

# OSTRICH EGGSHELL ISOTOPE DATA FROM BUNDU FARM, SOUTH AFRICA AND NEW EVIDENCE ON MIDDLE STONE AGE ENVIRONMENTS IN THE UPPER KAROO

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## ABSTRACT

*Pans and diatomaceous deposits along dry river beds in the Karoo of central South Africa attest to the presence of wet climatic phases in the region during the Pleistocene. Data for these wet climatic phases is largely restricted to the Late Pleistocene and Holocene, with little evidence for earlier periods. This study presents the results of new stable isotope analysis ( $\delta^{18}O$  and  $\delta^{13}C$ ) and amino acid racemisation analysis of ostrich eggshell samples from Middle Stone Age (MSA) site of Bundu Farm, associated with a Florisian faunal assemblage >200ka. Data from the site is compared to previously published data from Wonderwerk Cave and Equus Cave to provide the first direct evidence of Mid-Pleistocene climates and environments in the upper Karoo and their relationship with Middle Stone Age occupation of the region, 150 ka -300 ka, indicating MSA hominins occupation in the region during cooler and wetter phases associated with an expansion of productive grassland.*

Keywords: Palaeoclimates, Palaeoenvironments, Isotope analysis, AAR dating, Early Middle Stone Age, Karoo, Bundu Farm

## INTRODUCTION

The extensive network of pans and diatomaceous deposits along now dry river beds in the Karoo region of central South Africa, attest to the presence there of palaeolakes and rivers during the Pleistocene. While many of these pans sites are associated with Later, Middle and Early Stone Age archaeological material available evidence for wet climatic phases in the Karoo is largely restricted to Late Pleistocene and Holocene data. The lack of data for the Mid- Pleistocene has meant that the region is generally absent in debates on earlier climatic and environmental change and archaeology within the central interior of South Africa. One of only a handful of archaeological sites excavated in the upper Karoo is the site of Bundu Farm. Excavations at Bundu Farm, between 1998 and 2005, identified MSA lithic material associated with a Florisian faunal assemblage, subsequently dated by electron spin resonance (ESR) to 150 ka - 400 ka (Kiberd 2006). The Bundu Farm site in the upper Karoo provides a rare opportunity to investigate Mid-Pleistocene palaeoenvironments in the region (Figure 1). Relative dating of the site makes Bundu Farm of interest as one of few sites dating to the early Middle Stone Age, within a time range associated with the earliest appearance in southern Africa of genetic markers for *Homo sapiens* (Schlebusch et al. 2017) and fossil markers (Scerri et al. 2018, Grün et al. 1996). In this paper we present the results of new stable isotope analysis ( $\delta^{18}O$  and  $\delta^{13}C$ ) and amino acid racemisation (AAR) analysis of ostrich eggshell (OES) samples from MSA horizons at Bundu Farm and discuss the implications of the results for understanding past environments at the site and MSA occupation of the Karoo region and to provide new relative dating

of the Bundu Farm assemblages. OES analysed in the research, commonly found on archaeological sites due to its resistance to decay, has been shown to be a valuable resource for investigating palaeoclimates where other organic remains have either not been preserved or are limited (Brook et al. 1990). The results of OES analysis at Bundu are compared to data from the site to that for Wonderwerk Cave and Equus Cave to address three research questions: How do palaeoenvironments during the Mid-Pleistocene in the upper Karoo compare to other areas of the central interior? Did MSA and LSA hominins occupy the Karoo under different palaeoclimatic regimes? Does data from Bundu Farm therefore support earlier proposals that MSA occupation of the Karoo was restricted to periods of climatic amelioration? Our evidence suggests that the MSA horizons at Bundu Farm experienced cooler and wetter climates and environments and that hominin occupation of the upper Karoo was associated with periods of increased rainfall and an expansion of productive grassland.

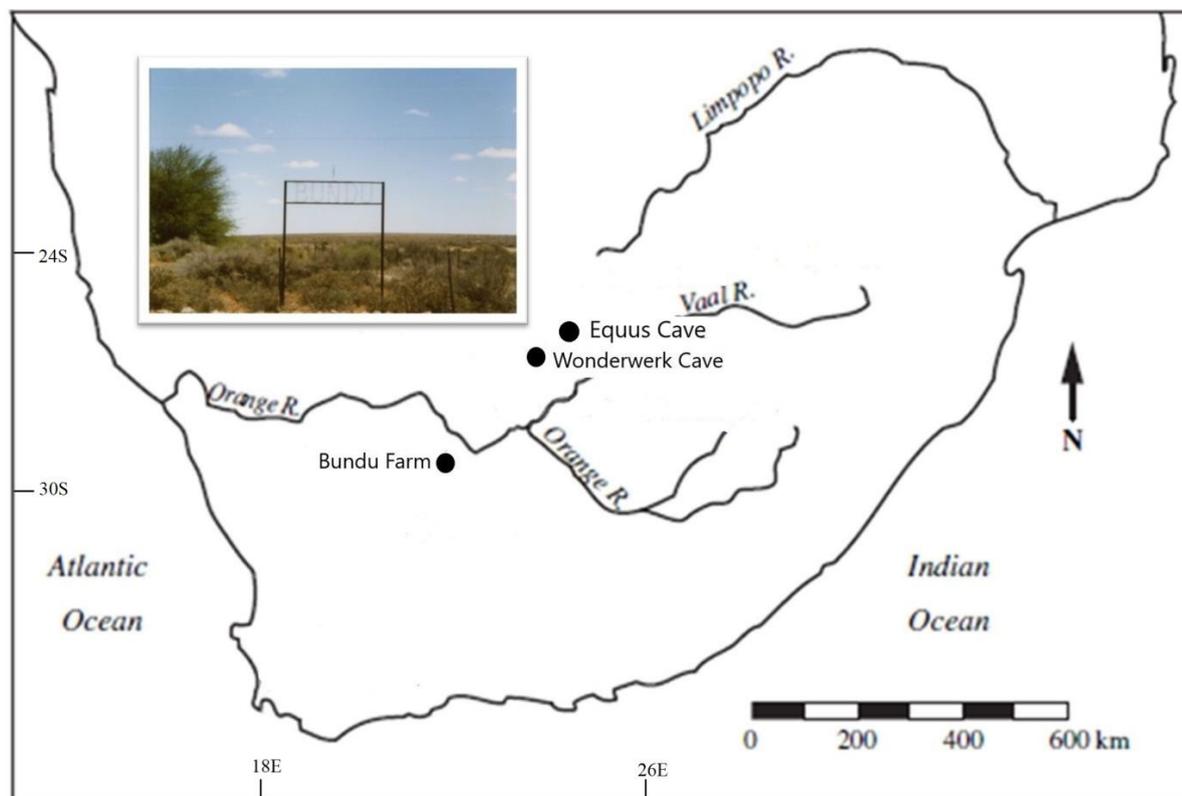


Figure 1. Map of South Africa showing the location of Bundu Farm and two main comparative sites, Wonderwerk Cave and Equus Cave. Inset image of the entrance Bundu Farm.

#### BACKGROUND TO THE SITE

Bundu Farm is an open-air archaeological site, located on the edge of the Bushmanland region, in the upper Karoo, Northern Cape, South Africa (29°44'54.3"S 22°12'13.2"E). The farm lies at an elevation of 1100m, within 25km of Doringberg/Asbesberg ranges, rising to 1363m and separating Bundu from the town of Prieska, on the banks of the Orange River to the east. Westward the landscape grades to <1000m within 20km of Bundu where it joins the Verneukpan / Grootvloer Complex of arid vleis.

The main site is one of several 'pan' sites that were observed during fieldwork on the farm and adjacent land, as well as handaxes in the wider landscape, together indicating widespread hominin activity in the area, suggesting that the Bundu Farm site was not an isolated find spot (Kiberd 2006).

