Expansion of stable carbon and oxygen isotope analysis of Ostrich Eggshell (OES) fragments from MSA levels at Blombos Cave and Klipdrift Shelter, South Africa

We have analysed a limited series of ostrich eggshell (OES) samples from the sites of Blombos Cave, Klipdrift Shelter, and Klipdrift Cave on the southern Cape coast of South Africa. The results from these sites have so far demonstrated that the influence of winter and year-round/summer rainfall regimes, and amount of rainfall, fluctuated substantially in this region of southern Africa in the past. In particular, the results have provided quantifiable evidence for climatic and environmental change *directly* associated with the appearance of the Still Bay and Howiesons Poort technocomplexes at Blombos Cave and Klipdrift Shelter, respectively. Although this was a successful and very promising outcome, our sample numbers, per stratum, are relatively few (*c.* 4-5). We therefore seek permission to analyse further OES fragments from the MSA layers of both Blombos Cave and Klipdrift Shelter in order to obtain a statistically sound stratigraphic environmental sequence in association with important behavioural changes in the archaeological records of these sites. This should facilitate the development of a robust dataset required for publication in international journals.

OES fragments are ubiquitous in many southern African archaeological sites, making it an excellent material with which to construct palaeoenvironmental proxy records. Stable carbon ($\delta^{13}C$) values from the organic and inorganic fractions of OES reflect that of the ambient vegetation consumed by the ostrich in the breeding season and can therefore track changes in C_3 and C_4 palaeovegetation. Due to the strong links between the distribution of C_3 and C_4 grasses and rainfall seasonality, $\delta^{13}C$ data from a single stratified site provides information on changes in season/amount of precipitation through time. Oxygen isotope data (expressed as ($\delta^{18}O$) generated during the same analysis have been shown to indicate aridity and allow estimation of past changes in mean annual precipitation (Lee-Thorp & Ségalen, unpublished data). Importantly, OES calcium carbonate is well-preserved over the long time period of interest in this study. As previously suggested, the Blombos and Klipdrift Shelter sites have proven to be ideally placed for such a study, as they lie very near the boundary between the ñpureò winter rainfall zone with its solely C_3 fynbos vegetation, and the intermediate zone with less confined rain and the emergence of grassy vegetation. These grasses have been shown to include C_4 forms (Cowling and Procheк, 2005; Radloff 2008).

The aim is to follow up our previous isotopic analysis at Blombos Cave and Klipdrift Shelter with stable carbon and oxygen isotopic analysis of more OES samples from each stratigraphic layer, in order to establish a robust record of vegetation and aridity that spans from 100 to 50 ka on the southern Cape coast of South Africa. Our existing results are comparable, but also add to, previous palaeoclimatic records from this region. At Pinnacle Point, Bar-Matthews *et al.* (2010) interpret shifts in $\delta^{18}O$ within speleothem records as primarily driven by fluctuations in rainfall source (Cruz *et al.,* 2005). However, while OES $\delta^{18}O$ is also influenced by rainfall source, the water balance of ostriches results in a strong influence of plant water and metabolic water on OES $\delta^{18}O$ that is primarily controlled by plant evapotranspiration. As a result, while changes in the Pinnacle Point $\delta^{18}O$ speleothem records are often very subtle, OES $\delta^{18}O$ can provide additional insight into relative local aridity. Our OES record indicates that there was a significant shift from winter rainfall to C_3 dominance to increasing C_4 vegetation, year round/ summer rainfall, and potentially aridity, from 97 ka onwards at Blombos Cave. The Klipdrift Shelter OES sequence then demonstrates substantial instability in in C_3/C_4 composition, aridity and rainfall regime between 65 and 59 ka.

