Ageing fossil birds using high-resolution X-ray computed tomography

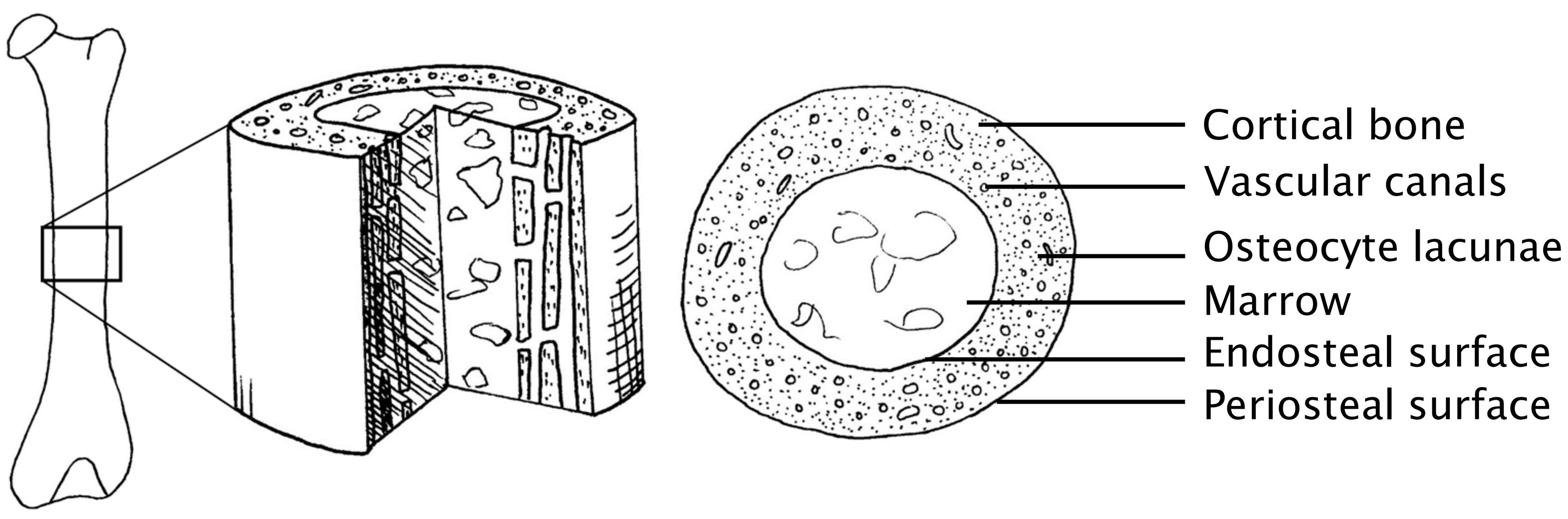
UNIVERSITY OF Southampton

Katherine Williams[1], Gareth Dyke[2], Neil Gostling[3], Richard Oreffo[4], Philipp Schneider[1]
[1] Faculty of Engineering and the Environment, University of Southampton. [2] Department of Evolutionary Zoology and Human Biology, University of Debrecen, Hungary. [3] Faculty of Natural and Environmental Sciences, University of Southampton. [4] Faculty of Medicine, University of Southampton.

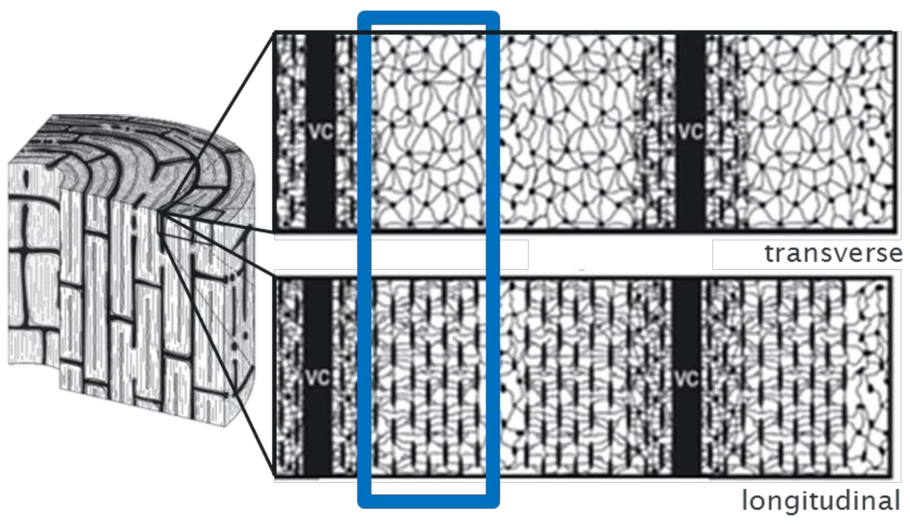
Introduction

Over 100 Mesozoic bird fossils are known, but with few exceptions only a single specimen is known for each species. Is it really the case that for so many species only a single specimen for each has been preserved? Or could some of this diversity actually represent different juvenile age stages of the same species?