Bundu Farm today sits within the modern Nama-Karoo biome with vegetation classified by Acocks (1965) as Arid Karoo veld type, characterised by desert grass species, small karroid shrubs and bushes and which falls under the categories of Bushmanland Arid (Nkb3) and Sandy Grassland (NKb4) (Mucina and Rutherford 2006). This part of the upper Karoo experiences high aridity and low annual precipitation, <200mm and there are few features in the landscape that provide shade where summer temperatures can reach 40°C. Rainfall here is not predictable and years without direct rainfall are not uncommon, embodying the local adage that while there is enough water for all it takes years to get around to everybody (Green 1975).

#### DATING AND STRATIGRAPHY

Excavation at Bundu focused on a series of sample trenches designed to understand the nature of the site, record the stratigraphic sequence and recover lithic and faunal assemblages for analysis (Figure 2). Trenches were excavated in 5cm spits within individual contexts. The depth of deposit varied across the site from a maximum of 1.98m in the central excavation area to 0.70m in the modern pan floor. All archaeological material was systematically collected and documented in relation to trenches and contexts. Individual contexts were grouped during analysis into seven (7) stratigraphic horizons (G1-7) within which four cultural horizons were identified, a LSA horizon in red sands at the top of the sequence (G1), a MSA assemblage within a calcified pebble/cobble horizon (G2/3), an EMSA assemblage in solid calcrete and calcified sands (G4-5), and a transitional/Fauresmith assemblage in clay rubble (G6) overlying sterile powder calcrete (G7).

Two attempts to date the Bundu Farm have been tried through application of electron spin resonance of animal teeth. The first was a series of dates from five animal teeth collected by Peter Beaumont from (G4-5) calcrete horizons associated with EMSA material (pers. obsv). ESR analysis at the Australian National University (ANU) produced a date range from  $270,357 \pm 22,603$  to  $364,032 \pm 30,339$ . These dates are complemented by a coupled ESR/uranium-series dating of an equid tooth from G2-3 horizons (T7) that produced a date of  $147,700 \pm 16,000$ . These dates bracket the age of the MSA assemblages between ~150 ka and 400ka, suggesting a deposition at the site occurred at points with marine isotope stages MIS 6-10 (Berger et al. 2016). The calcrete sequence at the site indicates that following environments in which fauna was deposited the area experienced significant periods of desiccation and deflation. Analysis of calcrete from G2-3 and G4-5 horizons identified that both experienced at least two desiccation events. The range between ESR dating of the MSA horizon (G2-3) and the EMSA horizon (G4-5) may therefore reflect a substantial gap between the

accumulations of MSA material, perhaps reflecting drier conditions in the region across MIS 7 and the majority of MIS 6, which may relate to wider regional aridification events (Sithaldeen et al.2015).

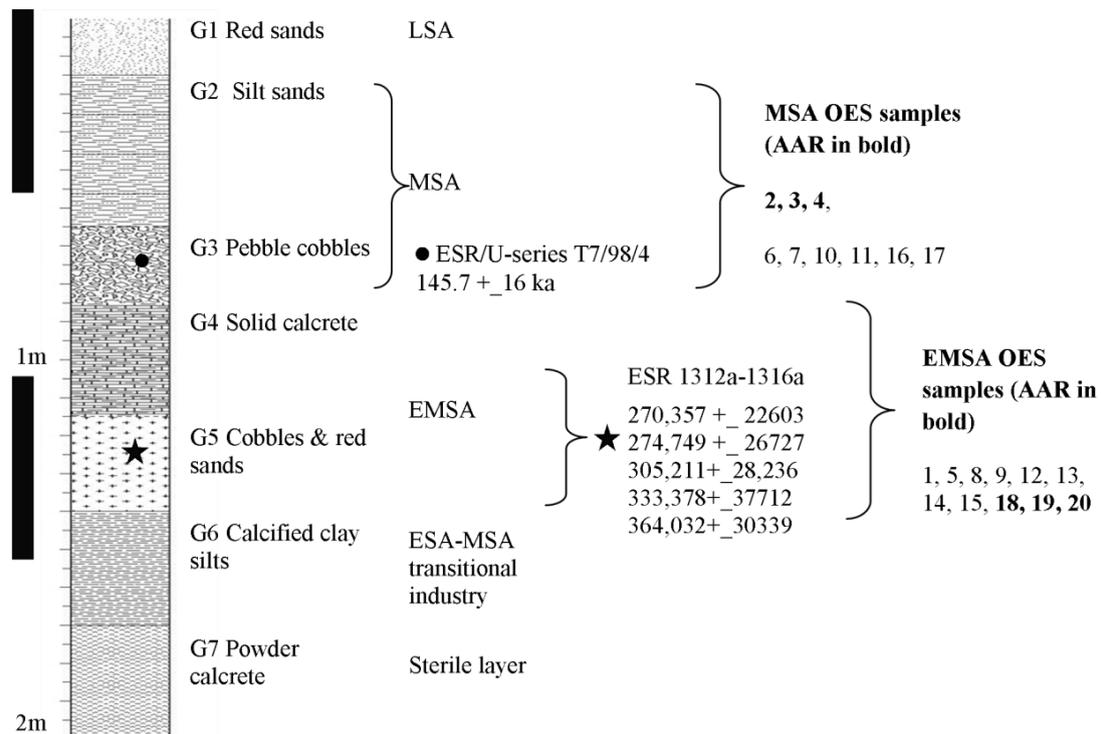


Figure 2. Bundu stratigraphy and chronology – OES samples were selected from MSA context within G2/3 and EMSA contexts G4/5

## FAUNA

The EMSA assemblage is directly associated with the majority of the faunal material recovered at Bundu as well as discrete areas of burning and burnt bone, possibly representing hearth features. While the majority of faunal material was recovered from EMSA horizons, occasional small fragments of bone and animal teeth were found in MSA horizon (G2-3) suggesting that animal bone may have been more extensive within the sequence and that the association of fauna with the earlier EMSA horizons at the site is likely therefore to be more a product of the conditions of preservation than to other behavioural factors.

Faunal material associated with the EMSA includes hippopotamus (*Hippopotamus amphibius*), wildebeest (*Connochaetes gnou*), warthog (*Phacochoerus* sp.), baboon (*Papio* sp.), zebra (*Equus* sp.), blesbok (*Damaliscus dorcas*), springbok (*Antidorcas* sp.), ostrich (identified from eggshell fragments), as well as the extinct giant alcephaline (*Megalotragus priscus*), Cape horse (*Equus capensis*) and an extinct *Damaliscus* sp. (Hutson 2012) (Table 1). The majority of faunal material was recovered from horizons G4 and G5, directly associated with EMSA material and exhibits varying

degrees of calcification. The G4 horizon includes a solid calcrete and silcrete band, which is primarily composed of cemented bone and lithic fragments, visible to the naked eye. Small bone fragments, shell fragments and traces of organic material indicative of standing water were observed in sections of calcretes from the G3 horizon, but large bone fragments are not evident in these calcretes (Nash 2005). Analysis of the fauna indicates at least some hominin role in the faunal accumulation, based on elements present and possible cut-marks, but with no clear pattern of a kill or camp site. This has led to the suggestion that Bundu may compare to an historic G/wi hunter-gatherer biltong hunt assemblage, implying logistically organised behaviour in the EMSA (Hutson 2018).