The development of MSA palaeoenvironmental records is particularly important given increasing discussion regarding the role climate, environment, and ecology have played in cultural shifts cited as representing ómodernô symbolic behavior (Ziegler *et al.,* 2013). However, with the exception of work at Pinnacle Point (Bar-Matthews *et al.,* 2010), most discussions of climatic influence have been based on remote records divorced from archaeological contexts. Furthermore, many researchers have assumed that contemporaneous change in detached environmental and cultural records facilitates simplistic causative generalisation (Blockley *et al.,* 2012). Application of isotopic OES methodology at Blombos Cave and Klipdrift Shelter provides an opportunity to compare directly associated environmental/climatic proxies and cultural records within the same chronological framework and process of anthropogenic formation. Blombos Cave has provided some of the earliest evidence for symbolic human behavior, in the form of perforated marine shell beads, bone tools and engraved ochre associated with Still Bay layers to *c.* 80 ka (Henshilwood *et al.,* 2011) while Klipdrift Shelter has yielded evidence for ochre use in contexts of the Howiesons Poort technocomplex (Hensilwood *et al.,* 2014). The initial OES results indicate that the emergence of the Howiesons Poort and Still Bay technocomplexes occurred during periods of climatic and environmental change at Klipdrift Shelter and Blombos Cave, respectively.

Our isotopic analysis of OES from Blombos Cave and Klipdrift Shelter remains the first high-resolution methodology to provide local environmental and climatic contexts for these sites. However, our own and previous work has shown a high level of variability in OES isotope values, even differing in one nest and certainly on inter-annual scales at the same location (Lee-Thorp and Ségalen, in prep). Consequently, multiple analyses are required per archaeological level. Although we have already sampled *c.* 5 OES fragments from a number of the Blombos Cave (CA, CB, CC, CFB/CFC, CIB, CJ, CK, CL, CN/CO) and Klipdrift Shelter (PAZ, PBA/PBB, PBD and PCA) MSA stratigraphic layers, we request to be allowed sample up to four more samples from each of the previously-sampled layers. We believe this will produce a dataset that is robust in the face of review at the highest level and can provide as high a confidence as possible in the trends observed. This is particularly important for layers CJ and CN/CO at Blombos Cave where a plot of $\delta^{13}C$ versus $\delta^{18}O$ has indicated that two samples in each layer may have come from the same egg, potentially reducing the statistical validity of results from these levels. We estimate that analysis of a further 50 OES samples from the two sites should provide a statistically robust dataset for international publication.

As was the case in our work over the last year, the analysis of OES fragments will follow the established protocol in our laboratory (the Research Laboratory for Archaeology, Isotope laboratory). It is destructive, but requires very minute samples (of the order of 0.5x0.5 cm^2, or less). Each fragment is cleaned with a soft brush and the surface is lightly abraded with a diamond-tipped drillbit attached to a microdrill, in order to remove surface contamination. Each sample is then crushed and homogenized. As extremely minimal amounts of protein remain in archaeological samples (if any), no further chemical treatment is required. The powders (about 30 µg each) are weighed and loaded into individual containers for H_3PO_4 acid hydrolysis and subsequent analysis of the clean, dry evolved CO_2 in a Thermo Delta V Advantage isotope ratio mass spectrometer, interfaced with a Kiel IV carbonate device, in the Earth Sciences Department, Oxford.

Our success in the pilot application of this methodology at Blombos Cave and Klipdrift Shelter, we believe, justifies the analysis of a further set of samples to increase the strength and statistical significance of our analysis and interpretations (MSc dissertation of Patrick Roberts available on request). This will allow the production of a robust and statistically sound data series that directly relates a local climatic and environmental record to the emergence of behavioural ñmodernityò in southern Africa. Publication of this methodology at these important sites should encourage its adoption at others. We thank the Iziko Museums for their already considerable support in the development of this project and we hope that we can continue to collaborate in the application of this emerging methodology to two of the most significant sites and periods in southern Africa prehistory.

References:

Bar-Matthews, M., Marean, C.W., Jacobs, Z., Karkanas, P., Fisher, E.C., Herries, A.I.R., Brown, K., Williams, H.M., Bernatchez, J., Ayalon, A., Nilssen, P.J. 2010. A high resolution and continuous isotopic speleothem record of paleoclimate and paleoenvironment from 90 to 53 ka from Pinnacle Point on the south coast of South Africa. *Quaternary Science Reviews* 29: 2131-2145.

Blockley, S.P.E., Lane, C.S., Hardiman, M., Rasmussen, S.O., Seierstad, I.K., Steffensen, J.P., Svensson, A., Lotter, A.F., Turney, C.S.M., Bronk Ramsey, C., INTIMATE members. 2012. Synchronisation of palaeoenvironmental records over the last 60,000 years, and an extended INTIMATE event stratigraphy to 48,000 b2k. *Quaternary Science Reviews* 36: 2-10.

