildebeest and zebras from the Serengeti ecosystem, we use stable isotope mixing models and ellipse-based metrics for the values of $\delta^{13}C$ and $\delta^{15}N$ from tail hair to draw inferences of seasonal dietary preferences and dietary niche interactions for the co-migrating species using isotopic dietary niche. Our findings suggest while both wildebeest and zebra have very similar diets in general, the isotopic dietary niche of wildebeest is larger than that of zebras during both wet and dry seasons. Wildebeest tend to supplement their diet with forbs particularly in the dry season, while zebra focus almost entirely on grass all year round. The highest dietary niche overlap between the two species occurs in August and the lowest occurs in May. Lactating females for both wildebeest and zebra tend to have a larger isotopic niche width than the non-lactating females, which likely reflects the high metabolic demand by lactating females. This study helps to understand species co-existence as well as the influence of reproduction on resource acquisition. For example, the high degree of isotopic dietary niche overlap between the two ungulates species during specific months suggests there may be strong competition for pasture, however grazers could mitigate this by supplementing their diet with forbs during lean or

demanding periods (such as lactation) or alternatively by spatially segregating over large areas.

Key words: Tanzania, wildebeest migration, East Africa, isotope niche, SIBER, SIMMR, trophic enrichment factor.

## 5.2 Introduction

One of the most fundamental questions in community ecology focuses on understanding how competing species co-exist in a resource limited environment (Rosenzweig & Abramsky, 1986). Understanding how, for instance, more than 100 ungulate species across different African ecosystems co-exist with a single food source (grass) and the carnivores that eat them (Cumming, 1982), provides an opportunity to explore the degree of interaction between species (e.g. competition or facilitation) and the drivers of niche dynamics in these systems (Sinclair et al., 2006). However, dietary preferences and niche partitioning across species and communities differ (Suriyamongkol et al., 2022), and quantifying this interaction is of great scientific significance.

Large mammalian herbivores offer an excellent opportunity for exploring the processes of resource interaction and niche partitioning among the co-habiting animal species. This is because, variation in their species composition can significantly affect structure and functioning of the entire ecosystem (Cumming, 1982). However, understanding dietary preferences and niche interaction is always costly in terms of both money and time. This is because the common methods for studying dietary niche and resource interactions have typically involved direct observation of foraging events (Slagsvold & Wiebe, 2007), visual morphological analysis of stomach, gut or fecal contents (Caryl et al., 2012) or through DNA metabarcoding analysis (de Sousa et al., 2019). Stable isotope ratio analysis offers an alternative and a powerful tool for studying resource partitioning and niche interaction for co-habiting animal species (Codron et al., 2005; Codron & Brink, 2007; Newsome et al., 2007; Tamburin et al., 2019).

The isotope values in animal tissues reflect the isotopic composition of diet (Hobson & Wassenaar, 2008; Kabalika et al., 2020) together with the associated temporal scale of the dietary information (Hobson et al., 2014). For example, isotope values measured from inert biological tissues such as hair or feathers have

been used in the studies of animal movement (Kabalika et al., 2020; Zazzo et al., 2011), migratory connectivity of birds (Clegg et al., 2003; Garcia-Perez & Hobson, 2014; Smith et al., 2003) and niche dynamics in ungulates (Burnik Šturm et al., 2017; Kaczensky et al., 2017). This is because these tissues grow for a long period of time and are inert once formed, enabling them to preserve their isotope composition indefinitely (Horacek et al., 2012; Kaczensky et al., 2017). Such tissues, therefore, present an excellent archive of animal's diet and habitat change information (Burnik Šturm et al., 2017). Stable carbon isotope ($\delta^{13}C$) ratios measured from animal tissues are normally utilized to infer features of eating behavior and habitat usage by animals (Janzen et al., 2020; Sealy et al., 1987; Tieszen & Boutton, 1988). This is possible due to differences in anatomical and physiological differences in plant photosynthetic pathways (Spasojevic & Weber, 2021), such that the values of $\delta^{13}C$ between $C_3$ and $C_4$ plants are consistently different (Dawson et al., 2002; Yan et al., 2020), differentiating $C_3$ versus $C_4$ regions (Spasojevic & Weber, 2021). Although the values of $\delta^{13}C$ change with trophic level of an organism, it is less marked than that of nitrogen stable isotope ratios ($\delta^{15}N$) (Zanden & Rasmussen, 1999, 2001). The values of $\delta^{15}N$ are commonly used to understand the trophic position (Codron & Brink, 2007; Middelburg, 2014; Sealy et al., 1987) or physiology of an animal (Rysava et al., 2016). More recently, $\delta^{15}N$ have been used to elucidate differences in dietary protein (Robbins et al., 2005) and starvation patterns (Rysava et al., 2016). $\delta^{13}C$ and $\delta^{15}N$ have been used together to study niche dynamics (Shaner & Ke, 2022; Tamburin et al., 2019), dietary preferences (e.g. Burnik Šturm et al., 2017; Codron et al., 2011; Kaczensky et al., 2017) as well as the ability to switch between diets following environmental changes (Codron et al., 2013).

In this study we use the variation of $\delta^{13}C$ and $\delta^{15}N$ measured along lengths of tail hairs of wildebeest and zebra from the Serengeti ecosystem to explore feeding ecology and niche interaction between the co-migrating ungulates. We explore seasonal composition of diet and variability of dietary isotope niche between wildebeest and zebra. Specifically, we are interested to understand the amount of isotopic niche[2] overlap between the two species across wet and dry

[2] Isotopic niche of a species refers to a multidimensional space in which the axes correspond to the isotopic values of different elements found in an animal's tissues. While there is a connection between the isotopic niche and the trophic niche, they are typically distinct. However, the isotopic data of consumers is primarily

seasons. Further, we want to know if the dietary isotope niche size is influenced by the physiological demands of lactation. We hypothesize that, if the migratory species are partitioning the grazing resources based on preference, then we expect the dietary isotopic niches of the two species to be consistently different, however if the partitioning is influenced by seasonal availability of resources, then we expect the dietary isotopic niches to be similar at least in one season. We also expect the dietary isotope niche to be different between lactating and non-lactating individuals because lactation presents an energetically demanding period of animal's life history (Rysava et al., 2016; Vailati-Riboni et al., 2016). By understanding diet partitioning and interaction of wildebeest and zebra, we can further explain the feeding ecology of these two dominant ungulate species of the Serengeti ecosystem and demonstrate how seasonal availability of resources helps to shape the functioning of the ecosystem.

## 5.3 Materials & Methods

### 5.3.1 Study area and study species

The study was located in the Serengeti-Mara ecosystem, in the southern part of Kenya and the northern part of Tanzania (Figure 5-1). The area is endowed with diversity rich habitat types comprising a mixture of both $C_3$ and $C_4$ plants (Reed et al., 2009), leading to highly variable stable carbon ($\delta^{13}C$) isotope ratios across the landscape. Grassland is the most abundant land cover, comprising more than 60% of the total land cover of the ecosystem (Anderson, 2008).

The ecosystem is home to more than 27 species of ungulates including migrating populations of blue wildebeest and plain zebra (Mcnaughton, 1985; Sinclair, 1995). Wildebeest are the most abundant and zebras are the second most abundant ungulates in the Serengeti (Hopcraft, 2015). Wildebeest are ruminants, getting more than 90% of their diet from $C_4$ grasses (Cerling et al., 1997) and the zebras are hindgut fermenters, obtaining approximately 92% of their diet from grass and 8% from dwarf shrubs and herbs (Furstenburg, 2009; Penzhorn, 2013).

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of ecological origin and it is therefore a suitable descriptor of ecological information regarding the individuals, populations, or communities they represent (Jackson et al., 2011).

Reproductive maturity as well as the timing for lactation between the two species differ. For example, a female wildebeest reach sexual maturity at two to three years of age and the gestation period for wildebeest lasts for ~240 days (8 months) (Hopcraft, 2015), while a female zebra may reach sexual maturity three years after they are born and their gestation period lasts for 11 months (Grange et al., 2004). Wildebeest exhibit a synchronized calving (Appendix D-1), as an adaptive strategy to enhance population recruitment (Hopcraft, 2015), but zebras do not. Thus zebra foals are born throughout the year (Furstenburg, 2009).

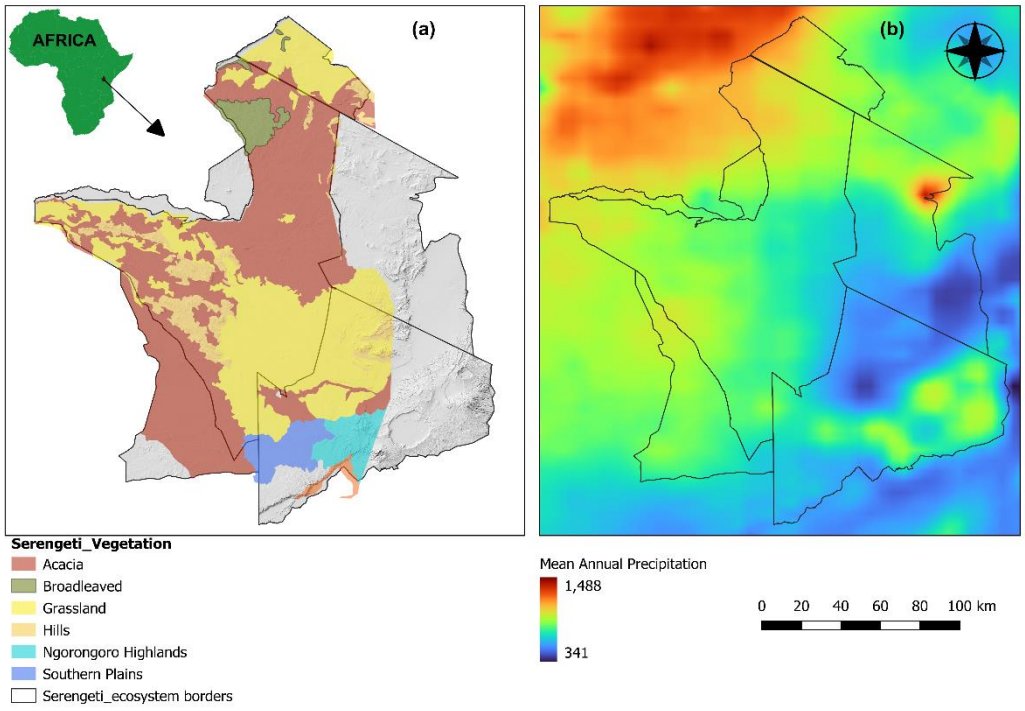



Figure 5-1. The Serengeti ecosystem in black solid lines showing a) different vegetation habitats across the ecosystem (Data from Serengeti data repository: (https://serengetidata.weebly.com/data.html) and b) showing the gradient of precipitation levels (Precipitation data from CHIRPS repository: https://www.chc.ucsb.edu/data/chirps).

### 5.3.2 Sample collection

We collected a total of 54 tail hair samples from both wildebeest (n=41) and zebra (n=13) between 2011 and 2019. Out of our 41 wildebeest tail hair samples, 14 of them were from lactating females and out of 13 zebra samples, 5 of them were

from lactating females (Appendix D-2). The samples were collected from a mixture of both live wildebeest and zebra that were immobilized for GPS collaring as well as unknown carcasses. Tail hair samples collected from GPS collared individuals were obtained by shaving the right side of the tail to a skin level of an immobilized animal. Tail hair samples collected from carcasses were obtained by pulling the hair which included the root. For every tail hair sample collected, we recorded the following additional information; date, animal ID, collar ID (for live animals), age, reproductive status (i.e. either lactating or non-lactating), and a possible cause of death (for carcass). We used the presence of a calf or a foal as evidence of a lactating female. For animals who might have recently lost their offspring, we checked their mammary glands. All tail hair samples were tied together at the proximal end to standardize time zero and stored in a paper envelop for laboratory analysis.

To collect vegetation samples, we first identified species composition in the diets of wildebeest and zebra using metabarcoding data from fecal samples (Pansu et al., 2022). The metabarcoding data suggested the diets of wildebeest and zebra to be composed of five different plant types, namely; $C_4$-grasses, legumes from family Fabaceae (*Acacia* species and Indigofera species), Commelina species, and 'other' $C_3$-plants (Pansu et al., 2022). We collected a total of 201 vegetation samples from 163 sites located within the ecosystem's boundaries and across the surrounding villages. Of these, 116 were of $C_4$-grasses, 22 of *Acacia* seedlings, 22 Commelina species, 22 Indigofera and 19 mixed forbs. To collect grass samples, a 4 x 4m plot was laid out and five sub-samples (one from each corner and one at the middle) within a 25 x 25 cm quadrat were clipped to ground level and pooled together to make one single grass sample from each site. To collect $C_3$ plants samples, we used the same 4 x 4m plot and clipped specific plant species belonging to the genera of Commelina, Acacia, and Indigofera within the plot and in its immediate vicinity to ensure we had sufficient sample size. To account for the contribution of the 'other $C_3$-plants' in wildebeest and zebra diets, we collected a 'mixed forb' sample, which comprised a mixture of all dominant $C_3$ plant species within the 4 x 4m plot and in the immediate vicinity, exclusive of the other four that we had already identified. For each vegetation sample collected, we recorded GPS location, date and time of collection as well as the plant species collected. All vegetation samples were kept in paper envelopes and

air dried at room temperature to prevent microbial activities and fungi development

### 5.3.3 Sample preparation for stable isotope analysis

Tail hair samples were washed for 10 minutes in 2:1 chloroform methanol solution to remove all the impurities and rinsed in double distilled water to remove the remnants of the chloroform solution. Samples were dried for 48 hours at room temperature and sectioned into 8mm segments, corresponding to ~16 days of growth for the wildebeest (based on a wildebeest tail hair growth rate of 0.511 mm per day estimated by (Kabalika et al., under review)), and 11 days for the zebra (based on mean growth rate of domestic horse of 0.72 mm per day estimated by averaging growth rate from different published studies[3]. Tail hair growth rate was assumed to be constant as suggested by several studies (e.g. Ayliffe et al., 2004; Burnik Šturm et al., 2015; Dunnett & Lees, 2003; Schlupp et al., 2004; West et al., 2004). To acquire time-series of diet history, hairs were sectioned in order from the most recent to the oldest sections. The clipped hair was placed in steel grinding tubes, dipped in liquid nitrogen ($LN_2$ ) for 60 seconds to embrittle the hair, and ground to a powder using a Retch MM400 ball mill for 90 seconds at 600 rpm. Powdered samples were weighed to 1 to 1.3mg using a Mettler Toledo MX5 microbalance with a readability of 0.001 mg.