Table 1. Fossil fauna identified at Bundu Farm (after Hutson 2012)

<b>Taxa</b>	<b>NISP</b>	<b>MNE</b>	<b>MNI</b>
<b><u>Bovid size 2</u></b>			
Springbok, <i>Antidorcas sp.</i>	3	3	1
Indet. bovid size 2	37	28	3
<b><u>Bovid size 3</u></b>			
Blesbok, <i>Damaliscus dorcas</i>	2	2	1
Damaliscus sp.	1	1	1
Wildebeest, <i>Connochaetes gnou</i>	47	33	6
Indet. bovid size 3	188	74	4
<b><u>Bovid size 4</u></b>			
Giant alcelaphine, <i>Megalotragus priscus</i>	15	15	3
Indet. bovid size 4	59	38	2
<b><u>Equid</u></b>			
Zebra, <i>Equus sp.</i>	20	20	2
Extinct Cape zebra, <i>Equus capensis</i>	12	10	2
<b><u>Suid</u></b>			
Warthog, <i>Phacochoerus sp.</i>	34	24	6
<b><u>Large Mammal</u></b>			
Hippopotamus, <i>Hippopotamus amphibius</i>	5	3	1
Indet. bovid	5	3	0
Indet. large ungulate	1	1	0
Indet. mammal	21	20	0
<b><u>Small mammal</u></b>			
Baboon, <i>Papio sp.</i>	3	3	1
Indet. small mammal	1	1	0
<b><u>Carnivore</u></b>			
Hyenid sp.	3	3	1
Indet. large carnivore	1	1	0

The fauna is of perhaps greatest value in that it evidences a westerly extension of the Florisian Faunal Suite, a Mid-Late Pleistocene land mammal stage dominated by grazing species and indicative of open grassland conditions (Brink 2016). The modern climate and rainfall at Bundu Farm is insufficient to sustain such populations of large grazers or wetland species, highlighting a potential marked difference between Mid-Pleistocene environments in the Karoo to arid Holocene times, and to proposed dry glacial periods of the Pleistocene (Esler et al. 2006). While some extant species present

at Bundu are known historically within the Karoo (Badenhorst et al. 2015, Dean and Milton 1999), most are only found in locations close to riparian habitats and in areas of more predictable or higher rainfall. This perhaps supports arguments that Pleistocene hominin occupation of the Karoo was associated with periods of climatic amelioration, and increased rainfall, allowing for grassland expansion in contrast to the ability of Holocene populations to survive under more arid regimes (Beaumont 1986).

## **MATERIALS AND METHODS**

To investigate questions of climate and environment at Bundu, isotopic analysis of inorganic carbonates of OES was identified as a method to generate new data on conditions during periods of hominin occupation. Ostrich eggshell fragments were present in small numbers across all MSA and EMSA contexts, as well within LSA red sands levels. A total of eighty (80) OES fragments have been identified within the Bundu Farm assemblage, sixty-four (64) OES fragments were reported from the faunal analysis (Hutson 2012, 2018) and a further sixteen (16) fragments have been identified as part of ongoing research. No assumption has been made that the presence of ostrich eggshell (OES) at Bundu is due to hominins. OES can be accumulated by various species, including hyenas, lions, and baboons, as well as being broken by ostriches themselves, either by hens removing unwanted eggs from a nest or fledglings hatching.

Isotope data from OES is known to reflect ambient vegetation and can be used to imply palaeoenvironments and climates (Johnson et al. 1998, Montanari 2018). The  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  data obtained provides an opportunity to compare climatic conditions in the Karoo, with data from previous studies of Pleistocene conditions in the region and the modern environment (Ecker et al. 2015, Johnson et al. 1997, 1998, Thackeray and Lee-Thorp 1992, Vogel 1983).

Ostriches are found in a wide range of habitats from semi-desert to thick woodland (Jarvis et al. 1985). This diversity illustrates a capacity to quickly adapt to changes in environmental conditions. As non-selective grazers ostriches eat the freshest leaves of green grasses available in their environment,  $\text{C}_3$ ,  $\text{C}_4$  or CAM, with a preference for  $\text{C}_3$  vegetation (Milton et al. 1994) meaning eggshell isotopic composition reflects the proportion of palatable plants in their environment (Johnson et al. 1998, Von Schirnding et al. 1982). Ostrich diet varies in response to environment and, in more arid contexts, higher levels of  $\text{C}_4$  grasses are consumed (Milton et al. 1994). It has to be taken into account that ostriches have a preference for  $\text{C}_3$  browse and increased  $\text{C}_4$  grass signal may not be apparent until such a point that  $\text{C}_3$  browse is no longer a primary food source. Ostriches are known to breed across the year, but peak during the wet season, across a 2-3 week period following rains, with eggs laid after 3-5 days (Bertram 1992, Jarvis et al. 1985, Sauer and Sauer 1966). Signatures from OES therefore provide a picture reflecting the portion of palatable plants in the environment at a specific period in time (Johnson et al. 1998); that is a snapshot of diet averaged over 3-5 days after

rains. Studies of modern OES samples have found good correlation between  $\delta^{13}\text{C}$  measures and diet and the relative distribution of  $\text{C}_3$  and  $\text{C}_4$  plants, as well as  $\delta^{18}\text{O}$  measures and relative humidity from plant-leaf water ingested by ostriches (Milton et al. 1994). OES  $\delta^{13}\text{C}$  is enriched by approximately 16‰ with respect to diet and reflects the proportion of  $\text{C}_3$  and  $\text{C}_4$  plants consumed during the period of eggshell formation (Johnson et al. 1997). As ostriches are non-obligate drinkers, OES  $\delta^{18}\text{O}$  will reflect the isotopic composition of plant leaf water ingested during the period of eggshell formation (Johnson et al. 1997). In turn, plant leaf waters are related to rain and soil water but modified substantially by evapotranspiration with high values reflecting high ambient temperatures and aridity and low values reflecting lower temperatures and/or wet and humid conditions. These measures can indicate the likely prevailing plant biomass at the time the eggshell formed and differences in climatic conditions from present (Hobson and Clark 1992).

Twenty (20) OES samples were selected under South African Heritage Resources (SAHRA) permit (no. 2778), from collections held at McGregor Museum, Kimberley (Table 2). OES was selected with the primary aim of collecting data from EMSA and MSA contexts, to compare these two horizons and represents 25% of the total number of OES fragments recovered from the excavations. Samples were selected from excavated contexts that were assigned to G2-3 MSA horizon (9 samples) and the G4-5 horizon (11 samples). OES samples were taken from different trenches and contexts within the groups to allow for the possibility that fragments at the site could derive from the same egg and therefore to provide a random sample for analysis. An effort was made to avoid any samples that showed possible signs of heating, burning or any other defects, or those that were heavily calcreted. One OES sample (no.10) was from the same context as the equid tooth that provided the 145.7 ka ESR/U-series date.

Following Ecker et al. (2015), a small fragment of shell was removed from each sample, cleaned by abrasion with fresh sandpaper and then crushed with an agate pestle and mortar. The powdered OES was analysed using a Kiel IV carbonate preparation system (using 106% phosphoric acid at 70°C) coupled to a dual inlet MAT253 isotope ratio mass spectrometer at the National Oceanography Centre in Southampton.  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  are reported with reference to VPDB and the precision is better than  $\pm 0.2\text{‰}$  for  $\delta^{18}\text{O}$  and better than 0.1‰ for  $\delta^{13}\text{C}$ . Most previous OES studies have reported  $\delta^{18}\text{O}$  values with reference to the VSMOW scale. Therefore, comparisons to existing data were facilitated by converting the Bundu data to the VSMOW scale using the equation  $\delta^{18}\text{O}_{\text{VSMOW}} = 1.03091 * \delta^{18}\text{O}_{\text{VPDB}} + 30.91$  (Coplen 1988, equation 11).

To complement isotope analysis histological and biometric analysis of OES was investigated with reference to methods used in the analysis of fossil dinosaur eggshell (Williams et al. 1984). Thirty (30) samples were measured using digital callipers to record thickness and a subset of 10 eggshell fragments were prepared for histological thin section analysis, following Ecker et al. (2015) to investigate pore density, complemented by surface observations of pore numbers across sixteen (16)

specimens. Eggs are discrete environments in which bird embryos develop. They are in this regard responsive to prevailing environmental conditions. Eggshell thickness relates to the functional conductance of the egg, while gas exchange (oxygen intake and carbon dioxide outtake) provides a measure of porosity of an egg (Tullett 1984). Thinner eggshells have higher rates of gas exchange, which are viable under more humid conditions, when risk of water loss is reduced. Conversely, thicker shells would be expected under more arid conditions to protect against water vapour loss by reducing gas exchange. However, while shell characteristics respond quickly to environmental conditions, they also reflect the physiology of the hen and significant variation is found between nests in the same environment (Tullett 1984). Lastly, six (6) OES samples (no.s 2, 3, 4, 18, 19, 20) were forwarded to the North East Amino Acid Racemization (NEaar), Palaeo Lab, University of York for amino acid racemisation dating.

