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Turbinate Development in Nasal-emitting Bat Species *Artibeus jamaicensis*

by Guenivere Starling

An Undergraduate Thesis Submitted in Partial Fulfillment

of the Requirements for the University Honors Program

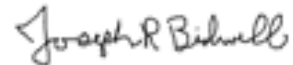
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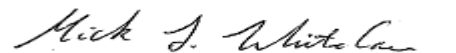
Guenivere Starling



Dr. R. T. Carter, Thesis Mentor



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Introduction

The olfactory capabilities of bats shape the way that they function. Many bats utilize their sense of smell and echolocator abilities to find meals and navigate across the landscape.

Echolocation calls are emitted either through the mouth or the nose and therefore, in these nasally emitting bat species, the nasal cavity is presumably under selective pressure for efficient olfaction and echolocation. Not surprisingly, some species of bat are known to have a complex nasal anatomy, while in others the anatomy is not well understood (Neuweiler, 2000). Much of the variation in mammalian nasal anatomy is due to differences in turbinal structure. These projections function to increase surface area of olfactory and respiratory epithelium in the nose (Giannini, Macrini, Wible, Rowe, & Simmons, 2012). This study is concerned with any changes in the growth and development of turbinals through various ages of a nasal-emitting bat species *Artibeus jamaicensis*. The terminology of mammal turbinals is not ubiquitous. For the purpose of this study, terminology will follow Giannini, et al. (2012).

Turbinals are sometimes described as “bony scrolls,” as they appear to be curled, ossified elements within the nasal cavity. There are several subtypes of turbinals, however this study is concerned with ethmoturbinals and maxilloturbinals (named after the bones from which they project) and how they may increase in size throughout ontogeny. The ethmoturbinals contain a large amount of sensory epithelia, aiding in the function in olfaction. They are located in the anterior portion of the nasal cavity. The maxilloturbinals are mainly covered in non-sensory epithelium. They are located posteriorly within the nasal cavity and function to heat and moisten inhaled air, as well as recovery of heat and water upon exhalation (Van Valkenburgh, et al., 2018). In figures 1 and 2, the ethmoturbinals can be seen in blue and the maxilloturbinals in red.

Most bats emit echolocation signals orally, while some species emit signals through their nasal cavity (Neuweiler, 2000). When examining bats of different ages, there are stark contrasts depending on species and method of echolocation emission. It is important to note that the method of the bat's emission can have an impact on sense of smell when discussing the differences between nasal and oral emitters of echolocation signals. A nasal emitter will have more nasal structures dedicated to the production of their echolocation signals, generally meaning diminished olfactory potential (Bhatnagar, 1975). This translates to more intricate respiratory turbinals in the posterior region combined with reduced olfactory turbinals, which are located anteriorly (Van Valkenburgh, et al., 2018). This study was conducted using *A. jamaicensis*, a species that emits echolocation signals nasally.

The turbinals of bats undergo the most pronounced changes prenatally, as their structures become more complicated (Ito, et al., 2021). Postnatally, development occurs in composition as they are comprised of cartilage early in life, and later ossify. Since young bats are exclusively reliant on their mothers for food, it is hypothesized that their turbinals are less developed because they are not yet being utilized. As they wean from their mother, their diet begins to shift from milk to other sources. They become more dependent on their echolocation and sense of smell and begin using them to locate other food sources to supplement their diet. As this shift occurs, many bat species begin to emit echolocation signals orally. In some species, such as *A. jamaicensis*, their echolocation emission shifts from oral to nasal as they mature into adults. Due to their lack of use in youth, it is hypothesized that the size of the ethmoturbinals will increase with age relative to the size of the maxilloturbinals, resulting in a stark contrast from youth to maturity. An important question to ask is how nasal echolocation and olfaction affect the anatomy of the

turbinals during this ontogenic shift and if the anatomical shift accompanies other behavioral shifts like flight development?

Methods

To test this hypothesis, I used previously collected specimens of an unrelated study. The bats in this study came from a captive breeding colony of approximately 60 Jamaican fruit bats (*Artibeus jamaicensis*) in Greeley, CO. The colony was fed a mixed fruit diet daily with a biweekly blended supplement of Harlan Global, 25% primate diet mixed with added sugar from corn syrup, calcium from milk, and dry Jell-O gelatin for flavor. Larger pieces of fruit were hung within the 200 m² enclosure to provide enrichment and combat competition at the feeding trays. A reversed 12h light/dark cycle within the enclosure ensured that the bats were active during daylight hours for observation purposes. Room temperature was maintained near 23°C with relative humidity kept between 50% and 65%.

All 60 individuals in the study were either found dead or humanely euthanized before being stored in the freezer prior to shipment from the University of Northern Colorado to East Tennessee State University in 70% ethanol solution. Extensive data on age, wing, and flight and body development exist for other individuals from this colony, allowing accurate estimation of postnatal age and flight ability using forearm length (Shaw, 2011). The postnatal developmental stages of flight established for this species by Shaw (2011) (Table 1) were used to assign specimens to stages. The adult stage was defined as individuals greater than 104 days old, the average age at which the epiphyseal gap of the 4th metacarpal closes in *A. jamaicensis*, completing limb skeletal growth (Ortega & Castro-Arellano, 2001). Prenatal individuals were assigned to embryonic stage using techniques developed by Cretokos et al. (2005) and Ventura et

al. (2018) (Table 1). These techniques place specimens in relative developmental order rather than assignment of an absolute prenatal age.