Vegetation samples were prepared by first removing all unwanted materials (e.g. soil, dust or unidentified plant species) and then oven warmed at $40^0$C for 3 hours. This was to harden them for easy grinding. The dried and embrittled plants were then powdered using the same Retch MM400 ball mill for 90 seconds at 600 rpm and weighed into tin capsules (2.0 to 2.3 mg each) using the same mini-balance as above, ready for laboratory isotope analysis.

[3] Table 5-0: Tail hair growth rate of a domestic horse obtained from the literature

| Growth rate (mm/day) | Sample size | Reference |
|---|---|---|
| 0.711 | 2 | (Ayliffe et al., 2004) |
| 0.814 | 8 | (Schlupp et al., 2004) |
| 0.72 | 6 | (West et al., 2004) |
| 0.79 | 5 | (Dunnett & Lees, 2003) |
| 0.57 | 1 | (Burnik Šturm et al., 2015) |
| **Mean ± SD** | **0.72 ± 0.094** | |

### 5.3.4 Laboratory analysis for stable isotope ratios

Samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ at the Scottish Universities Environmental Research Centre (SUERC) Stable Isotope Ecology Laboratory. Two systems were used, which were cross-calibrated using the international reference USGS40 (l-glutamic acid; (Coplen et al., 2006; Qi et al., 2003). The first was a Pyrocube elemental analyser (Elementar Analysensysteme) coupled to a VisION isotope ratio mass spectrometer (Elementar); this was optimised for $\delta^{34}S$ measurements (Kabalika et al., 2020). The second system was an identical elemental analyser interfaced with a Delta XP Plus (ThermoFisher Scientific); the second system was measuring C and N isotope ratios only. Internal laboratory standards were thus slightly different (though USGS40 was a component of both types of measurements as an independent check on accuracy and additionally used to track N and C concentration). For the VisION, these standards were methanesulfonamide/Gelatine(MSAG2), methionine/alanine/glycine/gelatine(M2) and sulfanilamide/ alanine/ gelatine (SAAG2) were repeated after every 10 samples and were used to correct for linearity and instrument drift over a 72-h analytical run (Kabalika et al., 2020; Werner & Brand, 2001). For the Delta, internal laboratory standards of GEL (gelatine), ALAGEL (alanine-gelatine spiked with 13C-enriched alanine) and GLYGEL (glycine-gelatine spiked with 15N-enriched alanine) were run every ten samples, and a suite of GELs of different sizes were used to correct samples for linearity and drift over a 22-hour run. GEL is a gelatin solution, ALAGEL an alanine-gelatine solution, and GLYGEL a glycine-gelatine solution. All data are reported in δ notation (McKinney et al., 1950) with respect to the international standards of AIR for $\delta^{15}N$ and V-PDB for $\delta^{13}C$. The analytical precision for nitrogen and carbon isotopes were 0.08‰ and 0.03‰ respectively.

### 5.3.5 Statistical analysis

All statistical analyses were performed in R software version 4.1.2 (R-Core-Team, 2022). To test if the values of $\delta^{13}C$ and $\delta^{15}N$ for wildebeest and zebra were significantly different, we used a two-sample unpaired t-test. To understand and visualise dietary preferences and percentage composition of $C_3$ versus $C_4$ plants for both wildebeest and zebra across length of their tail hairs, Stable Isotopes Mixing Models (SIMMS) were used with the package SIMMR in R. Our SIMMR model

was fitted with two bio tracers of $\delta^{13}C$ and $\delta^{15}N$ and two categorical variables of wildebeest and zebra. The trophic enrichment factor (TEF) for $\delta^{13}C$ and $\delta^{15}N$ in wildebeest tail hair was estimated from the SIDER package using the values derived from domestic cattle. TEF for zebra was adopted from that of domestic horse from the published literature (Sponheimer et al., 2003a; Sponheimer et al., 2003b). The TEF values were passed onto the SIMMR model as a correction for the isotope values in the tail hairs. We used uninformative standard priors of mean 0 and standard deviation 1 to model dietary composition for both wildebeest and zebra. The SIMMR model was run with four MCMC chains for 10,000 iterations with 1000 of them as burn-in. The chains were thinned by 2, to ensure posterior samples were independent. The model was checked for convergence using Gelman diagnostics of <= 1.1 for all parameters.

To estimate isotopic niche width and niche overlap across different months for wildebeest and zebra, we used a Bayesian approach based on bivariate, ellipse-based metrics for the values of $\delta^{13}C$ and $\delta^{15}N$ using SIBER package (Jackson et al., 2011; Parnell et al., 2013). Specifically, the SIBER package was used to quantify isotopic niche width for each month for both wildebeest and zebra as well as the amount of overlap between the two species for each month. The package was also used to quantify the variation in niche sizes during the energetically demanding period of lactation. The isotopic niche sizes are presented as the Standard Bayesian Ellipse Area (SEAB) in permil squared ($‰^2$) (Jackson et al., 2011). We used uninformative standard priors (i.e. the recommended default SIBER parameters and priors) (Jackson et al., 2011) to model the isotopic niche widths for both wildebeest and zebra. Our SIBER model was run with three chains for 50,000 iterations with 1000 of them as burn-in. The model was thinned by 2 to ensure posterior samples were independent. The model was checked for convergence using Gelman diagnostics of <= 1.1 for all parameters.

## 5.4 Results

### 5.4.1 Relative composition of $C_3$ and $C_4$ plants in the diet of wildebeest and zebra

Results from the interspecific comparison indicated that the means for $\delta^{13}C$ between wildebeest and zebra were not significantly different (wildebeest: mean

= -10.765 ± 1.080, zebra: mean = -10.824 ± 0.493) (t = 1.641, p-value = 0.101). On the other hand, wildebeest had significantly higher values of $\delta^{15}N$ (mean = 7.212 ± 1.009) than the zebras (mean 5.861 ± 0.782) (t = 24.64, p-value = 2.2e-16).

Results from the SIMMR package suggest that $C_4$ grass contributes an average of 96% of the total diet for both wildebeest (mean = 0.963, 0.958 - 0.969, 97.5% CI) and zebra (mean = 0.954, 0.943 - 0.965, 97.5% CI), indicating that only 4% of their diet comes from $C_3$ sources (Figure 5-2 Figure 5-3). The upper credible interval (i.e. 97% CI) for *Acacia* suggests that it contributes less than 2% of total diet for wildebeest (mean = 0.007, 0.001 - 0.018, 97.5% CI) and a maximum of 3% for zebra (mean = 0.014, 0.002 - 0.032, 97.5% CI). *Commelina* contributes less than 2% of total diet for both wildebeest (mean = 0.005, 0.001 - 0.013, 97.5% CI) and zebra (mean = 0.005, 0.001 - 0.01, 97.5% CI). *Indigofera* contributes a maximum of 2% of the total diet for wildebeest (0.012, 0.002 - 0.024, 97.5% CI) and no more than 4% for zebra (0.018, 0.004 - 0.039, 97.5% CI). Mixed forbs contribute a maximum of 2% of total diet for both wildebeest (0.012, 0.002 - 0.025, 97.5% CI) and zebra (0.01, 0.002 - 0.024, 97.5% CI) (Figure 5-3).

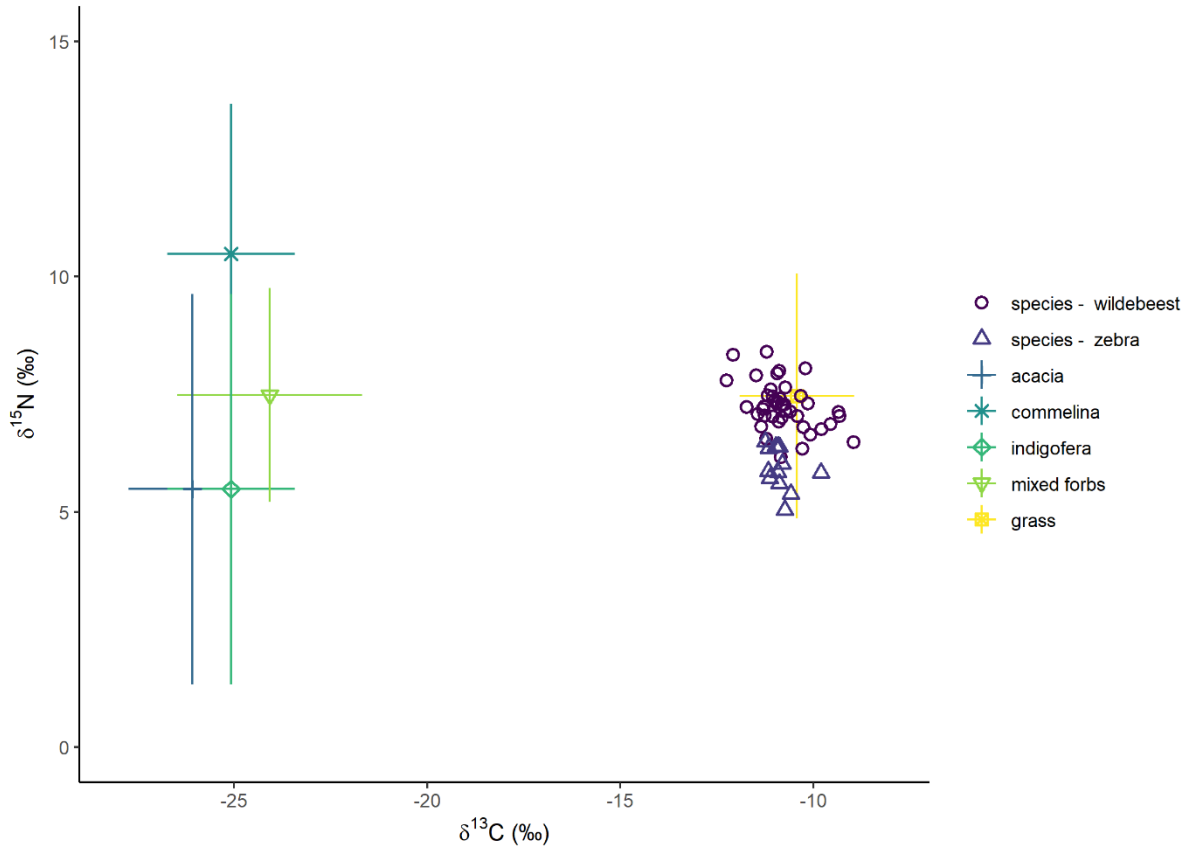


Figure 5-2: Isotopic signature of $C_3$ (*Acacia, Commelina, Indigofera* and mixed forbs) and $C_4$ plants (grasses) from the Serengeti ecosystem and the estimated dietary composition for wildebeest and zebra. Wildebeest tend to have more positive values for $\delta^{15}N$ as values of $\delta^{13}C$ become more negative (ie bending towards upper left), which suggests that the wildebeest might be supplementing their diet with the other $C_3$ sources especially when they are facing starvation. Conversely, $\delta^{13}C$ and $\delta^{15}N$ values for zebras rarely diverge, which suggests that they might be relying almost solely on the $C_4$ grass for their diet. Note that the $\delta^{13}C$ and $\delta^{15}N$ values for wildebeest and zebras presented on this plot are the calculated means for each animal (i.e. n=54) and not the for the individual segment of each tail hair. This was done to enable clear visualization of the plot.

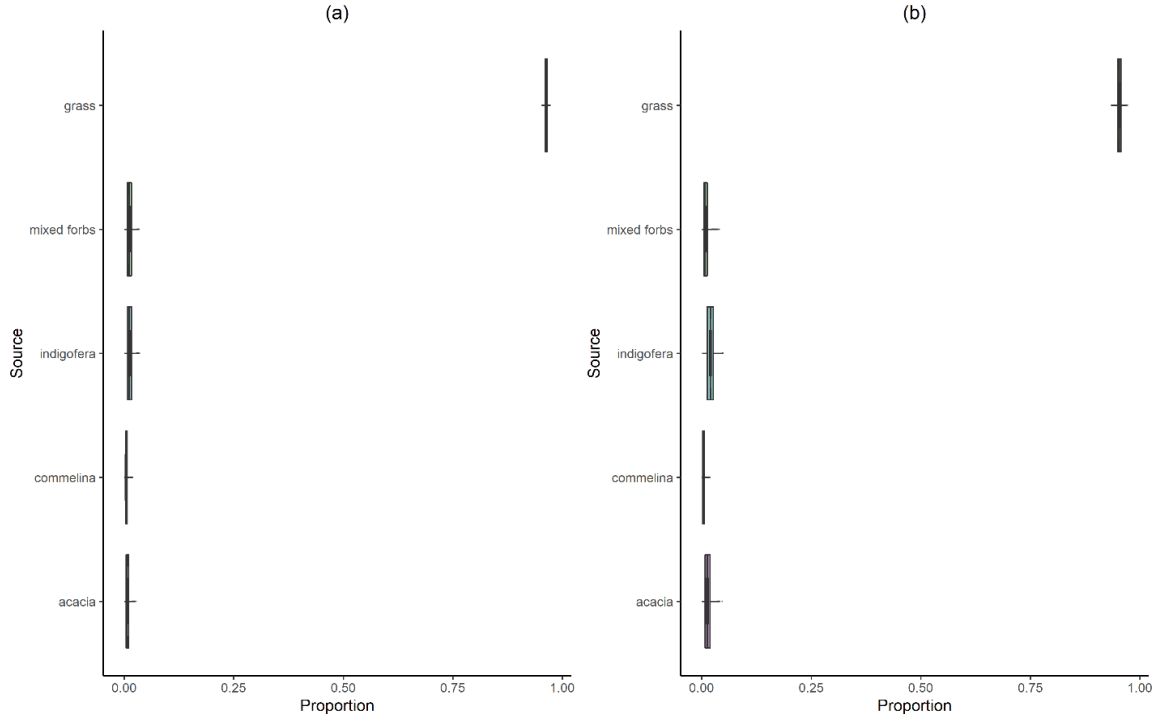



Figure 5-3: A proportional representation of diet contribution from different plant species (i.e. *Acacia* species, *Commelina* species, *Indigofera* species, mixed forbs and grass) for (a) wildebeest and (b) zebra across all months as returned by our SIMMR model, suggesting that the diet for both species is predominantly $C_4$ grass.

### 5.4.2 Seasonal variation in dietary isotopic niches between wildebeest and zebra

Results from SIBER suggested that wildebeest and zebra have different dietary isotopic niche widths. Generally wildebeest have larger niche width than zebras.