The six OES samples submitted to NEaar, were analysed using AAR, three fragments from the MSA horizon ( samples 2, 3, 4) and three from the EMSA horizon (samples 18, 19, 20), with each fragment subsampled twice (Sample references NEaar 12778-89; BF Up2-4.1-2 and NEaar 12766-77; BF low 18-20.1-2). All OES samples were prepared using the procedures of Crisp et al. (2013) to isolate the intra-crystalline protein by bleaching. Two subsamples were then taken from each sample; one fraction was directly demineralised and the free amino acids analysed (referred to as the 'free' amino acids, FAA), and the second was treated to release the peptide-bound amino acids, thus yielding the 'total' amino acid concentration, referred to as the 'total hydrolysable amino acid fraction (THAA, H\*)'. Samples were analysed in duplicate by reversed-phase high-performance liquid chromatography (RP-HPLC), with standards and blanks run alongside samples. During preparative hydrolysis, both asparagine and glutamine undergo rapid irreversible deamination to aspartic acid and glutamic acid respectively (Hill, 1965). It is therefore not possible to distinguish between the acidic amino acids and their derivatives and they are reported together as Asx and Glx respectively.

## RESULTS

### ISOTOPE ANALYSIS

The Bundu OES results fall mostly in the range of -7‰ to -10‰ in  $\delta^{13}\text{C}$  and between -0.5‰ and +6.5‰ in  $\delta^{18}\text{O}$  (Table 1). A Student's T-test detected no significant differences in  $\delta^{18}\text{O}$  or  $\delta^{13}\text{C}$  between the two MSA layers (P-values = >0.05). The intra-layer  $\delta^{18}\text{O}$  results are strikingly homogenous compared with OES assemblages investigated at other sites (e.g. Johnson et al. 1997; Ecker et al. 2015). Means values for the two horizons are -7.9‰ for  $\delta^{13}\text{C}$  and +4.6‰ for  $\delta^{18}\text{O}$  for the MSA horizon compared to means of -8.8‰ for  $\delta^{13}\text{C}$  and 3.7‰ for  $\delta^{18}\text{O}$  for the EMSA. Means values for the OES as a whole are -8.4‰ for  $\delta^{13}\text{C}$  and +4.1‰ for  $\delta^{18}\text{O}$ . Sample number 10 from the dated MSA context in Trench 7 provided a reading of -7.3‰ in  $\delta^{13}\text{C}$  and +3.17‰ in  $\delta^{18}\text{O}$ .

Table 2. Isotope data for the OES at Bundu Farm

Sample name	Horizon	Normalised $\delta^{13}\text{C}$ ‰ VPDB Mean	Normalised $\delta^{18}\text{O}$ ‰ VPDB Mean	$\delta^{18}\text{O}$ ‰ VSMOW conversion
BF 2	MSA	-7.31	3.66	34.69
BF 3	MSA	-7.41	4.5	35.54
BF 4	MSA	-9.18	5.19	36.26
BF 6	MSA	-8.69	6.46	37.57
BF 7	MSA	-7.08	3.13	34.13
BF 10	MSA	-7.28	3.17	34.17
BF 11	MSA	-8.55	3.32	34.33
BF 16	MSA	-5.72	6.41	37.52
BF 17	MSA	-9.89	5.06	36.13
BF 1	EMSA	-8.45	4.24	35.28
BF 5	EMSA	-9.95	5.45	36.53
BF 8	EMSA	-9.3	2.98	33.98
BF 9	EMSA	-7.58	4.88	35.94
BF 12	EMSA	-8.76	5.27	36.34
BF 13	EMSA	-9.2	1.62	32.58
BF 14	EMSA	-8.3	4.92	35.98
BF 15	EMSA	-9.11	2.35	33.34
BF 18	EMSA	-8.49	3.88	34.91
BF 19	EMSA	-8.17	-0.5	30.39
BF 20	EMSA	-9.79	5.91	37.01

The Bundu data indicate an isotopically homogenous leaf water pool for the ostriches that laid the eggs recovered at Bundu (Figure 3). Both the  $\delta^{13}\text{C}$  and the  $\delta^{18}\text{O}$  data at Bundu point towards a humid climate where plants experienced minimal water stress, leaf transpiration was low and the isotopic composition of plant leaf water did not change much during the daily cycle.  $\delta^{13}\text{C}$  results suggest a mix of  $\text{C}_3$  and  $\text{C}_4$  plants was available to ostriches near Bundu in the MSA and that there was sufficient  $\text{C}_3$  vegetation in the MSA environment to feed the ostriches. Given the diversity of grasslands faunal species found at Bundu, this suggests either a mixed habitat of  $\text{C}_3$  browse and  $\text{C}_4$  grasslands, or that the climate was cool enough to support  $\text{C}_3$  grasslands as well as  $\text{C}_3$  browse (Figure 4).

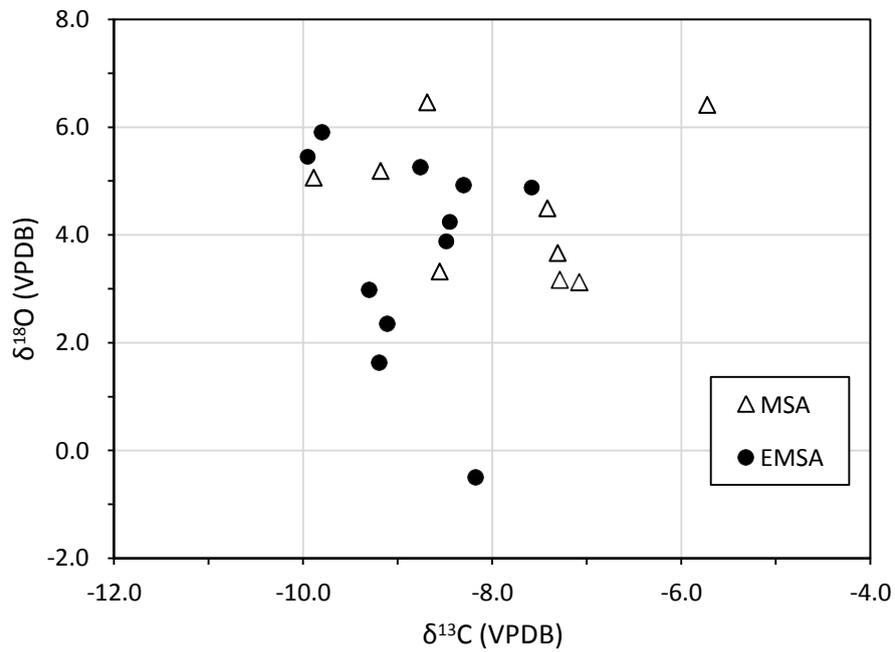


Figure 3. Scatterplot showing  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  isotope readings for Bundu Farm EMSA and MSA horizon ostrich eggshell (OES) samples. The chart shows the clustering and homogeneity of samples between earlier and later MSA horizons

