Dynamics of bone growth and remodeling in captive leopard geckos (*Eublepharis macularius*)

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Introduction

- Bone histology is the study of bones at the microscopic level to understand an animal's life history traits, such as life expectancy and growth rates. Skeletochronology examines microstructures, called Lines of Arrested Growth (LAGs) in bones to determine the somatic age of an individual[4].
- LAGs typically appear in wild animals annually due to hibernation or environmental stressors[2]. However, bone remodels through growth, and so a portion of the growth record may be lost in older individuals[3].
- It is thought that LAGs may not appear in captive, lab-reared animals that are not subject to these environmental stressors, so fluorescent markers can be injected into the animal to mark actively mineralizing tissue[4]. Fluorescent injections will be absorbed immediately into all the animals' mineralizing tissues[1] marking one point in time.
- By using fluorescent bone markers in captive animals, we can determine patterns of bone growth throughout the life of an animal in the absence of LAGs and determine whether patterns differ between elements from the same individual in the absence of environmental stressors[4].
- To examine the dynamics of bone growth and LAGs, embryonic, juvenile, and adult lab-reared leopard geckos (*Eublepharis macularius*, Fig. 1) previously injected for a different study were used in the current study[1].

Figure 1: Adult (left) and juvenile (right) leopard geckos [5]

Research Questions

1) **When do the leopard geckos stop growing in captivity?**
2) **Do fluorescent markers appear consistently in all of an individual's skeletal elements through growth, or is the growth record lost through remodeling?**
3) **Are Lines of Arrested Growth (LAGs) visible in captive leopard geckos?**

Methods

1) Removing left limb bones of 13 adult, juvenile, and embryonic leopard geckos (Fig. 2)
2) Placed bones in a series of acetone solutions to dehydrate them
3) Placed bones in resin
4) Used Hillquist Saw and IsoMet 1000 (Fig. 3) to create thin sections of bone on glass slides
5) Examined thin sections for LAGs
6) View slides under a fluorescent microscope equipped with a camera.
7) Analyzed number of fluorescent markers on images
8) Compared number of fluorescent markers between elements of individual animals
9) Compared fluorescent markers to health and growth data

Figure 2. Dehydrated left femur from leopard gecko LG 173

Figure 3. IsoMet 1000 precision saw [2]

Results

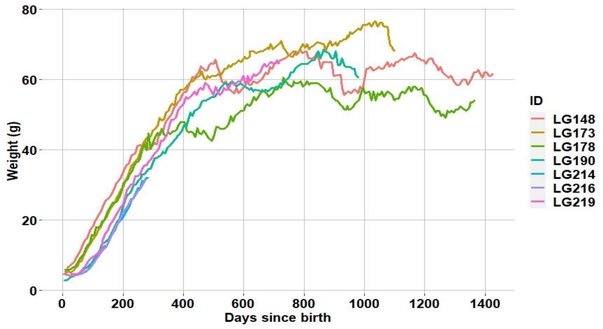

Figure 4. Days since hatching versus leopard gecko weight in grams.

- Examination of the health data (Fig. 4) shows that leopard gecko growth is rapid during the first 400 days of life, but afterwards levels off. However, the presence of fluorescent labels that bind to actively mineralizing tissue show that the bone is still being maintained and new bone is being deposited, just not enough to affect the size of the geckos.
- LG 214, LG 216, and LG 219 all had one *in ovo* injection each. LG 214 and LG 216 received three injections in total while LG 219 had four (Fig. 5).
- LG 219's humerus and radius have all four labels present (Fig. 5). In LG 219's tibia, the November label is not present, however, the other three are. The fluorescent labels that are present are yellow, which means that the calcein (green) and xylenol (red/orange) were mineralized in the same growing bone as they were injected only three weeks apart.
- All the labels for LG 214 and LG 216 are present in the radius and humerus. In the tibiae, only two labels are present, with specifically the *in ovo* label missing.
- Overall, the tibia is the least reliable bone for examining the growth record in the leopard gecko as it shows the most remodeling while the humerus and radius retain all fluorescent labels and specifically have the most preserved *in ovo* labels.