Metrics for wildebeest suggest the convex hull area (TA) is 33.66 ‰$^2$ and the Standard Bayesian Ellipse Area (corrected for small sample size) (SEAB) is 2.85 ‰$^2$ (2.70 - 3.00, 95% CI). Metrics for zebra suggest the TA is 6.66‰$^2$, and the SEAB is 1.04 ‰$^2$ (0.93 - 1.16, 95% CI). The difference in the dietary isotopic niche widths between the two species (i.e. wildebeest having larger niche than zebra) is consistent for both wet and dry seasons. The metrics for wildebeest during wet season are as follows: TA is 30.73‰$^2$ and SEAB is 3.03 ‰$^2$ (2.82 - 3.25, 95% CI). The metrics for zebras during wet season are as follows: TA is 4.64 ‰$^2$ and SEAB is 0.77 ‰$^2$ (0.66 – 0.88, 95% CI). The metrics for wildebeest during dry season are as follows: TA is 21.12‰$^2$ and SEAB is 2.45 ‰$^2$ (2.29 - 2.69, 95% CI). The metrics for zebras during dry season are as follows: TA 6.59 ‰$^2$ and SEAB = 1.33‰$^2$, (1.12 - 1.33, 95% CI).

Our results further suggest wildebeest have the biggest SEAB in February, March and June (mean = 3.327‰$^2$ ± 0.182), medium SEAB in January, April, May, July and August (mean = 2.693‰$^2$ ± 0.271) and the smallest SEAB in September through December (mean = 1.814‰$^2$ ± 0.062) (Table 5-1; Figure 5-4). For zebras, the biggest SEAB is observed in June through September (mean = 1.378 ‰$^2$ ± 0.268), medium SEAB in February, March, April, October and November (mean = 0. 805 ‰$^2$ ± 0.091) and the smallest SEAB in January, May and December (mean = 0.493 ± 0.056) (Table 5-1; Figure 5-4). Generally, an average niche overlap between wildebeest and zebras is 19.19%. The highest niche overlap between the two species is 30.633%, which is observed in August and the lowest is only 9.009% observed in May (Table 5-1;Figure 5-4).

Table 5-1: Ellipse area sizes for wildebeest and zebra in permille squared (‰$^2$) across different months of the year and their associated amount of overlap.

| | Ellipse area size (SEAB) in ‰$^2$ | | |
|---|---|---|---|
| Month | Wildebeest | Zebra | % overlap |
| Jan | 2.817 | 0.414 | 10.104 |
| Feb | 3.503 | 0.677 | 12.538 |
| Mar | 3.401 | 0.741 | 11.512 |
| Apr | 2.796 | 0.842 | 13.737 |
| May | 2.863 | 0.527 | 9.009 |
| June | 3.076 | 1.426 | 24.597 |
| Jul | 2.834 | 1.644 | 25.362 |
| Aug | 2.153 | 1.434 | 30.633 |
| Sept | 1.739 | 1.005 | 22.464 |
| Oct | 1.881 | 0.942 | 19.008 |
| Nov | 1.764 | 0.824 | 24.746 |
| Dec | 1.870 | 0.538 | 18.553 |

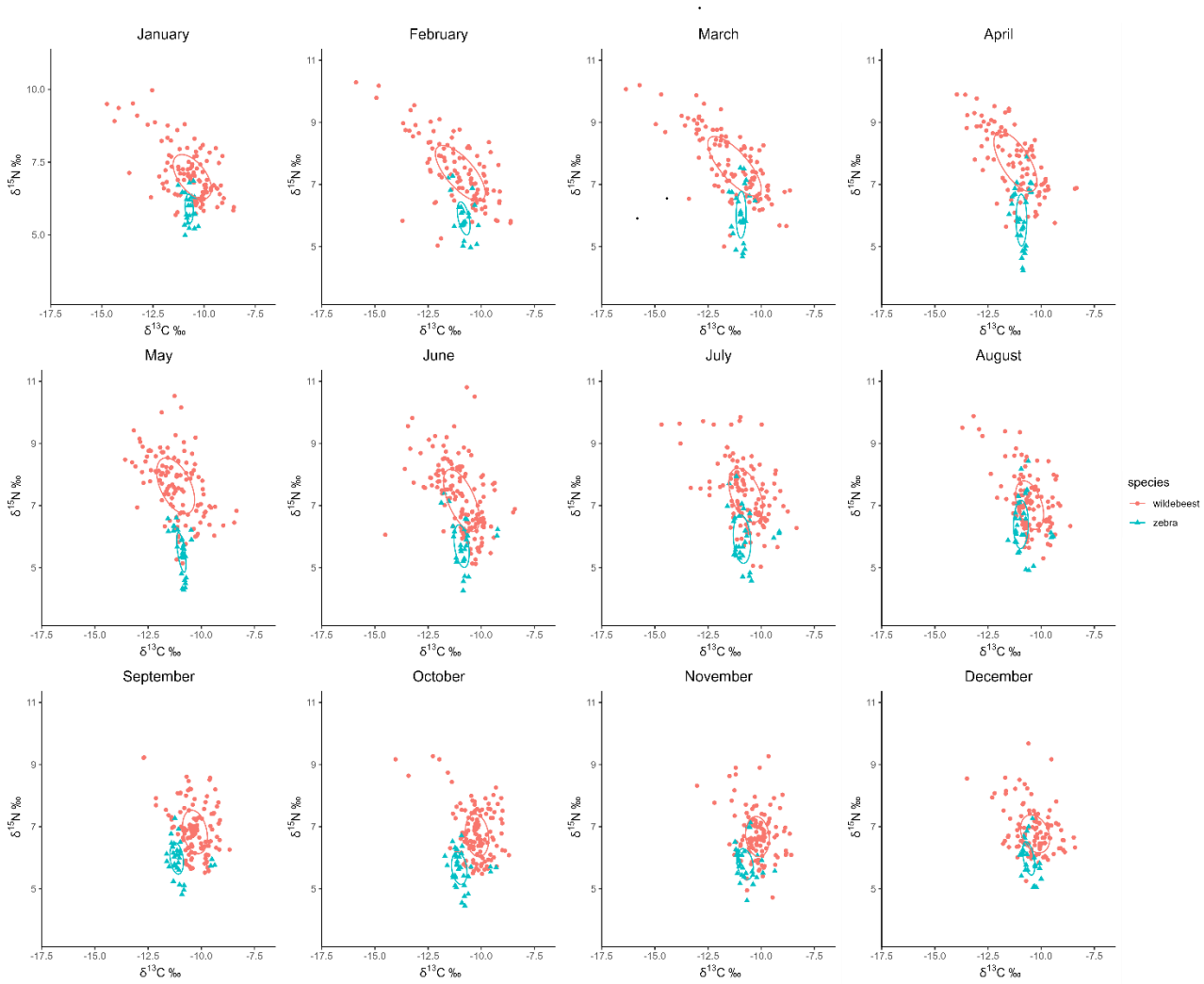



Figure 5-4: The area of the ellipse showing an isotopic niche overlap between wildebeest and zebra across different months, suggesting that the highest overlap is in August and the lowest in May. December to May represent rain season while June to November represent dry season in the Serengeti.

### 5.4.3 Dietary isotopic niche width during lactation

Our SIBER model for comparing niche widths between lactating and non-lactating females for both wildebeest and zebra suggested lactating females have smaller niche width (SEAB = 2.815‰$^2$, 2.607 - 3.028, 95%CI) than non-lactating females (SEAB= 3.081‰$^2$, 2.891 - 3.272, 95%CI). However, when niche sizes were compared independently within species, results were different. The lactating female wildebeest had slightly larger niche width (SEAB = 2.816‰$^2$, 2.569 - 3.066, 95% CI) than the non-lactating females (SEAB = 2.726‰$^2$, 2.541 - 2.913, 95% CI). Similarly, the lactating female zebra had larger niche width (SEAB = 1.172‰$^2$, 0.983 - 1.362,

95% CI) than the non-lactating females (SEAB= 0.752‰$^2$,0.644 - 0.862, 95% CI; Figure 5-5).

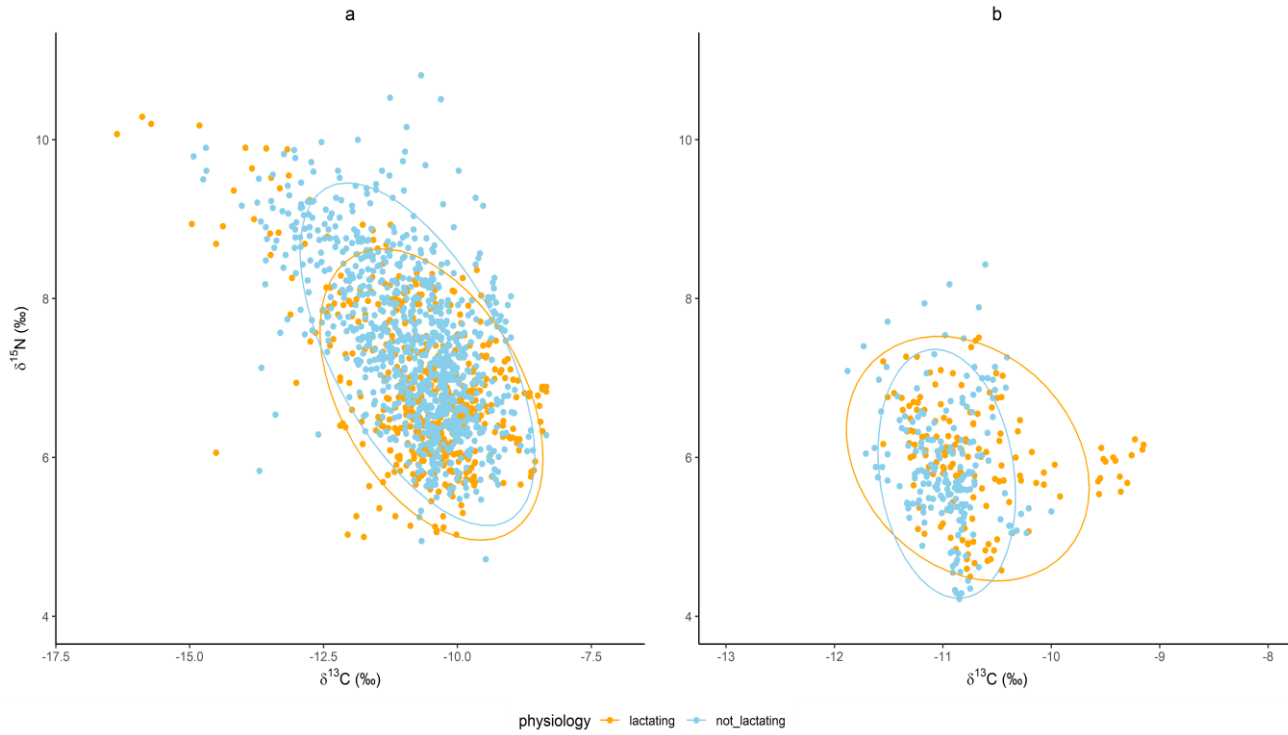



Figure 5-5: Standard Bayesian Ellipse Area(SEAB) for lactating (orange colour) and non-lactating females (light-blue colour) for (a) wildebeest and (b) zebra, showing that lactating females have slightly lower SEAB than non-lactating females which is an artefact of small sample size we have for the lactating females. There are twice as many samples for lactating females than non-lactating females (lactating, n=19; non-lactating, n=35). For the female wildebeest only (lactating, n=14; non-lactating, n=27).

## 5.5 Discussion

The main finding in this study suggests that wildebeest and zebra have very similar diet in general, in which we have identified that more than 96% of diet for both wildebeest and zebra from the Serengeti ecosystem is comprised of $C_4$-grass. The values of $\delta^{13}C$ are similar between the two species, but wildebeest have higher values of $\delta^{15}N$ than zebra. Although we have identified that wildebeest have consistently larger isotopic niche widths than zebra during both wet and dry seasons, the isotopic niche widths between the two species vary across different months of the year. For example, the largest isotopic niche widths for the wildebeest are observed in in February, March and June and for the zebras in June

through September, suggesting that the diet of wildebeest is most diverse in the wet season, while that of zebra is most diverse in the dry season. An overall isotopic niche overlap between wildebeest and zebra is only 19%, but it varies across months with the largest overlap of ~31% observed in August, and the lowest of only 9% observed in May. Furthermore, we have identified that isotopic niche widths are different between lactating and non-lactating females. For instance, we have observed a minimal difference in isotopic niche widths between the lactating and non-lactating female wildebeest, in which the lactating females have slightly larger isotopic niche widths than non-lactating females. But we have observed a clear difference in isotopic niche widths between the lactating and non-lactating females of zebra, such that the lactating females have larger isotopic niche widths than non-lactating females. This suggests that animals become less selective during periods of high physiological demand such as lactation and may supplement their diet with $C_3$ forbs.

The similarity in the values of $\delta^{13}C$ between wildebeest and zebra suggests a shared resource use between the two species (Tamburin et al., 2019), while high values of $\delta^{15}N$ for the wildebeest could be related to either diet quality (Robbins et al., 2005) or physiological and nutritional demands (Hobson et al., 1993; Rysava et al., 2016). The strong $\delta^{13}C$ similarity between wildebeest and zebra observed in this study has also been reported in similar studies in other African savannahs (e.g. Codron et al., 2005). Studies suggest that migrant zebra are closely associated with wildebeest especially during the wet season (Sinclair, 1985). Zebras are also less sensitive to resource limitations such as water availability (Ogutu & Owen-smith, 2003) or diet quality (Redfern et al., 2003). However, their abundance in Serengeti is likely limited by predation pressure (Sinclair, 1985), probably necessitating zebras to stay together with wildebeest as an anti-predatory strategy. Furthermore, wildebeest and zebras are known to be pure or near pure grazers (Owaga, 1975; Owen-smith, 1993), and they both prefer grassland habitats (Mcnaughton & Georgiadis, 1986; Sinclair, 1985). Therefore, similar habitat preferences as well as close association between the two species could be pushing them towards a common resource, causing the similarity of $\delta^{13}C$ values we observe.

The large isotopic niche width observed in wildebeest during both wet and dry seasons suggests that the wildebeest utilize a wider range of food resources compared to zebra. Both wildebeest and zebra have been reported to occupy

similar habitat types of woodlands, open-grasslands and semi-desert environments (Fischhoff et al., 2007; Skinner & Chimimba, 2005) which provide access to different food resources to both species. However, the digestive system of wildebeest (i.e. rumen) which is more efficient at resource absorption than the zebras' hindgut (Illius & Gordon, 1992; Mckie et al., 2004), could be influencing the broader niche we observe in wildebeest. Furthermore, wildebeest have been reported to supplement their diet by browsing on nutrient rich $C_3$ plants when under high physiological demand or limited grass availability (Zyl, 1965).

