ESRF	**Experiment title:** Life and Death in the Middle Jurassic - Metabolic Rates and Lifespans of Early Mammals Determined Through Phase Contrast Imaging of Tooth Cementum Increments	**Experiment number**: ES502
Beamline: ID19	**Date of experiment**: from: 01/02/2017 to: 05/02/2017	**Date of report**: 05/03/2018
Shifts: 12	**Local contact(s)**: Vincent Fernandez	*Received at ESRF:*

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Report: In this ID 19 experiment we used single distance phase contrast x-ray microtomography to study the molar teeth of a range of Bathonian stage, Middle Jurassic (~170 million years old) mammals and mammaliaforms. The purpose was non-destructive identification, mapping and quantification of growth increments (hereafter referred to as lines of arrested growth, LAGs) in order to reconstruct life history variables in the earliest mammals and their close relatives the mammaliaforms. The data collected has been presented at 2 international conferences, included in a completed PhD thesis (Elis Newham, University of Southampton, July 2018) and is currently having analysis completed for publication, alongside data from additional ESRF ID19 experiments e.g. **ES583**, for publication in a high profile journal. For this, the data will be combined with that from an earlier ESRF ID19 and ID16A experiment (**ES152**) that is currently in the revision stage of review for publication in Nature Communications, and available as a preprint on bioRxiv (Newham et al. 2019). Methods developed for study of cementum LAGs in this and other ID19 experiments will also be published in one or more separate methodological papers and a review of these methods is currently in review as a peer reviewed book chapter (Newham et al. 2020).

We used two different voxel resolutions, principally 0.33μm but also 0.66μm where larger specimens required an increased field of view, and additionally worked in half-acquisition mode to ~ double the field of view laterally when needed. Working distance was typically around 18mm. Energies used included 19keV, 26keV, 35keV and 53keV, depending on the size and density of the specimen. The recently developed accumulator acquisition mode was used throughout the experiment. Exposure times were 25,100, 180, 360ms, and 2999 or 6000 projections were made.

Cementum lines obtained through destructive thin sectioning have been used for ageing a range of taxa, including humans, and for archaeological, zoological, palaeontological and forensic purposes including conservation and identification of crime victims. However, limitations of the method have been identified, prinicipally due to difficulties in objectively quantifying LAG numbers from single histological thin sections of variable location and quality. We aim to improve the method through the use of tomographic acquisition of cementum LAGS, allowing more accurate quantification of LAG numbers due to the availability of whole root LAG data instead of single tooth root thin sections per specimen. Additionally, we are developing new methods for objective quantification of cementum LAG counts from tomographic data (e.g. Newham et al. 2019,2020).

More than 200 measurements/scans were completed during the 12 shifts, despite 3 losses of beam during the experiment, for an average of <25 minutes/scan. Since each fossil mammal/mammaliaform cheek tooth has two roots and the region of interest volume size at these high resolutions does not encompass the length of an entire root, many isolated teeth required multiple measurements per specimen for complete coverage. Specimens were discarded if initial reconstructions revealed a lack of LAG resolution due to internal damage or chemical diagenetic alteration during fossilisation. This resulted in 71 Bathonian fossil mammal/mammaliaform individuals being measured.

