

Ostrich Eggshell from the Far Eastern Steppe:
Stable Isotopic Exploration of Range, Commodification, and Extirpation

A Thesis Submitted to the Committee on Graduate Studies in Partial Fulfillment of the
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Abstract

Ostriches of the Far Eastern Steppe: Stable Isotopic Exploration of Range, Commodification, and Extirpation

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This study uses stable isotope analysis on both the organic and inorganic fractions of ostrich eggshell obtained from archaeological excavations in Mongolia, northern China, and southern Siberia. By establishing the $\delta^{13}\text{C}_{\text{org}}$, $\delta^{15}\text{N}$, $\delta^{13}\text{C}_{\text{inorg}}$, $\delta^{18}\text{O}$ isotopic compositions of the eggshell of the Asian ostrich (*Struthio asiaticus*), this study provides insight into the maximum northern range of the species, which I suggest reached Lake Baikal, Siberia through the late Pleistocene, up to the Last Glacial Maximum. Through these isotopic data, the interactions between *S. asiaticus* and human forager groups are explored, specifically the trade of ostrich eggshell by hunter/gatherer populations in the early/middle Holocene. Because of deviations from the correlation between $\delta^{18}\text{O}$ and latitude observed for other sites, the site of Shabarakh-usu may have been an aggregation point for ostrich eggshell from other locations. Movement of eggshell is observable from north to south but not from south to north on the basis of eggshell $\delta^{18}\text{O}$. Finally, I am able to develop a hypothesis regarding the drivers of species extinction. Specifically, I discuss trade in eggshell leading up to extinction as evidence for human pressure on *S. asiaticus* at a time when environmental shifts likely isolated populations in small regions of habitable landscape. I therefore implicate both changing environmental conditions and human pressure in my proposed explanation for the extinction of *S. asiaticus*.

Keywords:

Ostrich, *Struthio*, Steppe, Mongolia, Inner Mongolia, Siberia, East Asia, Pleistocene-Holocene Transition, Extinction, Megafaunal Extinction, Trade Networks, Stable Isotope Analysis, Biogeochemistry, Paleoenvironment

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“I think that, if required on pain of death to name instantly the most perfect thing in the universe, I should risk my fate on a bird’s egg.”

~Thomas Wentworth Higginson

Chapter 1: Introduction

In this study, samples of eggshell from extinct Asian ostrich (*Struthio asiaticus*) were analysed to determine their stable isotopic compositions. Samples were obtained from sites across the Far Eastern Steppe, including Siberia, Mongolia, and Northern China. The samples were grouped by three broad geographic designations: the north (Northern Mongolia and Siberia), the east (East Gobi and Eastern Steppe), and the south (South Gobi in Southern Mongolia and Northern China). The samples span a date range of >49,000 to 8,000 cal yr BP. The isotopic compositions of the organic carbon ($\delta^{13}\text{C}_{\text{org}}$), nitrogen ($\delta^{15}\text{N}$), inorganic carbon ($\delta^{13}\text{C}_{\text{inorg}}$) and oxygen ($\delta^{18}\text{O}$) of the eggshells were measured. The samples were obtained from human occupations and ranged from unmodified eggshell fragments (Fig. 1.1) to highly modified beads (Fig. 1.2).

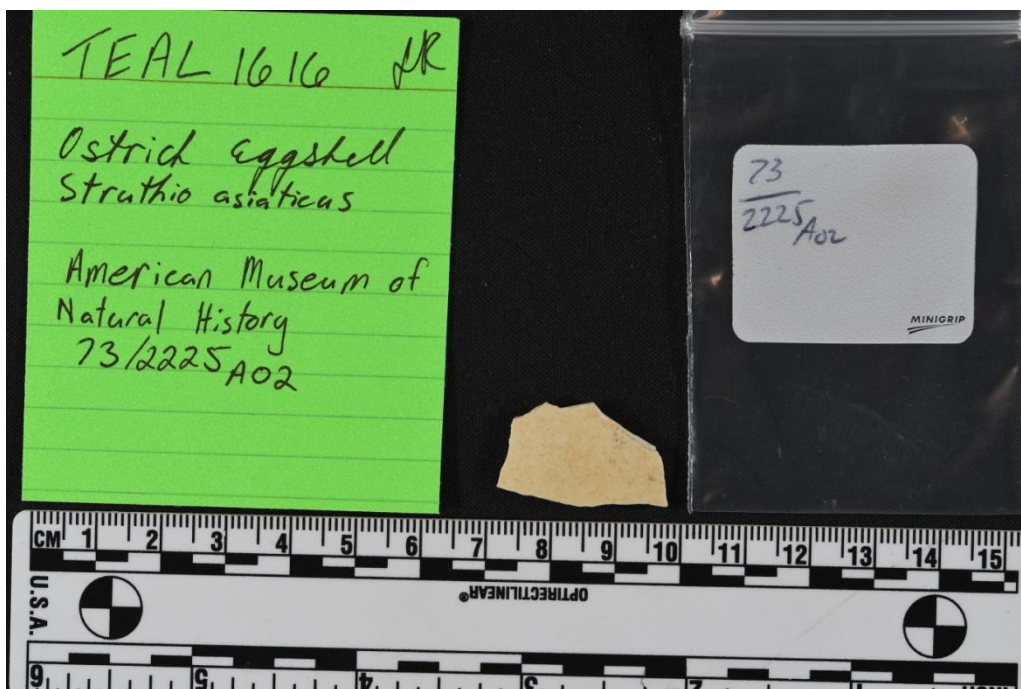


Figure 1.1: Example of an unmodified eggshell fragment.

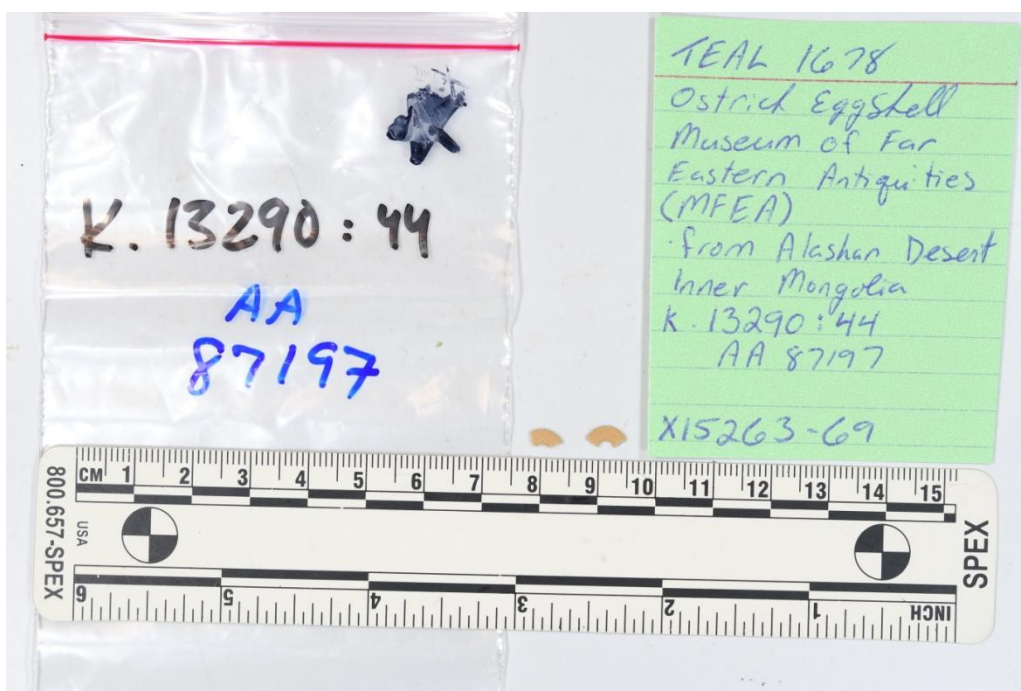


Figure 1.2: Example of eggshell bead sample.

This study addressed several research questions, governed by two broad themes: location and extirpation. The interpretations of the isotopic data required first asking whether the samples were local to the human occupation sites from which they were recovered. Given that the samples were not recovered from nesting sites (e.g., Newsome et al., 2011; Miller et al., 2005) and were therefore found in secondary locations, the question becomes, how far were they transported? This question was broken down into two, more specific, lines of inquiry:

1. Is the isotopic composition of samples recovered from Northern Mongolia and Siberia consistent with the environment of the north, thereby indicating that breeding ostriches extended as far north as Lake Baikal? Conversely, are these samples observed to originate outside the region, indicating significant relocation by foragers?
2. On the basis of eggshell isotopic compositions and known variation in regional isoscapes, is there evidence for anthropogenic movement of eggshell, potentially indicating trade networks?

The second theme of this study asked if the isotopic data could be used to elucidate the factors that contributed to the extirpation of the species from the steppe. Hypotheses informed by regional archaeological and palaeoclimatic literature were developed as a starting point for interpretation. The initial expectation was that the samples would be consistent with the locations of the human occupation sites and that there would not be evidence of trade. If evidence of trade was found, the expectation was that the northern samples would be of distant origin because it is counterintuitive that ostriches would have inhabited Northern Mongolia and the Trans-Baikal region. Today

these areas are characterized by forest-steppe and harsh winters averaging -24°C (Kharuk et al., 2013). The northern samples in this study date to the Late Pleistocene (approximately 26,000-44,500 cal yr BP), a time of less regional forestation but winter temperatures which averaged -17°C (Savinova, 2006). It would be significant to find that the eggshell samples had isotopic compositions that were consistent with a local northern origin as this would help to clarify the environmental tolerances and northern range of the species.

If evidence of trade was observed, it would help clarify the connections established among forager groups on the Far Eastern Steppe. Social networks established by hunter-gatherer groups have been inferred by the exchange of non-subsistence related materials, such as beads (Whallon, 2006) and ostrich eggshell has been identified as such a material in exchange networks in Africa (Stewart et al., 2020; Ndiema et al., 2010). These networks, either dyadic or less direct chains of connection, were based on reciprocity, allowing groups to create social safety-nets to improve resilience in lean times (Befu, 1977). Reciprocal social networks extend over vast distances and across a variety of ecosystems, facilitating access to a wider range of resources. It is rare to find direct evidence for forager social networks and tracking them spatially is significant for understanding past life ways.

The final theme addressed the extirpation of the Asian ostrich. My initial hypothesis was that the isotopic data would indicate a wet, possibly forested environment leading up to the loss of the species. These conditions would have driven extirpation through habitat loss and diminished egg hatchability (Gonzalez et al., 1999). The clarification of species extirpation is important not only for elucidating a specific instance

of megafaunal extinction, but also for creating strategies and improving communication related to conservation efforts directed toward extant African ostriches (Magige and Røskoft, 2017; Cooper et al., 2009).

1.2 Thesis Structure

This document is divided into seven chapters, including the Introduction. Chapter Two provides contextual background for the research. It begins by providing information on the climate and environment of the region during the periods relevant to the samples (i.e., Late Pleistocene through Early Holocene). The chapter also deals with ostrich ecology and the use of eggshell as an analytical substrate for stable isotope analysis. Chapter Three provides background on the principles of stable isotope analysis through discussion of each of the isotope systems utilised in this study. Chapter Four covers the methods used in the laboratory including pre-treatments, instrumentation, and analytical uncertainty. Chapter Five provides the results of the analyses and statistical comparisons of the data. Chapter Six, the discussion, has two foci, the first of which is the interpretation of the location of the samples, particularly pertaining to questions of northern extent and potential trade. Second, the chapter looks at what the isotope data reveal about the extirpation of ostriches from the Far Eastern Steppe. Finally, Chapter Seven concludes the thesis by summarizing the important findings of the study, discussing the limitations of the interpretation and explaining the significance of the research.

Chapter 2:

Environment, Ostrich Ecology and Eggshell

2.1: Regional Environment Through Time

This study utilises samples of ostrich eggshell from archaeological sites within the Far Eastern Steppe in what is now Mongolia, southern Siberia and northern China. Figure 2.1 gives an overview of the region as a whole, with site locations indicated. Climate in this region can be broadly characterised along a north-south transect, with consideration of the effects of elevation and hydrology on a more local scale (An et al., 2008; Ni et al., 2010; Feng et al., 2013). Calibrated dates for the samples in this study range from a radiocarbon-dead subset (i.e., greater than 45,000 BP) to the extirpation of *S. asiaticus* around 8,000 cal yr BP (Janz et al., 2015). This will be the temporal framework for the environment discussion below.



Figure 2.1: Sites of origin for archaeological eggshell samples: 1 Tri Skaly, 2 Podzvonkaya, 3 Ust'-Kyahta, 4 Bayangoliin am, 5 Tolbor, 6 Otson Tsokhio, 7 Chilian Hotoga Well, 8 Alkali Well, 9 Shara Murun Crossing, 10 Baron Shabaka Well, 11 Barun Daban, 12 Shabarakh-usu, 13 Mantissar. Sites 1-5 are classified as the northern region, 6-10 are the eastern region and 11-13 are the southern region. A Lake Baikal, B Lake Khubsugul, C Lake Gun Nuur. (Adapted from Snazzy Maps snazzymaps.com, Google Data SIO, 2020).

2.1.1: The Northern Region

Samples recovered from sites in northern Mongolia and Siberia date from approximately 26,000-44,500 cal yr BP (Table 2.1) and are therefore restricted to the late Pleistocene, up to and including the Last Glacial Maximum (LGM 26.5-20 ka, Clark et al., 2009). Precipitation in the northern region is dictated by the Westerlies, out of Central Asia (Herzschuh, 2006). A past hypothesis suggesting that periodic increases in moisture could be attributed to expanding and shifting monsoon has not been supported (Chen et al., 2008; Zhang et al., 2012). Chen et al. (2008) indicate that fluctuations in regional moisture are out of sync with moisture changes which occurred in regions known to be affected by the summer monsoon. Instead, it is likely that the moisture regime was a function of changing sea surface temperatures (SST) in the North Atlantic, with the Westerlies driving increased evaporative water westwards across Central Asia (Chen et al., 2008).

Arid Central Asia experienced cool and dry conditions, with high winds, during much of the late Pleistocene (Pei, 1985; Fedotov et al., 2004). Many lakes, throughout Mongolia were dry and larger bodies of water, like Lake Khubsugul (north of Bayangoliin am), were at very low levels, with high salinity toward the end of the Pleistocene (Fedotov et al., 2004; An et al., 2008). The pollen record for the central Lake Baikal region, taken from adjacent Lake Kotokel, indicates that the late Pleistocene, from 55,000 to 30,000 cal yr BP was a forest/steppe or forest/tundra environment (Shichi et al., 2009). Arboreal species included *Betula* sp. (birch), *B. nana* (dwarf birch), and *Alnus* sp. (alder). Herbaceous species included steppe and wetland plants in the genus *Artemisia*

(herbaceous plants and shrubs), and the Poaceae (grasses) and Cyperaceae (sedges) families.

During the LGM, the northern region had lower effective moisture and was a steppe or tundra ecosystem (Herzschuh, 2006). Evidence for arboreal species decreased to less than 5 % and pollen from herbaceous species dominated. Species including *Artemisia* and Poaceae were especially prevalent with additional increases in Rosaceae (rose family), Brassicaceae (mustard family) and Asteraceae (aster family) (Shichi et al., 2009).

Climate oscillations through the Older Dryas, Bølling Allerød and Younger Dryas were more impactful on the region than oscillations earlier in the Pleistocene (Fedotov et al., 2004). Subsequent to the LGM between 18,000-15,000 cal yr BP (Fedotov et al., 2004), deglaciation of the mountains north of Lake Baikal resulted in an influx of melt water to lakes in northern Mongolia and Siberia, as seen through increased carbonates in lake cores from Lake Khubsugul. Zhang et al. (2012) proposed that melt water from deglaciation may have been a significant factor in the increase of moisture to arid central Asia. Fedotov et al. (2004), moderates this interpretation, estimating that melt water would have increased the level of Lake Khubsugul by only about 15.2 m and would have had little impact on salinity. Lake levels continued to be low through the Bølling Allerød (approximately 14,700 to 12,900 cal yr BP based on stratigraphic timescale from the North Greenland Ice Core Project) (Rasmussen et al., 2006) relative to the Holocene (Fedotov et al., 2004). Nevertheless, from around 18,500 cal yr BP there was an increase in arboreal pollen as forestation took hold through the remainder of the Pleistocene, into the Holocene (Shichi et al., 2009).

Under the influence of the Westerlies, moisture was largely consistent from 12,100 to 2,700 cal yr BP with a brief increase between 7,500 and 6,800 cal yr BP (Herzschuh, 2006). It is understood that the Holocene was a moist environment relative to the Pleistocene, however, the timing of moisture increase is not universally agreed upon. A multi-proxy study conducted by Zhang et al. (2012) at Lake Gun Nuur, presents evidence for a wet regime through Mongolia at the onset of the Holocene. Feng et al. (2013) present opposing evidence from Lake Gun Nuur, indicating a cool, dry transition into the Holocene, with moisture increasing through the mid-Holocene. A moderate view indicates a dry initial Holocene across arid central Asia, with moisture increasing through the early and middle Holocene (Chen et al., 2008).

Between 11,500 and 9,000 cal yr BP there was rapid expansion of the Siberian peatlands (Smith et al., 2004). While peatlands are an effective carbon sink, they also became an important global source of atmospheric methane, causing feedback that amplified climate warming (Smith et al., 2004). Atmospheric feedbacks including increased methane and CO₂, led to increasing temperatures in the early Holocene (Richerson et al., 2001; Smith et al. 2004). This set the stage for increased plant productivity and forestation, with tree cover estimated to have been about 25 % of the total plant biomass in the early Holocene (Richerson et al., 2001; 000 et al., 2013). By 8,500 to 7,500 cal yr BP the region was under widespread, well established taiga forest (Tarasov et al., 2007).

2.1.2: East Gobi/Eastern Steppe

Two time periods are represented from the eastern extent of the study region. Samples from the desert-steppe transitional zone, in present day eastern Mongolia, were dated to approximately 35,700-37,700 cal yr BP. The majority of the samples were retrieved from archaeological sites in the East Gobi, in the Inner Mongolia Autonomous Zone, People's Republic of China (PRC) and date from approximately 10,700-17,400 cal yr BP placing them subsequent to the LGM, at the Pleistocene/Holocene transition.

The moisture regime of the eastern region is synchronous with the East Asian Monsoon (Wang and Feng, 2013). From 50,000 cal yr BP to the LGM, monsoonal Central Asia was relatively wet, with increasing aridity through the LGM, followed by amelioration during the terminal Pleistocene and early Holocene (Herzschuh, 2006). Pollen biomisation evidence suggests that, during the LGM, the eastern region was cold, with areas of evergreen, needle leaf forest (Ni et al., 2010), though pollen may travel significant distances, making pollen evidence regional rather than local. It is likely that, through the LGM, vegetation was unstable with significant expansion of sandy desert. (Lu et al., 2013).

As the region transitioned into the Holocene, the desert soil stabilized due to increased vegetation (Lu et al., 2013). The transition to the Holocene climate was warmer and wetter than it is currently and arboreal vegetation was dominated by pine, with increasing evidence for steppe, through plants such as *Artemisia* and *Ephedra* (Shi and Song, 2003). Through the early/middle Holocene there was a decrease in arboreal pollen as the landscape shifted to an arid desert/steppe with fewer plants overall (Shi and Song,

2003). Through the early Holocene, eastern Inner Mongolia was a forest-steppe with the principal arboreal taxa including *Pinus* (pine), *Picea* (spruce), *Abies* (fir), *Betula* (birch), *Carpinus* (flowering birch), *Quercus* (oak) and *Ulmus* (elm) (Shi and Song, 2003; Zhao et al., 2009). The herbaceous taxa were comprised of *Artemisia*, *Ephedra*, Chenopodeaceae (goosefoot family), Compositae (daisy family), Poaceae (grasses), Cyperaceae, Polygonaceae (knotweeds) Leguminosae (legumes), Labiatae (mints), *Nitraria* (kharmag or duneberries), Umbrelliferae (carrot/parsley family), and Ranunculaceae (meadow-rue family, e.g. butter cup) (Shi and Song, 2003). The abundance of plant-life, particularly the presence of multiple arboreal species speaks to the relative increase in effective moisture present in the early Holocene.

2.1.3: South Gobi

Samples from the South Gobi include samples from both the Pleistocene and the Holocene. One subset from Mantissar, in the Gurnai Depression is comprised of samples which are radiocarbon dead at greater than 45,000 BP. Other samples from the region span a date range of approximately 8,100-44,000 cal yr BP, spanning the late Pleistocene to the early Holocene Climatic Optimum. There is an absence of samples from the LGM.

Lake levels are synchronous with glaciation events and from 45,000 to 25,000 cal yr BP the area was a catchment for high altitude glacial melt water (Wünnemann et al., 2007). After the LGM, the region was impacted less by melt water and more by the summer monsoon. Moving into the mid-Holocene there is only intermittent effects from monsoon systems as the region shifted to be more synchronous with the Westerlies (Wünnemann et al., 2007).

The late Pleistocene, prior to the LGM was a warm wet forest/steppe environment with both coniferous and broadleaf trees including pine, spruce, oak, birch and elm at higher elevations and around water sources. Chenopodiaceae was characteristic of the low altitude deserts, indicating a moister steppe environment than in modern times (Liu et al., 2002; Shi and Song, 2003; Herzschuh and Liu, 2007). Loss of permafrost to the north allowed flowing water to cut rivers through the Gobi Desert landscape (Grunert et al., 2000). Subsequent to the LGM dry conditions ameliorated somewhat, likely as a result of monsoon influence (Herzschuh and Liu, 2007). From approximately 19,000-17,000 cal yr BP the vegetation profile was that of steppe and alpine forest; however vegetation through this period is unstable (Herzschuh and Liu, 2007).

The monsoon boundary persisted into the South Gobi through to the middle Holocene (Lee et al., 2013). Rivers fed wetlands, which were present across the Gobi Desert (Janz et al., 2016). Pollen evidence from the early Holocene (10,700 to 5,400 cal yr BP) indicates an arid environment with desert plants like Chenopodeaceae, *Artemisia*, and *Ephedra* (Herzschuh et al., 2004). There is evidence for arboreal taxa such as pine, spruce, and elm around lakes (Yang and Williams, 2003). Effective moisture was higher in the early Holocene but was interrupted by a cool/dry phase from 8,500 to 7,500 cal yr BP (Felauer et al., 2012).

Table 2.1: Summary table sample dates and environments

Region	Sample Radiocarbon Age Range (^{14}C yr BP)	Date Range (cal yr BP)	N	Sites	Environment
North	31,427 \pm 177 - 40,103 \pm 457	34,860-44,560	8	Tolbor, Bayangoliin am, Podzvonkaya, Tri Skaly, Ust'-Kyahta	Forest/Steppe or Forest Tundra: cool and dry with high winds. Low lake levels
North	21,845 \pm 79	25,875-26,255	1	Tolbor	Tundra or Steppe: Reduced arboreal species, <i>Artemesia</i> and <i>Poaceae</i> prevalent
East	32,366 \pm 193 - 32,890 \pm 196	35,780-37,700	4	Otson Tsokhio	Desert/Steppe: relatively wet. Under East Asian Monsoon Regime
East	12,450 \pm 74 - 14,129 \pm 80	14,200-17,475	4	Shara Marun Crossing, Baron Shabaka Well	Desert/Steppe: relatively wet. Under East Asian Monsoon Regime
East	9,562 \pm 51 - 10,586 \pm 56	10,710-12,685	3	Alkali Well, Chilian Hotoga Well	Desert/Steppe: arboreal species present (pine), warm and wet
South	>46,000		5	Mantissar	Undetermined
South	30,490 \pm 780 - 38,600 \pm 1000	32,985-44,345	2	Shabarakh-usu	Warm and wet: broadleaf and needle leaf arboreal species. Steppe at lower elevation
South	14,857 \pm 85	17,855-18,310	1	Mantissar	Improved moisture, unstable steppe with alpine forest
South	7483 \pm 47 - 8522 \pm 50	8,195-9,550	13	Shabarakh-usu, Barun Daban	Desert steppe. Arboreal taxa around lakes.

2.2: Hunter Gatherers of the Far Eastern Steppe

There is little in the way of physical human remains of early hunter/gatherers from the Far Eastern Steppe and occupations are usually located through lithic surface scatter (Janz et al., 2017). The Lake Baikal region is considered the origin of microblade technology in the Far Eastern Steppe with southward dissemination into Northern China before the LGM (Nian et al., 2014). Scant material culture with wide gaps in dates result in the interpretation of late Pleistocene sites being occupied for short, sporadic intervals (Janz et al., 2017). Faunal assemblages indicate exploitation of large bodied herbivores such as mammoth and antelope (Janz et al., 2017; Nian et al., 2014).