Cowling, R.M., Procheķ, ķ. 2005. Patterns and evolution of plant diversity in the Cape Floristic Region. *Biologiske Skrifter* 55: 273-288.

Henshilwood, C.S., Sealy, J.C., Yates. R., Cruz-Uribe, K., Goldberg, P., Grine, F.E., Klein, R.G., Poggenpoel, C., van Niekerk, K.L., Watts, I. 2001. Blombos Cave, Southern Cape, South Africa: preliminary report on the 1992-1999 excavations of the Middle Stone Age levels. *Journal of Archaeological Science* 28: 421-448.

Henshilwood, C.S., dôErrico, F., van Niekerk, K.L. Coquinot, Y., Jacobs, Z., Lauritzen, S-E., Menu, M., García-Moreno, R. 2011. A 100,000 Year Old Ochre Processing Workshop at Blombos Cave, South Africa. *Science* 334, 219-221.

Henshilwood CS, et al. (2014) Klipdrift Shelter, southern Cape, South Africa: Preliminary Report on the Howiesons Poort layers. *Journal of Archaeological Science*: DOI: 10.1016/j.jas.2014.01.033.

Jacobs, Z., Hayes, E.H., Roberts, R.G. Galbraith, R.F., Henshilwood, C.S. (2013). An improved OSL chronology for the Still Bay layers at Blombos Cave, South Africa: further tests of single-grain dating procedures and a re-evaluation of the timing of the Still Bay industry across southern Africa. *Journal of Archaeological Science* 40 (1): 579ï594.

Johnson, B.J., Gifford H. Miller, Marilyn L. Fogel, Peter B. Beaumont 1997. The determination of late Quaternary paleoenvironments at Equus Cave, South Africa, using stable isotopes and amino acid racemization in ostrich eggshell. Palaeogeography, Palaeoclimatology, Palaeoecology 136 (1997) 121- 137

Johnson, B.J., Fogel, M.L., Miller, G.H. 1998. Stable isotopes in modern ostrich eggshell: A calibration for palaeoenvironmental applications in semi-arid regions of southern Africa. *Geochimica et Cosmochimica Acta*, 62: 2451-2461.

Lee-Thorp, J.A., E.S. Talma 2000. Stable light isotopes and past environments in the Southern African Quaternary and Pliocene, In T.C. Partridge, R. Maud (Eds.) *The Cenozoic of Southern Africa*, Oxford: Oxford University Press, pp. 236-251.

Radloff FGT (2008) The ecology of the large herbivores native to the coastal lowlands of the Western Cape, South Africa. Ph.D. dissertation, Stellenbosch University, Stellenbosch

Rabett, R. 2012. *Human Adaptation in the Asian Palaeolithic: Hominin Dispersal and Behaviour during the Late Quaternary*. Cambridge: Cambridge University Press.

Scott, L. and Lee-Thorp, J.A. 2004. Holocene climatic trends and rhythms in southern Africa. *In:* R.W. Battarbee, F. Gasse and C.E. Stickley (Eds.). *Past Climate Variability through Europe and Africa*. Springer Dordrecht: the Netherlands, pp. 69-91.

Ségalen, L., M. Renard, J.A. Lee-Thorp, L. Emmanuel, L. Le Callonnec, M. de Rafélis, B. Senut, M. Pickford, J.-L. Melice. (2006) Neogene climate change and emergence of C_4 grasses in the Namib, southwestern Africa, as reflected in ratite ^{13}C and ^{18}O. *Earth and Planetary Science Letters* 244: 725-734.

Thompson, J. & Henshilwood, C. S. 2011. Taphonomic analysis of the Middle Stone Age larger mammal faunal assemblage from Blombos Cave, southern Cape, South Africa. *Journal of Human Evolution*, 60:746-767.

Ziegler, M., Simon, M.H., Hall, I.R., Barker, S., Stringer, C., Zahn, R. 2013. Development of Middle Stone Age innovation linked to rapid climate change. *Nature Communications* 4, doi:10.1038/ncomms2897.