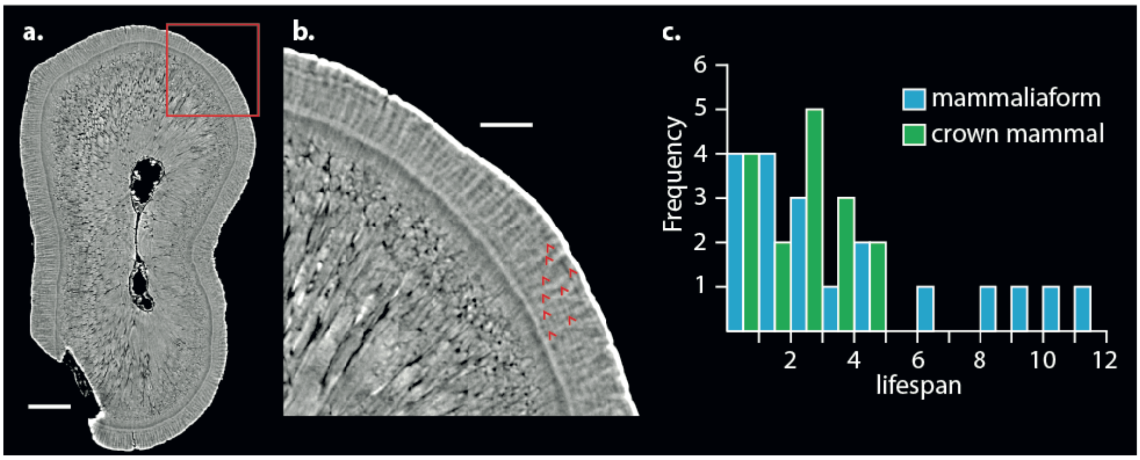

Figure 1. a) Virtual cross section of a Bathonian, Middle Jurassic docodont tooth root (*Krusatodon*)**.** Cementum lags visible in outer cementum layer. **b)** Detail of cementum highlighted by red box in (**a**), nine individual increments marked by red arrows **c)** Histogram of lifespan estimates for 35 individuals with readable cementum increments. Total sample split into mammaliaforms (basal stem mammals – comprising docodonts) and crown mammals (derived taxa bound by living mammals phylogenetically –comprising eutriconodonts and trechnotherians). Stem mammaliaforms show a maximium lifespan estimate double that of any crown mammal group, and significantly longer than any living mammal of similar body mass (ca. 30-100 grams).

Of the 71 individuals of Bathonian fossil mammals/mammaliaforms for which measurements were made, 46 individuals (67%) were of sufficient preservational quality to allow cementum LAGs to be resolved (**Fig. 1a,b**). This represents almost twice the percentage as were found to be of good preservation in our previous ID19 ES152 experiment looking at cementum LAGs in the older mammaliaform *Morganucodon watsoni* (39%), indicating either a preservational difference between Early and Middle Jurassic sites, or some other taphonomic properties of the *Morganucodon* specimens contributing to their poor preservation. The remainder typically showed heavy diagenetic chemical alteration and/or damage to the cementum, either or both of which served to obscure the cementum LAGs. Some otherwise well preserved specimens entirely lacked cementum, which was only determined by examination of the tomographic data.

Counts of cementum LAGs for the 35 of these 46 individuals identifiable to species level allows a graph of the distribution of the age at death for population sized samples of various Bathonian mammaliaforms and mammals (**Fig. 1c**). Results show that phylogenetically basal mammaliaform taxa like docodonts have longer lifespans than crown group mammals like eutriconodonts, which are similar to those of living mammals. This relatively high maximum cementum LAG number for docodonts (**Fig. 1b**) and hence maximum age/lifespan is outside the top of the range found for extant mammals of similar body mass, suggesting physiological differences between docodonts on one hand and crown fossil and living mammals on the other.

We additionally gathered pilot tomographic data from teeth of the fossil sauropod dinosaur *Euhelopus zdanskyi* and succesfully reconstructed dentine increments; this data has been presented at an international conference (ISDM, October 2017), used for a succesful beamtime application (**ESRF ES-753**), and will form the basis of a PhD (Seela Salakka, University of Helsinki) and associated pubications.

References: Newham, E.N., Robson-Brown, K., Gill, P.G., Brewer, P., Bayle, P., Schneider, P., and Corfe, I.J. 2020. (Invited book chapter, in review). Non-invasive 3D methods for the study of dental cementum. In: New studies in cementochronology (ed. Stephan Naji), Cambridge University Press.

Newham, E.N., Gill, P.G., Brewer, P., Benton, M.J., Fernandez, F., Gostling, N., Haberthur, D., Jernvall, J., Kakanpää, T., Kallonen, A., Navarro, C., Pacureanu, A., Zeller-Plumhoff, B., Richards, K., Robson-Brown, K., Schneider, P., Suhonen, H., Tafforeau, P., Williams, K., and Corfe, I.J. 2019. (Reviewed, in revision). Reptile-like physiology in Early Jurassic stem-mammals. *Nature Communications*. Also published as a preprint on bioRxiv: https://doi.org/10.1101/785360