During the LGM hunter/gatherers were likely to have receded from the Gobi, however, during the transition into the Holocene, established wetland/dunefield ecosystems were regularly, though not exclusively, utilised (Janz et al., 2017). Considered the Oasis 1 culture, hunter/gatherers continued to move regularly. Lithic technology was often expedient and informal. Pottery did not appear on the Far Eastern Steppe until 9,685-9,500 cal yr BP and was uncommon (Janz et al., 2015). Coincident with the extirpation of ostriches and the moisture/vegetative optimum, around 8,000 cal yr BP, utilisation of oasis habitation sites became more established and intensive (Janz et al., 2015). This transition marked the beginning of the Oasis 2 culture, with elaboration of plant-processing technologies including large grinding stones (Janz et al., 2017). The changes in life-ways associated with Oasis 2 helps confirm the adoption of broad-spectrum foraging subsistence strategies including greater exploitation of smaller, faster animals (Janz et al., 2017). All-in-all, over the period of the study, hunter/gatherers likely

transitioned from a highly mobile lifestyle and short-term habitations, to longer, more settled occupations.

2.3: Model of Megafaunal Extinction

Guthrie (1984) proposed a model for megafaunal extinction connected with the deterioration of the mammoth steppe. Through the LGM the mammoth steppe was an extensive biome spanning much of the breadth of the northern hemisphere with plant and animal biomass rivaling the African savannah (Zimov et al., 2012). In this model he proposed that climate change caused increased seasonality, a circumstance which reduces vegetative diversity. Rapid climate change and increased seasonality can limit the range of plant species and truncate the growing season, limiting the availability of edible vegetation for certain types of specialized, large-bodied herbivores (Morueta-Holme et al., 2013; Guthrie, 1984). This enhanced seasonality and vegetative change led to a significant loss of faunal diversity as well as a decrease in body size and range distribution (Guthrie, 1984).

Woolly mammoth (*Mammuthus primigenius*) was common and globally widespread until 12,000 BP after which the species ranged in more limited refugia through the Arctic, Siberia, and areas of eastern Europe until about 9,000 BP (Kuzmin, 2010), persisting in an isolated population on Wrangel Island in Siberia beyond 4,000 BP (Vartanya et al., 2008). Losses of large-bodied herbivorous species have cascading effects on landscape ecology with the absence of landscape modifying species like mammoth contributing to increased forestation (Janz et al., 2016). Analogous effects were seen in the area of Tsavo, Kenya, where trade in ivory led to a decrease in the

elephant population (Peterhans and Gnoske, 2001). Without elephants the vegetation pattern changed from grassland to dense woody and thorny plants, influencing the loss of ungulate biodiversity (Peterhans and Gnoske, 2001). Effects such as these cause habitat fragmentation which further isolate populations off from one another, limiting reproductive potential which frequently leads to extinction (Guthrie, 1984; Fahrig, 2003). Fragmentation of preferred ostrich habitat may have hampered species viability and factored into extirpation.

2.3.1 Application of the model to the current study

When the Asian ostrich (*S. asiaticus*) occupied the Far Eastern Steppe, this area supported a faunal mosaic without modern analogue, including: spotted hyena (*Crocuta crocuta*), red deer (*Cervus elaphus*), giant elk (*Megaloceros sp*), woolly rhinoceros (*Coelodonta antiquitatis*), Przewalski's horse (*Equus przewalskyi*), and Asiatic wild ass (*Equus hemionus*) (Pei, 1985). Climate oscillations through the mid-Pleistocene do not seem to have caused the widespread disruption to biome coherence or to have impacted sympatric species diversity on a regional level, to the extent that was observed at the Pleistocene-Holocene transition (Guthrie, 1984; Ni et al., 2010; Janz et al., 2016). Increased landscape fragmentation resulted in steppe that was separated by desert, wetland/dunefield interactions and forests (Janz et al., 2016).

This fragmentation of the landscape may have similarly fragmented ostrich populations causing population bottlenecks that may have been deleterious due to loss of genetic variability through inbreeding (Nei et al., 1975; Wright, 1931). Changes in the climate

and habitat restriction may also have impacted fertility through loss of egg hatchability, as will be explained below (Gonzalez et al., 1999).

2.4: Ostrich Ecology

2.4.1: Evolution and Habitat

Ostriches belong to the genus *Struthio* and are descendants of theropods (Mikhailov, 1992). Fossil bone and eggshell indicate the ancestor of the ostrich was a small, flightless bird that evolved on the Eurasian steppe in the Eocene and by the Pliocene had evolved into a large-bodied ratite (Bibi et al., 2006). Analysis of eggshell microstructure supports a Eurasian origin for ostriches with eventual dispersal into Africa, and ultimate distribution across much of Asia and Africa (Mikhailov, 1992). Modern ostriches evolved about 2 million years ago and have become increasingly gracile over time (Bibi et al., 2006). Lowe (1931) proposed recognising the Pleistocene *Struthio* species of China and Mongolia as two taxa: *S. anderssoni* in China and *S. mongolicus* in Mongolia on the basis of a difference in body size inferred through eggshell thickness. The species is now collectively classified as *S. asiaticus*. Evidence for *S. asiaticus* is found in India, People's Republic of China, Mongolia, and Siberia (Janz et al., 2017; Blinkhorn et al., 2015). There is also evidence for a species of *Struthio*, morphologically similar to *S. asiaticus* in Turkey (Janoo and Sen, 1998). It is approximated that *S. asiaticus* was 20 to 50 % more robust than common modern ostrich (*S. camelus*) though the two species are similar in height (Blinkhorn et al., 2015).

Extant ostriches occupy habitats ranging from semi-arid savannah and desert grassland to coastal regions, with a strong preference for short-grass plains and semi-

desert (Cooper et al., 2009). Habitats with grasses exceeding 1 m in height and dense woodland are selected against (Cooper et al., 2009). Paleontological evidence from a past expansion of ostriches into India confirms that evidence for *Struthio* is limited to the semi-arid ecosystem (Blinkhorn et al., 2015). It can, therefore, be inferred that ostriches preferentially select for arid steppe, savannah, and grassland habitats with little environmental plasticity.

2.4.2: Ostrich Foraging Ecology

Suggestions that wild ostriches are opportunistic feeders, consuming any and all C₃, C₄, and crassulacean acid metabolism (CAM) plants in their environment, were refuted by isotopic analysis which has shown that ostriches will consume C₄ grasses when new growth is abundant but have a strong preference for C₃ browse and actively select against CAM plants (Von Schirnding et al., 1982; Johnson et al., 1998; Segalen et al., 2002). Von Schirnding et al. (1982) found that within an ecosystem of mixed vegetation, C₄ plants comprised only a small part of the diet and CAM plants were not consumed. This suggests that $\delta^{13}\text{C}$ of ostrich eggshell samples are best interpreted as an indication of the availability of shrub browse in the environment and should not be used as a proxy for the proportion of C₄ and CAM plants in the ecosystem (Von Shirnding et al., 1982).

Extant ostriches are broadly herbivorous with approximately 4-5 % of the diet comprised of animal foods such as insect eggs and small mammals (Aganga et al., 2003). Stomach contents of *S. camelus* have revealed bones, teeth, and shell fragments (Milton et al., 1993). There is lack of agreement on the foraging patterns of ostriches with some

researchers classifying the species as omnivorous (Endo et al., 2010) and others classifying them as herbivorous, with limited intake of animal foods (Hove and Mukaratirwa, 2005). It has been demonstrated, however, that juvenile ostriches practice insectivory to fortify the protein contribution to their diet (Milton et al., 1993). Some ratites increase the animal component of their diet during the mating season (Newsome et al., 2011). The degree and timing of omnivory in *S. asiaticus* cannot be directly observed, but the possibility of omnivorous consumption, particularly around the time of eggshell formation is possible, if not likely.

Ostriches digest through hindgut fermentation, which allows them to maximise the utility of their low nutrient diet (Swart et al., 1993; Johnson et al., 1998). Hindgut fermentation utilizes large chambers, posterior in the digestive tract, where digestion is aided by a symbiotic relationship with the gut's microflora (Swart et al., 1993; Sponheimer et al., 2003). Differences between foregut and hindgut fermentation can impact $\delta^{15}\text{N}$, causing herbivores with foregut digestion to appear to be a trophic level above herbivores with hindgut digestion (Sponheimer et al., 2003). Microflora situated in the hindgut tends to be excreted from the digestive tract on a regular basis, preventing the elevation in $\delta^{15}\text{N}$ produced by foregut fermentation (Sponheimer et al., 2003). Johnson et al. (1993) reported the diet to tissue offset for $\delta^{15}\text{N}$ in ostrich eggshell as 3.3 ± 0.1 ‰ above the diet, which is in line with the majority of studies quantifying trophic discrimination factors in other vertebrate tissues (Post, 2002).

2.4.3: Water Consumption

As part of their adaptation to arid environments, ostriches are non-obligate drinkers, meaning they obtain adequate hydration through the consumption of their leafy diet (Johnson et al., 1998; Kingston, 2011). Johnson et al., (1998) found a linear correlation between imbibed water and ostrich eggshell $\delta^{18}\text{O}$, in controlled environments. Evapotranspiration results in significant fluctuation in leaf water $\delta^{18}\text{O}$ over the course of a day (Landais et al., 2006). The lowest $\delta^{18}\text{O}$ in leaf water will be observed early in the day and the highest $\delta^{18}\text{O}$ will be observed at the hottest, driest point, around midday (Landais et al., 2006). Ostriches are, therefore, considered evaporation sensitive animals and the $\delta^{18}\text{O}$ in their eggshells should more clearly differentiate changes in effective environmental moisture from fluctuations in the amount of meteoric water (Levin et al., 2006). That said, the assumption that $\delta^{18}\text{O}$ of ostrich eggshell is entirely reflective of leaf water may be overly simplistic given that, in the presence of adequate water sources, ostriches will drink frequently (Cooper et al., 2009). This change in behaviour should have an effect on the $\delta^{18}\text{O}$ observed in the eggshell.

2.5: Ostrich Reproductive Behaviour and Eggshell as an Analytical Substrate

A single male ostrich will father a clutch of eggs, which is contributed to by a harem of two to seven females (Von Schirnding et al., 1982). Dominant females lay their eggs within the territory of a single male, while secondary females may range between the territories of multiple males, potentially integrating the isotopic compositions of a more varied environment (Hodgkins et al., 2018). There may be as many as 30 to 40 eggs laid in a clutch (Von Schirnding et al., 1982). Only about 20 eggs will be incubated

while the excess eggs are pushed out, potentially retaining more eggs from the dominant hen (Von Schirnding et al., 1982). A single clutch may demonstrate a significant amount of isotopic variation, since eggs are contributed over several days by multiple females (Von Schirnding et al., 1982).

Ostriches breed subsequent to the rainy season, at a time of year when resources are adequate to support reproduction (Kingston, 2011). The new growth produced by the rainy season is of higher nutritive value and has a lower anti-herbivory toxin load relative to older growth (Guthrie, 1984). It is therefore the case that most herbivore growth occurs coincident with new plant growth, making it reasonable to breed at this time as well (Guthrie, 1984). It should be noted, therefore, that the environment impacting the isotopic compositions of the egg, reflects conditions specific to the days of shell accretion rather than the long-term averages provided by tissues like bone collagen which have protracted turnover (Hedges et al., 2007; Kingston, 2011).

An ostrich egg is produced over a 3 to 5 day period (Kingston, 2011). There is no evidence that calcium is remobilized from the bone tissues of birds during the production of eggshell, nor is there evidence of any impact from limestone grit, which the ostrich may consume during the mating season (Von Schirnding et al., 1982; Blum et al., 2001). The isotopic compositions of ostrich eggshell ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) have been studied and found to be in equilibrium with the bird's body for the 4 days of accretion (Johnson et al. 1995). It can, therefore, be assumed that ostrich eggshell reflects the diet consumed within the tissue accretion interval, with little lag time or averaging beyond the spatial and temporal parameters of production (Hobson et al., 1997; Johnson et al., 1998; Blum et al., 2001).

The composition of ostrich eggshell is 95 % inorganic, predominantly calcium carbonate (CaCO_3), 3.5 % organic matter and 1.5 % water (Von Schirnding et al., 1982; Dauphin et al., 1998). Ostrich eggshell is accreted from the shell membrane toward the outer surface and upon completion, is thick relative to the eggshells of non-ratite birds (Mikhailov, 1992, Harrison and Msuya, 2005, Blinkhorn et al., 2015). The pore apertures are 20 to 50 μm and eggshells can approach 2 mm in thickness (Von Schirnding et al., 1982; Gonzalez et al., 1999). Thick eggshells contain pore complexes which begin on the interior of the shell and branch as they extend toward the outer surface, forming pore pits (Ar et al., 1974; Von Schirnding et al., 1982; Blinkhorn et al., 2015).

Pores, as well as cracks and fissures in the shell, are the most susceptible to post-depositional alteration, through the incorporation of sediments, molecular exchange, and recrystallization (Dauphin et al., 1998; Hoppe et al., 2003). Microscopic examination of fossil eggshell has revealed good preservation and little alteration, provided visually apparent contamination is avoided when sampling (Dauphin et al., 1998, Hoppe et al., 2003). Many contaminants, such as burial sediments, can be removed through mechanical abrasion and chemical pre-treatment (Segalen et al., 2002; Hoppe et al., 2003; Kingston, 2011; Blinkhorn et al., 2015).

The morphology and arrangement of eggshell microstructure is largely biologically predetermined and analysis of microstructure has been used to taxonomically identify species with greater accuracy than macrostructural elements (Mikhailov, 1992). Over the course of ostrich evolution, the trend has been toward thinner shells with decreasing thickness, which has been interpreted as a derived trait in progressively modern ostriches (Mikhailov, 1992; Harrison and Msuya, 2005, Blinkhorn et al., 2015).

Environmental conditions also seem to play a proximal role in shell thickness. Modern ostrich eggshells are observed to be thicker when there is greater availability of water and high-quality food (Harrison and Msuya, 2005). The observation of decreasing shell thickness from the late Miocene into the Pleistocene is correlated with increasing aridity and may be an adaptation to the environmental conditions (Mikhailov, 1992). Overall, pore morphology and shell thickness seem to be optimised to maximise hatchability within specific climatic conditions.

Ostriches have relatively low hatch rates, which appears to be tied to conductance of water vapour and respiration (Ar et al., 1974; Gonzalez et al., 1999; Hassan et al., 2004). Ostrich eggs should lose 13 % to 15 % of their initial weight, over the course of incubation, through water loss, across the shell, through the pores (Ar et al., 1974; Gonzalez et al., 1999, Hassan et al., 2004). Ratite eggs are large and lose less water, relative to their size, than smaller eggs (Ar et al., 1974). This is associated with the observation that larger eggs have longer incubation periods, subjecting them to a protracted period of water loss (Ar et al., 1974). Water vapour, carbon dioxide, and oxygen, diffuse across the shell, bringing in what is needed and respiring out waste (Gonzalez et al., 1999). If the egg does not release sufficient water, the embryo may suffocate in the final days of incubation (Gonzalez et al., 1999).

Commercial ostrich producers have observed improved hatch rates from eggs with thinner shells and high numbers of large pores per square centimetre (Gonzalez et al., 1999). Ideally, the incubation temperature should be maintained between 35.0 and 36.9°C and the humidity maintained below 25 % (Hassan et al., 2004). An increase in temperature from 36.0 to 37.2°C corresponds with a decrease in hatchability from 73 %

to 44 % (Hassan et al., 2004). This indicates a narrow range of environmental conditions outside of which fertility is impacted. Given that ostrich eggshell is adapted to maximise hatchability in an arid environment, it may be the case that an increase in relative humidity and temperature may have impacted the reproductive capacity of ostriches on the Eurasian steppe in the early Holocene (Mikhailov, 1992; Cooper et al., 2009). The increase in effective moisture, in many regions, during the early Holocene may be a driver of ostrich extirpation from the steppe and this increase in moisture may be observed isotopically in the eggshell samples.

Chapter 3:

Stable Isotopes in Terrestrial Environments

3.1 Overview of Stable Isotope Analysis

The statement “you are what you eat, plus a few per mil” (DeNiro and Epstein, 1976) is a phrase so common in isotope science it has become a cliché. It resonates because of its simple articulation of the principals underpinning a suite of complex chemical processes. Plant and animal tissues incorporate their environment, through physiological processes related to consumption. Archaeologists use the isotopic compositions of plant and animal tissues to interpret past environments, climate change, and to assess migration and trade. In this project I employ analysis of nitrogen, carbon, and oxygen isotopes, which have been successfully used in reconstructions of movement and palaeoenvironment, particularly related to precipitation (Bowen et al., 2005), aridity (Bar-Mathews and Ayalon, 2011), temperature and vegetative composition (Johnson et al., 1997).

This project involved the isotopic analysis of the organic and inorganic fractions of archaeologically recovered ostrich eggshell to investigate questions of ostrich ecology

and human interaction. Ostriches are no longer present in this region. Their extirpation is likely to have occurred by 8,000 cal yr BP (Janz et al., 2009). Around the time of ostrich extirpation, the region underwent climatic and environmental changes that may have impacted the viability of the species (Janz et al., 2009; Zhang et al., 2012). Analysis of stable isotopes of nitrogen, carbon, and oxygen may shed light on a number of questions regarding the environment and stresses related to climate change, which may have factored in the extirpation of this species. It is, therefore, necessary to understand how these isotopes can be used to reconstruct past environments.

Stable isotope compositions are expressed as a ratio of heavy to light isotopes (Koch et al, 2009). The elements used in this study are carbon, nitrogen, and oxygen which are presented as isotope ratios $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, and $^{18}\text{O}/^{16}\text{O}$ respectively. Delta notation using the symbol δ is used to compare the isotope ratio of the sample with a known standard using the equation:

$$\delta = \left(\frac{R_x}{R_{std}} - 1 \right)$$

Where R represents the ratio of the heavy isotope to the light, x indicates the sample, and std indicates the standard (Sharp, 2017). These ratios are expressed in per mil (‰). For carbon and oxygen, the values are expressed relative to the standards Vienna Pee Dee Belemnite limestone (VPDB) and nitrogen is expressed relative to atmospheric N_2 or ambient inhalable reservoir (AIR).

The ratios of these stable isotopes vary among different parts of the biosphere, atmosphere, hydrosphere and geosphere because of a process known as fractionation.

Fractionation takes place when the bonds between elements are broken, often but not always, leaving the products relatively depleted of the heavier isotope compared to the reactant (Sharp, 2017). The processes resulting in fractionation may be reversible, as in the phase changes of water, or irreversible, as is the case when amino acids are synthesized and incorporated into body proteins (Sharp, 2017). Through various environmental and metabolic processes, the isotopic ratios of materials are indicative of climate, environment, diet source, and food-web structure (Schwarcz et al, 2010).

3.2 Stable Isotopes and Environmental Reconstruction – $\delta^{15}\text{N}$

Nitrogen isotopes are analysed from the organic fraction of tissues, including eggshell (e.g.: Johnson et al, 1997; Emslie and Patterson, 2007). The $\delta^{15}\text{N}$ of consumer tissues can be used to indicate trophic position and assess changes in food-web structure through an increase of approximately 3.4 ‰ with each trophic level increase (Post, 2002). Prior to attempting any trophic assessments, however, the nitrogen cycle at the base of the food-web must be understood because this baseline changes significantly with differences in environment and climate (Ambrose, 2000) as well as specific digestive strategies which may result in significant differences in $\delta^{15}\text{N}$ in animals consuming different vegetation within the same environment (Codron et al, 2012) .

Water availability is an important driver of variation in $\delta^{15}\text{N}$. Decreasing effective moisture in the environment is associated with an increase in $\delta^{15}\text{N}$, allowing paleoenvironmental researchers to monitor past increases and decreases in precipitation and regional water availability (Sealy et al., 1987). The early explanation for the correlation between water stress and $\delta^{15}\text{N}$ hinged on a supposed response in animal

physiology, wherein water is conserved and isotopically light urea is excreted (Ambrose, 2002). Subsequent research has shown that high animal $\delta^{15}\text{N}$ in arid conditions occurs independent of excretion and that it is in fact a product of interactions between plants and soils (Murphy and Bowman, 2006).

In arid and semi-arid environments, the nitrogen cycle is more open, with nitrogen being redirected from the soil to the atmosphere through ammonia volatilisation and denitrification (Handley et al, 1999). Fractionation occurs with these processes, resulting in a greater loss of ^{14}N to the atmosphere, leaving proportionally more ^{15}N in the soil (Handley et al., 1999). This produces elevated $\delta^{15}\text{N}$ in the biomass.

Interactions between plants and mycorrhizal fungi have an influence on $\delta^{15}\text{N}$. The majority of plants form symbiotic interactions with mycorrhizal fungi which make various micronutrients, including nitrogen, available to the plant (Read, 1991). Due to retention of ^{15}N on the part of mycorrhizae, symbiotic relationships between plants and these fungi generally result in lower $\delta^{15}\text{N}$ in associated plants, with the effect varying depending on the specific mycorrhiza involved (Hobbie and Högberg, 2012). Fungal profiles correlate with differences in vegetative composition; each interacting with nitrogen to produce a distinguishable isotopic ratio within primary production.

Arbuscular mycorrhizae predominate in open environments, while temperate and boreal forested environments are dominated by ectomycorrhizae (Read, 1991).

Ectomycorrhizae leave a lower $\delta^{15}\text{N}$ signature in the plants associated with them, relative to plants associated with arbuscular mycorrhizae (Hobbie and Högberg, 2012).

Environmental change resulting in a shift from grassland to forest or vice versa, may be

visible in the $\delta^{15}\text{N}$ data obtained from tissue samples of consumers within the environment.

The efficacy of using $\delta^{15}\text{N}$ in environment reconstruction is well established. The early study of $\delta^{15}\text{N}$ in consumer bone collagen demonstrated the ability to differentiate forest dwelling from savannah ranging species and also observed differences between obligate and non-obligate water consuming species, wherein the non-obligate drinkers presented $\delta^{15}\text{N}$ values 2-4‰ above the obligate drinkers (Ambrose, 1986). Through analyses of red deer bone collagen, Drucker et al. (2003) observed changes in $\delta^{15}\text{N}$ associated with vegetation changes through the Pleistocene/Holocene transition, where increased vegetation intensified the nitrogen cycle, causing higher $\delta^{15}\text{N}$ in herbivore bone collagen. The inverse relationship between $\delta^{15}\text{N}$ and aridity was employed in the study of ostrich eggshell from Equus Cave in South Africa to help reconstruct precipitation and temperature through the late Pleistocene and early Holocene (Johnson et al., 1997). In all of these cases the $\delta^{15}\text{N}$ was used in concert with either $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ as has been done in this study.

3.3 Stable Isotopes and Environment Reconstruction – $\delta^{13}\text{C}$

Carbon isotope compositions can be determined for both the organic and inorganic fractions of biological tissues (Schwarcz, 1991). In the case of ostrich eggshell, carbon is incorporated into both fractions through diet, however, due to differences in routing, there is an offset causing the inorganic fraction to be higher than the organic by 14 ± 2 ‰ (Johnson et al., 1997). The trophic discrimination of organic carbon is minimal, around 0-1 ‰ (DeNiro & Epstein, 1978) meaning carbon is more useful as an indicator

of the ultimate sources of primary production than of trophic position. There is a further diet to tissue offset for $\delta^{13}\text{C}_{\text{org}}$ of 1.5 ± 0.8 ‰ in ostrich eggshell (Johnson et al., 1998). Interactions between plants and their environment drive much of the resultant variation in $\delta^{13}\text{C}$ observed in consumer tissues (Farquhar et al., 1989). The $\delta^{13}\text{C}$ of plant and animal tissues is, therefore, a useful proxy of environmental conditions. Variation in $\delta^{13}\text{C}$, in primary production, occurs as the result of differences in photosynthetic pathway, the openness of the landscape, and aridity.

Plants utilize three different carbon fixation mechanisms during photosynthesis: C_3 , C_4 and CAM. Photosynthesis evolved early in Earth's history, first with an anoxic form which produced energy using elements other than oxygen (Hohmann-Marriott and Blankenship, 2011). Modern photosynthesis produces sugars for the plant and generates oxygen, as a by-product, through the decomposition of water, liberating electrons which can be used by the plant to build and repair cells (Hohmann-Marriott and Blankenship, 2011). Most plants, including most trees and shrubs, produce photosynthates through the C_3 (Calvin-Benson) cycle, without additional carbon fixation mechanisms (Osborne and Sack, 2012). In this process, atmospheric CO_2 is taken in through leaf stomata, where it is converted to an intermediary three-carbon compound, 3-phosphoglyceric acid (3-PGA) through the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), thus giving these plants the C_3 designation (Farquhar et al, 1980).