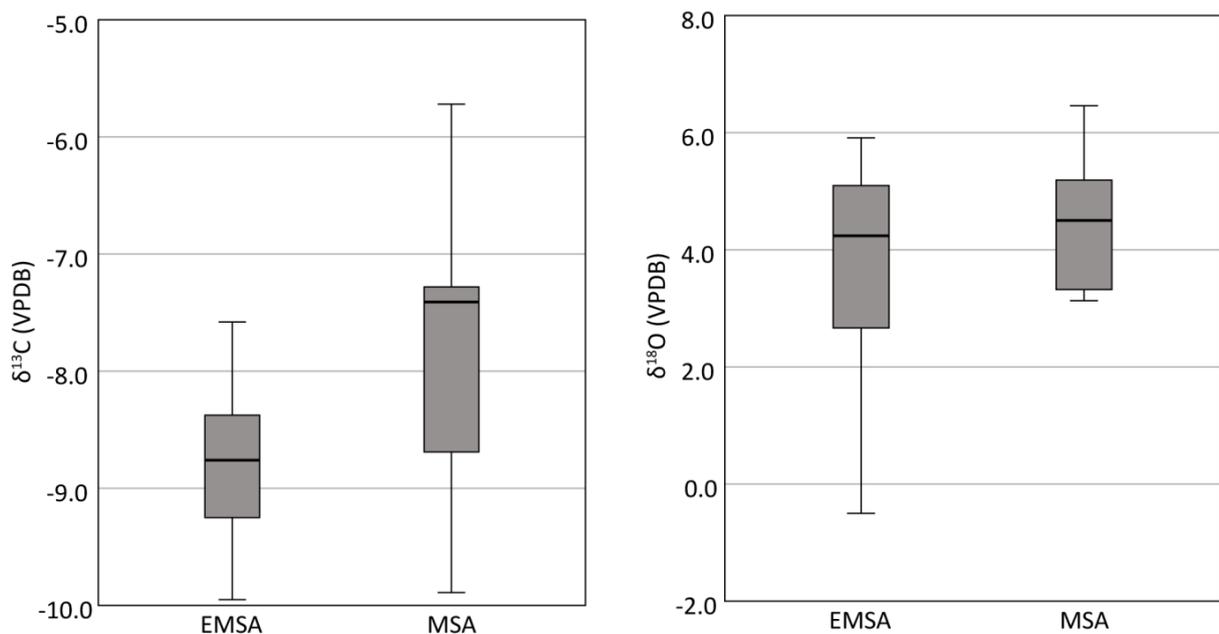


Figure 4. Boxplots of OES  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  showing close correlation between isotope measures across Bundu Farm EMSA and MSA horizons.

## EGGSHELL THICKNESS AND PORE DENSITY

Eggshell thickness and pore density data mirrors isotopic results indicating a homogeneous sample. Mean eggshell thickness in the MSA horizon is 1.68mm ( $\sigma = 0.04$ ) and 1.71mm ( $\sigma = 0.04$ ) in the EMSA horizon. This is matched by mean pore density, the number of pores per mm<sup>2</sup>, which is 0.13 ( $\sigma = 0.05$ ) for the EMSA horizon and 0.14 ( $\sigma = 0.03$ ) for the MSA. OES thickness is reported as averaging 2mm in modern birds and ranging from 1.48-2.77mm (Willoughby et al. 2016). Suggestions from the Wonderwerk Cave biometry and histology study are that thinner samples with lower pore density indicate more humid conditions (Ecker et al. 2015). Recent CT tomography study of OES raises questions of this interpretation, finding little correlation between shell thickness, pore diameter and the number of pores (Willoughby et al. 2016). The study highlighted the variation in pore density across the egg, with increased numbers of pores at the blunt end, where gas exchange takes place. This finding supports earlier observations that multiple branching of pores can be differentially expressed across the egg surface (Tullett 1984). Without knowing the part of the egg observed it is therefore difficult to ascertain whether the Bundu samples reflect variation within and between eggs or if they support the hypothesis that eggshells are thinner with fewer pores under mesic conditions. The best that can be stated is that they are at the lower end of the average range for modern ostrich eggs.

A further feature of the Bundu OES observed in thin-section samples is a degree of disturbance in the eggshell structure. This may correlate to crushing or heating of eggshell, reflecting the open nature of the site and trampling by animals, or possibly burning (Tyler and Moore 1965). Several specimens that feature disturbance show evidence of burning, but otherwise minimal edge rounding and little evidence of abrasion by transport (Oser and Jackson 2014). Micro-fragments of OES also occur with bone and lithics within the solid calcrete matrix suggesting contemporaneity with fauna and lithics that equally show minimal weathering.

## AMINO ACID RACEMISATION (AAR)

The range of data for the Bundu Farm sites was compared to existing data measured from the Pinnacle Point site, where the oldest material measured dated to 150 ka (Crisp, 2013). Asx, Ala and Phe are relatively fast racemisers, and their D/L ratios (D-form to L-form of each amino acid) are nearing equilibrium in the Bundu Farm material. For Phe, there is no real difference observed between the EMSA and MSA samples; for Asx D/L two samples from the EMSA are higher than those from the MSA, with one similar. For Ala D/L, the EMSA samples show higher FAA Ala D/Ls than the MSA samples.

Glx, Val and Ile are slow racemisers, and should therefore provide better resolution for samples of this age. For Glx D/L two samples from the EMSA are higher than those from the MSA, with one similar. Val shows a split in THAA for MSA samples. Ile D/Ls show quite a large spread in the data.

In general, the D/L values support the interpretation that the two MSA levels are relatively similar in age, with the EMSA horizon likely to be slightly older, as indicated by the FAA D/Ls in particular. For all the amino acids, all the Bundu Farm samples have significantly higher D/L values than the 150 ka samples from Pinnacle Point. Assuming there is a similar temperature history between the two regions this pattern indicates an age significantly older than 150 ka for the Bundu Farm material. This analysis supports previous ESR dates for the site which point to MSA occupation during Marine Isotope Stages (MIS) 6 for the MSA horizon and MIS 8-10 for the EMSA (Berger et al. 2016).

### **COMPARISON OF BUNDU OES TO OTHER OES SEQUENCES**

To aid interpretation, the isotopic data from Bundu were compared to available OES data for the Pleistocene and Holocene at Wonderwerk Cave, Northern Cape, (Lee-Thorp and Ecker 2015, Thackeray and Lee-Thorp 1992), Equus Cave (Johnson et al. 1997), and to Holocene and modern samples from the Northern and Western Cape and Free State, South Africa (Johnson et al. 1998). Both  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  results for Bundu are similar to the Early-Mid Pleistocene at Wonderwerk Cave, to late Holocene horizons at Equus Cave, and to modern OES samples from Postberg in the Western Cape (Figures 5 & 6 and Table 3). These comparisons further support the interpretation of relatively humid climates at Bundu during the MSA, with moderate to plentiful precipitation and cooler temperatures (Johnson et al. 1997; Ecker et al. 2015).

Wonderwerk Cave is situated in the Kuruman Hills, 200km to the north-east of Bundu. Vegetation around Wonderwerk is described as Vryburg Bushveld, with sourveld grasses and scrub (Avery 1981) and receives mean annual rainfall today of 455 mm (Thackeray and Lee-Thorp 1992). Isotope analysis of OES and animal teeth provide valuable comparative data. Bundu data more closely corresponds to OES results for stratum 10, and 11 (Excavation 1) and stratum 2 (Excavation 2) at Wonderwerk Cave, which imply moister conditions, and increase in  $\text{C}_3$  plant foods (Ecker et al. 2015). Increased  $\text{C}_3$  and more mixed  $\text{C}_3/\text{C}_4$  in ungulate diets than present for these strata is supported by isotope analysis of animal teeth from Wonderwerk (Ecker et al. 2018). Stratum 10 at Wonderwerk has a mean  $\delta^{18}\text{O}$  of  $36.0\text{‰} \pm 2.2\text{‰}$  (all ratios now quoted relative to VSMOW) with a mean of  $37.1\text{‰} \pm 4\text{‰}$  in stratum 2 (ibid). Similarly mean  $\delta^{13}\text{C}$  in stratum 10 is  $-7.5\text{‰}$ . Stratum 11 has a mean  $\delta^{13}\text{C}$  of  $-8.6\text{‰}$  and  $\delta^{18}\text{O}$  of  $35.2\text{‰}$  which matches closely with mean Bundu readings of  $-8.4\text{‰}$  for  $\delta^{13}\text{C}$  and mean  $\delta^{18}\text{O}_{\text{VSMOW}}$  of  $35.1\text{‰}$ .