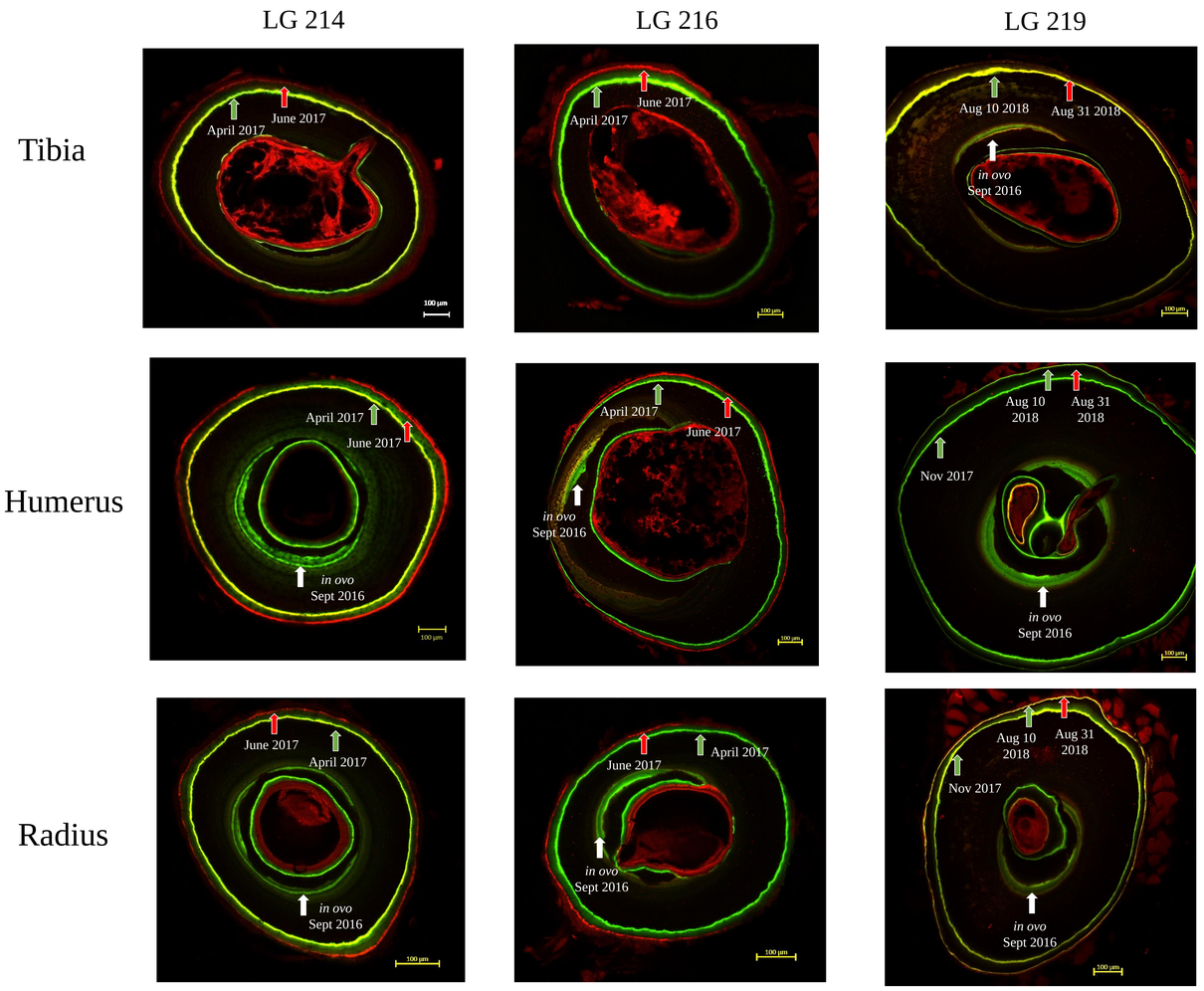

Figure 5. A comparison of the tibia, humerus, and radius in leopard geckos LG 214, LG 216, and LG 219. White arrows indicate calcein labels injected in the egg, green arrows indicate calcein injections in post-hatching animals, and red arrows indicate xylenol orange injections in post-hatching animals, with date indicated. Green and red labels in the centre of the bone mark endosteal remodeling.

Conclusions and Importance

- Fluorescent labels are visible in all the bones and appear when expected in the timeline of that animals' growth, however, some labels were not seen in certain elements. This differing bone deposition and loss of bone shows that leopard gecko bones remodel throughout life and loss of bone is unavoidable. By using fluorescent injections, bone remodeling is quantifiable and therefore accounted for when analyzing bone growth.
- It is recommended from these results that paleontologists and ecologists who research the life history traits of extinct and extant animals that are similar to lizards to use the humerus and radius for their studies. These two bones are least likely to remodel and will show the most accurate number of growth marks such as LAGs.
- Growth studies that use captive animals should be aware from these leopard gecko results that each bone element remodels and grows differently. Differing bone deposition and remodeling shows that the skeletal structure of an organism grows at varying rates and should be accounted for in bone histology research.
- The methods used thus far (fluorescent microscopy, confocal microscopy, and examination with a petrographic microscope under cross-polarized light) to uncover bone markings has not shown LAGs, only fluorescent injections (Fig. 6).

Future work

1) Determine bone growth rates during active growth and during specific time frames (e.g., 1 year of growth), then compare growth between animals.
2) Decalcify the bones and use stains to identify bone tissues such as LAGS, osteoblasts, osteocytes, and mineralized and unmineralized tissue (e.g., Fig. 6).

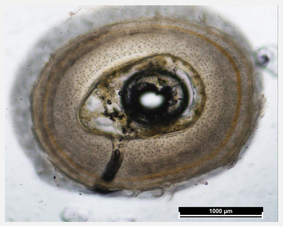

Figure 6. Tibia (LG 214) viewed under cross-polarized light reveals details of bone tissue not visible under fluorescence, however, better staining is needed.

References

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