The C_4 process involves a specialized leaf morphology and additional steps relative to the C_3 pathway. It has evolved more than 60 times as an adaptation for plants in arid open environments, (Hatch, 1987; Sage et al., 2011). Through the C_4 photosynthetic pathway, plants convert atmospheric CO_2 to a four-carbon acid, in the

mesophyll cells through phosphoenolpyruvate carboxylase (PEPC) (Hatch, 1987). The four-carbon molecule, malate or aspartate (Hatch & Slack, 1966; Meister et al, 1996), is transported to the bundle sheath cells, converted back to carbon dioxide and concentrated around RuBisCO and the plant completes photosynthesis. Plants in conditions of low atmospheric CO₂, open landscapes, and high temperatures experience hydraulic stress (Osborne & Sack, 2012). The sequestration of carbon in the bundle sheath cells allows the plant to control the degree and duration of openness of stomatal apertures and decreases the probability that RuBisCO will use O₂ as a substrate, thereby controlling photorespiration and increasing water efficiency (Osborne and Sack, 2012).

The least common form of photosynthesis is CAM, a process used by desert plants such as agave. Like C₄ plants, CAM plants utilize PEPC for CO₂ capture with photosynthesis being completed via RuBisCO as is done via the C₃ pathway (Borland et al., 2009). While C₄ plants utilize adaptive structures in the leaves to concentrate CO₂ around RuBisCO, CAM plants undertake the entire process in the mesophyll, using time to separate the reactions i.e., carrying out the PEPC sequestration of CO₂ at night while the stomata are open and converting CO₂ into sugars in the daylight, while the stomata are closed. By conducting photosynthesis in this way, CAM plants greatly increase their hydraulic efficiency, using water three times more efficiently than C₄ plants and six times more efficiently than C₃ plants (Borland et al., 2009).

Differing discrimination against ¹³C resulting from C₃ and C₄ photosynthesis make these plants isotopically distinct, with C₃ of modern plants having observably lower $\delta^{13}\text{C}_{\text{org}}$ (between -37 and -20 ‰) than C₄ plants (between -20 and -9 ‰, frequently sitting in the -16 to -12 ‰ range) (O'Leary, 1988, Kohn, 2010). CAM plants typically

have $\delta^{13}\text{C}_{\text{org}}$ values between -10 and -20 ‰, intermediate between their C_3 and C_4 counterparts (O'Leary, 1988). The $\delta^{13}\text{C}_{\text{org}}$ of archaeological samples cannot be directly compared to modern plants due to the effect of anthropogenic carbon and must, therefore, be normalised by a correction for the Suess Effect (Keeling, 1979). Within this dataset there was no correction needed for anthropogenic carbon.

Conditions within the environment can influence the amount of carbon fractionation occurring in the biomass, in several ways. First, in hot, dry, open environments the vegetative composition may contain a greater proportion of C_4 and CAM plants, thus elevating the $\delta^{13}\text{C}$ of the overall biomass. Second, C_3 plants experience variable fractionation based on environmental conditions (Kohn, 2010). Elevated $\delta^{13}\text{C}_{\text{org}}$, up to -20 ‰ is observed in C_3 plants under water stress. In closed canopy forests, $\delta^{13}\text{C}_{\text{org}}$ of C_3 plants will often be less than -29 ‰ due to CO_2 recycling i.e., isotopically light CO_2 emitted at the forest floor which is taken up by plants, where fractionation further lowers the $\delta^{13}\text{C}$ of the foliage (van der Merwe and Medina, 1989). Changes in aridity have minimal impact on the isotopic composition of C_4 plants, which instead provide a record of the isotopic composition of atmospheric CO_2 incorporated through photosynthesis (Marino and McElroy, 1991).

The $\delta^{13}\text{C}$ of consumer tissues and in particular, eggshell, have been used to track changes in aridity and vegetation (e.g.: Ecker et al., 2015; Ségalen et al., 2006) through the mechanisms discussed above. Lower $\delta^{13}\text{C}$ is anticipated with increased mean annual precipitation (MAP) or a shift from open to closed canopy landscapes. Increased $\delta^{13}\text{C}$ is expected from an environment impacted by water stress and a vegetative composition reflecting plants with aridity adaptations i.e., C_4 and CAM.

3.4 Stable Isotopes and Environment Reconstruction – $\delta^{18}\text{O}$

Measurements of $\delta^{18}\text{O}$ are typically obtained through analysis of inorganic material, that is, the inorganic fraction of tissues such as eggshell and bone, or mineral deposits such as speleothems (e.g. Johnson et al., 1998; Bar-Matthews and Ayalon, 2011). Oxygen measured through the analysis of consumer tissues, like eggshell, is a reflection of ingested water sources (Clarke et al., 2006; Levin et al., 2006). Variation in $\delta^{18}\text{O}$ can provide insight into environmental conditions by recording changes in aridity (Landais et al., 2006; Bar-Matthews and Ayalon, 2011). It can also be a geographic marker, potentially indicating migration and trade (Bowen et al., 2005).

Isoscapes of meteoric $\delta^{18}\text{O}$ have been generated on a global scale (e.g. Terzer et al., 2013). The $\delta^{18}\text{O}$ of meteoric water varies based on factors such as latitude, altitude, basin, and the amount of precipitation, especially in regions under monsoon influence where $\delta^{18}\text{O}$ may be particularly low. Melting glaciers will also influence the $\delta^{18}\text{O}$ of regional water sources, making these values lower (Epstein and Mayeda, 1953). These factors and their interactions create an isoscape driven by precipitation which can be mapped and used to predict the geographic origin of a sample (Bowen et al., 2005; Terzer et al., 2013). To understand how this is possible, it is important to understand the underlying mechanisms influencing fractionation.

In water, fractionation takes place through bidirectional phase transitions which occur in thermodynamic equilibrium, making the process a Rayleigh distillation (Gat, 1996). Through evaporation and cloud rain-out the heavier isotope, ^{18}O , preferentially remains in or reverts back to the more stable liquid state. During evaporation, source

water will preferentially retain ^{18}O resulting in greater release of ^{16}O to the atmosphere. Likewise, during precipitation events, clouds will retain a greater proportion of ^{16}O as ^{18}O preferentially returns to the liquid state and rains out. In general, this rain-out occurs as clouds, which form near the equator, move toward the poles and encounter cold air masses, in association with changes in topography and latitude (Dansgaard, 1964). The correlation between the isotopic composition of meteoric water and latitude helps assess the likelihood that a sample originated or was accreted in the region to which it has been attributed (Bowen et al., 2005).

The mechanisms of fractionation are also employed in elucidating past climate and environment. Increasing aridity will leave terrestrial water sources with elevated $\delta^{18}\text{O}$. Conversely, as water stress ameliorates, the system will have lower $\delta^{18}\text{O}$ (Landais et al., 2006; Bar-Matthews and Ayalon, 2011). Changes in climate regimes may result in earlier or later rain-out of clouds, altering precipitation patterns (Dansgaard, 1964).

When $\delta^{18}\text{O}$ is measured directly from inorganic calcite, as is done in analysis of speleothems, the resulting isotopic composition will be dictated by the isotopic composition of meteoric waters (Bar-Matthews and Ayalon, 2011). These data can then be used to estimate the amount of rainfall per year of speleothem accretion, as was done at Soreq Cave, Israel (Bar-Matthews and Ayalon, 2011).

Human and animal tissue samples reflect the $\delta^{18}\text{O}$ of consumed water sources, however, not all animals are obligated to drink from surface water sources. Non-obligate drinkers, like ostriches, consume water through dietary vegetation (Levin et al., 2006). The physiological processes of plants can result in significant oxygen isotopic

fractionation and highly variable foliar isotopic compositions (Augusti and Schleucher, 2007). The $\delta^{18}\text{O}$ of the foliage is based on the value of local precipitation (Cormie et al., 1994), however, leaf stomatal conductance permits considerable discrimination against ^{18}O through evapotranspiration, resulting in elevated $\delta^{18}\text{O}$ with decreasing relative humidity (Landais et al., 2006; Augusti and Schleucher, 2007). The $\delta^{18}\text{O}$ of leaf water will fluctuate significantly over the course of a 24 h period and may, therefore generate isotopic compositions quite different from other local water sources (Landais et al., 2006). The variable nature of $\delta^{18}\text{O}$ in leaves is not consistent with other plant tissues. Phloem, which transports photosynthates from the leaves to the rest of the plant, carries a $\delta^{18}\text{O}$ signal which averages the day-night cycle (Augusti and Schleucher, 2007). Animals that consume drinking water are considered to track meteoric water through $\delta^{18}\text{O}$, while non-obligate drinkers are considered to primarily track evaporation (Levin et al., 2006). Studies of the enamel of evaporation sensitive animals (non-obligate drinkers) have recorded a linear correlation with aridity (Clarke et al., 2006; Levin et al., 2006).

Chapter 4 Materials and Methods

4.1 Materials

This study involved the application of stable isotope analysis to 57 samples of ostrich eggshell obtained from twelve locations circumscribing the Far Eastern Steppe in Mongolia, northern China and southern Siberia. Appendix 1 gives the location and contextual information for each sample analysed in this study; all of the sites were located at approximately the same altitude. The samples were acquired through Dr. Lisa Janz, The American Museum of Natural History, and the Museum of Far Eastern Antiquities.

In addition to the archaeological samples, a specimen of modern ostrich eggshell was obtained from Ostrich Land, an ostrich farm located in Ontario, Canada. From this single eggshell, 9 aliquots were subsampled and treated with the same procedures as the archaeological samples to provide a measure of the repeatability of each of the analyses. The modern eggshell samples were incorporated into each analytical session, in duplicate. Incorporating the modern eggshell into the study allowed for an assessment of the homogeneity of the isotopic and elemental compositions of the organic and inorganic

fractions of the eggshell. This made it possible to assess the effectiveness of the protocols being used in that a deviation from the expected ratio may indicate that the fraction had not been isolated in its entirety.

4.2 Methods

4.2.1 Pretreatment

Aliquots in excess of 125 mg were taken from the museum and archaeological site samples with the exception of an archaeological bead-blank, recorded as TEAL 1678, which weighed only 92.7 mg and was consumed by this study in its entirety. All samples were pretreated to remove contaminants from the burial context (Protocols in Figure 4.1) following Johnson et al. (1998). All samples were sonicated in approximately 10 ml of Type I water (resistivity $>18.2 \text{ M}\Omega\cdot\text{cm}$) for 1 h. Some samples required mechanical abrasion with an NSK Ultimate XL-D dental drill equipped with a cutting wheel, to remove museum catalogue numbering. The samples were dried at room temperature. Once dry, samples were submerged in 2 M HCl for 60 to 100 s and then rinsed repeatedly with Type I water, until the solution was neutral. This acid pretreatment was done to expose the inner eggshell for analysis and to remove contaminants associated with the outer surfaces (Johnson et al., 1998). This treatment resulted in a moderate loss of material from the modern aliquots but was quite destructive to many of the archaeological samples, with losses on the order of 50 % of the original material.

Subsequent to treatment with 2 M HCl, some samples continued to exhibit obvious contamination, in the form of sediment in the eggshell pore complexes. Attempts to remove this material were unsuccessful. Sampling, therefore, concentrated

on areas lacking pore complexes, which resulted in significant loss of sample material. When pore avoidance was not a viable option, the sample was taken with the contamination in the pores noted (Appendix 1).

A second contaminant was identified by sight and smell in samples 4377 and 4380. These samples had a distinct hydrocarbon odour. In the case of 4380 the odour was accompanied by dark discolouration. An attempt was made to remove hydrocarbon contamination, following a solvent extraction protocol that combined the methods described in Coltrain et al. (2004) and Fuller et al. (2014). This method was used to remove similar contaminants from samples recovered from the La Brea tar pits. Subsequent to the usual decontamination pre-treatment, these two samples were placed in glass culture tubes, in 2:1 toluene:methanol (v/v) and sonicated for 5x1 h with the solution refreshed at each repetition. The samples were then left in the toluene:methanol solution overnight and then sonicated for 1 h in the morning. Subsequent to this sixth repetition, the solution was removed and methanol was added to the culture tubes to remove any remaining toluene. The samples were sonicated in the fume hood for 1 h. The solution was removed and Type I water was added, followed by sonication for 1 h. The Type I water was replaced and sonication was repeated twice. The culture tubes were covered with parafilm and freeze-dried. To test the efficacy of the extraction method, a second aliquot of samples 4377 and 4380 were prepared in a manner consistent with all other samples, with no attempt to remove the hydrocarbon contamination being made. These aliquots were numbered 4377a and 4380a.

Subsequent to these pre-treatments, all eggshell samples were powdered using a Plattner's mortar and pestle. The powder was transferred to micro-centrifuge tubes for storage.

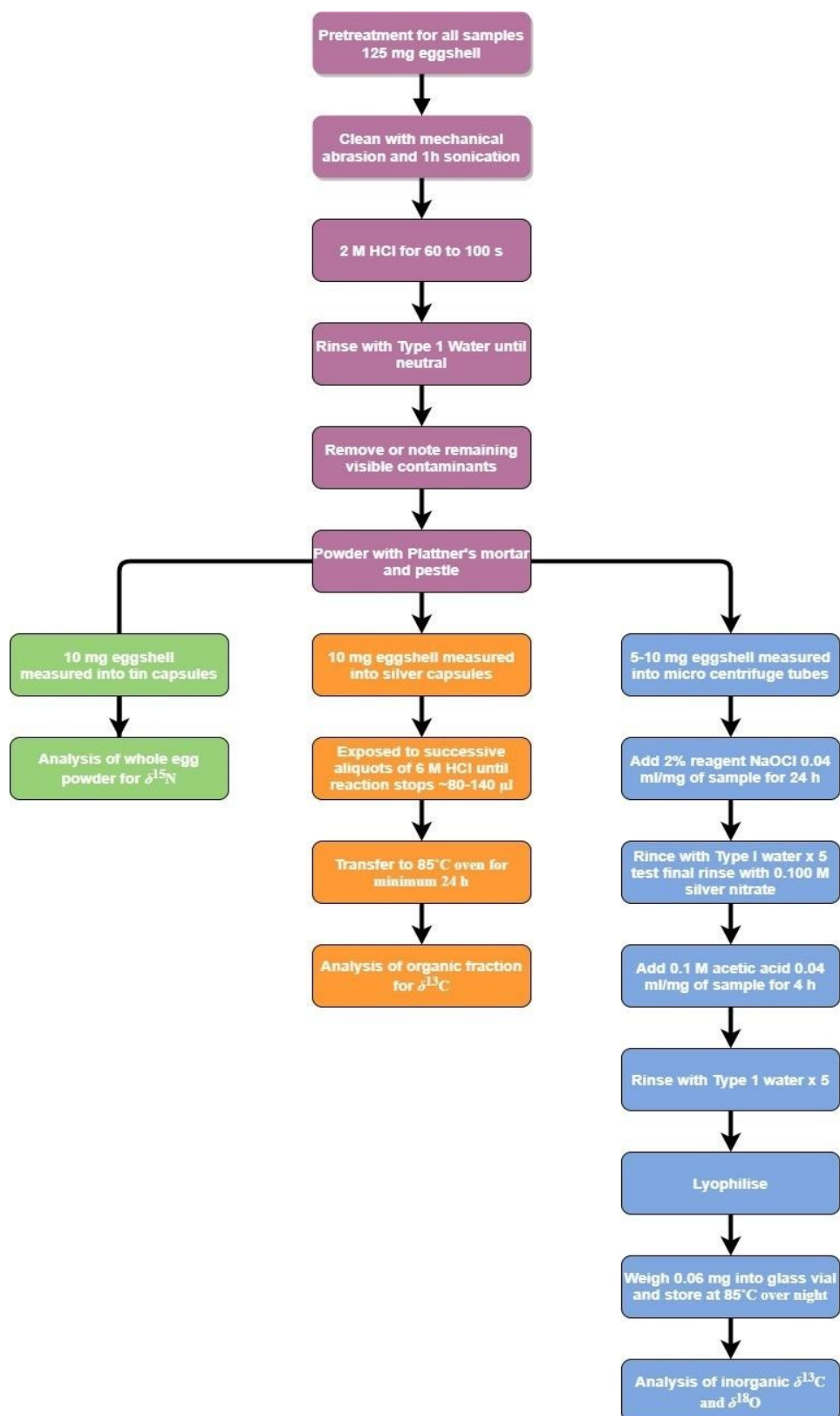


Figure 4.1 Flowchart of preparation protocol.

4.2.2 Preparation of Organic Fraction

Preparation of the organic fraction of the ostrich eggshell samples was adapted from the method described in Newsome et al. (2011). To obtain the $\delta^{13}\text{C}$ values of the organic fraction, samples of whole eggshell powder weighing 5-10 mg were placed into 10.5x9 mm silver capsules.

The inorganic fraction, of the samples, was dissolved in silver capsules using sequential aliquots of 6 M HCl. Initially, samples were subjected to 20 μl of 6 M HCl per repetition. This was increased to 60 μl , to ensure the entire sample was inundated with the acid. All samples were dissolved in 140 to 180 μl of 6 M HCl, to ensure that none of the inorganic fraction remained. The samples were left to dry overnight in the fume hood. Once the acid had evaporated at room temperature, the samples were transferred to the oven for a minimum of 24 h at 85°C. Samples remained in the silver capsules, which were folded in preparation for analysis.

A separate analysis was conducted to obtain the $\delta^{15}\text{N}$ of the organic fraction. In this analysis, 10 mg of powdered eggshell was placed into 4.25x5mm tin capsules. The samples were analysed as whole egg powder without separating the organic fraction from the inorganic fraction (Black et al., 2017).

4.2.2.1 Analysis of Organic $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

The samples were analysed for their carbon isotopic compositions through a EuroEA 3000 (Euro Vector SpA) Elemental Analyzer, coupled to a Nu Horizon (Nu Instruments) continuous flow isotope ratio mass spectrometer at the Water Quality Centre at Trent University.

Nitrogen is only present in the organic fraction of the eggshell, making it unnecessary to dissolve the carbonate. Carbon, however, is present in both the organic and inorganic fractions of the eggshell. There was potential for high CO₂ peaks to interfere with the relatively small N₂ peaks during analysis. To avoid this problem a carbon trap, containing NaOH (EMASorb) and magnesium perchlorate, was installed on the elemental analyser, in series with the water trap.

The analytical runs were set up to incorporate standards and duplicates in order to calibrate the data and assess the accuracy and precision of the instrument, throughout the day. The duplicates consisted of aliquots of modern eggshell supplied by Ostrich Land, prepared as discussed above. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were calibrated to VPDB and AIR using USGS40 and USGS41a. Internal standards SRM-1, SRM-2, SRM-14 (collagen), SRM-17 (phenylalanine), amygdalin and tyrosine (known values in Appendix 2) were used as quality assurance standards to assess accuracy and precision, and to monitor for instrument drift. The analytical sessions for organic carbon had an uncertainty of ± 0.11 ‰ for $\delta^{13}\text{C}$, calculated in accordance with the best practices outlined in Szpak et al. (2017). The analytical session for nitrogen had an uncertainty of ± 0.20 ‰ for $\delta^{15}\text{N}$.

4.2.3 Preparation of the Inorganic Fraction

Preparation for analysis of the inorganic fraction of the ostrich eggshells followed the procedure for preparation of enamel, obtained from Western University (Koch et al., 1997; Garvie-Lock et al., 2004). All samples were pretreated for removal of burial contaminants and powdered, as described above. The subsequent pretreatment was

conducted with the objective of eliminating the organic fraction of the eggshell as well as any secondary carbonates contaminating the samples.

Samples of archaeological ostrich eggshell weighing 5 mg and samples of modern ostrich eggshell weighing 10 mg were placed in micro-centrifuge tubes. Twice as much material was weighed for the modern samples so that they could be analysed in duplicate. To each micro-centrifuge tube, 0.04 ml of 2 % reagent grade NaClO was added for each mg of sample. The micro-centrifuge tubes were stored at room temperature, on their sides, to maximise the exposed surface area. Samples were agitated with a vortex mixer periodically to ensure adequate contact with the bleach. The samples remained in the solution for a minimum of 24 h.

Samples were rinsed five times with Type I water, centrifuging between repetitions and removing liquids with Pasteur pipette to prevent sample loss. To ensure the complete removal of the reagent bleach, the final rinse of Type I water was tested with a drop 0.100 M AgNO₃ (silver nitrate). If the liquid remained clear, the bleach removal was successful, if a cloudy precipitate formed the sample received an additional rinse with Type I water.

Once removal of the reagent bleach had been confirmed, 0.1 M acetic acid was added to the micro-centrifuge tubes in aliquots of 0.04 ml per mg of sample. The micro-centrifuge tubes were stored on their sides to maximise the surface area of the sample exposed to the solution. The micro-centrifuge tubes were occasionally agitated with a vortex mixer to ensure adequate contact between the sample and the acid. The samples remained in acetic acid for 4 h, after which they were rinsed five times with Type I water.

Samples were centrifuged between repetitions and liquids were removed by Pasteur pipette to minimise the risk of sample loss. The samples were covered with parafilm and freeze dried.

At Western University, 0.06 mg of sample were weighed into glass vials and placed in the oven at 72°C, overnight. The samples were then capped with septa and analysed for their isotopic compositions of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ with an Optima Dual Inlet mass spectrometer with GV Multiprep at the Laboratory for Stable Isotope Science at Western University. The analysis was conducted at 90°C. Equilibrium with phosphoric acid was maintained for 10 minutes. The reaction was open to the cold trap.

4.2.3.1 Analysis of Inorganic $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$

As with the organic preparations, the analytical runs for the inorganic fraction were designed to incorporate standards and duplicates to assess the accuracy and precision of the instrument and to calibrate the data. Duplicates were created from modern eggshell, with the same aims and following the same principles of consistency with the archaeological samples discussed above. The results of each analytical session were calibrated using NBS19 and NBS18 for $\delta^{18}\text{O}$ and NBS19 and LSVEC for $\delta^{13}\text{C}$. In-house standards WS-1 and SupraPur (known values in Appendix 2) were used as check standards to assess accuracy and precision over the course of the run. The total uncertainty for the analytical sessions of the inorganic fraction was ± 0.31 ‰ for $\delta^{13}\text{C}$ and ± 0.26 ‰ for $\delta^{18}\text{O}$.

Section 4.3: Radiocarbon Dates

Eleven new radiocarbon dates were determined as part of this study. Samples 1673, 1676, 2287, 4361, 4363, 4365, 4369, 4373, 4382, 4384, 4386 were directly ^{14}C dated at A. E. Lalonde AMS Laboratory at the University of Ottawa (Appendix 1). Dates were calibrated through Calib Rev 7.0.4 (Stuiver and Reimer, 1993).

4.4 Statistical Treatment

Statistical comparisons of the data (Shapiro-Wilk test, Wilcoxon rank sum test, and t-test) were completed using RStudio Version 3.6.2 (RStudio Team, 2019). Graphs and Pearson's r tests were completed using Microsoft Excel version 12.0 (Microsoft Corp., 2007). Principal components analyses were completed using PAST 4.03 (Hammer, 2020). The data collected for $\delta^{13}\text{C}_{\text{org}}$, $\delta^{13}\text{C}_{\text{inorg}}$, $\delta^{18}\text{O}$, and $\delta^{15}\text{N}$ (Appendix 3) were compared through statistical analyses to assess regional and temporal differences. In all statistical tests, significance was determined by a p -value <0.05 .

Chapter 5: Results

Section 5.1: Challenges Associated with Analyses

5.1.1: Organic

Carbon is a component of both the organic and inorganic fractions of eggshell but because of differences in macronutrient routing and the biochemical processes of tissue production, the two carbon fractions have different $\delta^{13}\text{C}$ values (Lee Thorp and Sealy, 1989). These two fractions must, therefore, be isolated from one another in preparation for analysis. Acid fumigation was used to digest the inorganic carbonate and isolate the organic fraction of the eggshell sample. The use of HCl, in this process, results in CaCl_2 as a by-product (Komada et al., 2008). This salt acts as a desiccant, causing the samples to absorb atmospheric moisture, leading to unstable weights, excessive sample moisture and difficulties with instrumentation (Komada et al., 2008). Additionally, digestion with HCl required the use of silver capsules rather than tin, the latter of which combusts more readily at a lower temperature. The first analytical session for organic carbon analysis required significant correction for instrumental drift. The second analytical session for organic carbon analysis was slightly modified by wrapping the silver capsules in tin to

improve combustion and by retaining the samples in an 85°C oven between preparation and analysis. Throughout the analysis, samples remained in the oven and were loaded into the auto sampler at 45 min intervals until the analytical session was complete. These slight modifications generated results which did not require drift correction.