The largest isotopic niche width for the wildebeest observed in February and March (Table 5-1) could be associated with ecological opportunity (Shaner & Ke, 2022) as a result of high food abundance in Serengeti during this time (Sinclair et al., 2000), while the highest niche width in June coincides with the limited food availability in the Serengeti (Sinclair et al., 2000), suggesting that wildebeest might be diversifying their diet during this time. The niche expansion for the wildebeest in February and March could also be related to an increased density of wildebeest after calving (Hopcraft, 2015; Sinclair et al., 2000) making them less selective in their dietary choices (Fretwell & Lucas, 1970). The largest isotopic niche width in zebra is observed in June through September, which is the core dry season for the Serengeti, supports the fact that zebras are bulky eaters (Grange et al., 2004; Petersen, 1972).

Smaller isotopic niche width observed in lactating females than in non-lactating females when both species combined (refer to result section) is an artefact caused by small sample size we have for the lactating females (lactating, n=19; non-lactating, n=35). During lactation, females experience high physiological and metabolic demands (Vailati-Riboni et al., 2016), forcing an animal to mobilize internal reserves which elevates their $\delta^{15}N$ (Rysava et al., 2016). After separating lactating females by species and assessing the isotopic niche sizes for each species separately, lactating females from each species had larger isotopic niche width than non-lactating females. Although the effect of small sample size was still evident in lactating wildebeest (i.e. lactating=14 versus non-lactating=27), yet their isotopic niche width was slightly larger compared to non-lactating.

The overall amount of isotopic niche overlap between wildebeest and zebra reported in this study is minimal, suggesting high resource partitioning between the two species. However, the highest amount of isotopic niche overlap between

the two species of ~31% observed in August might be an indication that in extreme resource limitation conditions, these species are towards the same resource. For example, August in the Serengeti is characterized by little or no rainfall, high temperature and low resource availability (Holdo et al., 2009; Sinclair et al., 2000). Nevertheless, the trend of high isotopic niche overlap between the two species is consistent over the entire dry season (see Table *5-1*).

## 5.6 Conclusion and recommendations

This study has demonstrated the patterns of feeding ecology of wildebeest and zebra across the Serengeti ecosystem using stable isotope technique. Similarity of the isotopic patterns and isotopic dietary niches indicate a shared resource use between the two species. Following this study, an interesting aspect to explore would be to apply metabarcoding analysis to either fecal samples or gut content for the two species to get a clear understanding of how different or similar their diets are in terms of plant species preferences. Also, an additional element like oxygen ($\delta^{18}O$) and hydrogen ($\delta^{2}H$) which vary by different water sources (Bowen et al., 2005), can help to give us a better understanding of their diet partitioning. This study has also identified that the high niche overlap between wildebeest and zebra is observed in August, which is the period of dry season and high tourism activities in the Serengeti. Future studies might want to explore how tourism activities influence wildebeest's and zebras' feeding ecology, which can help to shed some light into proper planning and implementing of tourism activities in such a way that they do not compromise welfare of all wildlife.

# Chapter 6: General discussion and conclusions

## 6.1 Future perspectives of movement research using stable isotope analysis technique

Stable isotope ratio analysis has demonstrated considerable potential in different animal movement studies for both marine and terrestrial habitats (Clegg et al., 2003; Haché et al., 2014; Hénaux et al., 2011; Hobson, 1990, 2008; Hobson, 1999; Rubenstein & Hobson, 2004; Trueman et al., 2012; Trueman & Glew, 2019). Specifically, the use of stable carbon (Cerling et al., 2006, 2009; Janzen et al., 2020), oxygen (Hobson & Koehler, 2015), sulfur (Sayle et al., 2013; Zazzo et al., 2011) and strontium (Britton et al., 2009; Evans et al., 2019) isotope ratios have been commonly used in this respect. Carbon has been used as a tracer of diet and movement over time and space due to the fact that the values of $\delta^{13}C$ are consistently different between marine and freshwater primary producers (Mizutani et al., 1990; Newton & Bottrell, 2007); and in terrestrial ecosystems, $\delta^{13}C$ varies across photosynthetic pathways (Philp, 2007; Smith & Epstein, 1971). This has made $\delta^{13}C$ useful in differentiating animal movements across distinct plant communities (i.e. $C_3$ versus $C_4$) (Ambrose & Sikes, 1991). Stable nitrogen isotopes ($\delta^{15}N$) are primarily used to assess the position of organisms in food chains (Codron & Brink, 2007; Deniro & Epstein, 1981; Herrera et al., 2003), because the value of $\delta^{15}N$ increases at higher trophic levels (O'Reilly et al., 2002). However, recently $\delta^{15}N$ has also been applied in movement studies, for example, to estimate time since migration in marine organisms (Shipley et al., 2021). $\delta^{15}N$ is generally considered a poor indicator of animal movement due to the fact that nitrogen is a building block of protein, which means that protein catabolism in the body might influence the $\delta^{15}N$ values (Hobson & Wassenaar, 2008).

Generally, sulfur is considered a good geolocator of animal movement for both terrestrial and marine habitats because $\delta^{34}S$ is consistently different between marine, freshwater and terrestrial ecosystems (Nehlich et al., 2011; Sayle et al., 2013; Thode, 1991) and the values of $\delta^{34}S$ reflect those of their local geology (Coplen et al., 2002; Tsuji, 1980). This has enabled ecologists to differentiate movement and diet across marine versus terrestrial habitats (Zazzo et al., 2011) as well as across different geological ranges of parent materials (Krouse, 1988, 1991; Krouse & Herbert, 1998). The findings from chapter two of this thesis, which

are consistent with previous studies (e.g. Fry & Chumchal, 2011; Zazzo et al., 2011), contribute greatly to Tanzanian ecological research by providing baseline information on the applicability of $\delta^{34}S$ to study animal movement across the Serengeti ecosystem. Although sulfur has been used to study life histories of marine organisms (Weber et al., 2002), the findings from chapter four of this thesis have also demonstrated that the same concept is applicable in terrestrial ecosystems. For example, using the temporal variation of $\delta^{34}S$ in tail hairs, I have been able to distinguish resident from migrant wildebeest as described in chapter four. The idea of using tail hairs, which is a less invasive way of recreating time-series information of an animal's history, provides a more sustainable approach for making a population-wide inference of animal movements and their migratory patterns.

## 6.2 Using $\delta^{34}S$ to geolocate migratory animals

Most applications of $\delta^{34}S$ have focused on archaeological materials (Britton et al., 2016; Nehlich et al., 2011; Richards et al., 2001, 2003; Sayle et al., 2013) to study either movement patterns (Richards et al., 2001; Sayle et al., 2013) or dietary preferences (Britton et al., 2016; Nehlich et al., 2011; Richards et al., 2001). These materials are mostly bones, teeth or hairs (Richards et al., 2001; Sayle et al., 2013; Schwertl et al., 2003), and they have retained their dietary information for a long period of time. Therefore, it has always been assumed that the $\delta^{34}S$ values from these materials are at equilibrium with the $\delta^{34}S$ values from their environment (i.e. diet), and they map simply straight on to time. However, the findings from chapter three of my thesis indicate that there is a delay between diet ingestion and isotope reflection. For example, I have explored the underlying mechanisms for sulfur metabolism and established a $\delta^{34}S$ turnover rate for the wildebeest tail hair to be an average of 78 days, which is consistent with previous studies (e.g. Bahar et al., 2009). This suggests that one should exercise caution while applying $\delta^{34}S$ to geolocate a live-migratory animal. This information is helpful for improving the accuracy of extracting and interpreting high resolution temporal records of dietary isotope variation from continuously growing tissues like hair for wildebeest and other ungulates of similar sizes. Studies suggest that the quality of the diet that an animal consumes affects the isotopic turnover rate, such that when an animal consumes a diet with low protein content (e.g. $C_4$ grass)

the isotopic signatures of the diet takes longer to be reflected in the tissues than when an animal consumes a diet with high protein content (e.g. $C_3$ grass) (Robbins et al., 2005). Therefore, this tells us that applying $\delta^{34}S$ to geolocate mobile animals might be less straight forward than other stable isotope mixing models (e.g. Jackson et al., 2011; Parnell et al., 2013; Stock et al., 2018) suggest.

## 6.3 The role of stable isotope ratios in the management of wildlife species

Stable isotope ratios play a significant role in the management of wildlife species. They have been used to delineate important information on the diet (Codron et al., 2005; Codron & Brink, 2007; Hobson et al., 1997; Hopkins & Ferguson, 2012; Tamburin et al., 2019), migration patterns (Garcia-Perez & Hobson, 2014; Rubenstein & Hobson, 2004) and habitat use of different wildlife species (Dale et al., 2011; Fry & Chumchal, 2011). By analyzing stable isotope ratios in animal tissues such as hair, feathers, and bone, researchers can gain insights into the animal's trophic level, life history strategies, food preferences, and the types of habitats they occupy. This information can then be used to inform conservation efforts and develop effective management strategies for wildlife populations. For example, Jackson et al (2011) demonstrate how stable isotope analysis has undergone a technological and mathematical revolution in the last 20 years and can now be used to quantify niche dynamics and interactions among populations and communities (Jackson et al., 2011). Chapter five of my thesis has explored the feeding ecology and niche dynamics for wildebeest and zebra using stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). One of the interesting findings from this chapter is that the highest amount of niche overlap between the two species is suggested to be in August and the lowest in May. This time of high overlap (i.e. August), is characterized by a low rainfall, high temperatures and low resource availability across the Serengeti (Holdo et al., 2009; Sinclair et al., 2000). From these findings, we might deduce that the feeding ecology of wildebeest and zebra across the Serengeti might be influenced either by the availability of food supply and access to their preferred grazing plant species, or by the tourism activities. Therefore, this implies that we can rely on stable isotope

ratio analysis of animals' tissues as a window on hidden processes caused by ecological changes or other external pressures.

Furthermore, the ability to utilize tail hairs to differentiate migrant from resident life history forms as well as the continuous time series information of diet partitioning that has been reported in this thesis, demonstrate that we can rely on stable isotope technique as means to understand past historical events that have been happening across systems using animal tissues. For example, we can create diet or movement history from dead animals using archived samples to get a better understanding of what happened in the past, which can help us in designing proper restoration programs or in the control of illegal wildlife trade.

## 6.4 Conclusion

Application of isotopic methods in ecology is not new (Cerling et al., 1997; Hobson, 1999; Peterson & Fry, 1987; Peterson et al., 1985; Rubenstein & Hobson, 2004; West et al., 2006). They have been applied in different aspects of ecology including animal migration (Hénaux et al., 2011; Hobson, 1999; Hobson, 1990; Hobson & Wassenaar, 2008), trophic level and niche interactions (Gannes et al., 1997; Jackson et al., 2011; Middelburg, 2014; Parnell et al., 2013; Stock et al., 2018; Trueman et al., 2017), niche partitioning (Burnik Šturm et al., 2017; Codron et al., 2005; Codron et al., 2011; Hopkins & Ferguson, 2012), biological invasion (McCue et al., 2020), and historical connectivity (Cerling et al., 2009; Coutu et al., 2016). While all these have been tested across different taxa and different places across the globe, there is limited information on the applicability of isotopic methods to understand the ecology of migratory animals such as wildebeest in East Africa. My PhD research demonstrates the potential for applying isotopic methods to understand movement and diet of migratory ungulates in Tanzania. Based on both laboratory experiments and statistical analyses, it can be concluded that we can apply variation of stable isotope ratios in tail hair as a tool for exploring movement patterns and feeding ecology of migratory ungulates. For example, I have established a sulfur isoscape for the Serengeti ecosystem for the first time, and used it to illustrate the scenarios in which the $\delta^{34}S$ in tail hairs might be used as a tool for animal geolocation as well as to differentiate between local versus non-local livestock. Furthermore, I have used the created $\delta^{34}S$ isoscape to illustrate that we can use the variation of $\delta^{34}S$ in the tail to

differentiate resident versus migrant life-history forms of wildebeest. In addition, through a series of mathematical models I was able to illustrate under what scenarios S isotopes in hair are useful in the analysis of movement. Using similar methods and analyses that I developed in earlier chapters, I show how tail hairs can provide a continuous time series information of animal diet which allows us to understand seasonal periods of diet convergence and partitioning. This approach is particularly useful for understanding niche partitioning and coexistence of species particularly in biodiverse regions where multiple species all focus on the same general resource.

This thesis has demonstrated that isotopic methods have a great potential for Tanzanian ecological research, methods that currently receive little or no attention from Tanzanian ecologists. The main reason for this being the lack of stable isotope analysis facilities (i.e. mass spectrometer) within the country or nearby countries. This limits both the knowledge and motivation to apply stable isotope techniques in ecological research. I believe that the knowledge and potential of stable isotope techniques demonstrated by my PhD work provide a callsign and encouragement to ecologists interested in utilizing isotopic methods in ecology, and that it will motivate ecological conservation stakeholders to provide more support to stable isotope related work in Tanzania.

## 6.5 Future works

This PhD research has contributed to the application of stable isotope ratios to understanding movement and dietary choices of co-migrating animal species. Specifically, using the Serengeti ecosystem as a case study, has demonstrated how variation of stable isotope ratios measured in tail hairs can contribute to understanding wildlife ecology across this system. However, the full potential remains unrealized and this work can act as a foundation to allow further exploration.

For example, future studies could apply isotopic methods to understand niche breadth and variation to compare the sexes of wildebeest. This can help to answer some difficult questions, for example whether the differences in body size between males and females is related to any differences in feeding behaviors or is simply an evolutionary adaptation to mating strategies (Ruckstuhl & Neuhaus, 2000).

Another interesting aspect to explore using isotopic methods across the Serengeti, would be to explore the level of niche interaction and dietary preference between wildebeest and their close relatives, topi and Coke's hartebeest. This could lead to an understanding of whether the seasonal migration of wildebeest is influenced by food competition with other non-conspecifics or is a genetically inherited trait as a means to cope with the big herd size which helps improve their condition and chance to breed. Generally, migration provides wildebeest access to mineral-rich nutritious young low-fiber herbage which only occurs for short periods after the first rains (Hopcraft et al., 2014; Hopcraft, 2015). If this is the case, it might imply that topi and hartebeest are adapted to low quality fibrous herbage and can stay in one place and be territorial.

Furthermore, future studies might need to focus on improving and further testing the classification of life history strategies as described in chapter four of this thesis. The key can be tested across different taxa, species or ecosystems where there are mixed populations with enough variation of $\delta^{34}S$. The improvement might be achieved by adding more classifying attributes such as a secondary isotope (e.g. $^{13}C$ which tends to vary regionally based on the plant types e.g. $C_3$ versus $C_4$ (Janzen et al., 2020; Spasojevic & Weber, 2021)). The approach of applying isotopic methods to identify residents and migrants is highly flexible and provides a useful and less invasive approach of making inferences of population wide movement.