Estimating developmental age in fossil birds is crucial for answering this question. We can estimate age using the structure of microscopic pores within bone, which change with age and bone deposition rate.



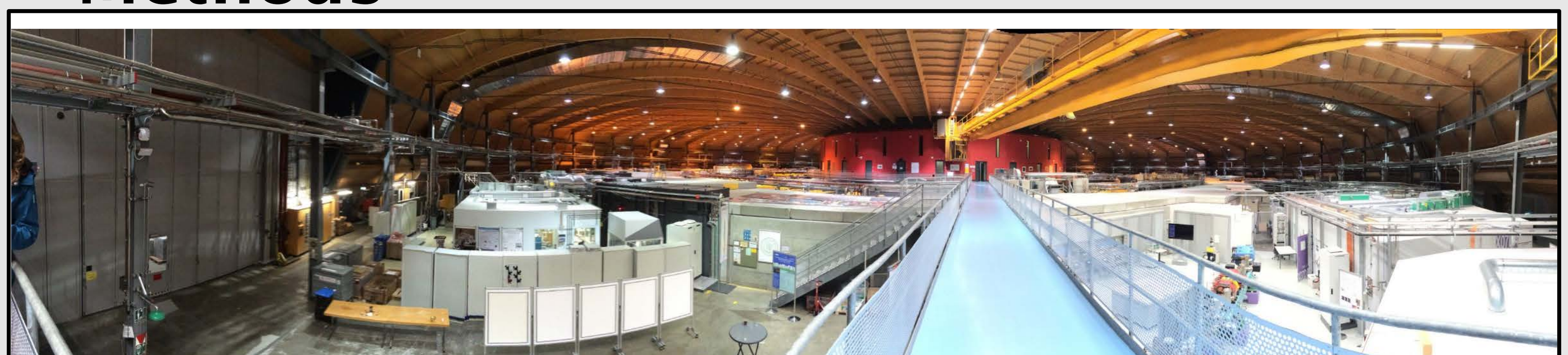
Current age estimation methods are qualitative, destructive and 2D: incapable of capturing complex 3D bone structure



Vascular canals and osteocyte lacunae can appear very different in differently oriented sections, so 2D imaging can misrepresent structure. Blue box highlights apparent levels of organisation in lacuno-canalicular networks in transverse and longitudinal sections. (Stein and Prondvai 2014)

A more effective method is needed: a high-resolution 3D imaging technique that is non-destructive and validated in living birds.

Methods



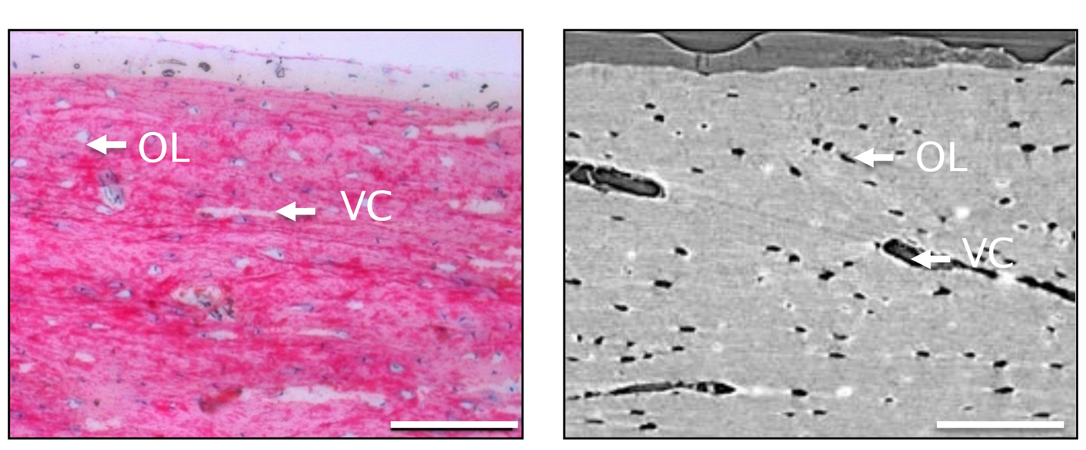
Internal view of the Swiss Light Source synchrotron facility, Switzerland.