Nitrogen is only a component of the organic fraction of eggshell. It was initially expected that the organic carbon and nitrogen analyses would be simultaneous; however, the acid fumigation pretreatment used on the samples results in fractionation of nitrogen, elevating the $\delta^{15}\text{N}$ (Harris et al., 2001). In order to obtain reliable $\delta^{15}\text{N}$ values, a separate nitrogen analysis was necessary. This analysis was conducted by weighing powdered eggshell into tin capsules and analysing the samples without isolating the organic from the inorganic fractions. This analysis also warranted a temporary modification of the instrument, to eliminate both the organic and inorganic carbon from the analysis, in order to avoid interference from an excessively large CO_2 peak bleeding into the N_2 measurement. This was achieved by installing a CO_2 trap, packed with NaOH, and magnesium perchlorate packed at each end of the tube. This protocol obtained reliable $\delta^{15}\text{N}$ measurements from the eggshell samples.

5.1.2: Inorganic

The pretreatment used to isolate the inorganic fraction of the eggshell may be associated with some problems. Bleach used to eliminate the organic fraction has been demonstrated to produce treatment-induced carbonates, from eliminated organics, which may affect the isotopic composition of the sample for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (Crowley and Wheatley, 2014). Pretreatment with acetic acid, intended to remove secondary

carbonates, has been demonstrated to result in lowered $\delta^{13}\text{C}$ and elevated $\delta^{18}\text{O}$ (Garvie-Lok et al., 2004). For both bleach and acetic acid, these outcomes have been demonstrated in enamel and bone, therefore there is an assumption of similar outcomes for eggshell although this has never been demonstrated.

The analytical method used has been demonstrated, through experimental study of enamel and bone, to have the potential to produce isotopic results for $\delta^{18}\text{O}$ that are variable and anomalously low (Metcalf et al., 2009). It is preferable to use a 'sealed vessel' reaction with duration of 25 minutes (Metcalf et al., 2009). Future research will therefore include repeat analysis of some of the samples using the preferred analytical method and without pretreatment, to assess to reproducibility of the results and evaluate the effects of the pretreatment and analytical method on archaeological eggshell.

Section 5.2: Criteria for Sample Inclusion/Exclusion

The isotopic compositions of 57 archaeological samples were determined as part of this study, however, some measurements were excluded. Post depositional alteration is a risk when conducting stable isotope analysis on any archaeological samples and consideration must be made of criteria for inclusion and exclusion of samples based on preservation and contamination. A metric for preservation used in eggshell studies conducted by Johnson et al. used an organic to inorganic $\delta^{13}\text{C}$ offset of 15 ± 2 ‰ (Johnson et al., 1998) and 14 ± 2 ‰ (Johnson et al., 1997). The $\delta^{13}\text{C}$ offsets collected in this study are reported in Appendix 3 and presented in Figure 5.1. This metric was not informed by experimental studies of eggshell diagenesis, however, and Johnson et al. (1998) acknowledge the possibility that dietary routing may result in different, specifically

tighter, offsets in birds with increased amounts of animal protein in the diet (Lee Thorp and Sealy, 1989). This effect is demonstrated in the data collected by Emslie and Patterson (2007). This study looked specifically at eggshell from Adélie penguins which are a carnivorous marine species. The data produced by this study demonstrated a much tighter organic to inorganic $\delta^{13}\text{C}$ offset, often around 4 or 5 ‰.

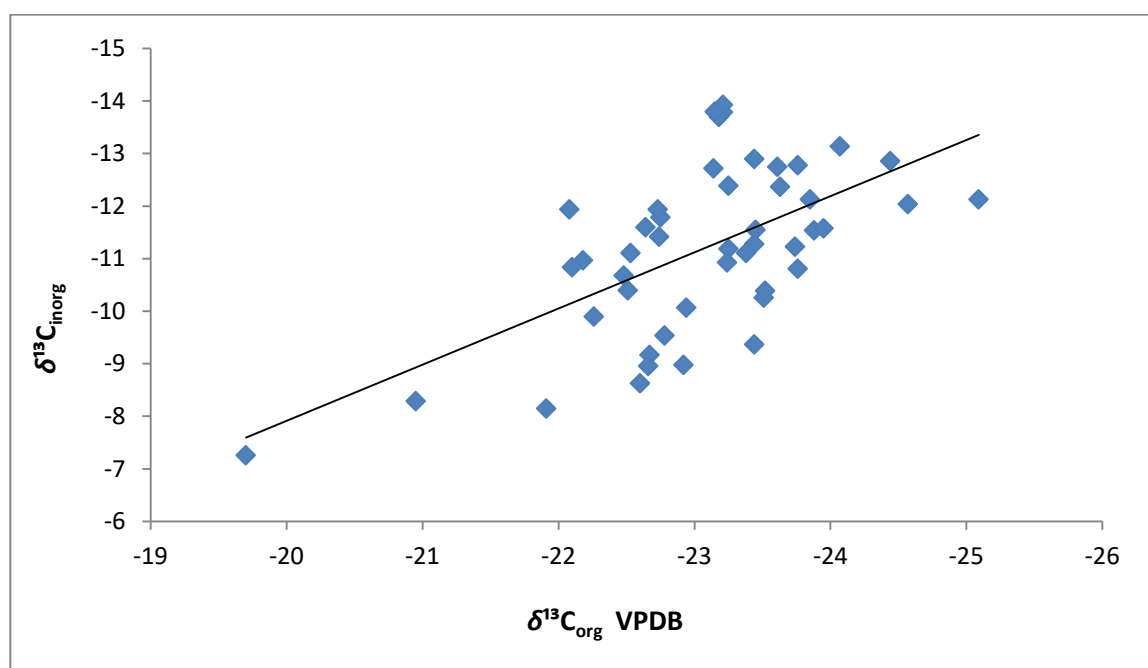


Figure 5.1. Offset between $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{13}\text{C}_{\text{inorg}}$. The trend line is based on a linear regression with Pearson's $R = 0.60$

Samples of modern ostrich eggshell used as a method check in this study were derived from a single eggshell, produced in an agricultural environment where humans provide the ostriches with food. These samples produced a mean organic to inorganic $\delta^{13}\text{C}$ offset of 15.05 ‰, in line with the expectations of Johnson et al (1997, 1998). The offset produced by a provisioned diet, consumed by extant, domesticated ostriches may not be the best comparison for an extinct species whose foraging patterns are unknown. As discussed in Chapter 2, ostriches do consume some amount of animal resources and this component of diet may have varied in the past (Milton et al., 1993, Milton et al.,

1994, Aganga et al., 2003, Endo et al., 2010, Newsome et al., 2011). For this reason, samples were not excluded from the study on the basis of having organic-inorganic $\delta^{13}\text{C}$ offsets less than 15 ‰.

In studies of tissues such as bone collagen, it is typical to report the %C, %N and the C:N ratios of the organic fractions of samples, as a method of assessing sample preservation. For eggshell, this is not a viable method for a number of reasons. In order to produce reliable results the $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ must come from separate analytical sessions. Additionally, as mentioned, the acid fumigation pretreatment for $\delta^{13}\text{C}_{\text{org}}$ produces salts which render the sample hygroscopic, making it impossible to obtain meaningful sample weights. Untreated eggshell weights are also not meaningful as the acid fumigation frequently caused capsule over flow and sample loss which would have caused the yields to appear artificially low.

Anomalies in the carbonate yields of the inorganic fraction of samples may be an alternate metric for assessing sample preservation. Plotting the $\delta^{18}\text{O}$ against the Wt % CO_3 produced by the analysis (Figure 5.2) indicates two possible outliers (1599 and 1604). The Wt % CO_3 of these two samples fell beyond two standard deviations of the mean, however, their isotopic compositions were not noteworthy, nor were there any other indicators of poor preservation. Notably, all of the samples removed from the study due to observable contamination, presented Wt % CO_3 values well within two standard deviations. Modern samples were also within the threshold of two standard deviations. There was no significant correlation between the inorganic isotopes and the Wt % CO_3 of the samples ($\delta^{18}\text{O}$, $R=0.0748$; $\delta^{13}\text{C}_{\text{inorg}}$, $R=0.0954$). Carbonate yield and its relationship

with isotopic compositions may not be effective metrics for assessing the preservation of eggshell.

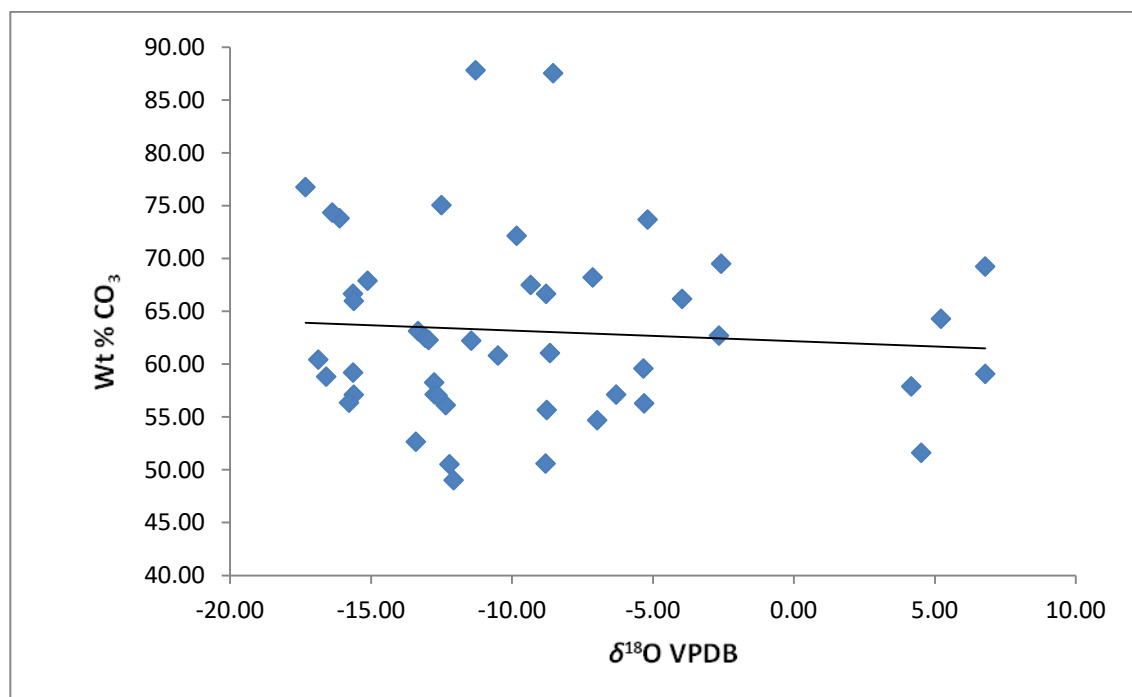


Figure 5.2. $\delta^{18}\text{O}$ plotted against Wt % CO₃ in an effort to assess preservation.

Samples were excluded on the basis of direct observation, either visual or olfactory (Noted in Appendix 1). In all, seven samples were excluded from the analysis, predominantly on the basis of visual confirmation of contamination in the pore complexes which could not be avoided during sampling. A further two samples were excluded on the basis of smell and staining resulting from hydrocarbon contamination. This form of contamination would impact the results through lowered organic $\delta^{13}\text{C}$ (Conrad et al., 1997). Lastly, sample 1677 was poorly preserved and did not provide data through any of the analyses as no gas was produced. The sample was, therefore, excluded. In total, 10 samples were excluded from interpretation. Appendix 3 tabulates all results, including those excluded from the analysis.

Three samples: 1681, 1682, 1683, smelled distinctly of campfire. Alteration by heating, either anthropogenic or natural, is common for eggshell samples in many locations (Miller et al., 2016; Collins and Steele, 2017). A study examining the effects of variable degrees of burning on *Genyornis* and *Dromaius* eggshells revealed that samples ranging from blackened to visually unburned exhibited no statistical alteration to the $\delta^{13}\text{C}_{\text{org}}$ or $\delta^{15}\text{N}$ (Miller et al., 2016). The heated samples encountered in this study were, therefore, included in interpretations.

Section 5.3: Radiocarbon Dates

Radiocarbon dates for samples 1673, 1676, 2287, 4361, 4363, 4365, 4369, 4373, 4382, 4384, 4386 are reported in Appendix 1.

Section 5.4: Regional Variation

Shapiro-Wilk tests were used to assess the normality of the distributions of each isotope in each region (Table 5.1). The assessment of normality was conducted with the aim of selecting the statistical test for each pair-wise comparison that would most robustly identify differences between regions and time periods.

TABLE 5.1: Shapiro Wilk tests of normality for regional isotope data (p values). Values in boldface indicate non-normal distributions.

Region	$\delta^{13}\text{C}_{\text{org}}$	$\delta^{13}\text{C}_{\text{inorg}}$	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$
Northern steppe (n=16)	0.66	0.51	0.008	0.73
South Gobi (n=21)	0.22	0.78	0.03	0.70
East Gobi/Steppe (n=11)	0.38	0.01	0.22	0.66

With some normal and some non-normal distributions, differences between regions were assessed through pair-wise comparisons using both the Student's t test and

the Wilcoxon rank sum test. The Student's t test was selected to test for differences between the means of parametric data, while the Wilcoxon rank sum test was used to ensure non-parametric data were robustly assessed for statistical difference. The results of these tests are tabulated in Tables 5.2 and 5.3.

TABLE 5.2: Wilcoxon rank sum test of difference between regions. Values in bold indicate significant differences.

Region	$\delta^{13}\text{C}_{\text{org}}$	$\delta^{13}\text{C}_{\text{inorg}}$	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$
North vs. East	0.44	0.004	<0.001	<0.001
North vs. South	0.08	<0.001	<0.001	>0.99
East vs. South	0.94	0.06	0.28	0.001

TABLE 5.3: Student's t test of difference between regions. Values in bold indicate significant differences.

Region	$\delta^{13}\text{C}_{\text{org}}$	$\delta^{13}\text{C}_{\text{inorg}}$	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$
North vs. East	0.23	0.012	<0.001	<0.001
North vs. South	0.08	<0.001	<0.001	0.80
East vs. South	0.65	0.23	0.10	<0.001

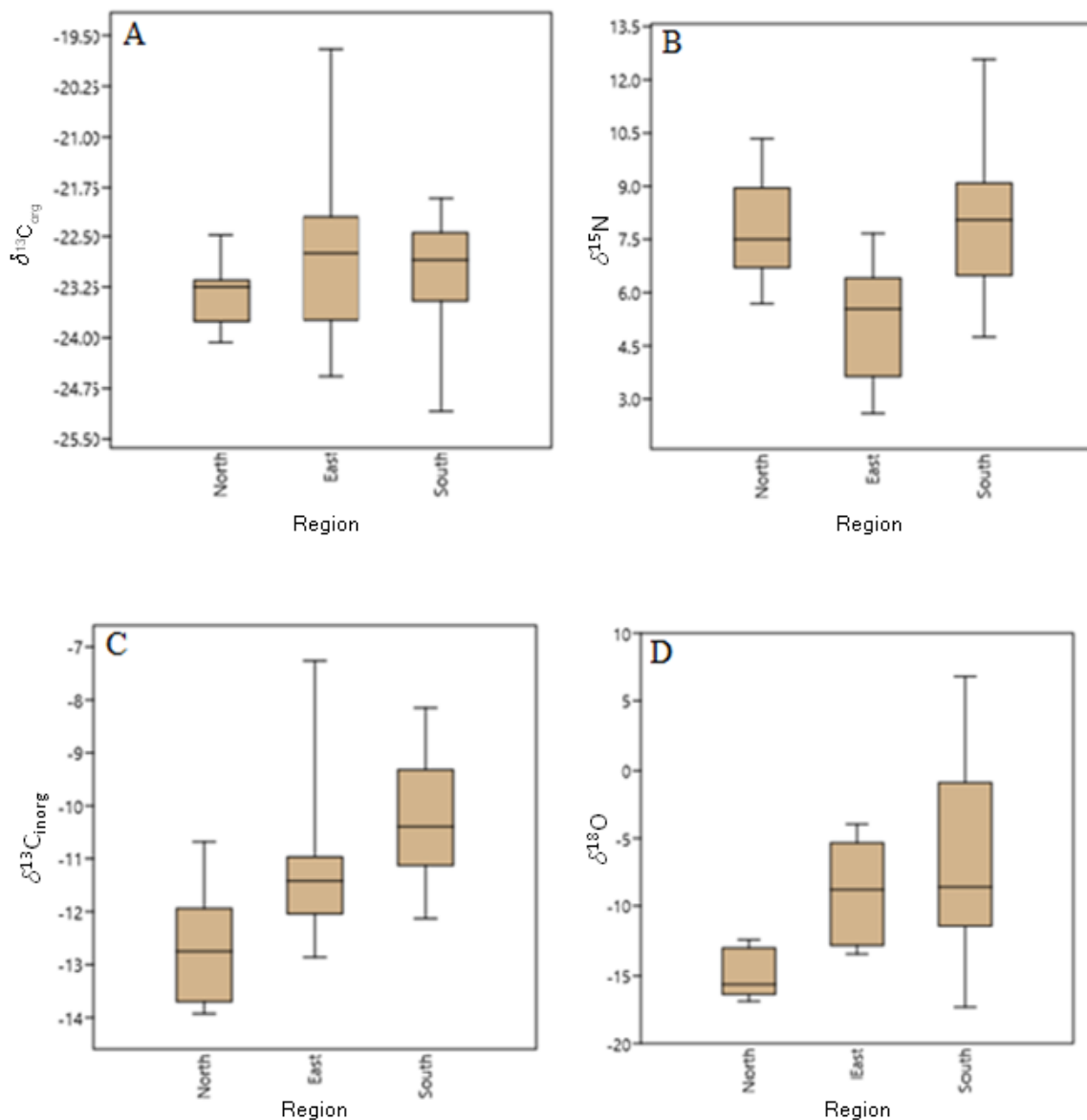


Figure 5.3. Isotopic compositions by region with mean and standard error: panel A $\delta^{13}\text{C}_{\text{org}}$, panel B $\delta^{15}\text{N}$, panel C $\delta^{13}\text{C}_{\text{inorg}}$, panel D $\delta^{18}\text{O}$

Though not all comparisons produced significant differences, the overall partitioning of stable isotope compositions among regions does not support the idea that all eggshell samples were drawn from the same population (Figure 5.3, Tables 5.2 and 5.3). The regional differences observed are more consistent with the hypothesis that the

eggshells were procured locally or that eggshells in each region were obtained from distinct geographic regions.

Section 5.5: Change Within Regions Over Time

Samples were allocated into time bins based on their radiocarbon dates. For reference, radiocarbon and calibrated dates are provided for all samples in Appendix 1.

Within the northern Mongolia/southern Siberian sample set, nine samples were directly ^{14}C dated at A. E. Lalonde AMS Laboratory in Ottawa. The dates indicate that the majority of the samples fall within a relatively narrow range which did not allow for statistical temporal comparisons to be completed. There was no apparent variation over time (Figures 5.4, 5.5, 5.6 and 5.7) which may be an artifact of small sample size.

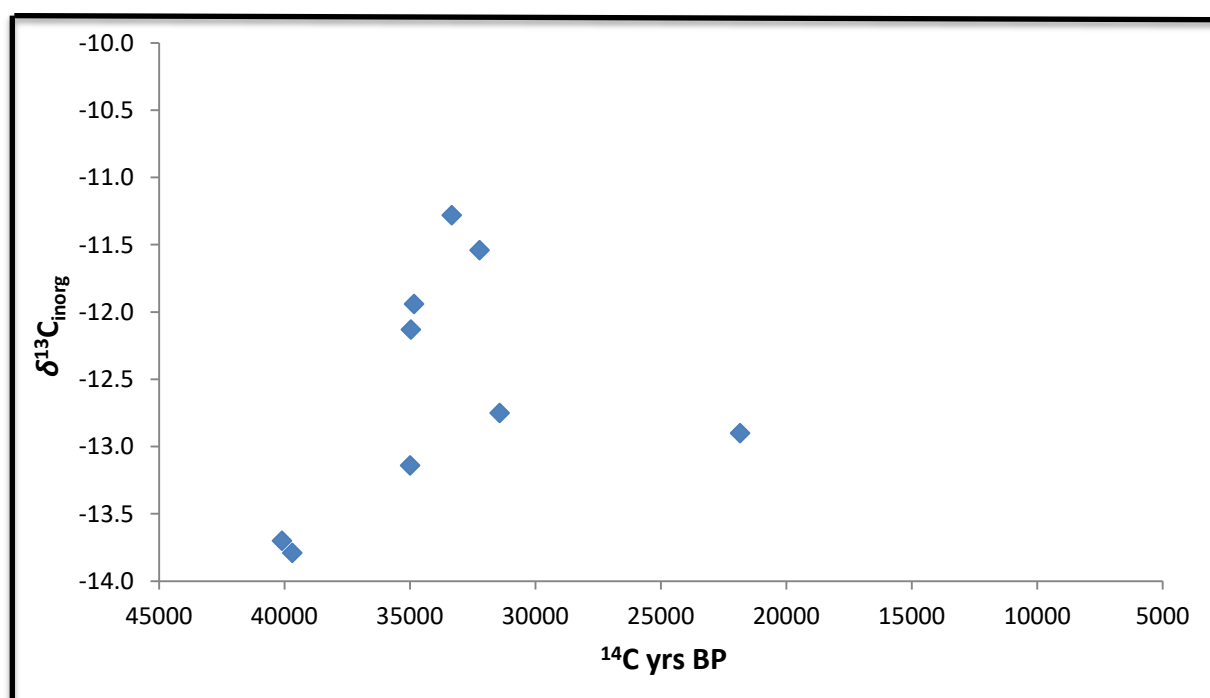


Figure 5.4. Variation in $\delta^{13}\text{C}_{\text{inorg}}$ for eggshells from the Northern region over time.

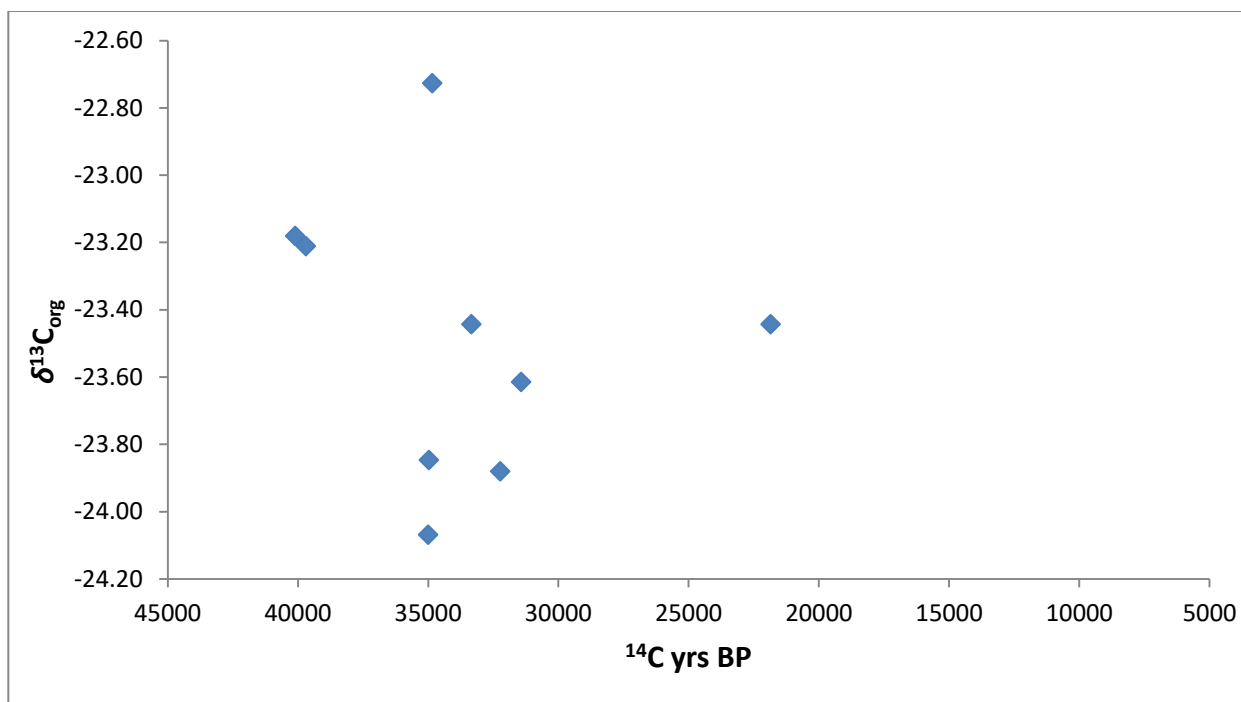


Figure 5.5. Variation in $\delta^{13}\text{C}_{\text{org}}$ for the eggshells from the Northern region over time.