In summary, there is scope to further focus our efforts to enhancing the applicability of stable isotope-related works across the Serengeti ecosystem. We can achieve this by establishing a foundation of isotope work through creating more and robust isoscapes of different elements. For example, further mapping of hydrogen (Bowen et al., 2005), oxygen (Bowen et al., 2005; Hobson & Koehler, 2015), strontium (Evans et al., 2019; Price et al., 2004) or carbon (Valenzuela et al., 2011) stable isotopes would add more classification axes for differentiating movement or diet of the wildlife dwellers of this system. We might also want to expand the coverage of our isoscape to include key corridors and buffer zones that animals might disperse across. This will help to show the extent of animal movement which can help to provide recommendations on the appropriate conservation actions to preserve such mobility.

# Appendix A: supplementary materials for chapter 2

**Appendix A-1: Summary statistics and model selection for the Serengeti sulfur isoscape.**

| | Model | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|
| **mod_gam11** | MAP + s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7) | 12 | -156.2 | 341.6 | 0 | 0.57 |
| **mod_gam12** | **s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7)** | 14 | -155.58 | 343.82 | 2.223 | 0.187 |
| **mod_gam10** | MAP + elevation + s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7) | 13 | -156.17 | 344.19 | 2.593 | 0.156 |
| **mod_gam8** | elevationMAP * elevation + N_isotope + s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7) | 16 | -154.52 | 347 | 5.402 | 0.038 |
| **mod_gam9** | elevationMAP * elevation + s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7) | 15 | -155.72 | 347.1 | 5.5 | 0.036 |
| **mod_gam7** | elevationMAP * elevation + C_isotope + N_isotope + s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7) | 18 | -153.14 | 349.95 | 8.348 | 0.009 |
| **mod_gam6** | elevationCEC + (MAP * elevation) + C_isotope + N_isotope + s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7) | 19 | -153.18 | 352.77 | 11.168 | 0.002 |
| **mod_gam5** | elevationCEC + (MAP * elevation) + C_isotope + N_isotope + (C_isotope * N_isotope) + s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7) | 17 | -156.1 | 353.77 | 12.169 | 0.001 |
| **mod_gam4** | elevation (MAP * elevation) + (MAP * CEC) + C_isotope + N_isotope + (C_isotope * N_isotope) + s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7) | 18 | -156.09 | 356.24 | 14.638 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **mod_gam1** | elevation (MAP * elevation * CEC)elevation (elevation * CEC) + C_isotope + N_isotope + (C_isotope * N_isotope) + s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7) | 12 | -194 | 417.33 | 75.73 | 0 |
| **mod_gam2** | MAP + elevation + CEC + (MAP * elevation * CEC) + (MAP * elevation) + (MAP * CEC) + C_isotope + N_isotope + (C_isotope * N_isotope) + s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7) | 12 | -194 | 417.33 | 75.73 | 0 |
| **mod_gam3** | MAP + elevation + CEC + (MAP * elevation) + (MAP * elevation * CEC) + C_isotope + N_isotope + (C_isotope * N_isotope) + s(y_proj) + s(x_proj) + te(x_proj, y_proj, k = 7) | 12 | -194 | 417.33 | 75.73 | 0 |

**Appendix A-2: List of questions asked to cattle owners for exploring movement history of cattle**

PARTICIPANT / ANIMAL INFORMATION SHEET

A) BASIC INFORMATION

Date……. District…… Site……

Tribe……... Animal's colour……. Sex…… Age (or estimated) ……

GPS readings: X…… Y…… Sample ID…….

B. SPECIFIC INFORMATION

1. (a) Did you buy your animal?

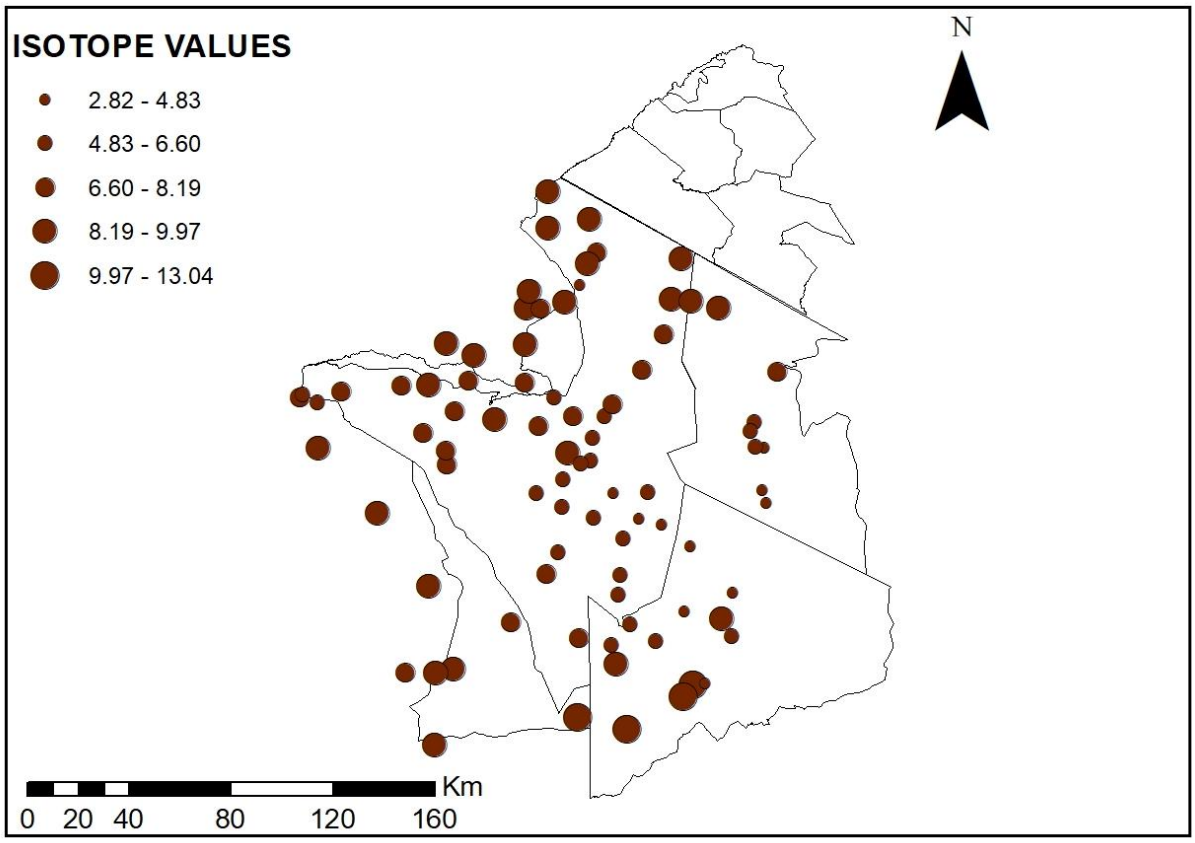
   (b) If yes, where and when?

   (c) If not, where did you get it?
2. For how long have you been keeping this animal?
3. Where do you normally graze your animal?
4. (a) Have you ever grazed this animal outside your normal grazing area?

   (b) If yes, where? And for how long? Can you remember the dates?
5. (a) Have you recently moved your animal to different place

   (b)If yes, where? Can you remember the dates?
6. Why did you move it?
7. When did you bring it back? And why?
8. If a need arises, can you allow us to re-sample your animal next time?

**Appendix A-3: Laboratory results for grass sulfur isotopes across the Serengeti ecosystem showing $\delta^{34}S$ value**



**Appendix A-4: Distance in Km moved by cattle as per questionnaire report. Distance is the straight-line measure between two points**

| ID | Village_ sampled | X-coordinate | Y-coordinate | Date-moved | Sample_ collected | Village_ moved | X-coordinate | Y-coordinate | Movement Period | Cardinal direction (radians) | Distance Travelled (Kms) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A5 | Robanda | 686907 | 9763049 | 11/05/2017 | 05/08/2017 | Machochwe | 688141 | 9815346 | 2.9 Months | 0.023 | 52.311 |
| A7 | Bwitengi | 684832 | 9790956 | 23/04/2107 | 05/08/2017 | Miseke | 688575 | 9790360 | 3 Months | 1.728 | 3.790 |
| A13 | Bonchugu | 699163 | 9792946 | 20/03/2017 | 06/08/2017 | Mugumu | 690458 | 9796270 | 5 Months | -1.206 | 9.318 |
| A22 | Isenye | 648172 | 9781707 | Feb, 2017 | 06/08/2017 | Iharara | 647788 | 9777718 | 5 Months | -3.045 | 4.007 |
| A24 | Isenye | 648413 | 9781854 | June, 2017 | 06/08/2017 | Iharara | 9777718 | 647788 | 2 Months | -0.785 | 12.914 |
| A25 | Isenye | 648413 | 9781854 | Aug, 2017 | 06/08/2017 | Iharara | 9777718 | 647788 | Few Days | -0.785 | 12.914 |
| A27 | Sapa | 660020 | 9618602 | Mar, 2017 | 08/08/2017 | Ilindwa | 656240 | 9616487 | 5 Months | -2.080 | 4.331 |
| A28 | Sapa | 659839 | 9618378 | Mar, 2017 | 08/08/2017 | Mwanhuzi | 648212 | 9611252 | 5 Months | -2.120 | 13.636 |
| A30 | Sakasaka | 652669 | 9650201 | July, 2017 | 09/08/2017 | Butuli | 653221 | 9656138 | 1 Month | 0.092 | 5.962 |
| A59 | Endulen | 752635 | 9644142 | July, 2017 | 02/10/2017 | Olepesi | 753903 | 9647217 | 3 Months | 0.391 | 3.3261 |
| A64 | Esere | 746519 | 9639601 | Aug, 2017 | 02/10/2017 | Endulen | 753903 | 9647217 | 2 Months | 0.769 | 10.607 |
| A65 | Esere | 756247 | 9639293 | Aug, 2017 | 03/10/2017 | Endulen | 753903 | 9647217 | 2 Months | -0.287 | 8.263 |

# Appendix B: supplementary materials for chapter 3

## Appendix B-1: Function used to estimate the lag time for $\delta^{34}S$ in the tail hair of wildebeest

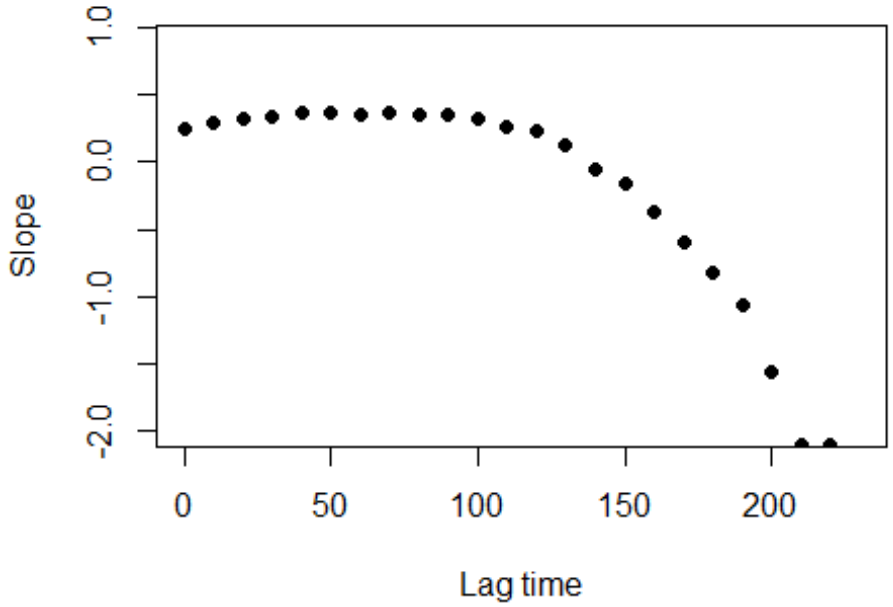
Author: Zabibu Kabalika description: Function for isotope estimation from animal's tail hair

```
# libraries
require(lubridate)
require(openair)
require(raster)
library(rgdal)
library(rgeos)
library(dplyr)
library(sp)

latlong <- ('+proj=longlat +datum=WGS84')
utmproj <- ("+proj=utm +zone=36 +south")

#First, load in the CSV file of an individual wildebeest's tail hair
get_tail_data <- function(animal_id){
  tail_data <- read.csv(animal_id, header = T)
  return(tail_data)
}

#Then, get location for each segment of a loaded tail hair
get_location_data <- function(file_name){
  location_data <- read.csv(file_name)
  latlong <- ('+proj=longlat +datum=WGS84')
  utmproj <- ("+proj=utm +zone=36 +south")
  location_data$Date <- as.POSIXct(location_data$Date, tz = 'GMT', forma
t = '%d/%m/%Y')
  location_data <- SpatialPointsDataFrame(location_data[, 5:6], location
_data, proj4string = CRS(latlong ))
  location_data <- spTransform(location_data, CRSobj = utmproj)
  location_data <- as.data.frame(location_data)
  return(location_data)
}
```