Data for Wonderwerk Stratum 2 (Excavation 2) is of further interest in that this horizon is also associated with EMSA lithic material dating to circa ~200ka (Chazan et al. 2020). Interpretation of

Wonderwerk Cave OES data, isotope data from animal teeth and phytoliths indicate a cooler, wetter Succulent Karoo regime during Stratum 10-11 times (Ecker et al. 2018). Stratum 10-11 are contemporary with evidence from the Mamatwan Mine, 40 km northwest of Wonderwerk Cave, for the presence of a large stable and shallow body of water, dated to 1.0-1.78 Ma, during a period of fluctuating climate with spells of increased moisture and aridity (Matmon et al. 2015). The close correlation between the Bundu and Wonderwerk data provides a possible similar scenario for the Karoo, 200 ka -300 ka.

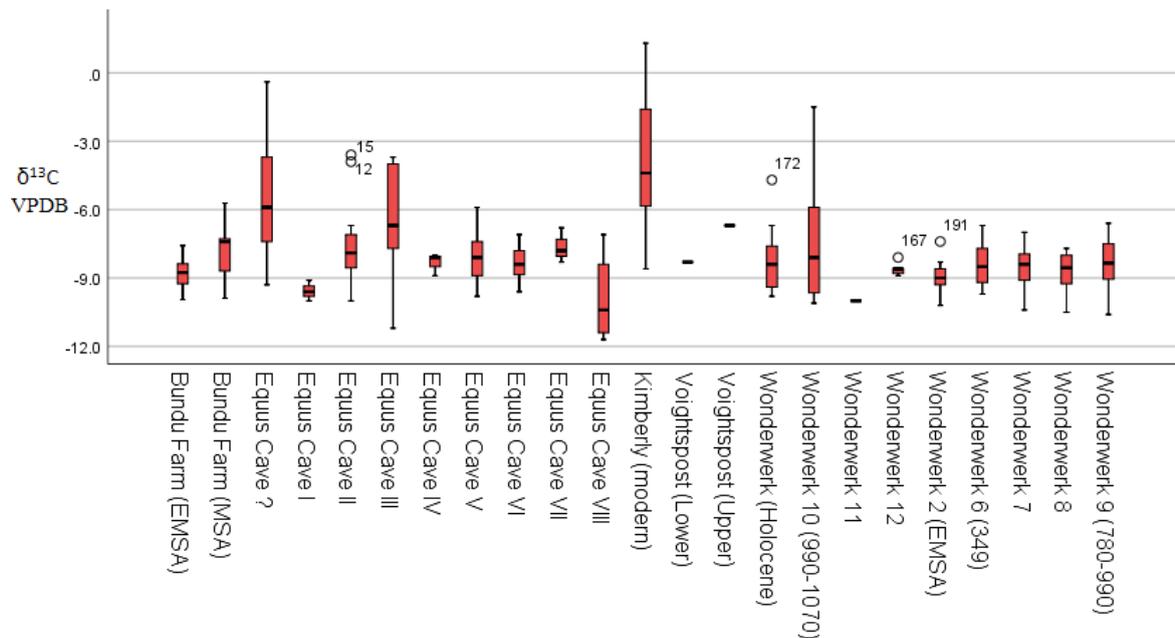


Figure 5. Comparative carbon data showing boxplot for Bundu Farm against data for Equus Cave (Johnson et al. 1997), Wonderwerk Cave (Ecker et al. 2015) and modern OES from Kimberley (Johnson et al. 1998). Open circles indicate statistical outliers.

The second site with OES isotope data suitable for comparison to Bundu data is Equus Cave. The site lies 130km north of Kimberley in the Northern Cape Province, South Africa, in an area classified as semi-arid with Mean Annual Precipitation (MAP) of 420mm and mean temperature of 16°C (Johnson et al. 1997). A correlation to Bundu OES isotope data is found in samples from Holocene layers, dating 11.4, 6.2 and 4.2 ka yrs. These relate to Johnson et al's Groups 4, 5 and 6 and Layer 1b at the site, with means of -7‰ to -8‰ in  $\delta^{13}\text{C}$  and 34-35‰ in  $\delta^{18}\text{O}$  (Johnson et al. 1997). Temperature and relative humidity for 6 ka and 4 ka age data suggest low evaporation and temperature with above modern average humidity (Johnson et al. 1997).  $\delta^{15}\text{N}$  isotope data was also obtained from *Equus* tooth samples and used to suggest annual rainfall estimates of between 550-590±150 mm/yr and  $\text{C}_3/\text{C}_4$  plant intake ratio of 85:15 (Johnson et al. 1997).

Pollen data from hyena coprolites and isotopes from equid teeth support the interpretation of more open vegetation at Equus Cave during accumulation of Layer 1b, with increased proportions of  $C_3$  grasses (Scott 1987). These data imply greater year-round rainfall resulting from cooler temperatures and increased winter rainfall (Lee-Thorp and Beaumont 1995). Dating of Equus samples to 4.2 ka correlate to reported pulses of occupation in the Karoo 4.5-4.3 ka and 11.7-7.5 ka (Beaumont 1986, Scott et al. 1995), periods which appear to have witnessed cooler and wetter grassland (Coetzee 1967, Mitchell 2002) and comparable fauna to that at Bundu Farm, including keynote grassland species *Megalotragus priscus*, *Equus capensis* and *Connochaetes gnou* (Lee-Thorp and Beaumont 1995, Klein 1979, Brink 2016).

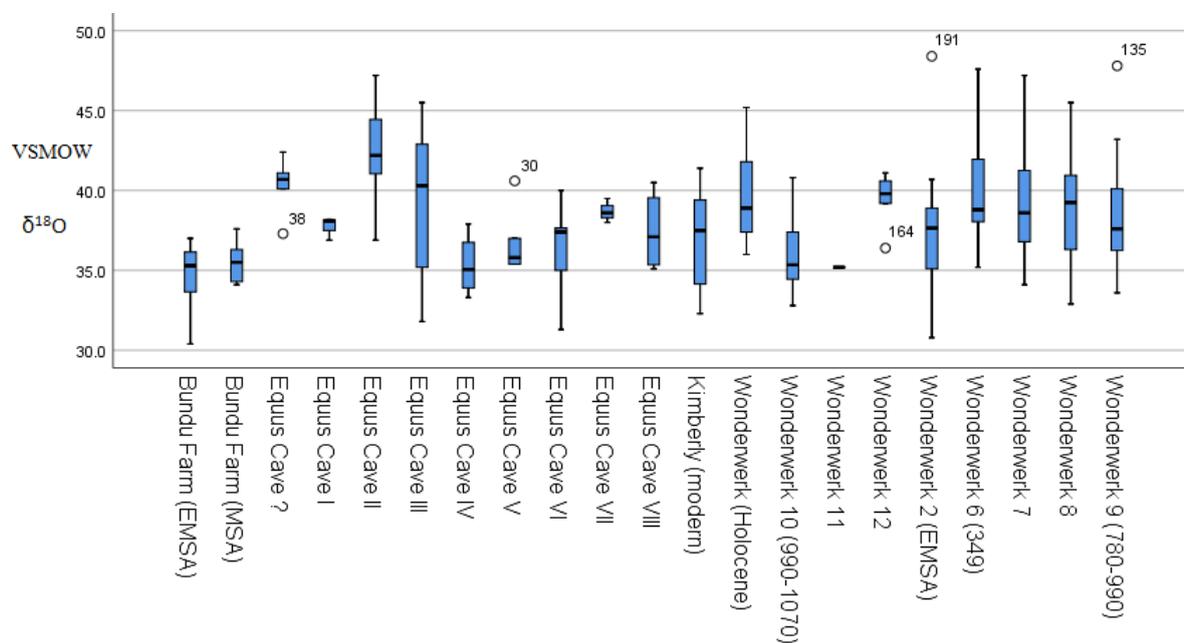


Figure 6. Comparison oxygen data showing boxplot for Bundu Farm against data for Equus Cave (Johnson et al. 1997), Wonderwerk Cave (Ecker et al. 2015, Chazan et al. 2020) and modern OES from Kimberley (Johnson et al. 1998). Open circles indicate statistical outliers.