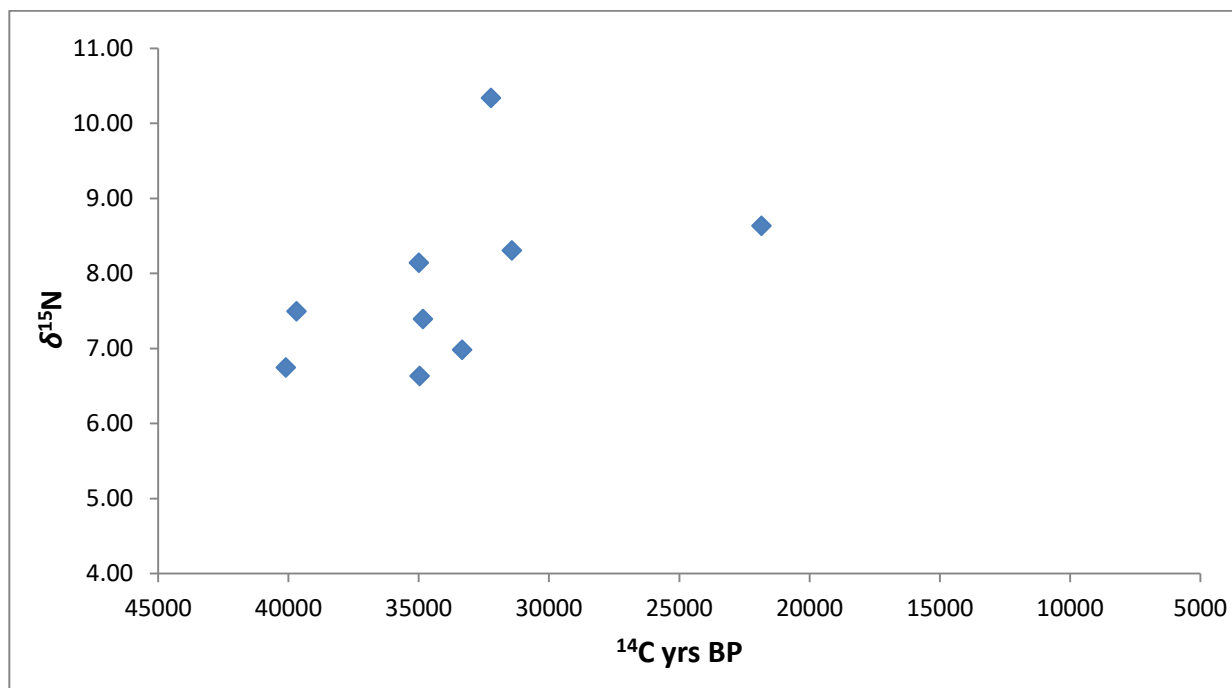


Figure 5.6. Variation in $\delta^{15}\text{N}$ for the eggshells from the Northern region over time.

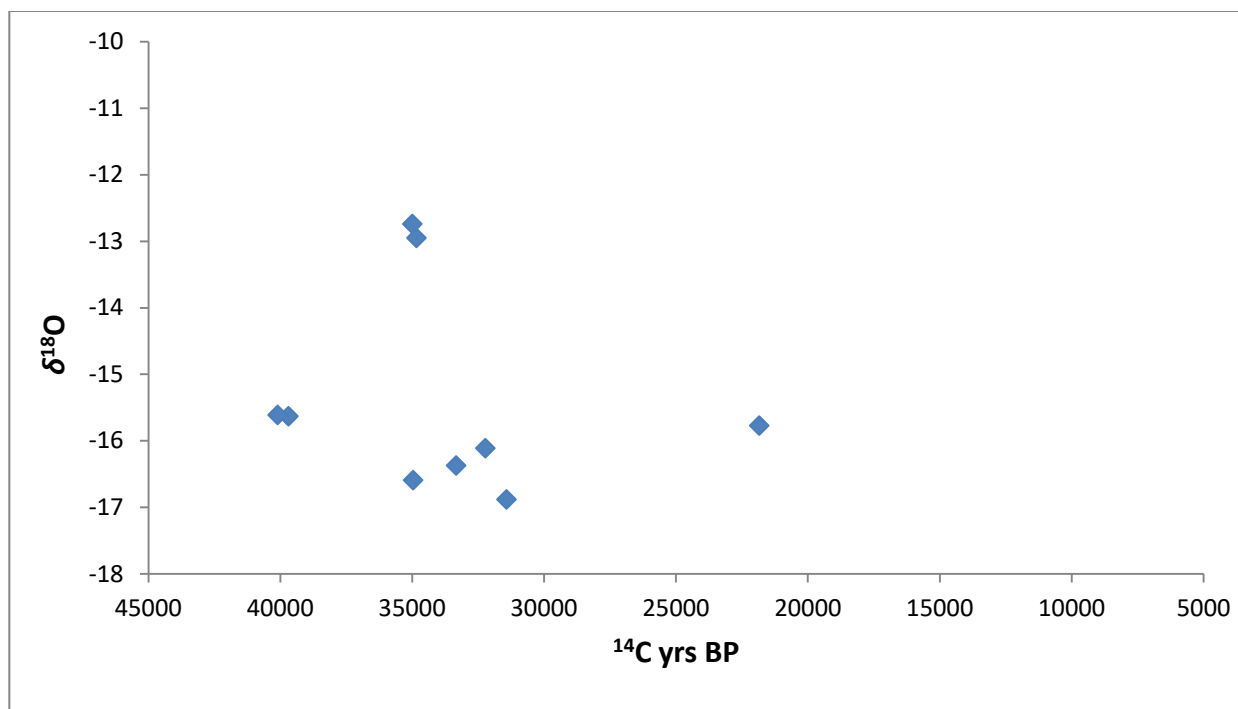


Figure 5.7. Variation in $\delta^{18}\text{O}$ for the eggshells from the Northern region over time.

Within the Eastern region and the Southern Region, samples were dated through previous research (Janz et al., 2015) and temporal differences were tested. The data were grouped into time bins, which were qualitatively assigned based on the availability of data, and the normality of the distribution of each bin was tested with pair-wise Shapiro Wilk test (Table 5.4), followed by Student's t tests when distribution was normal and Wilcoxon rank sum tests when the distribution was non-normal (Tables 5.4 and 5.6).

Table 5.4: Shapiro Wilk test of normality for time bin pairings. Values in Bold Indicate Non-Normal Distributions.

Region	Time Bin ^{14}C yr BP	$\delta^{13}\text{C}_{\text{org}}$	$\delta^{13}\text{C}_{\text{inorg}}$	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$
South	>40,000	0.89	0.77	0.21	0.24
South	39,000-10,000	0.76	0.37	0.93	0.24
South	<9,000	0.78	0.90	0.26	0.79
East	>30,000	0.17	0.97	0.82	0.22
East	29,000-12,000	0.21	0.90	0.54	0.99
East	<12,000	0.99	0.52	0.009	0.90

TABLE 5.5: Student's t test of temporal difference in the Southern Region (p values).

Variable		39,000-10,000 (n=3)	<9,000 (n=13)
$\delta^{13}\text{C}_{\text{org}}$	>40,000 (n=5)	0.48	0.33
	39,000-10,000	–	0.66
$\delta^{13}\text{C}_{\text{inorg}}$	>40,000	0.001	<0.001
	39,000-10,000	–	0.02
$\delta^{18}\text{O}$	>40,000	0.009	<0.001
	39,000-10,000	–	0.12
$\delta^{15}\text{N}$	>40,000	0.01	0.002
	39,000-10,000	–	0.62

In the Southern region there were no differences in $\delta^{13}\text{C}_{\text{org}}$ among the three time bins (Figure 5.9). In $\delta^{13}\text{C}_{\text{inorg}}$ there was a decrease of 2.89 ‰ between the means of the >40,000 and 39,000-10,000 BP bins and an increase of 1.26 ‰ between the means of the 39,000-10,000 BP and <9,000 BP bins (Figure 5.8). There was a decrease of 18.99 ‰ in $\delta^{18}\text{O}$ between the >40,000 and 39,000-10,000 BP bins and an increase of 5.15 ‰ between

the 39,000-10,000 BP and <9,000 BP bins (Figure 5.11). In $\delta^{15}\text{N}$ there was a decrease of 3.69 ‰ between the >40,000 and 39,000-10,000 BP bins which remained relatively consistent through the <9,000 BP bin (Figure 5.10).

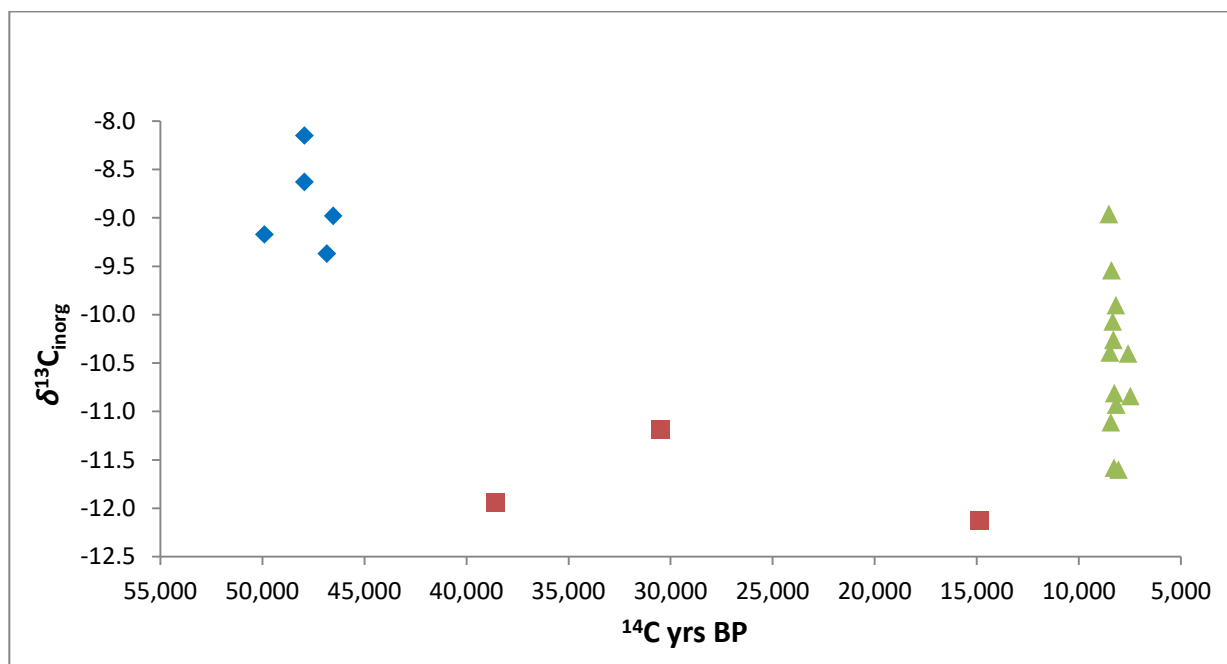


Figure 5.8. Variation in $\delta^{13}\text{C}_{\text{inorg}}$ for the eggshells from South Gobi over time. >40,000 BP (blue diamonds), 39,000-10,000 BP (red squares), <9,000 BP (green triangles).

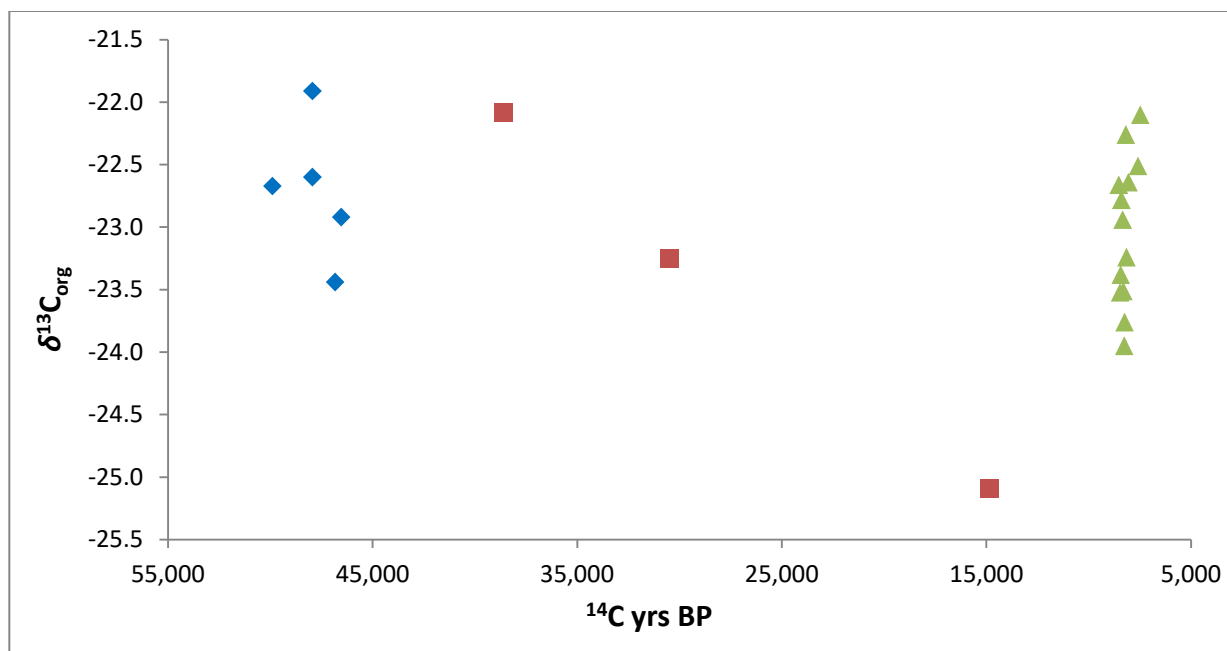


Figure 5.9. Variation in $\delta^{13}\text{C}_{\text{org}}$ for the eggshells from South Gobi over time $>40,000$ BP (blue diamonds), $39,000-10,000$ BP (red squares), $<9,000$ BP (green triangles).

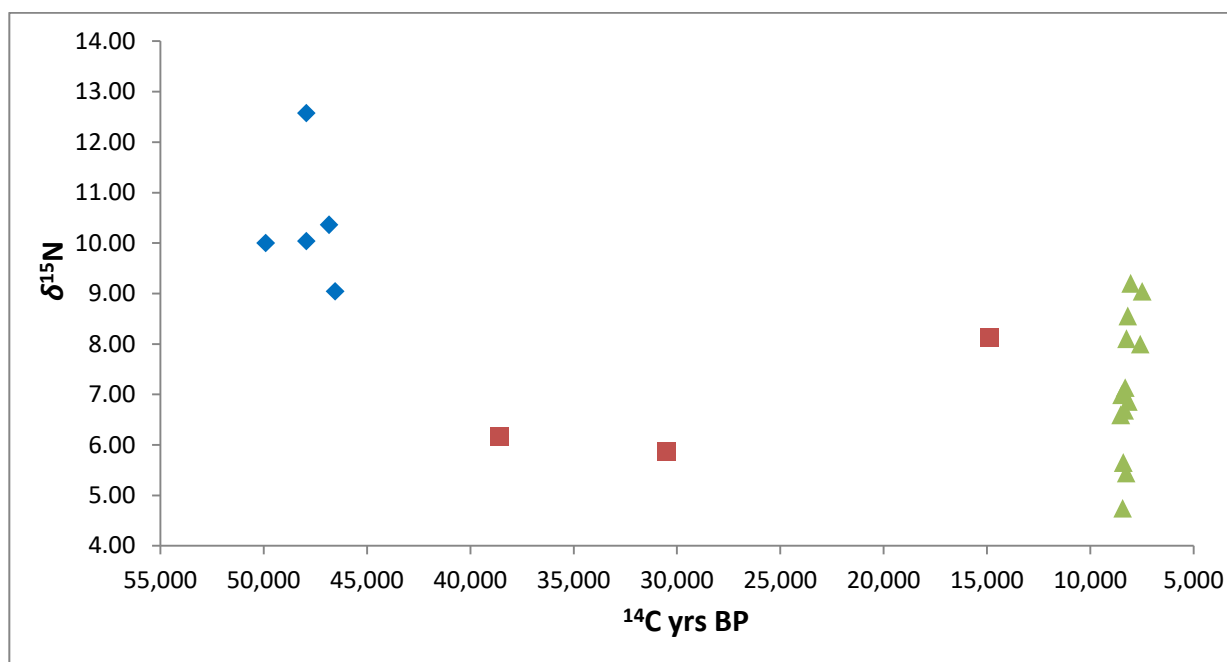


Figure 5.10. Variation in $\delta^{15}\text{N}$ for the eggshells from South Gobi over time $>40,000$ BP (blue diamonds), $39,000-10,000$ BP (red squares), $<9,000$ BP (green triangles).

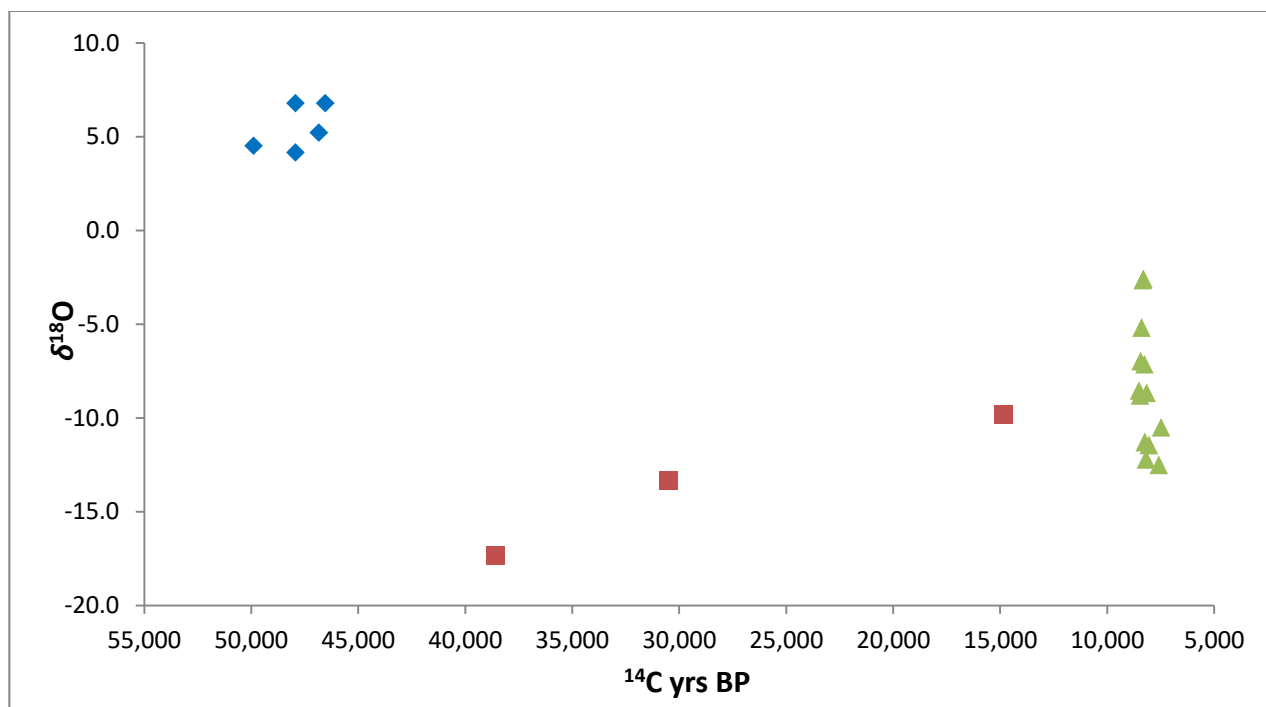


Figure 5.11. Variation in $\delta^{18}\text{O}$ for the eggshells from South Gobi over time. >40,000 BP (blue diamonds), 39,000-10,000 BP (red squares), <9,000 BP (green triangles).

A principal component analysis was conducted of the South Gobi data over time, to better depict the temporal clustering within the samples (Figure 5.12).

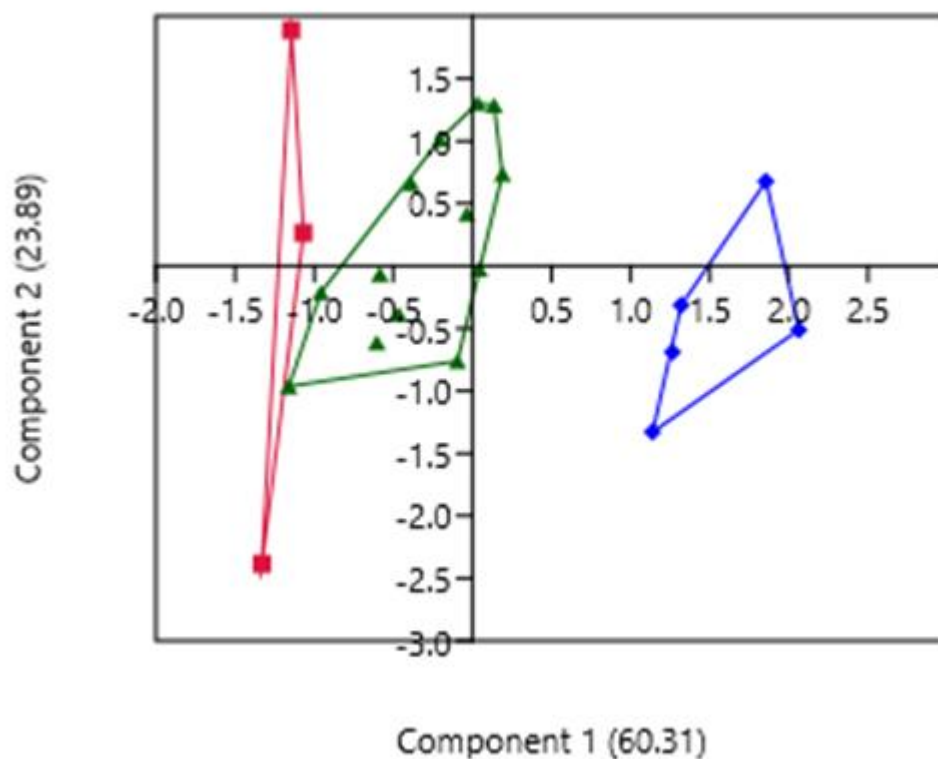


Figure 5.12. Principal component analysis depicting differences in the South Gobi, over time. The variation on the x axis is most affected by $\delta^{13}\text{C}_{\text{inorg}}$ and the y axis is most affected by $\delta^{13}\text{C}_{\text{org}}$. In total, 84.19 % of the variation in the data is accounted for by the principal component analysis. >40,000 BP (blue diamonds), 39,000-10,000 BP (red squares), <9,000 BP (green triangles).

Similar statistical approaches and figures were undertaken for the Eastern region data. Once normality had been assessed, Student's t tests were used on all data except pair-wise comparisons that included the 3rd time bin for $\delta^{18}\text{O}$, for which the Wilcoxon test was used. The 3rd time bin for $\delta^{18}\text{O}$ was the only data set which was determined to have a non-normal distribution.

Table 5.6. Student's t/Wilcoxon test of temporal difference in the East Gobi/Eastern Steppe (*p* values). Values in Bold Indicate Significance.

Variable		29,000-12,000 (n=4)	<12,000 (n=3)
$\delta^{13}\text{C}_{\text{org}}$	>30,000 (n=4)	0.008	0.11
	29,000-12,000		0.04
$\delta^{13}\text{C}_{\text{inorg}}$	>30,000	0.15	0.14
	29,000-12,000		0.09
$\delta^{18}\text{O}$	>30,000	0.02	0.06
	29,000-12,000		0.86
$\delta^{15}\text{N}$	>30,000	0.002	0.46
	29,000-12,000		0.02

In the East region there was change over time in $\delta^{13}\text{C}_{\text{org}}$, with a 1.2 ‰ decrease between the >30,000 and 29,000-12,000 BP time bins and an increase of 3.15 ‰ between the 29,000-12,000 and <12,000 BP time bins (Figure 5.14). There was no change in $\delta^{13}\text{C}_{\text{inorg}}$ over time (Figure 5.13). There was a 2.63 ‰ increase in $\delta^{18}\text{O}$ between the >30,000 and <12,000 BP time bins (Figure 5.16). The $\delta^{15}\text{N}$ values fluctuated between time periods with a 3.18 ‰ decrease between the >30,000 and 29,000-12,000 BP time bins and a 3.18 ‰ increase between the 29,000-12,000 and <12,000 BP time bins (Figure 5.15).