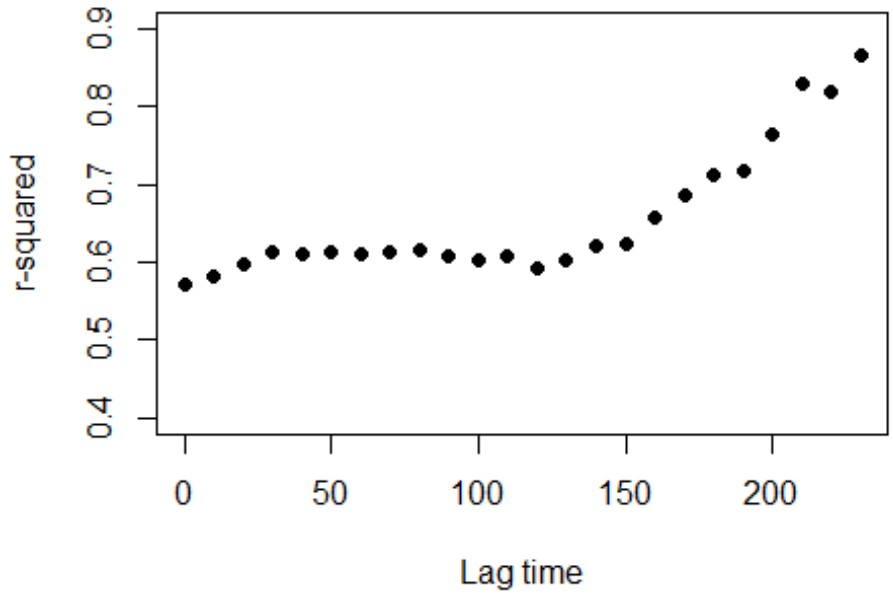
```
#Extract the isotope reading from isoscape and get the r squared between
the isotope reading in the tail hair and the isotope reading from tail h
air.
getr2 <- function(Location_data, sample_date, nof_segment, Ts_values, is
o_map, lag_tail, segment_days){
  LS_values = matrix(0,nrow = 0,ncol = 2)
  for (i in 1:nof_segment) {
    end_date <- sample_date - lag_tail - (i-1)*segment_days
    start_date <- end_date - segment_days
    selection <- subset(Location_data, Location_data$Date >= start_date
& Location_data$Date < end_date)
    #This gives the averaged isotope value from a landscape over the 15
days growth period
   if (nrow(selection)>0){
      coordinates(selection) <- ~ Longitude + Latitude
      crs(selection) <- latlong
      selection <- spTransform(selection, CRSobj = crs(iso_map))
      row_add<-c(mean(extract(iso_map, selection)),Ts_values[i])
      LS_values<-rbind(LS_values,row_add)
      }
  }
 return(cor(LS_values[,1],LS_values[,2]))
}

#Add lag time into the equation
get_plot <- function(Location_data, sample_date, nof_segment, Ts_values,
iso_map, lag_tail, segment_days, animal, history_days){
  LS_values = matrix(0,nrow = 0,ncol = 3)
  for (i in 1:nof_segment) {
    end_date <- sample_date - lag_tail - (i-1)*segment_days #where i is
the segment number
    start_date <- end_date - segment_days - history_days
    selection <- subset(Location_data, Location_data$Date >= start_date
& Location_data$Date < end_date)
    #Here we are trying to add a four days to the start date to allow di
et incorporation time
    pass<-0
```

```
    if (nrow(selection)>0){
       pass<-0
       for (j in 1:nrow(selection)){
          if ((as.Date(selection$Date[j])-start_date) < 4) {
            pass<-1
          }
       }
    }

    if ((nrow(selection)>0) && (pass>0)){
      coordinates(selection) <- ~ Longitude + Latitude
      crs(selection) <- latlong
      selection <- spTransform(selection, CRSobj = crs(iso_map))
      row_add<-c(mean(extract(iso_map, selection)),Ts_values[i],animal)
      LS_values<-rbind(LS_values,row_add)
    }
  }
  return(LS_values)
}

#Here we add more lag, 'history days'
Consider_a_herd <- function(lag_tail,segment_days,history_days) {
  iso_map <- raster('S_map_two.tif')
  animal_names <- c('WB_417.csv', 'WB_418.csv', 'WB_419.csv', 'WB_420.cs
v', 'WB_422.csv')
  tail_names <- c('WB_417t.csv','WB_418t.csv', 'WB_419t.csv', 'WB_420t.c
sv', 'WB_422t.csv')
  GLS_values = matrix(0,nrow = 0,ncol = 3)
 for (i in 1:5) {
   Location_data <- get_location_data(animal_names[i])
   Tail_data <- as.data.frame(get_tail_data(animal_id = tail_names[i]))
   sample_date <- as.Date(Tail_data$sdate[1],'%d/%m/%Y' )
   nof_segment <- as.numeric(Tail_data$n[1]) #Tail_data$V1[2]
   Ts_values <- matrix(data = 0, nrow = 0, ncol = 1)
   for (j in 1:nof_segment) {
    Ts_values <- rbind(Ts_values,Tail_data$values[j])
   }
GLS_values<-rbind(GLS_values,get_plot(Location_data, sample_date, nof_se
```

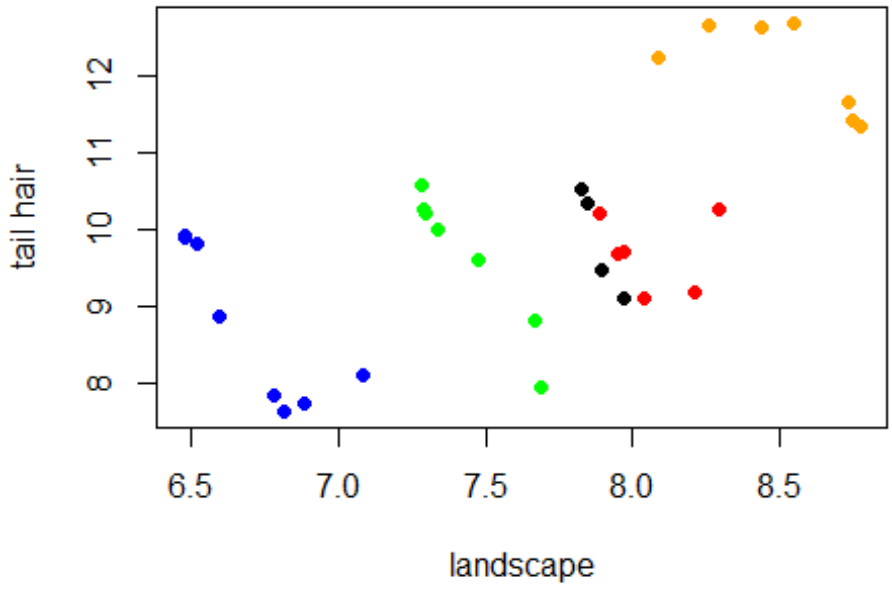
```
gment, Ts_values, iso_map, lag_tail, segment_days,i, history_days))

 }
return(GLS_values)
}

#Get the best correlation between the isotope reading from the diet and
the isotope reading in the tail hair.
span_parameters <-function(){
 r_scan<-matrix(data=0,nrow=0,ncol=2)
 TL_cor <- Consider_a_herd(lag_tail = 13, segment_days = 16,history_days
=0)
 r_scan<-rbind(r_scan,c(lag_tail,mean(TL_cor)))
}
#Fit the model to test for correlation.
x<- seq(0,230, 10)
y<- NULL
y_row <- matrix(0,1,3)
for (j in 1:length(x)){
  plot_data <- Consider_a_herd(lag_tail=13, segment_days = 15,history_da
ys=x[j])
  md<-summary(lm(plot_data[,2]~ plot_data[,1] + as.factor(plot_data[,3])
))
  y_row[1,1]<-md$coefficients[2,1][]
  y_row[1,2] <- md$r.squared
  y_row[1,3]<-x[j]
  y <- rbind(y, y_row)
}

#Plotting the output from a linear model
plot(y[,1] ~ y[,3], ylab='Slope', xlab='Lag time', pch=16, ylim=c(-2, 0.
9))
```



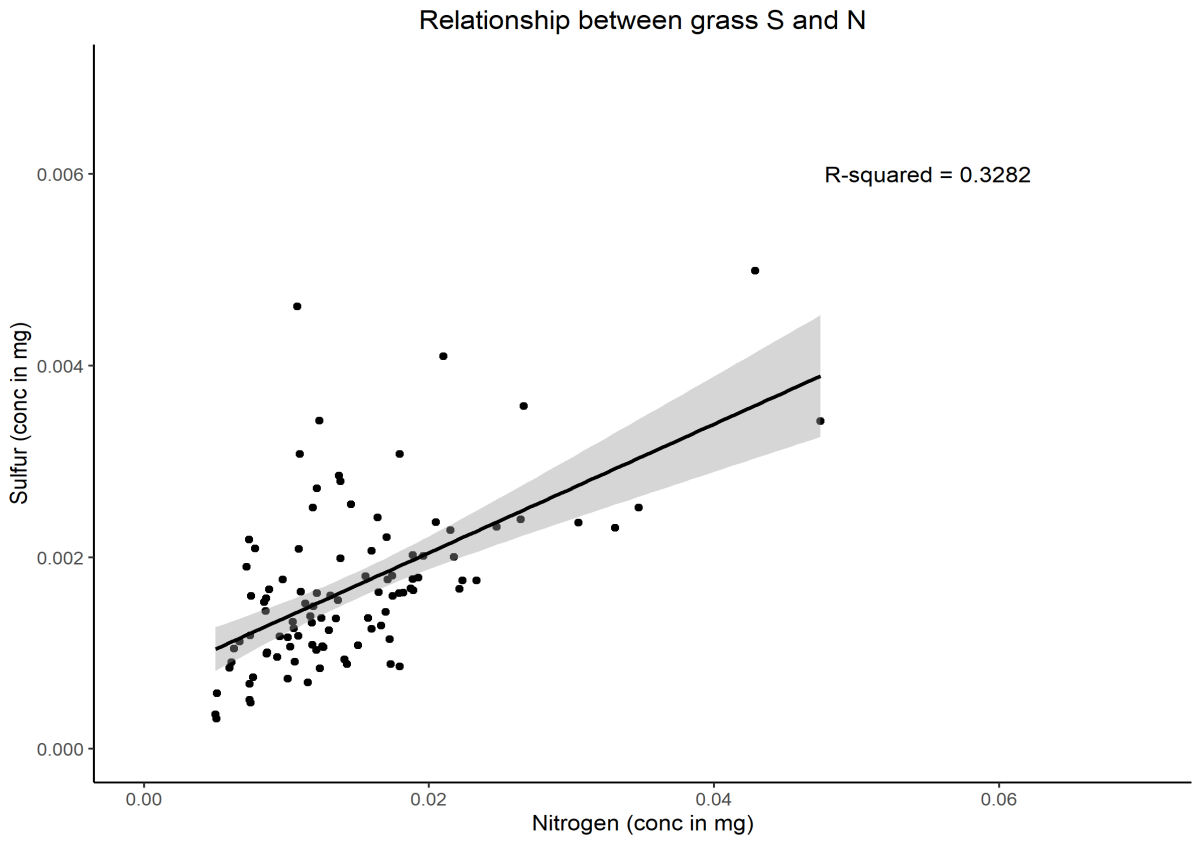

```
plot(y[,2] ~ y[,3], ylab='r-squared', xlab='Lag time', pch=16, ylim=c(0.
4, 0.9))
```




```
plot(plot_data[,2] ~ plot_data[,1], ylab='tail hair', xlab='landscape',
pch=16, col= col_dots[plot_data[,3]])
```

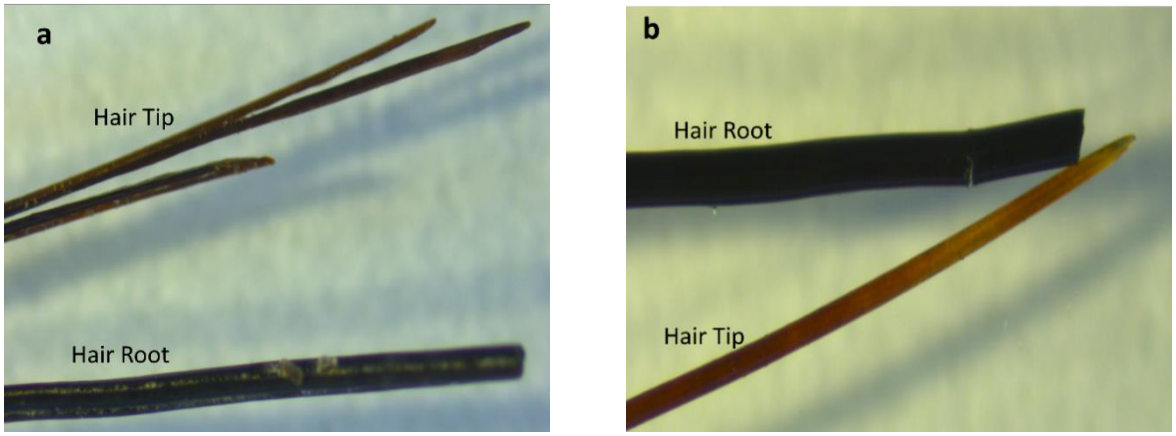



```
y_values <- data.frame(y)
colnames(y_values) <- c("slope", "rsquared", "days_back")
#write.csv(y_values, "third_trial.csv")
```




**Appendix B-2: Relationship between S and N concentrations in grass from the Serengeti ecosystem, suggesting that S is correlated with the protein content**

**of the grass. The units are in concentration per unit mg of grass (data source: Kabalika et al – unpublished data).**




**Appendix B-3: Microscopic view of a tail hair structure for root and tip ends showing that the hair root is thicker and more stable compared to the tip which is thinner and fragile, suggesting that the hair tip erodes as it grows. a) is a representative tail hair strand for W580 which grew for longer than 13 months and b) is a representative tail hair strand for W422 which grew for less than 13 months (Data source: Personal observation).**

# Appendix C: supplementary material for chapter 4

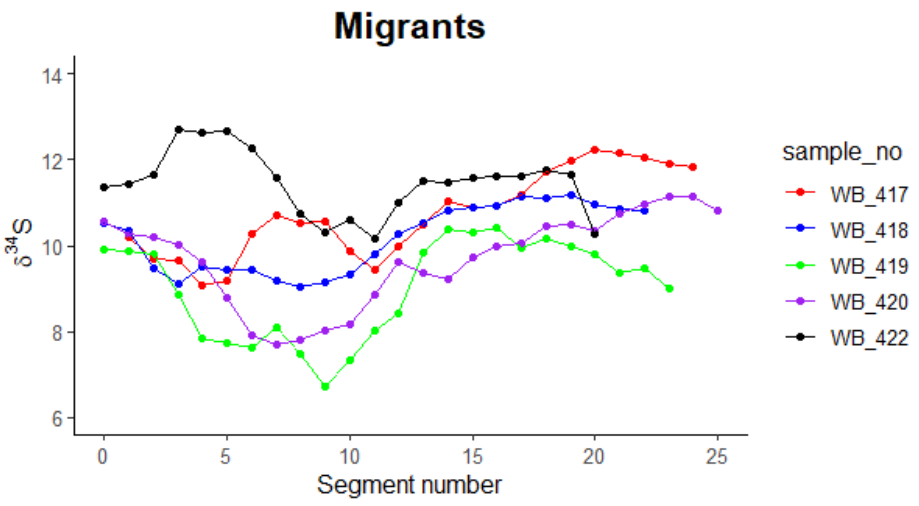
## Appendix C-1: State-space model of animal movement for differentiating resident and migrant life-history strategies using $\delta^{34}S$ in the tail hair.

We will explore a state-space framework for modeling time-series. The idea behind state-space models is to separate the process model, which describes how the system evolves in time or space, from the observation error model.