Table 3. Summary comparative data for Bundu Farm, Equus Cave and Wonderwerk Cave in the Northern Cape, Postberg and sites in the Western Cape. Data from Ecker et al. 2015, Lee-Thorp & Ecker 2015, and Johnson et al. 1997, 1998.

Site	Stratum	Age	n	Mean $\delta^{13}\text{C}$ VPDB	sd	Min	Max	Mean $\delta^{18}\text{O}$ VSMOW	sd	min	max
Bundu Farm	MSA	>200ka	9	-7.9	0.01	-9.98	-5.72	35.6	0.07	34.1	37.6
Bundu Farm	EMSA	>300ka	11	-8.8	0.01	-9.95	-7.58	34.8	0.07	30.4	37.0
Wonderwerk	6	>0.349	11	-8.4	1	-9.7	-6.7	40	3.5	35.2	47.6
Wonderwerk	7		19	-8.5	0.9	-10.4	-7	39.1	3.2	34.1	47.2
Wonderwerk	8		20	-8.7	0.8	-10.5	-7.7	38.8	2.9	32.9	45.5
Wonderwerk	9	0.99-0.78	16	-8.3	1.2	-10.6	-6.6	38.7	3.7	33.6	47.8
Wonderwerk	10	0.99-1.07	16	-7.5	2.5	-10	-1.5	36	2.2	32.8	40.8
Wonderwerk	11	1.07-1.78	1	-10				35.2			
Wonderwerk	12	1.78-1.96	5	-8.6	0.3	-8.9	-8.1	39.4	1.8	36.4	41.1
Kleinsee (Port Nolloth)	1	Modern	-	-5.6	-	-	-	31.2	-	-	-
Postberg (Langebaanweg)	1	Modern	-	-9.1	-	-	-	34.4	-	-	-
Cape Point (D.F. Malan)	1	Modern	-	-12.6	-	-	-	30.2	-	-	-
Burgervilleweg (Victoria West)	1	Modern	-	-11	-	-	-	37	-	-	-
Equus Cave (Welkom)	1	Modern	-	-7.3	-	-	-	41	-	-	-
Kimberley (Kimberley)	1	Modern	7	-4.6	-	-	-	37.3	-	-	-
Voightspost	upper	1220 BP	1	-6.7	-	-6.7	-6.7	-	-	-	-
Voightspost	lower	6350 BP	1	-8.3	-	-8.3	-8.3	-	-	-	-
Equus Cave	I	0	3	-9.5	0.2	-9.1	-10	37.7	0.7	36.9	38.2
Equus Cave	II	0.8	15	-7.8	1.4	-0.4	-10	42.2	3	36.9	47.2
Equus Cave	III	1.9	5	-6.6	2.1	-4	-11.2	39.1	5.6	31.8	45.5
Equus Cave	IV	4.2	4	-8.27	0.4	-8	-8.9	35.3	1.9	33.3	37.9
Equus Cave	V	6.2	6	-8	1	-5.9	-9.8	36.6	2	35.4	40.6
Equus Cave	VI	11.4	7	-9.5	0.7	-7.1	-9.6	36.3	2.9	31.3	40
Equus Cave	VII	14.3	3	-7.6	1.7	-6.8	-8.3	38.7	0.8	38	39.5
Equus Cave	VIII	16.6	4	-9.9	1.4	-7.1	-11.7	37.1	3	35.1	40.5

## DISCUSSION

The close correlation of the OES data from Bundu to later Pleistocene wet phases in the upper Karoo and central interior suggests that the region experienced comparable conditions in the Mid-Pleistocene between MIS 8-10, to those during MIS 2-3 (Siddall et al. 2017) (Table 4). At Grootvloer and Swartkolkvloer in the upper Karoo high lake levels 30 ka-14 ka, suggest periodic seasonal lacustrine conditions (Kent and Gribnitz 1985). These correlate to similarly high lake levels at Alexandersfontein, near Kimberley, and active tufa springs along the Ghaap Escarpment dated to 30 ka -13ka (Butzer et al. 1973). These findings document increased precipitation and/or moisture retention associated with cooler climates during MIS 3-2 across the central interior (Meadows and Watkeys 1999, Beaumont 1986). Karoo palaeolakes dating to the last glacial maximum feature higher lake boundaries that are likely to be of much earlier Pleistocene date (Cowling and Pierce 1999). The age of these earliest lakes is unclear, but potentially correlate to evidence for wetter conditions in the central interior during MIS 8-7 (Brink 2016, Matmon et al. 2015). The correlation between MIS 8 dates for Mid-Pleistocene Bundu events and MIS 3 is of interest given the close similarity between global ice volumes between these two stages (Siddall et al. 2007). Data for earlier wet phases include that from Haaskraal Pan and Florisbad circa, 279 ka (Partridge and Dalbey 1986, Scott and Brink

1992), the Mamatwan mine, Hotazel, 286 ka (Bateman et al. 2003), tufa formation on the Ghaap Escarpment,  $248 \pm 37$  ka associated with Florisian fauna and an ESA-MSA transitional assemblage (Curnoe et al. 2006), an expanded Vaal River 200 ka -250 ka (Butzer et al. 1978, Helgren 1978), a shrinking desert Kalahari 200 ka -186 ka (Brook et al. 1996), wetter conditions at Rossling Cave, Namibia, 260 ka (Thomas 1987), very large lake basins in the Makgadikgadi region of Botswana, circa ~250 ka (Burrough 2016,) and a large shallow lake in the Namib Desert, dated to 240 ka -210 ka, (Burrough et al. 2009). Collectively, these data point to extensive wet phases during the Mid-Pleistocene, across Namibia, Botswana, and South Africa, which extended a productive grassland ecosystem across southern Africa (Brink 2016). The isotope data from Bundu Farm reported here extends this Mid-Pleistocene wet phase into the upper Karoo, filling in a geographic gap and which, if Bundu can be considered contemporary with Duinefontein 2 (Klein et al. 1999), implies episodic grassland/wetland stretching from the Cape coast to the Okavango, 200 ka - 300 ka years ago.

While *Equus* and Wonderwerk caves provide palaeoclimatic fixes, the closest correlation to Bundu isotope data from modern OES is with that from Postberg, with a mean  $\delta^{18}\text{O}$  of 34.4‰ and mean  $\delta^{13}\text{C}$  of -9.1‰ (Johnson et al. 1998). Average modern temperatures in and around Bundu are 19.2 C with rainfall averaging 150 mm per annum, but with most years far below this. Precipitation follows a broadly summer rainfall pattern, with most rain likely in March and least in September, although very occasional winter rainfall is known. Temperatures in January and February average close to 26.9 C. Evapotranspiration above 2000mm per annum far outstrips precipitation meaning that standing water is rare. Occasional higher than average rainfall can lead to water-filled pan features, often accompanied by irruptive fauna and flora. The nature of such events means that they are short-lived, as resources are quickly consumed, and are not sustained due to returning dry-phases. This irruptive nature is characteristic of the Karoo where the floristic component of karoo vegetation responds optimally and differently to rainfall and temperature (Roux and Theron 1987).  $\text{C}_3$  shrubs and grasses are optimal when rainfall occurs in cooler months,  $\text{C}_4$  when summer rainfall occurs (Roux and Theron 1987). By contrast to the Karoo, the Postberg area has average annual temperatures at 17.8C and rainfall averages 374mm, nearly double annual rainfall at Bundu. This is a winter rainfall area with January driest month and greater amounts of rainfall in June (averaging 60mm). Average summer temperatures are 4 degrees lower than those of Bushmanland, while winter temperatures stay slightly higher and are related to winter rainfall.

