Histologic Analysis of Bone Development of the Florida manatee, *Trichechus manatus latirostris*

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Introduction

The amount of bone tissue present in an organism does not remain constant throughout a lifetime. Peak bone volume is understood to be achieved during biological development, with bone degradation to follow in adulthood¹. Aquatic mammals generally have more dense bones than their terrestrial counterparts² and there is little to no information on aquatic mammal bone development. The Florida manatee is a fully aquatic mammal which represents a good example of dense, amedullary bones³. The abundance of prominent bone tissue histological features such as osteocytes, mesenchyme, and trabeculae provide a mechanism to determine how bone tissue of the same species differs due to age. The ultimate goal of this study was to evaluate key histological differences between bone samples, from manatees of various ages, in order to approximate the age using characteristics of the bone tissue.

Figure 1. Intramembraneous ossification: (a) Ossification center is formed from grouping mesenchymal cells. (b) Osteoblasts become trapped within osteoid and turn into osteocytes. (c) Trabecular matrix and periosteum are formed. (d) Compact bone develops surrounding trabeculae, blood vessels become red bone marrow.⁴ As published in Biga Anatomy and Physiology

Materials and Methods

- **Samples:** Nasal tissue including bone from manatees in the perinatal, calf, juvenile, and adult stages of development as determined by the animals length⁵. Collected under permit MA067116-2.
- Paraffin blocks sectioned at 5 micrometers using a microtome.
- Sections mounted, dried, and stained with trichrome stain.
- Slides observed with light microscopy and photographed at 40X and 100X magnification.
- Osteocyte lacunae were counted in the field of view of each photograph.

Results

Figure 2. Trichrome stained nasal tissue and bone.

Number of Osteocytes at 40x & 100X Total Magnification

Discussion

Analysis of the osteocyte quantity using a chi square test for goodness of fit shows a significant trend ($p < 2.2 \times 10^{-16}$) directly corresponding to the age of manatee. As the age of manatee increased, greater amounts of osteocytes were counted. Further observations indicate trabeculae are more abundant and prominent in early developmental stages (perinatal and calf) compared to the latter stages (juvenile and adult) in which mature or secondary bone is predominant. Using visual approximation, adult bone contained the largest bone volume, but showed minor signs of degradation and degeneration by osteoclast activity. These results are in agreement with the available information regarding intramembraneous bone development, and successfully show a proportional relationship between age and osteocyte number. More specific data, such as the exact age of manatee and computer calculated bone volume/osteocyte lacunae count, would allow us to further model this determined correlation between bone volume and age.

Conclusions

In this work, we investigated the relationship between the relative age of a manatee and bone volume. Our findings agree with prior research on osteocyte population as a determinant of bone volume⁶. We conclude that as age increases, osteocyte population (thus bone volume) increases. Future research will include an increased samples size to codify these trends and allow for detailed comparison between developmental ages.

References

1. Boskey, A. L., & R. Coleman. Aging and bone. J. of dental research 89.12 (2010): 1333-1348.
2. Wall, W. The correlation between high limb density and aquatic habitats in recent mammals. J. of Paleontology. 57.2 (1983): 197-207.
3. Bazzini M.D., J.E. Reynolds, & R.A. Essman. Erythropoiesis and Granulopoiesis in the West Indian Manatee, *Trichechus manatus*. Acta Anatomica. 126 (1986). 150-152.
4. Biga, Lindsay M., et al. "Anatomy & Physiology." 6.4 Bone Formation and Development | Anatomy & Physiology, Open Oregon State, Oregon State University, library.open.oregonstate.edu/aandp/chapter/6-4-bone-formation-and-development/
5. Schwarz L.K. & M.C. Runge. Hierarchical Bayesian analysis to incorporate age uncertainty in growth curve analysis and estimates of age from length: Florida manatee (*Trichechus manatus*) carcasses. Canadian J. of Fisheries and Aquatic Sciences. 66.10 (2009) 1775-1789.
6. Vashishth, D., et al. "Determination of bone volume by osteocyte population." The Anatomical Record: An Official Publication of the American Association of Anatomists 267.4 (2002): 292-295.