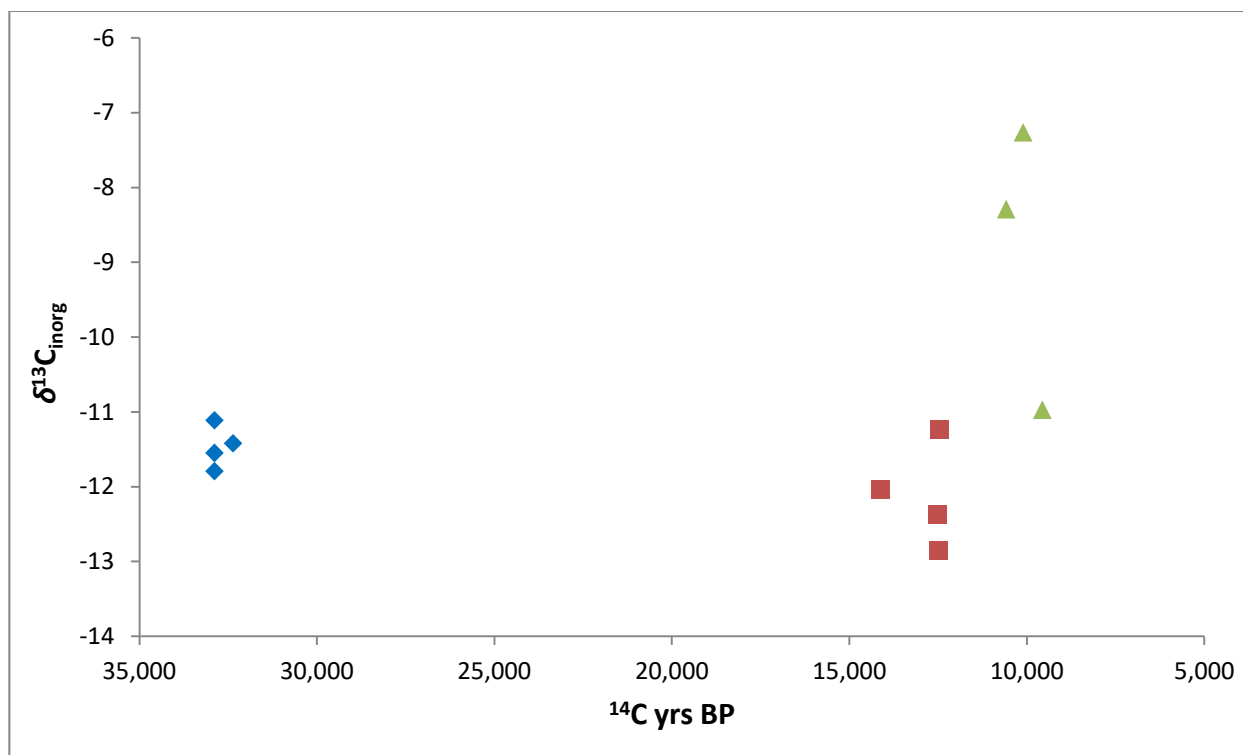


Figure 5.13. Variation in $\delta^{13}\text{C}_{\text{inorg}}$ for eggshells from the East over time. $>30,000$ BP (blue diamond), $29,000-12,000$ BP (red square), $<12,000$ BP (green triangle).

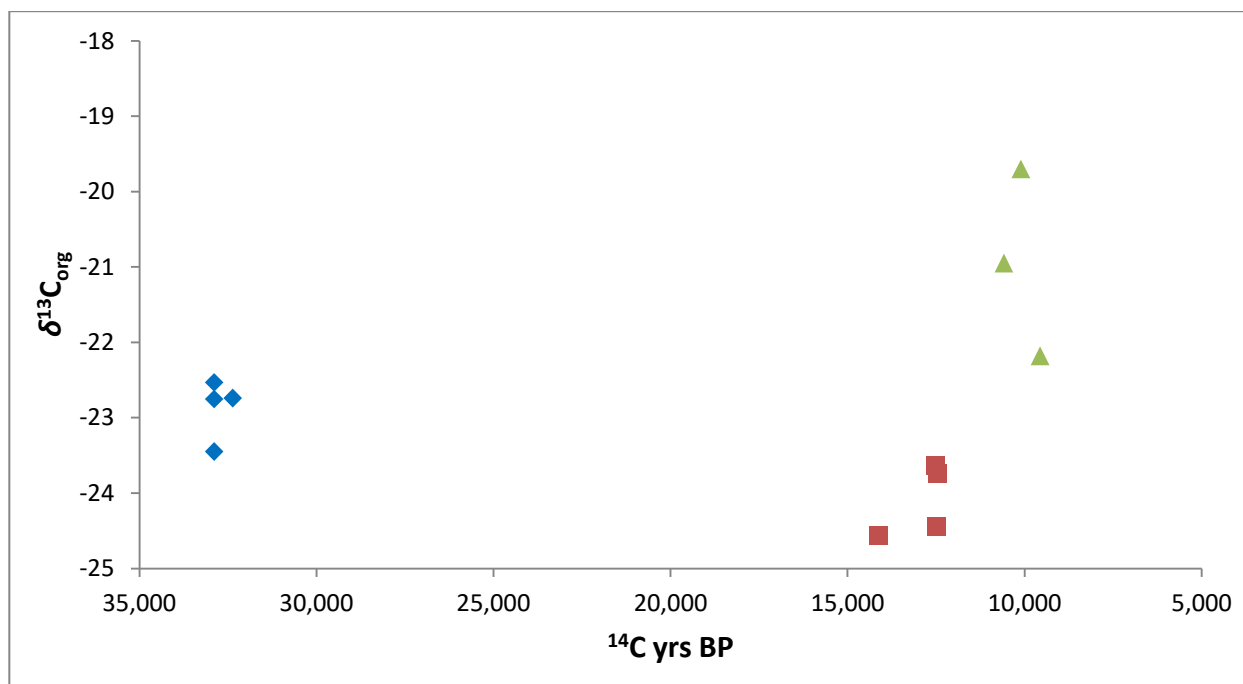


Figure 5.14. Variation in $\delta^{13}\text{C}_{\text{org}}$ for eggshell from the East over time. $>30,000$ BP (blue diamond), $29,000-12,000$ BP (red square), $<12,000$ BP (green triangle).

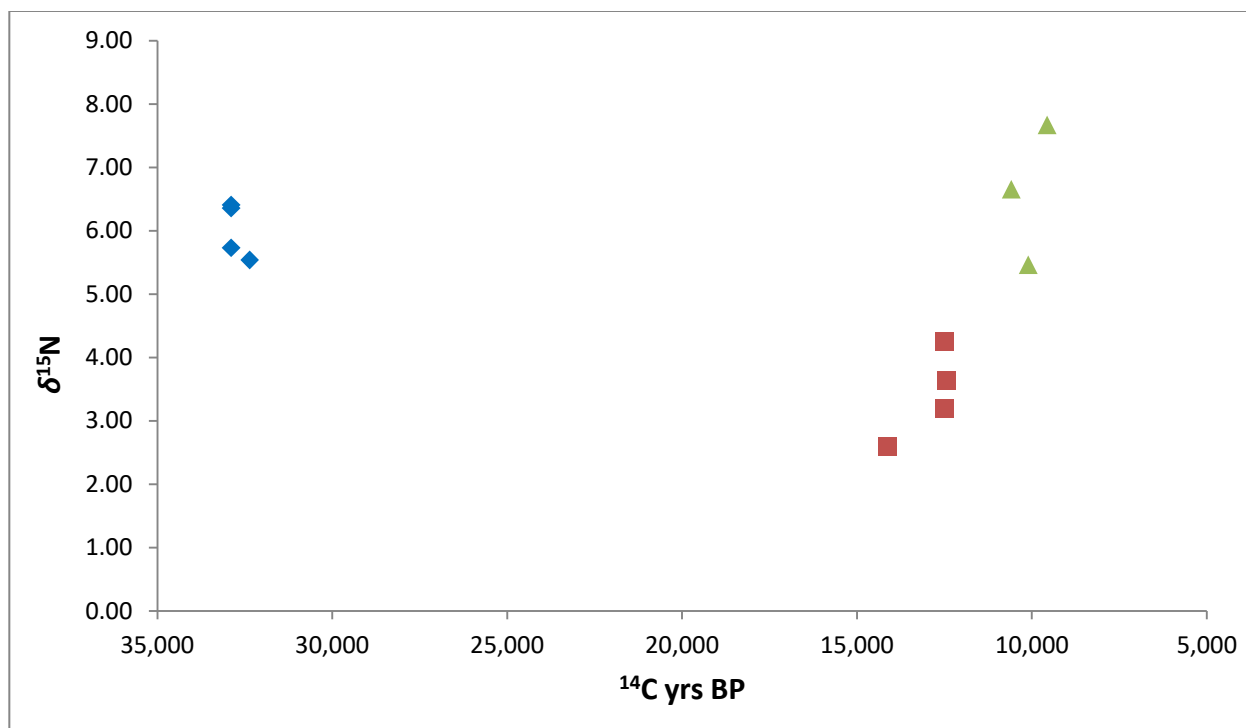


Figure 5.15. Variation in $\delta^{15}\text{N}$ for eggshell from the East over time $>30,000$ BP (blue diamond), $29,000-12,000$ BP (red square), $<12,000$ BP (green triangle).

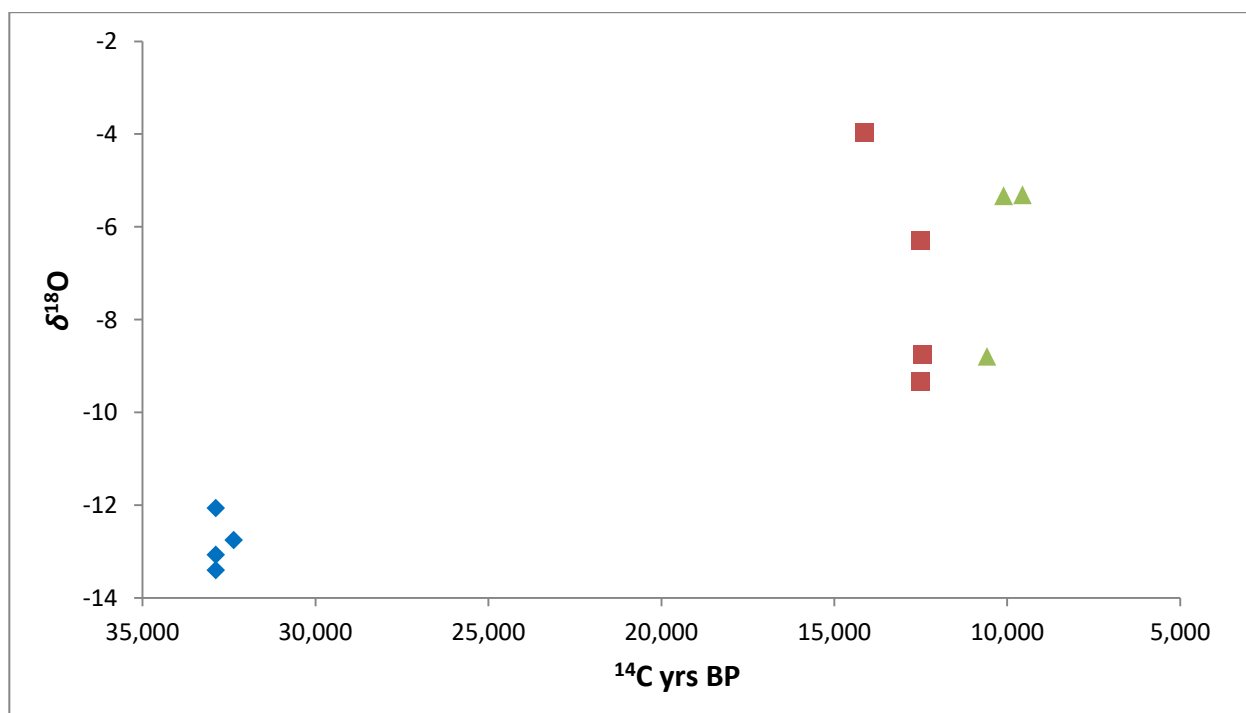


Figure 5.16. Variation in $\delta^{18}\text{O}$ for eggshell from the East over time $>30,000$ BP (blue diamond), $29,000-12,000$ BP (red square), $<12,000$ BP (green triangle).

A principal component analysis was conducted for the Eastern region data over time, to better depict the temporal clustering within the samples (Figure 5.17).

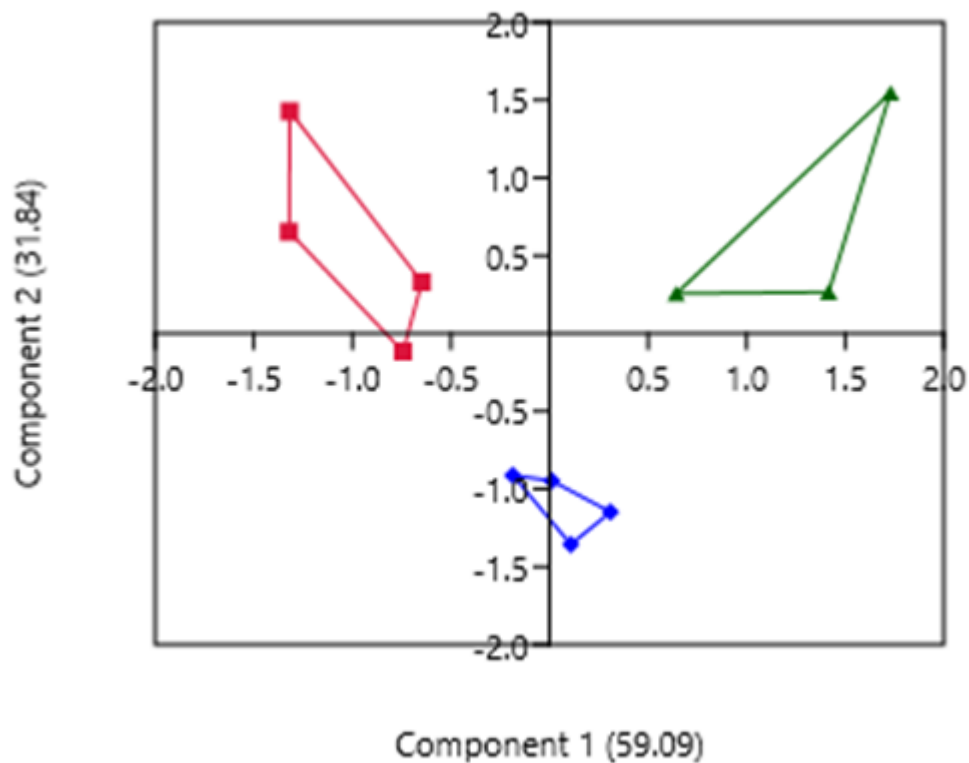


Figure 5.17. Principal component analysis depicting change in the Eastern region, over time. The variation on the x axis is most affected by $\delta^{13}\text{C}_{\text{org}}$ and the y axis is most affected by $\delta^{18}\text{O}$. In total, 90.93 % of the variation in the data is accounted for by the principal component analysis. >30,000 BP (blue diamond), 29,000-12,000 BP (red square), <12,000 BP (green triangle).

Section 5.6: Latitudinal Variation in $\delta^{18}\text{O}$

There was a significant correlation between eggshell $\delta^{18}\text{O}$ and latitude (Figure 5.18).

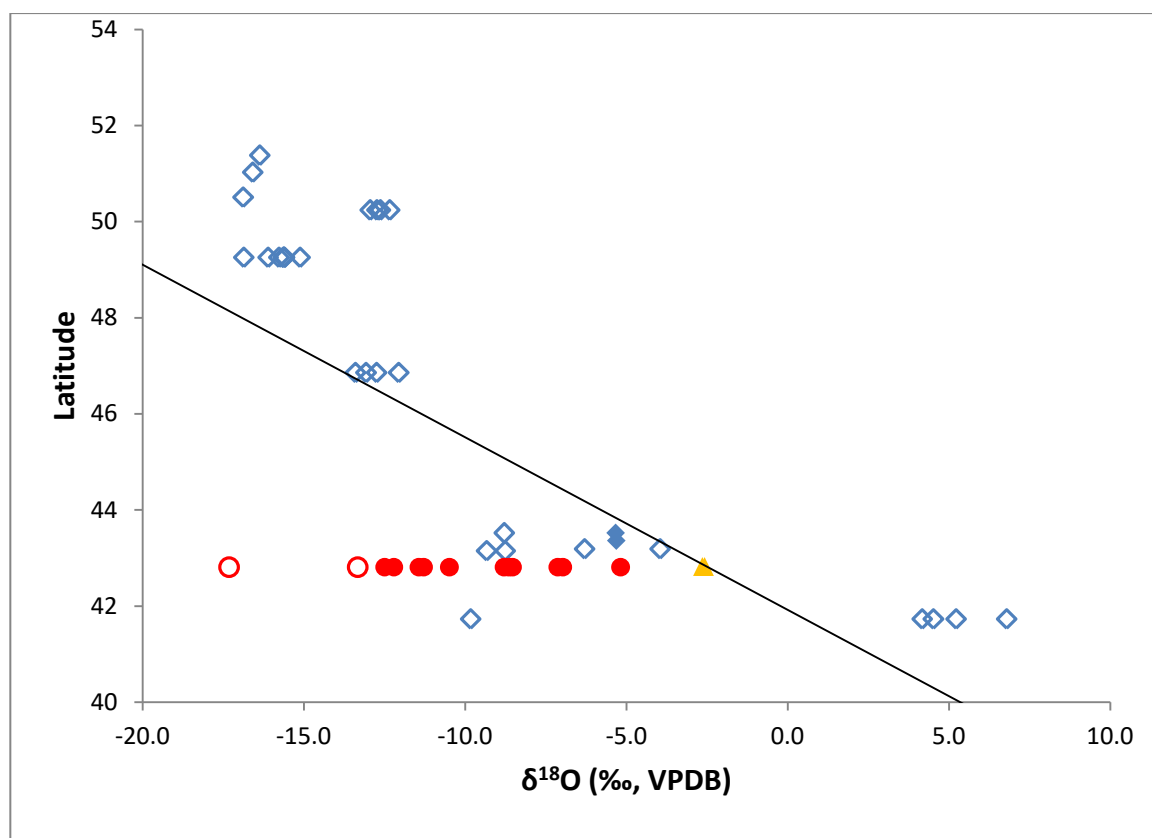


Figure 5.18. Variation in eggshell $\delta^{18}\text{O}$ and latitude for all samples included in this study. Samples from Shabarakh-usu (red filled circles: Holocene, red open circles: Pleistocene), Barun Daban (yellow triangles). All other sites (blue filled diamonds: Holocene, blue open diamonds: Pleistocene). Pearson's r indicated a moderate correlation $r = 0.698$, $p < 0.001$.

There was a great deal of variation at the site of Shabarakh-usu, which may indicate variation in sample origin. The nearby site of Barun Daban is contemporary with the majority of the samples at Shabarakh-usu. The two samples at Barun Daban fall on the trend line, indicating that the $\delta^{18}\text{O}$ results of these samples are consistent with the overall pattern. By removing samples from Shabarakh-usu, the correlation between $\delta^{18}\text{O}$

and latitude becomes much stronger (Pearson's $r = 0.859$, $p = <0.001$). This suggests the presence of eggshell derived from non-local areas at Shabarakh-usu.

Five samples from the Gurnai Depression (Figure 5.18) were ^{14}C indeterminate and presented $\delta^{18}\text{O}$ values that were unusually high (1679 = +4.52 ‰, 1680 = +5.22 ‰, 1681 = +6.79 ‰, 1682 = +6.79 ‰, 1683 = +4.17 ‰). The $\delta^{18}\text{O}$ of these samples are difficult to interpret but may be caused by long-term evaporation of standing water (Gat, 1996). Ostrich eggshell $\delta^{18}\text{O}$ is typically assumed to reflect the $\delta^{18}\text{O}$ of leaf-water, as discussed in Chapter 3. The anomalous values of these five samples may reflect a difference in behaviour, away from the exclusive consumption of leaf-water, toward consumption of standing water sources. As discussed in Chapter 4, these samples were determined to have been subjected to fire and as such, consideration was given to the possibility that fractionation through heat, may have elevated the $\delta^{18}\text{O}$. Experiments conducted on bone apatite and mollusc shell (Milano et al., 2016; Munro et al., 2008) indicate that the application of low heat (below 250°C) has little to no effect on the isotopic integrity of biogenic carbonate and increasing heat (250°C and above) progressively lowers the $\delta^{18}\text{O}$. Given that this subset of samples (1679-1683) have unusually *high* $\delta^{18}\text{O}$, it is unlikely that heat has affected the isotopic integrity and their elevated $\delta^{18}\text{O}$ is due to a difference in water acquisition on the part of the ostriches. When these samples were removed, the correlation between latitude and eggshell $\delta^{18}\text{O}$ was similarly strong (Pearson's $r = 0.869$, $p = <0.001$).

Section 5.7: Results of Hydrocarbon Extraction Experiment

A final result to be discussed pertains to the experiment conducted on two samples determined to be contaminated with hydrocarbons (4377 and 4380). Subsequently, samples 4377a and 4380a were prepared in a way consistent with all other samples, without the extraction attempt. The focus of this experiment was the $\delta^{13}\text{C}_{\text{org}}$, since this measurement should be lowered by the contaminant, as hydrocarbons may have a $\delta^{13}\text{C}_{\text{org}}$ around -30‰ (Conrad et al., 1997).

Table 5.7. Results of hydrocarbon extraction experiment.

Sample	$\delta^{13}\text{C}_{\text{org}}$ (+ Solvent extraction)	$\delta^{13}\text{C}_{\text{org}}$ (No Solvent extraction)
4377	-23.16‰	-23.87‰
4380	-23.63‰	-23.46‰

If the solvent extraction step was successful, one would expect to see the treated sample with a higher $\delta^{13}\text{C}_{\text{org}}$ value than the untreated sample. Sample 4377 did have a slightly higher $\delta^{13}\text{C}_{\text{org}}$ value than 4377a (by about 0.7‰), however, Sample 4380, actually had a slightly lower $\delta^{13}\text{C}_{\text{org}}$ result after the solvent extraction (Table 5.7). There can, however, be considerable overlap between the isotopic compositions of hydrocarbon contaminants and vegetation, making the assessment of decontamination through stable isotopes ambiguous. Indeed, in this instance the hydrocarbon contaminated samples are not isotopically inconsistent with other uncontaminated samples in the study (Conrad et al., 1997). Nevertheless, based on this limited experiment, and persistent discolouration

and odour of sample 4380, this extraction protocol appears to be insufficient for removing hydrocarbons from contaminated eggshell.

Chapter 6: Discussion

Section 6.1: Introduction

This study set out to examine questions regarding ostriches and ostrich eggshell in the Far Eastern Steppe. One question relates to species extirpation. *S. asiaticus* persisted on the Far Eastern Steppe, particularly in the South Gobi until about 8,000 cal yr BP which coincides with a moisture and vegetative optimum (Janz, 2015). This study aimed to clarify if the regional extirpation was forced by climate and environmental changes, human influence or some combination of both. My hypothesis from the outset was that changes in environment and climate forced ostrich extirpation as increased forestation would have reduced habitat and increased moisture would have reduced egg-hatch success, affecting species fertility and therefore viability in the region (Gonzalez et al., 1999; Janz et al., 2009). This question cannot be investigated; however, without making efforts to confirm the origins of the samples.

To that end, this research asks if the ostrich eggshell samples could be securely attributed to the local environment from which they were recovered. Eggshell samples were recovered from secondary locations, specifically from excavations of human

occupations, rather than nesting sites (Tashak, 2005; Janz, 2009). Ostrich eggshell is persistent and while samples often present dates that are consistent with the human occupation site of recovery (Tashak, 2000; Gao et al, 2008; Zwyns et al., 2014) there are instances of eggshells which present dates that are much older (Janz, 2015). This study tested the hypothesis that the eggshell was incorporated into the archaeological sites through local, opportunistic procurement by past populations, not by trade.

Identifying or negating the possibility of interregional eggshell movement is important for understanding the ancient territorial range of this extinct species, and investigating the possibility of trade. If eggshell isotopic compositions are inconsistent with their location of recovery, this may be indicative of interregional trade networks. Conversely, if eggshell is consistent with the region from which it was recovered, this could better inform our understanding of the geographic range of the species, particularly with respect to the samples from northern Mongolia and Siberia. If these specimens have isotopic compositions more consistent with a local origin, it would provide support for the presence of breeding ostriches in the north, clarifying the maximum expansion of *S. asiaticus*. If the samples were found to be inconsistent with northern origin, this would again speak to interregional trade.

Section 6.2: Origins of Sampled Eggshell and the Northern Expansion of Ostriches

The possibility of eggshell being relocated over significant distances is suggested by the interactions of past cultures with ostrich eggshell in Africa. Ndiema et al. (2010) suggest that pastoralists and hunter-gatherers around Lake Turkana, Kenya, interacted through exchange networks involving obsidian and ostrich eggshell beads, among other

commodities in the early Holocene. Kandel and Conrad (2005) suggest that in Western Cape, South Africa, ostrich eggs may have had a varied use life. Beginning as a food source, the shell might then have transitioned to a storage container and ultimately, when its utility as a vessel had ended, the shell was crafted into beads. It is unknown if ostrich eggshell in Asia shared analogous histories. The persistence of ostrich eggshell in the environment and the potential for a long use-life, with possible exchange networks, creates the possibility that the samples in this study originated some distance from the excavations that recovered them.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data collected do not provide a solid metric for assessing location of origin. Most differences in these data speak more to environmental variation over time than over space (Figures 5.1, 5.10, 5.15). The $\delta^{18}\text{O}$ data represent the most effective measurement among those collected in this study for assessing location of origin. As outlined in Chapter 3, variation in eggshell $\delta^{18}\text{O}$ is driven primarily by two factors: evaporation affecting consumed water, usually from leaves, and the latitude of the environment of origin.