Table 4: ESR dates for Bundu Farm with possible correlations to Marine Isotope Stages (MIS)

Sample number	Sample type	Lab	Results			Date Range		Marine Isotope Stage
			Coupled ESR/ U-series	Early U-Uptake	Linear U-Uptake	Min	Max	
T7/98/4	Equus tooth	University of Heidelberg	145.7 ± 16 k yrs			129.7	161.7	6a/b glacial?
1312a	?	ANU		158,833 ± 14,779		144,054	173,612	
					270,357 ± 22,603	247,754	292,960	8b glacial?
1313a	?	ANU		161,042 ± 16,764		144,278	177,806	
					274,749 ± 26,729	248,020	301,478	8c glacial?
1314a	?	ANU		184,949 ± 18,911		166,038	203,860	
					305,211 ± 28,236	276,975	333,447	9b interglacial trough?
1316a	Large bovid equus?	ANU		194,071 ± 23,375		170,696	217,446	
					333,378 ± 37,712	295,666	371,090	10a transition?
1315a	Small bovid	ANU		207,532 ± 18,617		188,915	226,149	
					364,032 ± 30,339	394,371	333,693	10c glacial?

Reduced temperature appears to have a more significant influence on water supply than rainfall amount alone. Water balance models further support a shift to increased winter rainfall. High lake levels at the last glacial maximum at Alexandersfontein would have required a 123% increase in regional rainfall from present 397mm / year, along with temperature reductions of 6°C (Butzer et al. 1973). Likewise, data for Equus Cave correlating to Bundu suggest a MAP increase of 129%. Swartkolkvloer in Bushmanland would have required a similar temperature reduction and 5-6 times increase in rainfall to 750-900mm/year, to fill the lake (Kent and Gribnitz 1985).

Active tufa formation, such as that at Groot Kloof in the Pleistocene, is reported as taking place where rainfall exceeds 600mm per year and within a range of 600-1000mm. MIS 3 precipitation for southern Africa has been estimated as averaging 600-700mm, an increase that would have provided more than adequate rainfall in the Karoo to sustain rivers and lakes (Blome et al. 2012). Heine (1992) has inferred that during MIS3/2 wet phases with increased rainfall affected a region from the Cape to the Makgadikgali palaeo-mega-lake that would have been in perennial flow in the presently dry

tributaries of the Orange river, as well as in the Molopo river. An earlier wet phase along the Molopo River valley, possibly contemporary to that at Bundu is suggested by the presence of Fauresmith-type archaeological deposits in association with fossil rhinoceros bones at Koopan-Suid (Beaumont pers comm.).

Thornthwaite-type water balance models for Bundu suggest a reduction in average temperatures of 3-5°C degrees and an increase of winter rainfall allows for standing water, requiring only a shift in precipitation from 156mm precipitation to 312mm. By contrast summer rainfall needs to increase close to 800mm to achieve similar results. A year-round rainfall regime could therefore have increased rainfall above 300mm, perhaps closer to 600mm allowing for the pool at Bundu to have sustained water all year and accounting for the presence of hippopotamus in the pan 100km from their historical presence in the Orange River.

Hippopotamus at Bundu clearly imply standing water and sufficient grazing areas. Grassland is further supported by the presence of equids, wildebeest and springbok, whose abundance from modern game-park observations is correlated to rainfall (Shorrocks and Bates 2015). However, whereas springbok, black wildebeest and equids, imply open short grass and high visibility, blesbok at Bundu may be an indicator of mixed open tree, shrub grassland. Warthog too have a preference for scrub environments and the presence of baboon indicates trees in the locality. Baboon, hippo and warthog are all water dependent species, and respond positively to year-round rainfall, implying at least seasonal rivers and pools. This mixed faunal composition adds weight to the idea of mosaic C<sub>3</sub> shrub and C<sub>4</sub> grassland at Bundu, implied by the isotope data. Isotopic assessment of modern ungulate diets for hippopotamus, blesbok and warthog in wetter modern environments indicate a concordance with Bundu δ<sup>13</sup>C OES data (Sponheimer et al. 2003, Vogel 1978) where readings -9.1‰ to -8.0‰ suggest percentages of browsing between 11-19% in response to increased grassland. The absence of keynote bushveld species such as kudu and buffalo in the fauna may be significant in confirming the dominance of grasses over shrub.

The modern ecology at Bundu is dominated by C<sub>4</sub> grasses and karroid shrubs. This is in keeping with C<sub>4</sub>/C<sub>3</sub> composition at the latitude for Bundu within South Africa (Werger and Ellis 1981). Isotope data indicative of increased C<sub>3</sub> plants at Bundu and correlation to ESR dates points to occupation during cooler MIS stages (8-10), during which circulation systems shifted northwards bringing winter rainfall regimes into the interior (Urrego et al. 2015). There is no suggestion of a complete transition to C<sub>3</sub> vegetation as may be expected in a winter rainfall regime and on the basis of comparison to other sites a degree of aridity was experienced. As has been suggested at Equus Cave for MIS 2, temperature may have been depressed by 3-4°C and therefore summer maximum and minimum temperatures would have remained at levels suitable for C<sub>4</sub> grasses to flourish (Lee-Thorp and Beaumont 1995). In parallel with data for Equus Cave and Wonderwerk a more likely scenario is that Bundu experienced

an increase in winter rainfall and reduction in rainfall seasonality. This would have led to a climate of more frequent year-round rainfall, resulting in proportional shifts in C<sub>3</sub>/C<sub>4</sub> plants, implied by the OES isotopes.

While the precise nature of vegetation cover experienced at Bundu during the Pleistocene requires further investigation the cooler and moister climates suggested hint at an EMSA environment at Bundu comparable to that of the central Karoo along a latitude of Calvinia to Richmond (Werger and Ellis 1981), conditions that supported the expansion of merino sheep in historic times and which correlated to a large cooling event in southern Africa in the late C17th and C18th (Sundqvist et al. 2013). These areas today experience rainfall >300mm, cooler temperatures, including frost, and optimal conditions for C<sub>3</sub> and C<sub>4</sub> grasses and shrubs (Cowling 1983)

## CONCLUSION

In summary, isotope data point to palaeoclimate and palaeoenvironment at Bundu in the Mid-Pleistocene that was cooler and wetter than present and which allowed for standing water and functioning grassland, with occasional trees. The closeness of Bundu OES isotopes to that of Equus Cave and Wonderwerk Cave during the Pleistocene and Holocene and to modern Postberg, raises the strong likelihood of increased winter rainfall, less seasonality and more year-round precipitation in the Karoo, 200 ka -300 ka years ago.

New isotope data from ostrich eggshell recovered at the Bundu Farm site provides the first direct evidence of Pleistocene environments, associated with the Middle Stone Age assemblages, in the upper Karoo, circa ~300ka. Homogeneity of the ostrich eggshell sample data strongly suggests that multiple hominin occupation throughout the MSA at Bundu occurred under similar conditions, when rainfall and temperatures allowed for expanded grassland. These periods of greater humidity were interspersed with periods of extreme aridity during which calcrete deposits formed and the region was presumably unoccupied. Data accords with proposed phases of global cooling in the Mid-Pleistocene (MIS 8-10) and support the presence of a wet phase associated with the presence of EMSA assemblages in the Karoo >200 ka (Berger et al. 2016).

The new isotope data provides a basis to investigate the Pleistocene environment, ecology and foraging opportunities open to MSA hominins in the Karoo. That both earlier and later MSA occupations appear to have occurred under similar environmental and ecological conditions adds further weight to the pattern of hominin occupation of the central interior during the Pleistocene (Brink 2016).

The apparent fluctuating occupation of the Karoo during the MSA, driven by wetter climatic phases raises the question as to the adaptability of early *Homo* and when past populations were first able to

occupy the Karoo under more arid conditions. New Bundu data provides an opportunity to re-evaluate the cultural and faunal assemblages at Bundu, to explore foraging behaviour and temporal differences at the site during the MSA. Of particular interest for further work is whether any correlation can be found between changes in MSA technologies between horizons and fluctuating climates, as has been suggested elsewhere in relation to the expansion and contraction of the greater Kalahari (Barham 2000) and the later MSA (Henshilwood 2008).

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