X-ray computed tomography (CT) permits **3D imaging** which reveals structural detail not visible in 2D

Synchrotron radiation-based computed tomography (SR CT) uses high-energy electrons to produce X-rays at high flux. SR CT provides:

- **Sub-micron resolutions,** enabling a **virtual histological** approach which is minimally destructive
- Fast scans (minutes rather than the hours needed using a lab-based CT system) mean high throughput for a **quantitative** study
- Good image contrast

Structures seen in SR CT images are validated using conventional stained histological sections, e.g. nuclear stains to check that pores visible in SR CT images do in fact contain osteocytes, as interpreted.



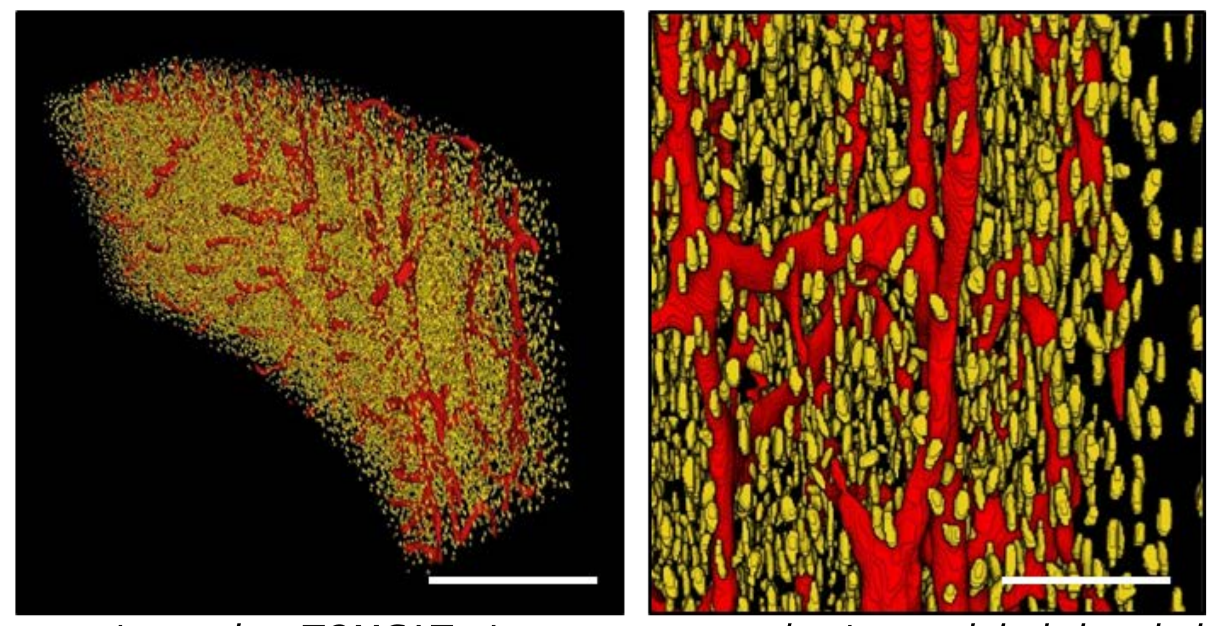
(Left) Histological section of duck cortical bone, stained with Alcian blue and Sirius red (A/S), (Right) SR CT image of a different section from the same bone. OL osteocyte lacunae, VC vascular canals. Scale bars 100µm.

A morphometric approach is used to quantify cortical bone porosity, including:

- Number density of osteocyte lacunae
- Volume of vascular canals

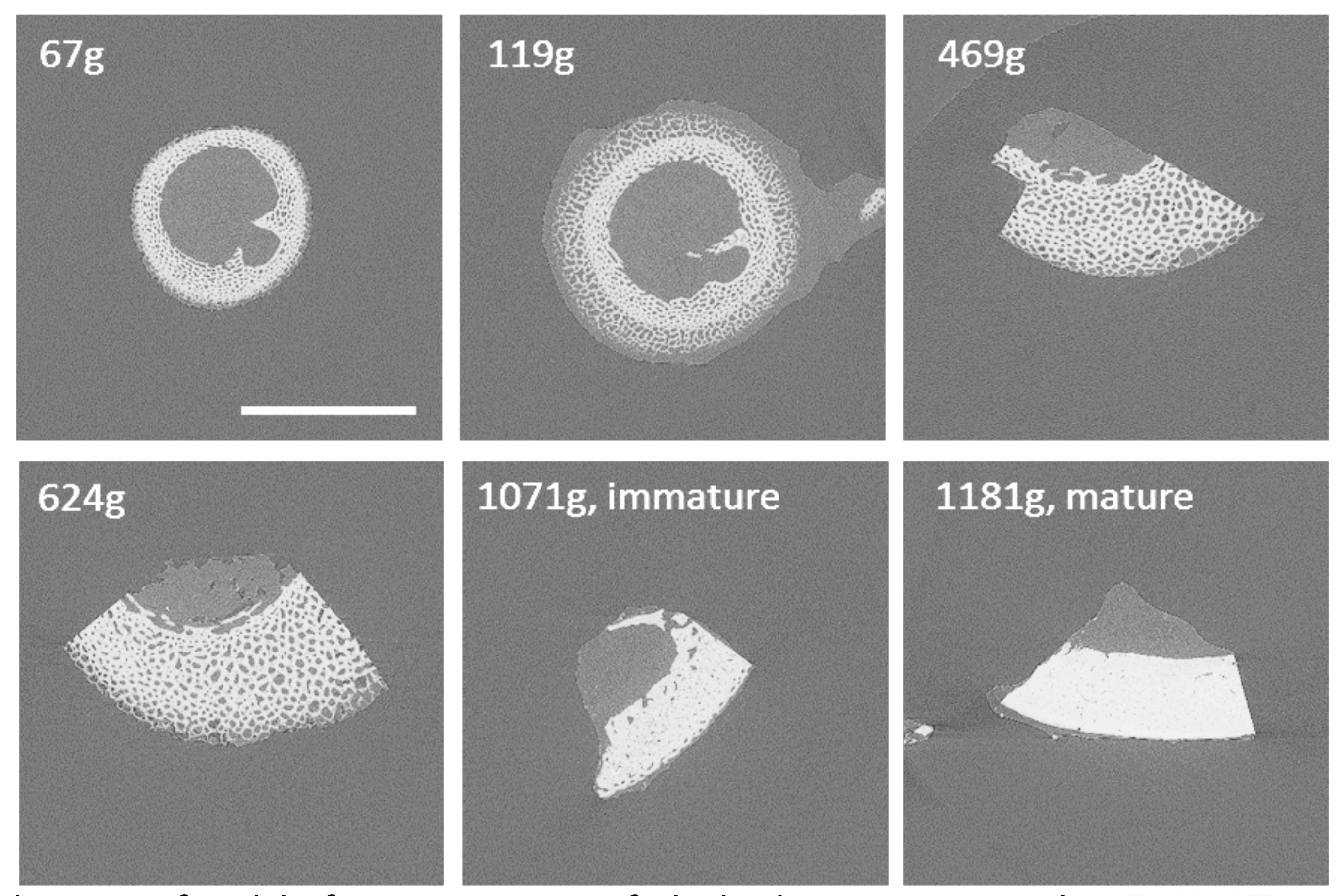
Results

1. SR CT imaging has been optimised to produce images that are high-contrast, high-resolution and relatively straightforward to segment
- Greyscale-based thresholding optimised to isolate pore spaces



Duck tibiotarsus imaged at TOMCAT . Image segmented using a global threshold. Porosity extracted, osteocyte lacunae (yellow) and canals (red). Scale bar (left) 200µm, (right) 50µm

2. SR CT images of a growth series of duck long bones show that juvenile bone is very porous and mineral density increases with age. Bones also continue to remodel after the bird reaches full size



Growth series of midshaft cross sections of duck tibiotarsus imaged at TOMCAT. Voxel size 1.2µm, scale bar 1mm.

3. Comparing images from the femur, tibiotarsus and humerus shows that different limb bones grow at different rates and have different microstructural appearances

Further work

Morphometric analysis of volume and number density of vascular canals and osteocyte lacunae will be used to test whether the observed changes in bone porosity are quantifiable and follow consistent patterns e.g. pore volume fraction decreasing with age. If so, this will be used to model bone microstructural age both in ducks and other birds, in order to estimate age in unknown samples.

Ageing methods developed will be tested by estimating developmental age in a blind sample of additional duck material and compared with existing 2D methods.

Once validated, these methods will then be applied to fossil material in order to estimate developmental age and contribute to species identification and understanding avian evolution.

Conclusions

Bone microstructure in domestic ducks changes through development. Understanding and quantifying this change will form the basis for a quantitative model of bone growth which could be used to estimate age in fossil birds. This novel application of techniques from engineering and biomedical sciences will help to develop a tool which, applied to fossil material, will aid interpretation of avian fossils and enhance our understanding of the evolution and biology of birds.

References

Stein, K. and E. Prondvai (2014). Biological Reviews **89**(1): 24-47.
Erickson, G. M., et al. (2009). Plos One **4**(10).
Castanet, J., et al. (1996). Comptes rendus de l'Académie des Sciences. Série 3, Sciences de la vie. **319**: 7.

Author contact details
Email: k.a.williams@soton.ac.uk