Trabecular structure through ontogeny in the Florida manatee (*Trichechus manatus latirostris*)

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BACKGROUND

The vertebral column is made mostly of trabecular (spongy) bone. Trabecular morphology and orientation is dynamic and changes in response to load direction and magnitude[1]. Previous literature has investigated the structural role of trabeculae in terrestrial mammals (humans, cattle, canines, rodents).

No published work comprehensively addresses the structural role of trabeculae throughout development in mammals that live submerged in water, whose bodies are supported against gravity through water's buoyant forces rather than limb support.

Manatees belong to one of two orders of fully aquatic mammals (obligate swimmers) that evolved from terrestrial counterparts to invade watery habitats[2]. They swim by dorsoventrally undulating their vertebral columns and producing forward thrust with their paddle-shaped flukes[3].

This project's companion study found that manatee vertebral bone was stronger (resist failure), stiffer (resist deformation) and more resilient (absorb energy) in more mature animals. Additionally, bone was stronger and more resilient in posterior regions of the vertebral column in the calves and subadults.

HYPOTHESES

H1: Trabecular structure will vary with ontogenetic stage. Greatest differences will be between the perinatal and calf stages, which is the period of the most body growth.

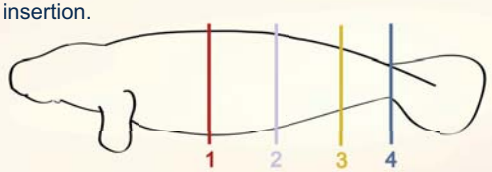
H2: Trabecular structure will vary among regions of the vertebral column in calves and subadults. Trabeculae in the posterior region will be structured for greater strength (resist failure) and resilience (absorb energy).

H3: Trabecular structure will not vary among regions of the vertebral column in perinatal manatees. We predict that bone has not yet adapted to habitual loads placed on the vertebral column in this life stage.

SAMPLING

Vertebrae were sampled from eight manatees total, with two animals from each life stage (perinatal, calf, subadult, and adult).

The following regions were sampled: (1) thoracic, (2) lumbar, (3) caudal, aligned with the anus, and (4) caudal, aligned with the fluke insertion.

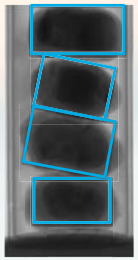


Soft tissue was removed from vertebrae with standard dissection equipment. Spinous and transverse processes were removed or reduced using a band saw.

Vertebral bodies were soaked in mammalian Ringer's solution, frozen at -20^{O}C, and shipped to Friday Harbor Laboratories (FHL), WA.

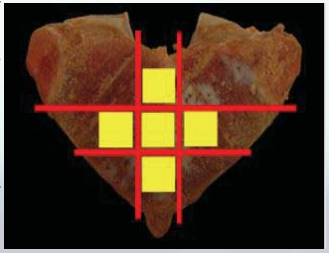
METHODS

Vertebrae were micro-computed tomography (microCT) scanned at 50-85 kV, 109-123uA, and 25-35 um with a Bruker SkyScan 1173 at FHL.



Bruker Reconstruction and DataViewer softwares were used for post-scan alignment corrections and for isolating vertebral bodies from one another (blue).

Using Fiji BoneJ software, we will select five regions of interest from vertebral bodies to measure trabecular morphology[4]. ROIs will be cubes.



The sides that make up the cubed ROIs will equal 20% of the vertebral body's length.

Bone volume fraction (BVF), trabecular thickness (TT), trabecular number (TN), and degree of anisotropy (DA) will be quantified from the ROIs.

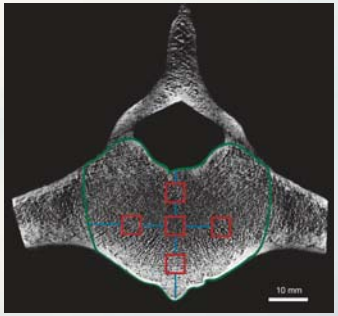
RESULTS AND FUTURE DIRECTIONS

We are quantifying and analyzing the trabecular morphology of all manatee vertebra microCT scans (N=32) and will be finished by Summer 2018.

Trabecular morphology (BVF, TT, TN, and DA) will be paired with bone material properties (strength, stiffness, and resilience) from a companion study to quantify the form-function relationship of vertebral trabecular bone among body regions and through ontogeny in manatees.

We are investigating the support vertebral bone provides to undulatory swimmers who are not subjected to gravitational forces and do not use limbs to support body weight.

Material properties and trabecular morphology will also be quantified in fully aquatic mammals with different swimming modes (dolphins and whales).



A microCT stack image of a male subadult manatee vertebral body (green) with five ROIs selected (red). ROIs are selected based off the center and four midpoints between the center and vertebral body edge.

ACKNOWLEDGEMENTS

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