Increasingly, regional distinctions in $\delta^{18}\text{O}$ of waters are used in archaeology and forensics as a tool for assessing the origin of biological samples but the results are coarse and should be used with an understanding of their limitations (Hobson et al., 2004; Kennedy et al, 2011; Lightfoot and O'Connell, 2016). In particular, the relationship between ingested water and eggshell $\delta^{18}\text{O}$ is unknown, therefore, it is not possible to pinpoint the location of sample origin on an isoscape for meteoric waters (Hobson et al., 2004; Hobson and Koehler, 2015). Nevertheless Bowen et al. (2005) has shown that a correlation between $\delta^{18}\text{O}$ and latitude is consistently observable, making it feasible to

appraise the north/south origin of samples, relative to one another. The data presented in this study show a strong and significant correlation between $\delta^{18}\text{O}$ and latitude ($r = 0.698$, $p = <0.001$), when all data are included (Figure 5.16). Broadly speaking, the $\delta^{18}\text{O}$ of the samples is driven by latitude sufficiently to be considered consistent with the hypothesis that the samples from the northern region are of more northern origin than the samples from the southern region. This result indicates that it is likely that the samples from northern Mongolia and Siberia were derived from local eggshells.

The $\delta^{18}\text{O}$ values from one particular site, Shabarakh-usu, are characterized by much more variability than any other site. There are thirteen samples from Shabarakh-usu, eleven of which date to the early/middle Holocene (approximately 9,500 to 8,200 cal yr BP). The other two samples, date to (1603) 32,985-36,095 cal yr BP and (1605) 41,140-44,345 cal yr BP. The $\delta^{18}\text{O}$ values from across the region (Figure 5.16) indicate that latitude largely determines the $\delta^{18}\text{O}$ of the samples and suggests that the eggshells from Shabarakh-usu have variable geographic origins. Alternatively, the highly variable $\delta^{18}\text{O}$ values from Shabarakh-usu may result from climate variability and day-to-day evapo-transporative processes which, through the early Holocene, may have produced a wide range of $\delta^{18}\text{O}$ values. The origin of waters entering the region impact the $\delta^{18}\text{O}$ values observed. Glacial melt-water from more northern latitudes or from mountains to the west may result in local hydrology with significantly lower $\delta^{18}\text{O}$ than would be expected on the basis of site latitude alone (Terzer et al., 2013; Epstein and Mayeda, 1953). If the eggshell found at Shabarakh-usu were local, rather than imported, the $\delta^{18}\text{O}$ of the local hydrology would be the variable factor. This may be an alternate explanation for the variation observed at Shabarakh-usu.

Shabarakh-usu, was a complex of recurrent, temporary occupations which allowed Holocene forager groups to exploit a lowland, wetland/dune-field ecosystem (Janz, 2006; Janz et al., 2017). One of the distinguishing factors of this site is the copious evidence for bead manufacture, using both ostrich eggshell and freshwater bivalve shell, some of which were incised with geometric patterns (Nelson, 1926). Relative to other sites in this study, Shabarakh-usu provided a large number of samples, most of which span a narrow range of dates, a circumstance which one might expect to provide a narrow range of $\delta^{18}\text{O}$ values if the eggshell was local. The large quantity of eggshell present at the site and the wide range of $\delta^{18}\text{O}$ values may indicate the importance of the site as a locus of eggshell aggregation in the early Holocene. The impermanence of the occupations at Shabarakh-usu makes it plausible that people acquired ostrich eggshell through travel and possibly interaction with other groups, carrying the material with them until they reached the location and period in their foraging cycle when bead-manufacture took place. That said, at least some of the samples from Shabarakh-usu coincide with site intensification consistent with Oasis 2, at a time when hunter/gatherers may have persisted at occupation sites for longer intervals, moving less frequently (Janz et al., 2017).

Two samples, 1603 and 1605, the only ones dated to the Pleistocene at Shabarakh-usu, were found in association with the samples dating to the early/middle Holocene, presenting a temporal anomaly at the site (Figure 5.18). Sample 1603 ($\delta^{18}\text{O} = -13.33\text{‰}$) is consistent with the oxygen isotope compositions of eggshell from sites in northern Mongolia. Sample 1605 ($\delta^{18}\text{O} = -17.32\text{‰}$) is the lowest $\delta^{18}\text{O}$ observed in the sample set, implying an origin even farther north than the excavations in southern Siberia

(Tri Skaly, Podzvonkaya, Ust'-Kyahta). The dates for both of these samples are coherent with dates obtained from northern samples, (Appendix 1). It is, therefore, likely that these two samples are particularly apparent examples of north-south anthropogenic movement of ostrich eggshell.

Alternatively, samples 1603 and 1605 may reflect the environment in the southern region around 30,000-40,000 cal yr BP. Wünnemann et al. (2007) indicates that through this period the southern region likely experienced a combination of increased precipitation and influxes of glacial melt water coming from the mountains into catchments in the region. Temperature through the late Pleistocene was also lower than the Holocene, with brief, sporadic exceptions (Wünnemann et al., 2007). This combination of factors could be responsible for the low $\delta^{18}\text{O}$ values observed in these Pleistocene, southern samples, meaning 1603 and 1605 may be of more local origin than the explanation of trade suggests.

Barun Daban, a site in close proximity to Shabarkh-usu, produced two samples with dates which are contemporary with the Holocene dates at Shabarakh-usu: (1607) 9,135-9,460 cal yr BP and (1608) 9,140-9,480 cal yr BP. These two samples produced $\delta^{18}\text{O}$ values of -2.58‰ and -2.65‰ respectively. These values are on or near the trend line in Figure 5.16 and so are in line with the trend observed between $\delta^{18}\text{O}$ and latitude across the study region. These data indicate a southern origin as they are the highest $\delta^{18}\text{O}$ values in the data set, apart from the values obtained from samples beyond the range of ^{14}C dating obtained in the Gurnai Depression (1679 to 1683) further south. The Gurnai Depression samples were interpreted in Chapter 5 as indicating a difference in water consumption on the part of ostriches at that time and place. The high $\delta^{18}\text{O}$ values of the

Barun Daban samples support the interpretation that the Shabarakh-usu samples originate in a variety of locations, with several likely originating to the north.

The evidence presented here indicates that broadly speaking, ostrich eggshell samples are consistent with a correlation between latitude and $\delta^{18}\text{O}$, along a north/south axis and most of the samples in this study likely originate near the excavation sites of recovery. This means that the samples collected in the north are consistent with the hypothesis of a local origin and a maximum species expansion extending north as far as Southern Siberia. There is also convincing evidence for anthropogenic movement of eggshell over considerable distance, from north to south, in the early/middle Holocene, aggregating at Shabarakh-usu. Given that ostrich eggshell represents a brief 3-5 day snapshot of environmental conditions; it is surprising that the relationship between $\delta^{18}\text{O}$ and latitude is as apparent as is observed in this study. It is possible that the climatic differences between the Pleistocene and the Holocene, in conjunction with small sample size have created a circumstance wherein elevated $\delta^{18}\text{O}$ in the north is representative of the Pleistocene environment on the steppe and is not proof of a northern origin. Differentiating the Pleistocene data from the Holocene data (Figure 5.16) indicates that, while somewhat less clear, broadly speaking, the Pleistocene data on its own are consistent with the relationship between $\delta^{18}\text{O}$ and latitude and it is likely that the northern samples are of northern origin. Nevertheless, the possible geographic origins of the eggshell requires further investigation through techniques such as strontium isotope analysis, which has the potential to match a sample more precisely with its place of origin and is not sensitive to temporal variation in environmental conditions (Bentley, 2006). It would also be beneficial to acquire additional samples as further analysis may add

resolution to this pattern, or alternatively, expose the pattern as an artifact of small sample size.

The evidence provided by this study for a breeding population of *S. asiaticus* in northern Mongolia and Siberia gives nuance to our understanding of the species' environmental tolerances. The period of the late Pleistocene that gives evidence for northern ostriches is marked by less forest and more steppe than the same region today but an average annual low temperature of -17°C (Savinova, 2006). *S. asiaticus*, therefore, likely required a fairly specific vegetative composition but could tolerate significant changes in temperature.

The evidence suggesting that Shabarakh-usu was a centre for ostrich eggshell trade is important for understanding hunter/gatherer subsistence strategies. Exchange in materials unrelated to subsistence, (eggshell beads) has been used archaeologically as an indication of social trade networks (Whallon, 2006). Social exchange networks such as these may involve simple trade between two groups or may take the shape of chains of exchange through multiple groups (Befu, 1977). Social networks were established by hunter/gatherers in an effort to foster reciprocity between groups, thereby increasing access to greater quantities and types of resources (Befu, 1977). In a fragmented environment such as the Far Eastern Steppe, during the early/middle Holocene, hunter/gatherer groups may have created buffers against short-term regional shifts in the productivity of subsistence resources through trade.

Section 6.3: Examining Ostrich Extirpation

Statistical analysis of the isotope data collected for this study indicates that there is some distinction, in the eggshell, between regions (Figure 5.1) however the magnitude of these differences is small, with considerable overlap. A striking feature of the $\delta^{13}\text{C}_{\text{org}}$ data is the lack of variation which may be the result of the specificity of environmental requirements for *S. asiaticus*. Assuming that C_3 plants are characterized by a modern range of $\delta^{13}\text{C}$ values between -37 and -20 ‰ (Kohn, 2010), it would appear that ostriches were consuming an exclusively C_3 diet. Plant $\delta^{13}\text{C}$ values < -29 ‰ indicate a closed canopy forest environment and $\delta^{13}\text{C}$ values > -23 ‰ indicate plants under water stress (van der Merwe and Medina, 1989). The $\delta^{13}\text{C}_{\text{org}}$ values in this study range narrowly between about -24 and -22 ‰, with a scant few exceptions. Assuming a diet to eggshell offset for $\delta^{13}\text{C}_{\text{org}}$ of 1.5 ± 0.8 ‰, (Johnson et al., 1998) this range of values is consistent with an open, semi-arid steppe environment with ample available C_3 plants for consumption. It is worth noting that studies like Johnson et al. (1998) have demonstrated, with samples from Africa, that it is possible to observe a wide range of $\delta^{13}\text{C}$ values from ostrich eggshell and that the narrow range obtained for *S. asiaticus* in this study is meaningful.

As mentioned, there were three $\delta^{13}\text{C}_{\text{org}}$ results which fell slightly outside the normal range. Sample 1678, from the Gurnai Depression in the south Gobi, dates to 17,855-18,310 cal yr BP. This post LGM period saw some degree of moisture amelioration coinciding with the onset of monsoon circulation (Herzschuh and Lui, 2007). The vegetation was unstable steppe with evidence for alpine forest (Herzschuh and Lui, 2007). The $\delta^{13}\text{C}_{\text{org}}$ value of -25.09 ‰ is the lowest in the sample set, and likely

represents a wet environment, potentially reflecting influxes of glacial melt water or elevated precipitation relative to the other samples in the study (Stewart et al., 1995). The corresponding $\delta^{15}\text{N}$ of the sample is +8.12 ‰ which is an intermediate value within the regional data. Ectomycorrhizal fungi are abundant in forested environments and result in lower $\delta^{15}\text{N}$, in their associated plants, relative to the arbuscular mycorrhizae associated with open environments (Read, 1991; Hobbie and Högberg, 2012). The $\delta^{15}\text{N}$ value of sample 1678 does not support the hypothesis of a change in plant/fungal associations resulting from a shift from an open to a forest ecosystem, as this change would likely result in a lower $\delta^{15}\text{N}$ value relative to other samples in the region (Högberg et al., 1996). The low $\delta^{13}\text{C}_{\text{org}}$ value can therefore not be explained by forest habitat. There are no other samples dating to 17,855-18,310 cal yr BP with which to compare this sample to assess the degree to which it reflects environmental conditions and it may simply be a product of a rapid environmental fluctuation over the very short time interval of egg production.

Samples 1610 and 1611 from Shara Murun Crossing, in the eastern Gobi Desert, date to 14,275-15,080 and 16,930-17,475 cal yr BP respectively. This region also experienced an increase in moisture associated with a transition from desert to steppe with increased vegetation including *Artemisia*, *Ephedra*, and pine (Shi and Song, 2003). The $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ values of these samples are low for this region compared with earlier dated samples ($\delta^{13}\text{C}$: 1610 = -24.44 ‰, 1611 = -24.57 ‰; $\delta^{15}\text{N}$: 1610 = +3.20 ‰, 1611 = +2.59 ‰). These low $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are consistent with the interpretation of a wet environment in this period.

At the site of Chilian Hotoga Well, in the East Gobi, samples 1613 (12,420-12,685 cal yr BP) and 1614 (11,400-11,975 cal yr BP), coincide with the Pleistocene/Holocene transition and present the highest $\delta^{13}\text{C}_{\text{org}}$ values in the study at -20.95‰ and -19.70‰ respectively. The $\delta^{15}\text{N}$ values for these samples are intermediate relative to the region at $+6.66\text{‰}$ (1613) and $+5.46\text{‰}$ (1614). The relatively high $\delta^{13}\text{C}$ may indicate an arid open steppe environment, consistent with the terminal Pleistocene/early Holocene, and some atypical contribution of C_4 plants in the diet (Von Schirnding et al., 1982). Though ostriches prefer C_3 plants, they will consume C_4 in small quantities, particularly over a short interval such as the 3-5 days needed to produce an egg.

Sample 4373 dates to 25,875-26,255 cal yr BP and is the only sample in the dataset associated with the Last Glacial Maximum (LGM). The sample was recovered at the site of Tolbor 17 in northern Mongolia where the LGM was characterized by a dry steppe or tundra environment, dominated by herbaceous plants (Shichi et al., 2009). This environment would have suited *S. asiaticus* better than regions further south during this extreme dry phase, where it may have been too arid to successfully breed. In Inner Mongolia, to the east, the LGM environment was cold and dry, with areas of needle-leaf forest and expanding desert, not at all the preferred environment for ostriches (Ni et al., 2010; Lu et al., 2013). In the South Gobi, the LGM was an extremely arid period, indicated by substantial aeolian deposits resulting from remobilisation of sand (Hülle et al., 2010). It is possible that neither the south nor the east met the resource requirements (food/water) of ostriches through the LGM, and the north likely acted as a refuge for the species. Stewart et al. (2010) defined Quaternary refugia as the region inhabited by a

species during a glacial/interglacial cycle which represents the maximum range contraction a species can tolerate and remain viable. Cryptic northern refugia are limited regions which meet the specific requirements of a species but are circumscribed by uninhabitable ecosystems (Stewart and Lister, 2001). This circumstance will sometimes result in the unexpected residency of a temperate species at a more northerly latitude than may be expected (Stewart et al., 2010), such as the case of *S. asiaticus*. This observation further supports the hypothesis that the northern samples are local rather than imported from farther south.

The consistency of the isotope data across time and space highlights a preference among *S. asiaticus* for open steppe, as opposed to desert or forest and suggests the exploitation of different geographic regions consistent with this preference throughout late Pleistocene and early Holocene climate shifts. The specific requirements of *S. asiaticus* imply low plasticity in ecosystem exploitation, resulting in low resilience in the face of dramatic environmental change. Low plasticity in environmental preference, therefore, likely underpins the extirpation of the species during the early to middle Holocene. The extirpation of ostriches around 8,000 cal yr BP coincides with the region's Holocene vegetative and moisture optimum. My hypothesis was that increased environmental moisture inhibited the loss of water through the pore complexes of the ostrich eggshell. This would have resulted in suffocation of the chick and loss of hatchability, therefore, reducing the viability of the species (Gonzalez et al., 1999). It was expected that the isotopic compositions of the eggshell samples, leading up to extirpation, would correspond with a wetter environment and as a proxy for this, would present the lowest $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ in the sample set.

The expected shifts in $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ were not present in the data, which suggests that ostriches continued to find their preferred conditions for nesting. There are 13 samples leading up to the extirpation of *S. asiaticus* dated 8,195-9,550 cal yr BP. These samples were recovered from the southern sites of Barun Daban and Shabarakhusu. The environment reflected in these samples does not distinguish itself as anything other than semi-arid open steppe. The samples do not present the highest or lowest values of either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, but are in fact, comparable to the isotopic compositions of earlier samples (Figure 6.1). This data set may not be well suited to capture abrupt environmental shifts as ostriches may only breed when conditions are suitable, meaning eggshell will not be produced when environmental conditions are unsuitable. In that case a slow, incremental shift in moisture may be observable (i.e., the broad shifts in eggshell $\delta^{18}\text{O}$ over thousands of years presented in this thesis), but an abrupt change would not.

There is some disagreement among researchers pertaining to the timing of the shift from monsoon to Westerlies-dominated moisture regimes in the South Gobi. Herzschuh (2006) argues the South Gobi was under the influence of the Westerlies, at the transition to the Holocene. Lee et al. (2013) assert that the monsoon boundary persisted in the region through to the middle Holocene. In areas under monsoon influence, conditions were much warmer and wetter, beginning 11,500 cal yr BP and extending to 3,000 cal yr BP (Herzschuh, 2006). The arid region of the Gobi became constrained to a swath between 38°N - 48°N, where conditions were extremely dry through the early/middle Holocene (Feng et al., 2007). This is supported by data from Southern Mongolia indicating that 8,500 to 7,500 cal yr BP was a period of cold/dry conditions (Felauer et al., 2012).

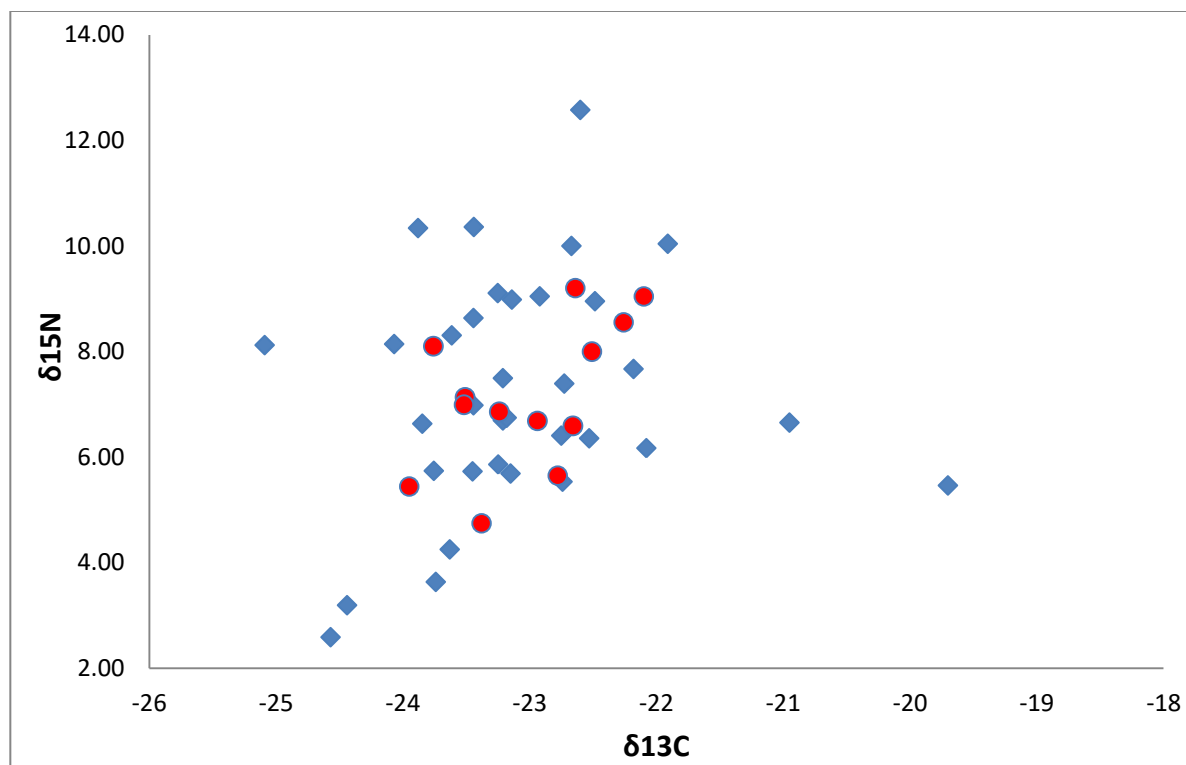


Figure 6.1. $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ values for Asian ostrich eggshell analyzed in this study: 10,700 to >49,900 BP cal yr BP (blue diamonds) and 8,100 to 9,500 cal yr BP (red circles).

Though aridity was prevalent, there is evidence for herbaceous wetland plants along riparian floodplains (Feng et al., 2007). In the north 8,500 to 7,500 cal yr BP is marked by elevated precipitation and the presence of well-established forest (Tarasov et al., 2007). At this time in the east, around Otson Tsokhio, the environment experienced increased humidity, and active river and lake systems (Bazarova et al., 2019). Small birch/alder forests became established in hospitable areas while sandy terraces were dominated by chenopods (Bazarova et al., 2019). Pollen records from Inner Mongolia indicate the presence of pine, birch, oak, and *Artemisia*, indicating a forest-steppe environment (Zhao et al., 2009). Western Inner Mongolia, through this time period, saw the elimination of evidence for needle leaf trees to greater dominance by desert-steppe (Zhao et al., 2009). Palynological studies of lakes from the Badain Jaran Desert show

evidence for pine, spruce and elm as well as grasses and sedges through the early/middle Holocene (Yang and Williams, 2003).

All told this indicates a fragmentation of the environment creating a patchwork of ecosystems, some beneficial to ostriches and some restricting. Guthrie's model (1984), which anticipates that Holocene ecosystem fragmentation led to megafaunal extinction, is likely relevant to ostrich extirpation in Asia. Species with greater environmental plasticity exhibit corresponding variability in the isotopic composition of their tissues (Cormie and Schwarcz, 1994). Ostriches appear to express little environmental plasticity on the basis of their invariant eggshell $\delta^{13}\text{C}$ values. Considering the mid-Holocene patchwork of environments in the Far Eastern Steppe, it is likely that populations of ostriches were sequestered and segregated into environmentally viable enclaves. The separation of populations creates genetic bottlenecks which, if persistent over a protracted period of time, will eventually reduce the viability of the species through inbreeding, causing a loss of genetic diversity (Nei et al., 1975; Wright, 1931). Isolated populations of ostriches would therefore have become increasingly fragile.

In addition to the detrimental, separating effects of the vegetative/moisture optimum, the proposition of eggshell movement during the early Holocene implies human pressure. As detailed above, the $\delta^{18}\text{O}$ of samples at Shabarakh-usu suggest that some or all of the eggshells recovered were acquired at other locations and aggregated at an occupation site that specialized in bead manufacture. Humans were, therefore, in proximity to and interfering with ostrich nesting sites, applying pressure to isolated populations. The relocation of eggshell from other locales implies that the local area did not or could not supply sufficient eggshell to meet human demand.

One of the most straightforward ways for a hunter/gatherer to locate an ostrich nest is to follow an adult bird and be led to the site (Hitchcock, 2012). This means the nest would still be active when raided. Accounts from the Kalahari indicate that once a nest is located, hunter/gatherers may wait for the adult ostrich to leave in order to avoid confrontation, or may attempt to frighten the adult off the nest. At this point the hunter/gatherer would procure several eggs, potentially leaving behind only one, in the hope that the female ostrich would be prompted to lay more and replenish the supply (Hitchcock, 2012). Clearly, interference of this type would be a detriment to a species that was already under strain from loss of habitat. It was therefore likely that environmental and human pressures worked in concert to contribute to the extirpation of the species.

Chapter 7: Conclusion

This research sought to use stable isotope data and radiocarbon dating to clarify questions concerning ostrich ecology, eggshell commodification, and ostrich extirpation. Specifically, the study addressed three questions.

1. Were the samples recovered in the north produced by breeding ostriches in Northern Mongolia and Siberia, or were the samples recovered at these sites a product of anthropogenic movement of eggshell?
2. Can the eggshell recovered from human occupations be attributed to the local area or is there evidence of anthropogenic movement/trade of eggshells?
3. What insights can stable isotope measurements of $\delta^{13}\text{C}_{\text{org}}$, $\delta^{15}\text{N}$, $\delta^{13}\text{C}_{\text{inorg}}$, and $\delta^{18}\text{O}$ provide about the extirpation of the species?