Before imaging, the specimens were soaked in contrast, a 70% ethanol 5% phosphomolybdic acid (PMA) by weight solution, for 1 week (Gignac et al., 2016). This provided contrast enhancement of the cartilage and other soft tissue that lines the nasal cavity. The specimens had their mouths propped open during contrast staining to ensure that the contrast penetrated the tissues that lined the posterior nasal cavity. The *A. jamaicensis* specimens were then imaged using micro-computed tomography on a Scanco μ CT 50 (Scanco Medical, Brüttisellen Switzerland) at the Vanderbilt Center for Small Animal Imaging, Vanderbilt University, Nashville, TN, USA. These image data were generated using a 0.75s exposure, through 360° of rotation with an X-ray tube voltage set at 70 kV with a 200 μ A anode current and using a 0.55 mm thick aluminum filter. This resulted in an isotropic voxel size of 11.4 μ m. The data were reconstructed using the associated Scanco image processing algorithms. Segmentations were then done in Dragonfly v 4.0 (Object Research Systems, Montreal, Quebec, Canada) and from these, surface area was calculated (Figures 1 and 2).

A Spearman's rank correlation was used to test for a correlation between surface area and developmental ranking. All statistical analyses were run using NCSS Statistical Software (East Kaysville, UT, USA).

Results

Due to inadequate uptake of the contrast only five specimens yielded image data that could be used in my analysis. However, I was still able to acquire data from a fetus, neonate, juvenile, subadult, and adult. Absolute surface areas of the maxillo- and ethmoturbinals were

converted into relative ratios for each specimen in order to account for differences in size (Figure 3). The ethmoturbinals constituted 82% to 89% of the turbinate surface area measured, while the maxilloturbinals constituted 10% to 17%. There was no significant association between either the ethmoturbinal ($P = 1$, $df 3$, $\alpha = 0.05$) or maxilloturbinal ($P = 1$, $df 3$, $\alpha = 0.05$) surface areas and developmental stage, indicating isometric growth of these surfaces.

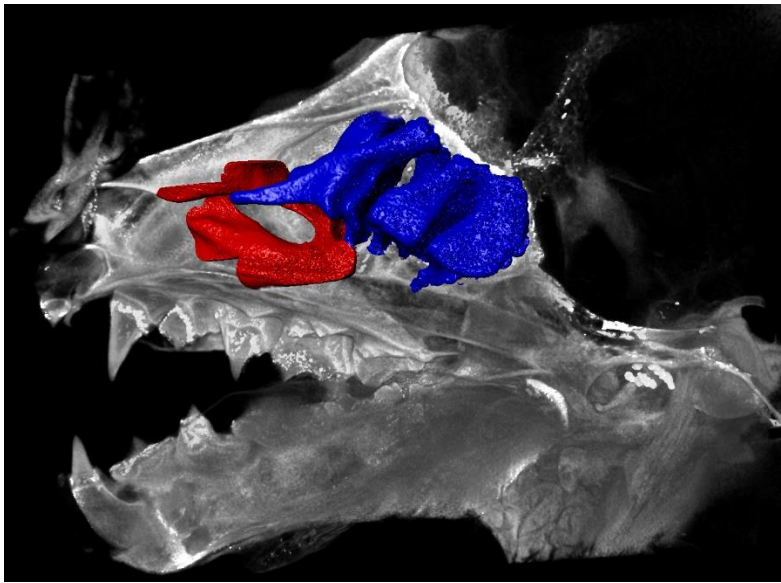


Figure 1: Volume rendered sagittal view from the subadult showing maxilloturbinate (red) and ethmoturbinate (blue) segmentations.

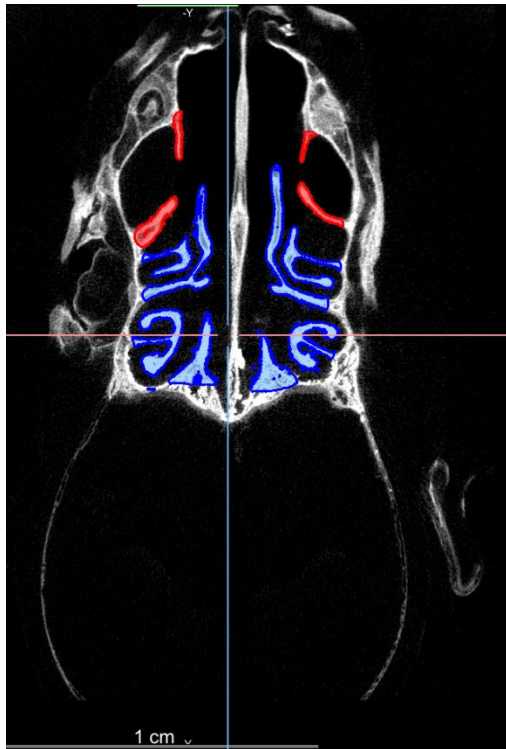


Figure 2: A transverse section from the subadult showing maxilloturbinals (red) and ethmoturbinals (blue) segmentations.