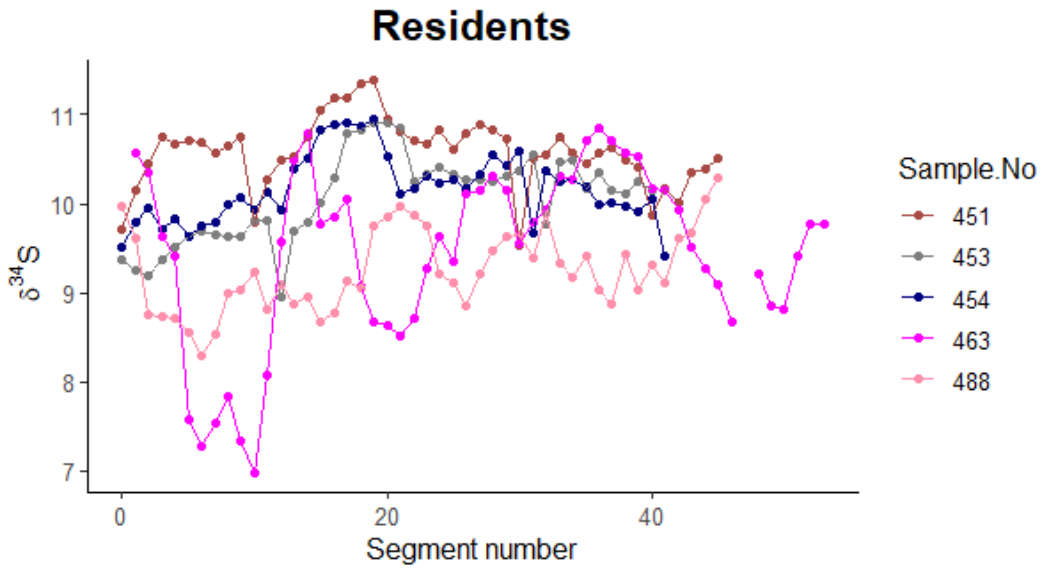
```
library(jagsUI)
library(dplyr)
```

Let's load the data and make exploratory plots

```
Sdata = read.csv("migrants.csv")
dat <- read.csv("residents.csv")
res_dat <- dat[dat$movement=='resident',]
switchers <-  read.csv('switchers.csv')
id <- unique(res_dat$Sample.No)
ids <- unique(Sdata$Sample.No)
idx <- unique(switchers$sample_no)

plot(-1,-1, xlim = c(0, 26), ylim = c(6, 13),
     xlab = "section", ylab = ~ paste(34, "S isotope"), main = 'MIGRANTS')

for (i in 1: length(ids)){
  lines(Sdata$Section.No[Sdata$Sample.No==ids[i]],
        Sdata$d34S[Sdata$Sample.No==ids[i]],
        type = "o", col = i, lwd = 3)
  legend('bottomright', legend = ids, pch=16, col = ids, ncol = 2, bty = 'n')
}
```



```
plot(-1,-1, xlim = c(0, 55), ylim = c(6, 13),
     xlab = "section", ylab = ~ paste(34, "S isotope"), main = 'RESIDENTS')
for (i in 1: length(id)){
  lines(res_dat$Section.No[res_dat$Sample.No==id[i]],
        res_dat$d34S[res_dat$Sample.No==id[i]],
        type = "o", col = i, lwd = 3)

  legend(30, 9, legend = id, pch=16, col = id, ncol = 2, bty = 'n')
}
```




We know there is some measurement error that we want to incorporate in the analysis. We also want to consider that hair sections that are next to each other are expected to be similar. Also, there is variability in the actual process that gives rise to the data.

Now, let's write a `JAGS` code to model this. The code has three parts: the observation model, the process model, and the priors.

```
arima1.bug = "
model{
  #### Data Model
  for(t in 1:n){
    y[t] ~ dnorm(x[t], tau_obs)
#tau=precision
#rho = correlation coefficient
  }

  #### Process Model
  for(t in 2:n){
    m[t] <- x[t-1] * rho + mu
    x[t] ~ dnorm(m[t], tau_proc)
  }
```

```
  #### Priors
  x[1] ~ dnorm(x_ic, tau_ic)
  sd_obs ~ dnorm(m_sd_obs, tau_sd_obs)
  sd_proc ~ dunif(min_proc, max_proc)
  tau_obs <- 1/(sd_obs*sd_obs)
  tau_proc <- 1/(sd_proc*sd_proc)
  rho ~ dbeta(1.1,1.1)
  mu ~ dnorm(m_mu, tau_mu)
}
"
```

Now lets write a model where there is a change in the mean with time

```
arima_t.bug = "
model{
  #### Data Model
  for(t in 1:n){
    y[t] ~ dnorm(x[t], tau_obs)
  }

  #### Process Model
  for(t in 2:n){
    mu[t] <- a + b * t
    m[t] <- x[t-1] * rho + mu[t]
    x[t] ~ dnorm(m[t], tau_proc)
  }

  #### Priors
  x[1] ~ dnorm(x_ic, tau_ic)
  sd_obs ~ dnorm(m_sd_obs, tau_sd_obs)
  sd_proc ~ dunif(min_proc, max_proc)
  tau_obs <- 1/(sd_obs*sd_obs)
  tau_proc <- 1/(sd_proc*sd_proc)
  rho ~ dbeta(1.1,1.1)
  a ~ dnorm(0,1)
  b ~ dnorm(0,1)
}
"
```

Model three: Cyclic model

```
arima_cirle_t.bug = "
model{
  #### Data Model
  for(t in 1:n){
    y[t] ~ dnorm(x[t], tau_obs)
```

```
  }

  #### Process Model
  for(t in 2:n){
    mu[t] <- a + beta * cos(b + 2* pi * 16/365*t)
    m[t] <- x[t-1] * rho + mu[t]
    x[t] ~ dnorm(m[t], tau_proc)
  }

  #### Priors
  x[1] ~ dnorm(x_ic, tau_ic)
  sd_obs ~ dnorm(m_sd_obs, tau_sd_obs)
  sd_proc ~ dunif(min_proc, max_proc)
  tau_obs <- 1/(sd_obs*sd_obs)
  tau_proc <- 1/(sd_proc*sd_proc)
  rho ~ dbeta(1.1,1.1)
  a ~ dnorm(0,1)
  b ~ dnorm(0,1)
  pi <- 3.141593
  beta ~ dnorm(0,1)T(0, )
}
"
```

Now let's try some data; a) migrants model 1; Stable mean

```
tab <- data.frame()
for (h in 1:length(ids)) {
  y = Sdata$d34S[Sdata$Sample.No==ids[h]]
  m_data <- list(y = y - mean(y, na.rm=T),
              n=length(y),
              x_ic = 0,
              tau_ic = 100,
              m_sd_obs = 0.7,
              tau_sd_obs = 500,
              min_proc = 0,
              max_proc = 10,
              m_mu = 0,
              tau_mu = 100
              )

nchain = 3

inits <- function() list(mu = runif(1,10,14))

params <- c("mu", "rho", "sd_obs", "sd_proc")
```

```
m1.sim <- jags(data = m_data,
               inits = inits,
               parameters.to.save = params,
               model.file = textConnection(arima1.bug),
               n.chains = 3,
               n.thin = 1,
               n.iter = 5000,
               n.burnin = 2500)
tab <- rbind(tab, data.frame(m1.sim $summary, DIC = m1.sim $DIC, ID=h,model = 'arima1.bu
g',  movement = 'migrant', sample_no = paste(ids[h])))
}

##
## Processing function input.......
##
## Done.
##
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 24
##    Unobserved stochastic nodes: 28
##    Total graph size: 113
##
## Initializing model
##
## Adaptive phase.....
## Adaptive phase complete
##
##
##  Burn-in phase, 2500 iterations x 3 chains
##
##
## Sampling from joint posterior, 2500 iterations x 3 chains
##
##
## Calculating statistics.......
##
## Done.
##
## ## Done.
```

Model 2; mean changes over time

```
tab_1 <- data.frame()
movement <- c('migrant', 'migrant', 'migrant', 'migrant', 'migrant')
```

```
ID <- ids
for (h in 1:length(ids)) {
 y = Sdata$d34S[Sdata$Sample.No==ids[h]]
m_data <- list(y = y - mean(y, na.rm=T),
               n=length(y),
               x_ic = 0,
               tau_ic = 100,
               m_sd_obs = 0.7,
               tau_sd_obs = 500,
               min_proc = 0,
               max_proc = 10
               )

nchain = 3

inits <- function() list(a = 0)

params <- c( "rho", "sd_obs", "sd_proc", "a", "b")

m1t.sim <- jags(data = m_data,
                inits = inits,
                parameters.to.save = params,
                model.file = textConnection(arima_t.bug),
                n.chains = 3,
                n.thin = 1,
                n.iter = 5000,
                n.burnin = 2500)
tab_1 <- rbind(tab_1, data.frame(m1t.sim $summary, DIC = m1t.sim $DIC, ID=h,model = 'ari
ma_t.bug',  movement = 'migrant', sample_no = paste(ids[h])))
}
## Processing function input.......
##
## Done.
##
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 24
##    Unobserved stochastic nodes: 29
##    Total graph size: 182
##
## Initializing model
##
## Adaptive phase.....
## Adaptive phase complete
```

```
##
## Burn-in phase, 2500 iterations x 3 chains
##
## Sampling from joint posterior, 2500 iterations x 3 chains
##
## Calculating statistics.......
## Done.
```

Model 3; cyclic mean

```
tab_2 <- data.frame()
for (h in 1:length(ids)) {
 y = Sdata$d34S[Sdata$Sample.No==ids[h]]
m_data <- list(y = y - mean(y, na.rm=T),
               n=length(y),
               x_ic = 0,
               tau_ic = 100,
               m_sd_obs = 0.7,
               tau_sd_obs = 500,
               min_proc = 0,
               max_proc = 10
               )

nchain = 3

inits <- function() list(a = 0)

params <- c( "rho", "sd_obs", "sd_proc", "a", "b", 'beta')

m2t.sim <- jags(data = m_data,
                inits = inits,
                parameters.to.save = params,
                model.file = textConnection(arima_cirle_t.bug),
                n.chains = 3,
                n.thin = 5,
                n.iter = 50000,
                n.burnin = 25000)
tab_2 <- rbind(tab_2, data.frame(params = row.names(m2t.sim$summary), m2t.sim$summary, D
IC = m2t.sim$DIC, id=h, model ='arima_cirle_t.bug', movement = 'migrant', sample_no = pa
ste(ids[h])))
}
## Processing function input.......
##
## Done.
```

```
##
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 24
##    Unobserved stochastic nodes: 30
##    Total graph size: 256
##
## Initializing model
##
## Adaptive phase.....
## Adaptive phase complete
##
##
##  Burn-in phase, 25000 iterations x 3 chains
##
##
## Sampling from joint posterior, 25000 iterations x 3 chains
##
##
## Calculating statistics.......
##
## Done.
```

b) Residents model 1; Stable mean

```
tab_3 <- data.frame()
for (h in 1:length(id)) {
  y = res_dat$d34S[res_dat$Sample.No==id[h]]
  m_data <- list(y = y - mean(y, na.rm = T),
              n=length(y),
              x_ic = 0,
              tau_ic = 100,
              m_sd_obs = 0.7,
              tau_sd_obs = 500,
              min_proc = 0,
              max_proc = 10,
              m_mu = 0,
              tau_mu = 100
              )

nchain = 3

inits <- function() list(mu = runif(1,10,14))

params <- c("mu", "rho", "sd_obs", "sd_proc")
```

```
m3.sim <- jags(data = m_data,
               inits = inits,
               parameters.to.save = params,
               model.file = textConnection(arima1.bug),
               n.chains = 3,
               n.thin = 1,
               n.iter = 5000,
               n.burnin = 2500)
tab_3 <- rbind(tab_3, data.frame(m3.sim$summary, DIC = m3.sim$DIC, ID=h,model = 'arima1.
bug',  movement = 'resident', sample_no = id[h]))
}
## Processing function input.......
##
## Done.
##
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 46
##    Unobserved stochastic nodes: 50
##    Total graph size: 201
##
## Initializing model
##
## Adaptive phase.....
## Adaptive phase complete
##
##  Burn-in phase, 2500 iterations x 3 chains
##
## Sampling from joint posterior, 2500 iterations x 3 chains
##
## Calculating statistics.......
##
## Done.
```

Model 2; mean changes over time

```
tab_4 <- data.frame()
for (h in 1:length(id)) {
 y = res_dat$d34S[res_dat$Sample.No==id[h]]
m_data <- list(y = y - mean(y, na.rm = T),
               n=length(y),
               x_ic = 0,
               tau_ic = 100,
               m_sd_obs = 0.7,
```

```
              tau_sd_obs = 500,
              min_proc = 0,
              max_proc = 10
              )

nchain = 3

inits <- function() list(a = 0)

params <- c( "rho", "sd_obs", "sd_proc", "a", "b")

m4t.sim <- jags(data = m_data,
               inits = inits,
               parameters.to.save = params,
               model.file = textConnection(arima_t.bug),
               n.chains = 3,
               n.thin = 1,
               n.iter = 5000,
               n.burnin = 2500)
tab_4 <- rbind(tab_4, data.frame(m4t.sim$summary, DIC = m4t.sim$DIC, ID=h,model = 'arima
_t.bug',  movement = 'resident', sample_no = id[h]))
}

##
## Processing function input.......
##
## Done.
##
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 46
##    Unobserved stochastic nodes: 51
##    Total graph size: 336
##
## Initializing model
##
## Adaptive phase.....
## Adaptive phase complete
##
##
##  Burn-in phase, 2500 iterations x 3 chains
##
##
## Sampling from joint posterior, 2500 iterations x 3 chains
```

```
##
##
## Calculating statistics.......
##
## Done.
```

Model 3; cyclic mean

```
tab_5 <- data.frame()
for (h in 1:length(id)) {
 y = res_dat$d34S[res_dat$Sample.No==id[h]]
m_data <- list(y = y - mean(y, na.rm=T),
               n=length(y),
               x_ic = 0,
               tau_ic = 100,
               m_sd_obs = 0.7,
               tau_sd_obs = 500,
               min_proc = 0,
               max_proc = 10
               )

nchain = 3

inits <- function() list(a = 0)

params <- c( "rho", "sd_obs", "sd_proc", "a", "b", 'beta')

m5t.sim <- jags(data = m_data,
                inits = inits,
                parameters.to.save = params,
                model.file = textConnection(arima_cirle_t.bug),
                n.chains = 3,
                n.thin = 5,
                n.iter = 50000,
                n.burnin = 25000)
tab_5 <- rbind(tab_5, data.frame(params = row.names(m5t.sim$summary), m5t.sim$summary, D
IC = m5t.sim$DIC, id=h, model ='Arima_cirle_t.bug', movement ='resident', sample_no = id
[h]))
}

##
## Processing function input.......
##
## Done.
##
## Compiling model graph
##    Resolving undeclared variables
```

```
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 46
##    Unobserved stochastic nodes: 52
##    Total graph size: 476
##
## Initializing model
##
## Adaptive phase.....
## Adaptive phase complete
##
##
##  Burn-in phase, 25000 iterations x 3 chains
##
##
## Sampling from joint posterior, 25000 iterations x 3 chains
##
##
## Calculating statistics.......
##
## Done.
##
```