The $\delta^{18}\text{O}$ values were useful for assessing sample origin. Since the $\delta^{18}\text{O}$ values varied with latitude, as one would expect given the mechanisms influencing meteoric water $\delta^{18}\text{O}$ values, these data showed that, broadly speaking, the samples were likely to be relatively local to their site of recovery. The eggshells from the site of Shabarakh-usu,

however, showed an unusual amount of variation in $\delta^{18}\text{O}$. There were also two samples recovered at this site that were dated to the Pleistocene and were, therefore, far older than the majority of samples, which dated to the Holocene (approx 8,000-9,500 cal yr BP). These temporal outliers also presented very low $\delta^{18}\text{O}$ and were interpreted to have originated in the north, but could reflect environmental variation representing higher moisture and lower temperature in the late Pleistocene. Environmental variation and daily evapo-transpirative processes, and non-local hydrology cannot be ruled out as causes of the wide variation in $\delta^{18}\text{O}$ values at Shabarkhusu. Nevertheless, given the strong, significant correlation between the $\delta^{18}\text{O}$ and latitude of most samples in the study, I propose that Shabarakh-usu was a site where eggshell from other locations was aggregated for the purpose of bead manufacture. Eggshell movement may be the product of the commodification of eggshell, through trade and exchange, as a means of creating reciprocal networks and fostering improved resilience for groups across the region.

The $\delta^{18}\text{O}$ values indicate the movement of eggshell from north to south but do not provide evidence of the reverse. It is, therefore, likely that the eggshell recovered from northern Mongolia and Siberia is consistent with a northern origin. The $\delta^{18}\text{O}$ values from the northern samples are the lowest in the study. Additionally, the only sample in the study dating to the LGM is from the Tolbor 17 site, a region that would have been suitable for ostriches at a time when other regions may have lacked resources. There are no northern dates after the LGM, which is consistent with the environment shifting to taiga forest. The evidence, therefore, fits well with an expansion of ostriches into northern Mongolia and Siberia from at least 44,000 cal yr BP and extending through the LGM before likely retreating from the region due to habitat loss.

The stable isotope data indicate that *S. asiaticus* had little plasticity in terms of diet or habitat use, at least with respect to variation that would manifest isotopically in eggshell. The species required open steppe with no apparent tolerance for forest or desert and had a strong preference for C₃ vegetation, as demonstrated by Von Schirnding et al., (1982), with little incorporation of C₄ plants. The hypothesis that the eggshell isotopic compositions would indicate a wet, possibly forested environment approaching extirpation was not supported by the results. Samples with dates approaching extirpation were recovered from the southern region from the sites of Barun Daban and Shabarakh-usu. All of the samples had $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ values that were consistent with an open steppe environment, very typical of the results from other times and places in the study. These data did not support the hypothesis of a wet, possibly forested environment, which indicates that even at a time when the environment was fragmented into a variety of biomes and preferred habitat was at a minimum, ostriches found refugia. This study also indicates that there was likely some degree of anthropogenic movement of eggshell, aggregating at the site of Shabarakh-usu, where bead manufacture took place. This may imply that through the early/middle Holocene, human interference, to procure the eggshell, put pressure on isolated and fragile populations.

There are several important caveats that must be discussed in relation to the interpretations of these isotopic data. While the $\delta^{18}\text{O}$ values do give an indication of relative north vs. south origin, they do not pin down the location with any specificity. Additionally, any movement of eggshell in an east/west interaction, at similar latitude, cannot be observed since, broadly speaking, the $\delta^{18}\text{O}$ of meteoric water is not expected to vary with longitude in the study regions. Therefore, while this study has contributed

strong evidence for movement of ostrich eggshell across a north-south axis, I have been unable to identify east-west movement, but this absence of evidence should not be taken as evidence of absence. Anthropogenic movement of eggshell may, therefore, have been more prevalent than the data shows. The possibility that the pretreatment or analytical method used to obtain data from the inorganic fraction may have produced variability in the results, reducing reliability, cannot be excluded. Some amount of repeated analysis should be undertaken to assess the reproducibility of the results, varying pretreatment and analytical methods on the same set of samples to determine their impact on eggshell isotopic compositions.

Further investigation, particularly strontium isotope analysis, may help to clarify the interpretations of this study and attribute sample locations of origin with greater precision. More precise regional attribution would permit the reconstruction of hunter/gatherer trade networks, allowing researchers to understand the scope and directionality of the interactions. Strontium isotope analysis is, however, not immediately feasible without first establishing an appropriate baseline of bioavailable strontium across the region. Furthermore, the small sample size of this study may present an incomplete picture. Increased sampling may reveal more distinct temporal variation which could not adequately be discerned given the small sample size used in this study. A greater sample size may also allow for more direct cross regional comparisons by evaluating samples from different contemporaneous locations.

This study was the first to use stable isotope analysis to investigate the geographic origins and ecology of *S. asiaticus*. It provides evidence supporting the hypothesis that ostriches had a maximum northern expansion that reached at least as far north as Lake

Baikal in Siberia, during the Late Pleistocene. This study also presented evidence consistent with the anthropogenic movement of eggshell, which may lead to reconstructions of forager trade networks with further analyses of this type. Ostrich eggshell beads were likely a non-subsistence trade item used to forge reciprocal relationships across space, improving resource access and buffering against regional resource shortfalls.

Current ostrich conservation efforts note that the dwindling extant population of *S. camelus*, in Africa is subject to a heavy burden of obstacles including division by the equatorial forest zone and environmental and human factors including drought, war, mechanised agriculture, and over-hunting/trade in live specimens (Cooper et al., 2009). In Tanzania, wild ostriches are hunted to procure a variety of primary and secondary commodities including meat, eggs, skin, feathers, and oil (Magige and Røskaft, 2017). These products are acquired for sustenance, ornamentation, and medication. Population separation, shifting environmental conditions, and human pressures are once again putting an ostrich species at risk.

Conservationists can use the example of the past to better understand the real-time risks to extant ostriches and work to ease some of the pressures. One method that may lessen human pressure on wild populations is the farming of domestic ostriches, allowing ostrich goods to be acquired cheaply, conveniently and with measures of control over production (Cooper et al., 2009; Magige and Røskaft, 2017). Human pressure is, however, only one component of a complex web of driving factors, the sum of which can be better understood in light of research into the drivers of past ostrich extirpation.

This research suggests that loss of habitat, population isolation, and human interference came together to cause the extirpation of *S.asiaticus* from the Far Eastern Steppe. If subsequent research supports this hypothesis, the threats to wild extant populations may be more readily communicated by conservationists. Extinction is less abstract when its mechanisms are more apparent.

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Appendix 1. Contextual information for samples analyzed in this study.

TEAL Number	Museum or Excavation ID	Region	Site	AMS Date BP¹	Calibrated yr BP¹	Contamination
1599	73/98A	South Gobi	Shabarkh-usu Lat: 42.81807°N Long: 104.1379°E	8,254 ± 47	9,035-9,408	
1600	73/648A	South Gobi	Shabarkh-usu	7,483 ± 47	8,196-8,382	
1601	73/790A01	South Gobi	Shabarkh-usu	8,396 ± 52	9,296-9,519	
1602	73/790A02	South Gobi	Shabarkh-usu	8,268 ± 44	9,094-9,424	
1603	73/790A03	South Gobi	Shabarkh-usu	30,490 ± 780	32,984-36,093	
1604	73/648B	South Gobi	Shabarkh-usu	8,522 ± 50	9,455-9,552	
1605	73/1034A02	South Gobi	Shabarkh-usu	38,600 ± 1000	41,142-44,346	
1606	73/1034A03	South Gobi	Shabarkh-usu	8,439 ± 60	9,306-9,537	
1607	73/1790A	South Gobi	Barun Daban Lat: 42.81797°N Long: 104.13787°E	8,307 ± 56	9,134-9,457	
1608	73/1790B	South Gobi	Barun Daban	8,338 ± 55	9,140-9,480	
1609	73/1034A01	South Gobi	Shabarakh-usu	8,054 ± 43	8,769-9,088	
1610	73/2303	East Gobi	Shara Murun Crossing Lat: 43.3938°N Long: 111.0334°E	12,497 ± 70	14,276-15,078	
1611	73/2403A	East Gobi	Shara Murun Crossing	14,129 ± 80	16,928-17,477	
1612	73/2646A	East Gobi	Alkali Well Lat: 43.3647°N Long: 112.3540°E	9,562 ± 51	10,712-11,121	
1613	73/2800A	East Gobi	Chilian Hotoga Well Lat: 43.5342°N Long: 114.3142°E	10,586 ± 56	12,421-12,683	Noted and Avoided
1614	73/2800C	East Gobi	Chilian Hotoga Well	10,103 ± 55	11,401-11,976	Noted and Avoided
1615	73/2225A01	East Gobi	Baron Shabaka Well Lat: 43.3929°N Long: 112.2920°E	12,509 ± 59	14,320-15,083	
1616	73/2225A02	East Gobi	Baron Shabaka Well	12,450 ± 74	14,198-15,013	
1617	73/984A	South Gobi	Shabarakh-usu	8,473 ± 64	9,314-9,548	
1618	73/894A	South Gobi	Shabarakh-usu	7,589 ± 47	8,325-8,513	
1619	73/763A01	South Gobi	Shabarakh-usu	8,159 ± 43	9,009-9,254	
1620	73/763A02	South Gobi	Shabarakh-usu	8,184 ± 44	9,019-9,270	

TEAL Number	Museum or Excavation ID	Region	Site	AMS Date BP ¹	Calibrated yr BP ¹	Contamination
1673	ots.18.B2.L:4 (wall)	Eastern Steppe	Otson Tsokhio Lat: 46.8651°N Long: 103.8330°E	32,366±193	35,780-36,718	
1674	ots.18.A2.L:bone	Eastern Steppe	Otson Tsokhio	32,890±196	36,319-37,702	
1675	ots.18.A2.L1.bone	Eastern Steppe	Otson Tsokhio	32,890±196	36,319-37,702	
1676	ots.18.A2.L2:35	Eastern Steppe	Otson Tsokhio	32,890±196	36,319-37,702	
1677	K.13212:184 AA 87198	South Gobi	Yingen khuduk – Alashan Gobi	41,900±1500	42,770-48,269	No Gas
1678	K.13290:44 AA 87197	South Gobi	Mantissar Lat: 41.7294°N Long: 105.8168°E	14,857±85	17,855-18,310	
1679	K.13293:29 AA 87200	South Gobi	Mantissar	>49,900	carbon dead	Noted and Avoided
1680	K.13298:60-03 AA 87203	South Gobi	Mantissar	>46,840	carbon dead	Noted and Avoided
1681	K.13298:60-02 AA 87199	South Gobi	Mantissar	>46,540	carbon dead	Noted and Avoided Smelled burned
1682	K.13298:60-01 AA 87202	South Gobi	Mantissar	>47,940	carbon dead	Smelled burned
1683	K.13298:55 AA 87201	South Gobi	Mantissar	>47,940	carbon dead	Smelled burned
2287	Tolbor 7342 site P.2 Sq.Id:3 Level 3B	Northern Steppe	Tolbor Lat: 49.2564°N Long: 103.4012°E	32,228±209	35,621-36,564	
4361	Tulbur – 21	Northern Steppe	Tolbor 21	39,692±439	42,696-44,215	
4362	Tulbur – 21	Northern Steppe	Tolbor 21			
4363	Tulbur – 21	Northern Steppe	Tolbor 21	40,103±457	42,943-44,562	
4364	Tulbur – 21	Northern Steppe	Tolbor 21			
4365		Northern steppe	Bayangoliin am Lat:50.2404°N Long: 100.0967°E	34,839±252	38,740-39,963	
4366		Northern steppe	Bayangoliin am			Contamination was unavoidable
4367		Northern steppe	Bayangoliin am			
4368		Northern steppe	Bayangoliin am			Contamination was unavoidable
4369		Northern steppe	Bayangoliin am	34,996±252	38,877-40,141	

TEAL Number	Museum or Excavation ID	Region	Site	AMS Date BP ¹	Calibrated yr BP ¹	Contamination
4370		Northern steppe	Bayangoliin am			
4371		Northern steppe	Bayangoliin am			Contamination was unavoidable
4372	T17-372	Northern Steppe	Tolbor 17			Contamination was unavoidable
4373	T17-312	Northern Steppe	Tolbor 17	21,845±79	25,876-26,253	
4374	T17-259	Northern Steppe	Tolbor 17			
4375	T17-551	Northern Steppe	Tolbor 17			Contamination was unavoidable
4376	T17-130	Northern Steppe	Tolbor 17			
4377	T17-340	Northern Steppe	Tolbor 17			Contamination was unavoidable (hydrocarbon)
4378	T17-362	Northern Steppe	Tolbor 17			Contamination was unavoidable
4379		Northern Steppe	Bukhyn Mankhan			Contamination was unavoidable
4380		Transbaikal Northern steppe	Podzvonkaya, Eastern Complex			Contamination was unavoidable (hydrocarbon)
4382		Transbaikal Northern steppe	Podzvonkaya, Eastern Complex Lat: 51.3809°N Long: 108.9023°E	33,336±224	36,803-38,369	
4384		Northern steppe	Tri Skaly (Three Rocks) Lat: 51.1155°N Long: 107.0431°E	34,966±280	38,804-40,154	
4386	Ust'-Kyahta - 16	Northern steppe	Ust'-Kyahta Lat: 50.5136°N Long: 106.2722°E	31,427±177	34,862-35,740	
2475		Ostrich Land	Ontario, Canada	Modern	2018-2019	
2476		Ostrich Land	Ontario, Canada	Modern	2018-2019	
2477		Ostrich Land	Ontario, Canada	Modern	2018-2019	
2478		Ostrich Land	Ontario, Canada	Modern	2018-2019	

TEAL Number	Museum or Excavation ID	Region	Site	AMS Date BP¹	Calibrated yr BP¹	Contamination
2479		Ostrich Land	Ontario, Canada	Modern	2018-2019	
2480		Ostrich Land	Ontario, Canada	Modern	2018-2019	
2481		Ostrich Land	Ontario, Canada	Modern	2018-2019	
2482		Ostrich Land	Ontario, Canada	Modern	2018-2019	
2483		Ostrich Land	Ontario, Canada	Modern	2018-2019	

1. Dates obtained from Janz, 2015 and A.E Lalonde AMS Laboratory, calibrated through Calib Rev 7.0.4 (Stuiver and Reimer, 1993).

Appendix 2. Accepted or long-term average values of standards used in the isotopic analyses

Standard	$\delta^{13}\text{C}_{\text{VPDB}}/\text{‰}$	$\delta^{15}\text{N}_{\text{AIR}}/\text{‰}$
USGS40	-26.39±0.04	-4.52±0.06
USGS41a	+36.55±0.05	+47.60±0.11
SRM-1	-19.36±0.08	+1.82±0.11
SRM-2	-14.77±0.06	+15.59±0.14
SRM-14	-13.67±0.07	+21.6±0.15
SRM-17	-12.44±0.05	+3.17±0.05
Amygdalin	-24.39±0.08	+0.58±0.21
Tyrosine	-22.97±0.04	+7.65±0.16
	$\delta^{13}\text{C}_{\text{VPDB}}/\text{‰}$	$\delta^{18}\text{O}_{\text{VPDB}}/\text{‰}$
NBS19	+1.95	+28.65
NBS18		+7.20±0.01
LSVEC	-46.60±0.20	
Ws-1	+0.76±0.14	+26.23±0.14
SupraPur	-35.55±0.21	+13.30±0.13

Appendix 3. Isotopic compositions of all samples analyzed in this study.

TEAL	Region	Sample Site Region	Calibrated yr BP	$\delta^{13}\text{C}_{\text{org}}$	$\delta^{13}\text{C}_{\text{inorg}}$	org:inorg offset	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$
1612	East Gobi	East Gobi - Alkali Well	10,712-11,121	-22.18	-10.97	11.21	-5.31	+7.67
1616	East Gobi	East Gobi - Baron Shabaka Well	14,198-15,013	-23.74	-11.23	12.51	-8.76	+3.64
1615	East Gobi	East Gobi - Baron Shabaka Well	14,320-15,083	-23.63	-12.37	11.26	-9.33	+4.25
1614	East Gobi	East Gobi - Chilian Hotoga Well	11,401-11,976	-19.70	-7.26	12.44	-5.33	+5.46
1613	East Gobi	East Gobi - Chilian Hotoga Well	12,421-12,683	-20.95	-8.29	12.66	-8.79	+6.66
1610	East Gobi	East Gobi - Shara Murun Crossing	14,276-15,078	-24.44	-12.86	11.58	-6.30	+3.20
1611	East Gobi	East Gobi - Shara Murun Crossing	16,928-17,477	-24.57	-12.04	12.53	-3.96	+2.59
1673	Eastern Steppe	Otson Tsokhio (Eastern Steppe)	35,780-36,718	-22.74	-11.42	11.32	-12.75	+5.54
1674	Eastern Steppe	Otson Tsokhio (Eastern Steppe)	36,319-37,702	-22.75	-11.79	10.96	-13.40	+6.41
1675	Eastern Steppe	Otson Tsokhio (Eastern Steppe)	36,319-37,702	-22.53	-11.11	11.42	-13.07	+6.36
1676	Eastern Steppe	Otson Tsokhio (Eastern Steppe)	36,319-37,702	-23.45	-11.55	11.90	-12.06	+5.73
4365	Northern Steppe	Bayangoliin am	38,740-39,963	-22.73	-11.94	10.79	-12.95	+7.39
4367	Northern Steppe	Bayangoliin am		-22.48	-10.68	11.80	-12.34	+8.95
4369	Northern Steppe	Bayangoliin am	38,877-40,141	-24.07	-13.14	10.93	-12.74	+8.14
4370	Northern Steppe	Bayangoliin am		-23.14	-12.72	10.42	-12.63	+8.99

TEAL	Region	Sample Site Region	Calibrated yr BP	$\delta^{13}\text{C}_{\text{org}}$	$\delta^{13}\text{C}_{\text{inorg}}$	org:inorg offset	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$
4382	Northern Steppe	Podzvonkaya	36,803- 38,369	-23.44	-11.28	12.16	-16.37	+6.98
4361	Northern Steppe	Tolbor	42,696- 44,215	-23.21	-13.79	9.42	-15.63	+7.50
4362	Northern Steppe	Tolbor		-23.21	-13.93	9.28	-15.63	+6.70
4363	Northern Steppe	Tolbor	42,943- 44,562	-23.18	-13.7	9.48	-15.61	+6.75
4364	Northern Steppe	Tolbor		-23.15	-13.8	9.35	-15.60	+5.69
4373	Northern Steppe	Tolbor	25,876- 26,253	-23.44	-12.9	10.54	-15.77	+8.64
4374	Northern Steppe	Tolbor		-23.25	-12.39	10.86	-16.87	+9.11
4376	Northern Steppe	Tolbor		-23.76	-12.78	10.98	-15.12	+5.74
2287	Northern Steppe	Tolbor	35,621- 36,564	-23.88	-11.54	12.34	-16.11	+10.34
4384	Northern Steppe	Tri Skaly	38,804- 40,154	-23.85	-12.13	11.72	-16.59	+6.63
4386	Northern Steppe	Ust'-Kyahta - 16	34,862- 35,740	-23.61	-12.75	10.86	-16.88	+8.31
1607	South Gobi	Barun Daban - Gobi Altai	9,134-9,457	-23.51	-10.26	13.25	-2.58	+7.14
1608	South Gobi	Barun Daban - Gobi Altai	9,140-9,487	-22.94	-10.07	12.87	-2.65	+6.69
1679	South Gobi	Gurnai Depession - Mantissar	Carbon Dead	-22.67	-9.17	13.5	+4.52	+10.00
1680	South Gobi	Gurnai Depession - Mantissar	Carbon Dead	-23.44	-9.37	14.07	+5.22	+10.36
1681	South Gobi	Gurnai Depession - Mantissar	Carbon Dead	-22.92	-8.98	13.94	+6.79	+9.05

TEAL	Region	Sample Site Region	Calibrated yr BP	$\delta^{13}\text{C}_{\text{org}}$	$\delta^{13}\text{C}_{\text{inorg}}$	org:inorg offset	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$
1682	South Gobi	Gurnai Depession - Mantissar	Carbon Dead	-22.60	-8.63	13.97	+6.79	+12.58
1683	South Gobi	Gurnai Depession - Mantissar	Carbon Dead	-21.91	-8.15	13.76	+4.17	+10.04
1678	South Gobi	Gurnai Depession - Mantissar	17,855-18,310	-25.09	-12.13	12.96	-9.83	+8.12
1603	South Gobi	Shabarakh-usu	32,984-36,093	-23.25	-11.19	12.06	-13.33	+5.87
1605	South Gobi	Shabarakh-usu	41,142-44,346	-22.08	-11.94	10.14	-17.32	+6.17
1600	South Gobi	Shabarakh-usu	8,196-8,382	-22.1	-10.84	11.26	-10.49	+9.05
1618	South Gobi	Shabarakh-usu	8,325-8,513	-22.51	-10.40	12.11	-12.5	+8.00
1609	South Gobi	Shabarakh-usu	8,769-9,088	-22.64	-11.6	11.04	-11.44	+9.20
1619	South Gobi	Shabarakh-usu	9,009-9,254	-23.24	-10.93	12.31	-8.65	+6.86
1620	South Gobi	Shabarakh-usu	9,019-9,270	-22.26	-9.90	12.36	-12.21	+8.55
1599	South Gobi	Shabarakh-usu	9,035-9,408	-23.76	-10.81	12.95	-11.29	+8.10
1602	South Gobi	Shabarakh-usu	9,094-9,424	-23.95	-11.58	12.37	-7.13	+5.44
1601	South Gobi	Shabarakh-usu	9,296-9,519	-22.78	-9.54	13.24	-5.18	+5.65
1606	South Gobi	Shabarakh-usu	9,306-9,537	-23.38	-11.11	12.27	-6.97	+4.75
1617	South Gobi	Shabarakh-usu	9,314-9,548	-23.52	-10.39	13.13	-8.80	+6.99
1604	South Gobi	Shabarakh-usu	9,455-9,552	-22.66	-8.96	13.7	-8.53	+6.59

TEAL	Region	Sample Site Region	Calibrated yr BP	$\delta^{13}\text{C}_{\text{org}}$	$\delta^{13}\text{C}_{\text{inorg}}$	org:inorg offset	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$
Excluded								
1677	South Gobi	Yingen khuduk	46,320-40,821	-26.89	-		-	-
4366	Northern Steppe	Bayangoliin am		-23.86	-13.86	10.00	-13.44	+10.16
4368	Northern Steppe	Bayangoliin am		-24.14	-13.15	10.99	-12.88	+7.65
4371	Northern Steppe	Bayangoliin am		-22.77	-12.83	9.94	-12.36	+8.49
4372	Northern Steppe	Tolbor		-23.39	-12.28	11.11	-15.05	+8.10
4375	Northern Steppe	Tolbor		-23.02	-11.84	11.18	-13.55	+6.95
4377	Northern Steppe	Tolbor		-23.16	-13.40	9.76	-15.60	+7.20
4378	Northern Steppe	Tolbor		-23.43	-13.07	10.36	-14.95	+8.53
4379	Northern Steppe	Bulgan aimag		-24.03	-12.92	11.11	-13.05	+6.59
4380	Northern Steppe	Podzvonkaya		-23.63	-12.11	11.52	-21.26	+8.20
Method Check								
2nd org C 2475		Ontario	Modern	-22.11	-7.72	14.39	-8.99	+5.67
2nd org C 2475		Ontario	Modern	-22.14	-7.29	14.85	-8.78	+5.78
2nd org C 2476		Ontario	Modern	-22.18	-7.14	15.04	-8.78	+6.13
2nd org C 2476		Ontario	Modern	-22.07	-7.28	14.79	-8.66	+6.34

TEAL	Region	Sample Site Region	Calibrated yr BP	$\delta^{13}\text{C}_{\text{org}}$	$\delta^{13}\text{C}_{\text{inorg}}$	org:inorg offset	$\delta^{18}\text{O}$	$\delta^{15}\text{N}$
2477		Ontario	Modern	-22.51				+6.18
2477		Ontario	Modern	-22.51	-6.78	15.73	-8.59	+6.10
2nd org C 2477		Ontario	Modern	-22.13				
2nd org C 2477		Ontario	Modern	-22.16				
2478		Ontario	Modern	-22.5	-7.27	15.23	-8.83	+6.13
2479		Ontario	Modern		-7.00		-8.89	+6.15
2479		Ontario	Modern		-7.52		-9.32	+6.55
2480		Ontario	Modern	-22.29	-7.06	15.23	-8.58	+6.84
2480		Ontario	Modern	-22.37	-7.30	15.07	-9.04	+6.22
2481		Ontario	Modern	-22.31				
2481		Ontario	Modern	-22.48				
2482		Ontario	Modern	-22.16				
2482		Ontario	Modern	-22.23				
2483		Ontario	Modern	-22.41				
2483		Ontario	Modern	-22.27				