ESRF	**Experiment title:** Getting to the Root of Mammalian Evolution - Lifespans and Metabolic Rates of Cretaceous Mammals Determined Through Phase Contrast Imaging of Tooth Cementum Increments	**Experiment number**: ES583
Beamline: ID19	**Date of experiment**: from: 15/02/2018 to: 17/02/2018	**Date of report**: 09/03/2020
Shifts: 6	**Local contact(s)**: Vincent Fernandez	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Ian Corfe, Institute of Biotechnology, University of Helsinki, Finland
Pamela Gill*, School of Earth Sciences, University of Bristol, UK
Elis Newham*, National Oceanographic Centre, University of Southampton, UK
Heikki Suhonen, Department of Physics, University of Helsinki, Finland

Report:

In this ID19 experiment we used single distance phase contrast x-ray microtomography to study the molar teeth of a range of Mesozoic mammal fossils. 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 fossil mammals throughout the evolution of the group. 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 previous ESRF ID19 experiments e.g. **ES502**, 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 prior 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 principally 0.33μm voxel resolution, and additionally worked in half-acquisition mode to ~ double the field of view laterally when needed. Working distance was typically around 20mm, exposure time 100ms, 2999 projections were used, and energy used was 26keV. The recently developed accumulator acquisition mode was used throughout the experiment.

We concentrated tomographic imaging mainly on the cementum LAGs found in the tooth roots of the various Mesozoic mammal species studied. Cementum is a collagenous mineralised tissue that connects tooth roots to the periodontal ligament, supporting dentition within the alveolus against forces applied through mastication. Cementum LAGs have been shown to reflect seasonal changes, creating alternating bands within the tissue. As cementum grows continuously through life, these bands offer an absolute record of annual seasonal change, and hence an estimate of annular age and season of death.

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.

More than 160 measurements/scans were completed during the 6 shifts, for an average of ~18 minutes/scan. Since each fossil mammal 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 (**Fig 1a**), 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 81 Mesozoic fossil mammal or mammaliaform (close mammal relative) individuals being measured, the majority of which we were able to identify well preserved cementum LAGs in (**Fig 1b,c**). Some were represented by single tooth roots and even single scan volumes, while most were jaws with teeth in situ, requiring multiple measurements to cover all teeth in the tooth row, all roots of a tooth, the entire volume of a tooth root, plus multiple regions of the bony dentary.

In general, the number of cementum LAGs was extremely consistent between both roots of single teeth and between teeth along the jaw. However, LAG preservation varied substantially along individual roots in many specimens. This highlights a key advantage of using non-destructive tomographic imaging rather than conventional destructive histological thin sectioning, as we show in our ID19 work for the first time. Limited numbers of thin sections can be made from a single tooth root, and resulting LAG counts can vary depending on where these are taken. In contrast, tomographic data allows an overview of cementum LAGs across the whole root, multiple areas with the clearest LAG preservation can be compared for consistency, and 3D LAGs can be traced through the entire volume of the root ensuring accurate counts can be made (Newham et al. 2019).

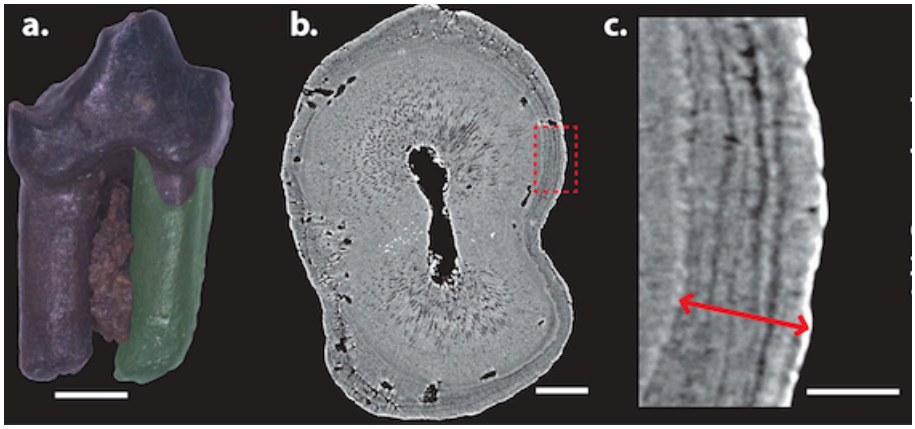

Figure 1. (**a**) Late Jurassic mammaliaform *Haldanodon* molar tooth with root cementum (highlighted in green in anterior root) wrapping around root dentine. (**b**) Transverse synchrotron µCT single propagation distance phase contrast virtual slice of a *Haldanodon* tooth molar root shows darker/denser incremental cementum wrapping around lighter/less dense root dentine. Scan details: 0.33µm voxel resolution, 26keV, exposure time 100ms, working distance 20mm, 2999 projections. (**c**) Detail of cementum highlighted in dashed red box in (**b**), displaying highly contrasting increments; cementum highlighted by arrowed red line. Scale bars: 500 µm in (**a**), 100 µm in **(b)**, and 30 µm in (**c**).

We additionally gathered pilot tomographic data of snail radula from which, if high quality images can be produced from the reconstructions, further beamtime proposals will be made.

Refs

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