THE UNKNOWNS; cyclic mean

```
unknowns <- read.csv('S_data.csv')
unknowns <- unknowns[unknowns$Sample.No %in% c('50', '108', '224', '238', '259', '266',
'274'),]

uid <- unique(unknowns$Sample.No)
tab_x <- data.frame()
for (i in 1:length(uid)) {
 y = unknowns$d34S[unknowns$Sample.No==uid[i]]
m_data <- list(y = y - mean(y, na.rm=T),
               n=length(y),
               x_ic = 0,
               tau_ic = 100,
               m_sd_obs = 0.7,
               tau_sd_obs = 500,
               min_proc = 0,
               max_proc = 10
               )

nchain = 3

inits <- function() list(a = 0)
```

```
params <- c( "rho", "sd_obs", "sd_proc", "a", "b", 'beta')


mxt.sim <- jags(data = m_data,
                inits = inits,
                parameters.to.save = params,
                model.file = textConnection(arima_cirle_t.bug),
                n.chains = 3,
                n.thin = 5,
                n.iter = 50000,
                n.burnin = 25000)
tab_x <- rbind(tab_x, data.frame(params = row.names(mxt.sim$summary), mxt.sim$summary, D
IC = mxt.sim$DIC, id=i, model ='Arima_cirle_t.bug', movement ='unknown'))
}
## Processing function input.......
##
## Done.
##
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 43
##    Unobserved stochastic nodes: 61
##    Total graph size: 506
##
## Initializing model
##
## Adaptive phase.....
## Adaptive phase complete
##
##  Burn-in phase, 25000 iterations x 3 chains
##
## Sampling from joint posterior, 25000 iterations x 3 chains
##
##
## Calculating statistics.......
##
## Done.
```

THE UNKNOWNS; Linear mean

```
tab_y <- data.frame()


unknowns <- read.csv('S_data.csv')
unknowns <- unknowns[unknowns$Sample.No %in% c('50', '108', '224', '238', '259', '266',
'274'),]
uid <- unique(unknowns$Sample.No)
```

```
for (i in 1:length(uid)) {
 y = unknowns$d34S[unknowns$Sample.No==uid[i]]
m_data <- list(y = y - mean(y, na.rm = T),
               n=length(y),
               x_ic = 0,
               tau_ic = 100,
               m_sd_obs = 0.7,
               tau_sd_obs = 500,
               min_proc = 0,
               max_proc = 10
               )

nchain = 3

inits <- function() list(a = 0)

params <- c( "rho", "sd_obs", "sd_proc", "a", "b")

myt.sim <- jags(data = m_data,
                inits = inits,
                parameters.to.save = params,
                model.file = textConnection(arima_t.bug),
                n.chains = 3,
                n.thin = 1,
                n.iter = 5000,
                n.burnin = 2500)
tab_y <- rbind(tab_y, data.frame(myt.sim $summary, DIC = myt.sim $DIC, ID=i,model = 'ari
ma_t.bug',  movement = 'unknown'))
}
## Processing function input.......
##
## Done.
##
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 43
##    Unobserved stochastic nodes: 60
##    Total graph size: 357
##
## Initializing model
##
## Adaptive phase.....
## Adaptive phase complete
##
```

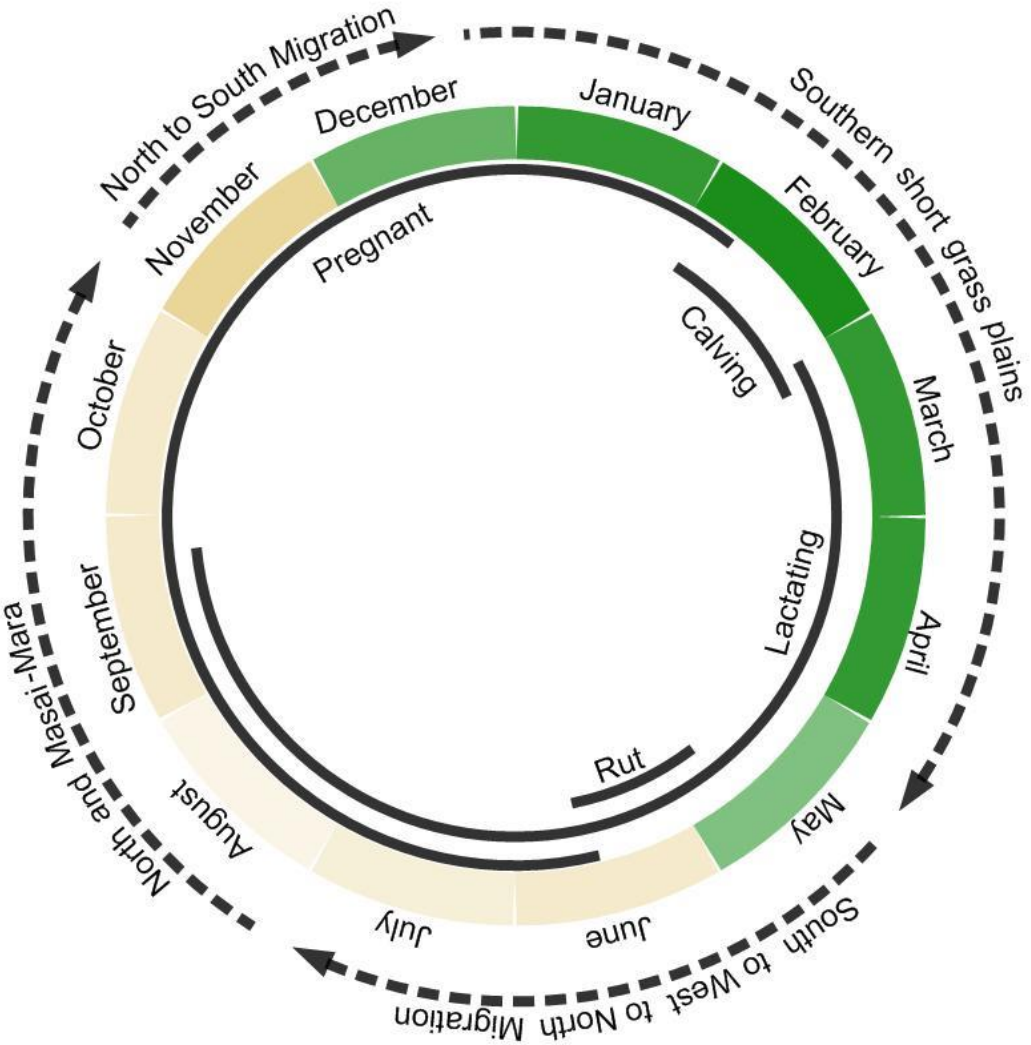
```
##  Burn-in phase, 2500 iterations x 3 chains
##
## Sampling from joint posterior, 2500 iterations x 3 chains
##
## Calculating statistics.......
##
## Done.
```

THE UNKNOWNS: stable mean

```
tab_z <- data.frame()
for (i in 1:length(uid)) {
  y = unknowns$d34S[unknowns$Sample.No==uid[i]]
  m_data <- list(y = y - mean(y, na.rm = T),
              n=length(y),
              x_ic = 0,
              tau_ic = 100,
              m_sd_obs = 0.7,
              tau_sd_obs = 500,
              min_proc = 0,
              max_proc = 10,
              m_mu = 0,
              tau_mu = 100
              )

nchain = 3

inits <- function() list(mu = runif(1,10,14))

params <- c("mu", "rho", "sd_obs", "sd_proc")

mz.sim <- jags(data = m_data,
               inits = inits,
               parameters.to.save = params,
               model.file = textConnection(arima1.bug),
               n.chains = 3,
               n.thin = 1,
               n.iter = 5000,
               n.burnin = 2500)
tab_z <- rbind(tab_z, data.frame(mz.sim$summary, DIC = mz.sim$DIC, ID=i,model = 'arima1.
bug',  movement = 'unknown'))
}

##
## Processing function input.......
##
## Done.
```

```
##
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 43
##    Unobserved stochastic nodes: 59
##    Total graph size: 213
##
## Initializing model
##
## Adaptive phase.....
## Adaptive phase complete
##
##  Burn-in phase, 2500 iterations x 3 chains
##
## Sampling from joint posterior, 2500 iterations x 3 chains
##
## Calculating statistics.......
##
## Done.
```

# Appendix D: Supplementary material for chapter 5




**Appendix D-1: The reproductive cycle of Serengeti wildebeest in relation to the seasonal annual migration. Female wildebeest are either pregnant or lactating (or both) all year round. Dark shading indicates wet season months, light shading indicates dry season months. source** (Hopcraft, 2015; Watson, 1967)

**Appendix D-2: Tail hair samples for lactating versus non-lactating females for wildebeest and zebra.**

| UID | Sample_no | Date | $\delta^{15}N$ | $\delta^{13}C$ | species | Life-history strategy | Physiology |
|---|---|---|---|---|---|---|---|
| 1 | 50 | 19/05/2013 | 9.19 | -10.27 | wildebeest | western corridor | not_lactating |
| 2 | 108 | 27/11/2013 | 4.72 | -9.47 | wildebeest | mara | not_lactating |
| 3 | 224 | 15/02/2014 | 9.21 | -13.74 | wildebeest | migrant | not_lactating |
| 4 | 238 | 07/02/2014 | 7.57 | -9.87 | wildebeest | migrant | not_lactating |
| 5 | 259 | 04/03/2014 | 8.65 | -12.93 | wildebeest | migrant | not_lactating |
| 6 | 266 | 12/03/2014 | 6.77 | -9.86 | wildebeest | migrant | not_lactating |
| 7 | 274 | 24/03/2014 | 9.21 | -13.74 | wildebeest | migrant | not_lactating |
| 8 | 417 | 28/05/2014 | 6.06 | -14.51 | wildebeest | migrant | lactating |
| 9 | 418 | 12/06/2014 | 6.75 | -10.91 | wildebeest | migrant | not_lactating |
| 10 | 419 | 14/06/2014 | 6.07 | -10.14 | wildebeest | migrant | lactating |
| 11 | 420 | 15/06/2014 | 6.11 | -10.53 | wildebeest | migrant | lactating |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12 | 422 | 15/06/2014 | 6.49 | -10.7 | wildebeest | migrant | lactating |
| 13 | 441 | 25/05/2014 | 7.62 | -11.51 | wildebeest | migrant | not_lactating |
| 14 | 451 | 19/05/2016 | 6.45 | -8.45 | wildebeest | western corridor | lactating |
| 15 | 453 | 19/05/2016 | 5.83 | -11.07 | wildebeest | western corridor | lactating |
| 16 | 454 | 19/05/2016 | 7.8 | -9.95 | wildebeest | western corridor | lactating |
| 17 | 463 | 20/05/2016 | 7.97 | -11.67 | wildebeest | migrant | lactating |
| 18 | 488 | 12/08/2016 | 7.01 | -11.01 | wildebeest | western corridor | lactating |
| 19 | 493 | 03/03/2016 | 9.9 | -14.7 | wildebeest | mara | not_lactating |
| 20 | 494 | 27/10/2016 | 6.38 | -10 | wildebeest | migrant | not_lactating |
| 21 | 496 | 20/03/2016 | 7.31 | -11.76 | wildebeest | migrant | not_lactating |
| 22 | 497 | 21/01/2016 | 8.8 | -10.95 | wildebeest | migrant | not_lactating |
| 23 | 508 | 06/04/2017 | 8.24 | -10.45 | wildebeest | western corridor | not_lactating |
| 24 | 511 | 04/06/2017 | 6.53 | -10.4 | wildebeest | migrant | not_lactating |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 25 | 521 | 28/02/2017 | 7.63 | -9.19 | wildebeest | mara | not_lactating |
| 26 | 533 | 06/07/2017 | 6.6 | -9.84 | wildebeest | migrant | not_lactating |
| 27 | 534 | 06/07/2017 | 5.81 | -10.51 | wildebeest | migrant | not_lactating |
| 28 | 535 | 06/07/2017 | 5.91 | -11.14 | wildebeest | migrant | lactating |
| 29 | 536 | 07/07/2017 | 7.15 | -11.39 | wildebeest | migrant | not_lactating |
| 30 | 539 | 06/11/2017 | 6.87 | -9.87 | wildebeest | migrant | not_lactating |
| 31 | 540 | 07/11/2017 | 6.69 | -10.82 | wildebeest | migrant | not_lactating |
| 32 | 541 | 07/11/2017 | 6.35 | -10.51 | wildebeest | migrant | not_lactating |
| 33 | 544 | 08/11/2017 | 6.9 | -9.9 | wildebeest | migrant | lactating |
| 34 | 551 | 16/11/2017 | 9.27 | -9.66 | wildebeest | western corridor | not_lactating |
| 35 | 552 | 16/11/2017 | 8.03 | -9 | wildebeest | western corridor | not_lactating |
| 36 | 553 | 01/11/2017 | 7.91 | -9.8 | wildebeest | western corridor | lactating |
| 37 | 561 | 11/04/2018 | 9.89 | -13.57 | wildebeest | migrant | lactating |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 38 | 562 | 03/12/2018 | 6.92 | -10.28 | wildebeest | migrant | not_lactating |
| 39 | 563 | 03/12/2018 | 6.05 | -9.82 | wildebeest | migrant | not_lactating |
| 40 | 565 | 04/12/2018 | 7.1 | -10.09 | wildebeest | western corridor | not_lactating |
| 41 | 566 | 05/12/2018 | 6.04 | -10.51 | wildebeest | migrant | lactating |
| 42 | 474 | 16/05/2016 | 5.9 | -10.46 | zebra | western corridor | lactating |
| 43 | 513 | 17/02/2017 | 5.65 | -11.27 | zebra | western corridor | lactating |
| 44 | 537 | 05/11/2017 | 5.72 | -11.04 | zebra | migrant | not_lactating |
| 45 | 538 | 06/11/2017 | 5.39 | -10.81 | zebra | western corridor | not_lactating |
| 46 | 542 | 08/11/2017 | 6.22 | -11.09 | zebra | western corridor | not_lactating |
| 47 | 543 | 08/11/2017 | 5.44 | -10.39 | zebra | western corridor | lactating |
| 48 | 555 | 03/12/2017 | 5.32 | -10 | zebra | western corridor | not_lactating |
| 49 | 569 | 07/12/2018 | 5.41 | -10.93 | zebra | western corridor | not_lactating |
| 50 | 571 | 20/04/2019 | 6.04 | -11.51 | zebra | western corridor | not_lactating |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 51 | 572 | 20/04/2019 | 6.87 | -11.24 | zebra | western corridor | not_lactating |
| 52 | 459 | 20/05/2016 | 6.17 | -11.55 | zebra | western corridor | lactating |
| 53 | 490 | 25/09/2016 | 6.15 | -11.32 | zebra | carcass | not_lactating |
| 54 | 567 | 06/12/2018 | 6.09 | -11 | zebra | migrant | lactating |
| Total lactating | | | | | | | 19 |
| Total non-lactating | | | | | | | 35 |

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