Table 1: Staging protocol

Definitions for pre- and postnatal individuals. Approximate age postpartum is provided in days postpartum (dpp) as estimated by forearm length (Shaw 2011). Prenatal staging protocol is from Cretekos *et al.* (2005) and Ventura *et al.* (2018). From Carter, *et al.* 2019.

Pre- and Postnatal Stages	Stage Definition
Stage 21	nose leaf is conspicuous with triangular shape and primordial midrib present along spear, large keratinized toe and thumb nails
Stage 22	muzzle well-formed and nostrils show, nose leaf is more developed than previous stage, umbilical cord and blood vessels present, genital tubercle present
Stage 23	nose leaf shows conspicuous and well-defined midrib, eyelids are open, calcar is visible, genital tubercle is undifferentiated

Stage 24	skin covered in fur, genitals are conspicuous below the umbilicus
Flutter (2-18 dpp)	individuals fell straight to the pad with no horizontal displacement but with wing movement
Flap (19-32 dpp)	individuals fell to the pad with horizontal displacement (within 200 centimeters of the perch)
Flight (33-104 dpp)	individuals achieved sustained flight with adult-like maneuverability
Adult	> 104 days old postpartum

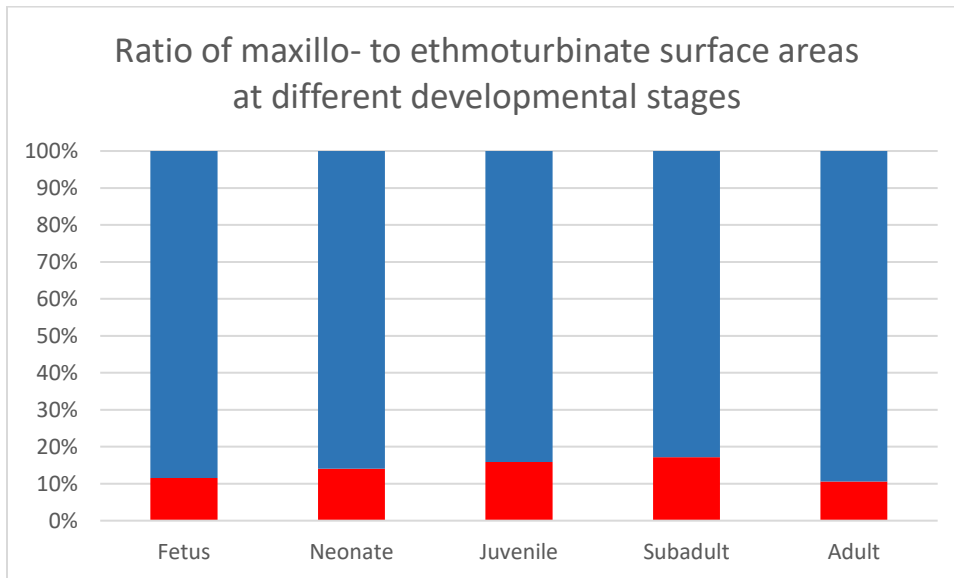


Figure 3: Ratio of maxilloturbinal (red) and ethmoturbinal (blue) surface areas for each developmental category.

Discussion

No significant correlation was found between the surface areas of the ethmoturbinals and maxilloturbinals relative to developmental stages. It was hypothesized that the relative surface area of the ethmoturbinals would increase with age compared to the surface area of the maxilloturbinals, as they are responsible for olfaction. The ethmoturbinals were found to have a much larger surface area across all specimens, meaning that they comprise a majority of the nasal cavity as compared to the maxilloturbinals. This data suggests that bats are already relying on their sense of smell soon after birth and are not developing this sense as they wean from their mother, as we had hypothesized.

The importance of olfaction in a frugivorous species such as *A. jamaicensis* is known to increase as young bats weaned from their mothers and began to emit echolocation signals from the oral to nasal cavity. It was predicted that this would be reflected by an increase in size of the turbinals around this age. Though *A. jamaicensis* are known nasal emitters of echolocation, they rely heavily on smell to find food. Their nasal anatomy seems to be more adapted for olfaction than for echolocation signal emission, indicating that their ethmoturbinals contain a wealth of olfactory receptors and nerve bundles (Bhatnagar et. al, 1974). Our data suggest that there seems to be no difference in size of the nasal cavity in terms of anatomy during the developmental range present in this study, though there are known changes in composition and function. We can infer from this study that there may be such strong selection for olfaction from birth that development of nasal emission for echolocation does not alter it. Furthermore, *A. jamaicensis* produces a relatively unsophisticated form of echolocation, therefore perhaps not requiring a highly derived nasal cavity (Carter et al., 2019).

This study may have failed to find statistically significant results because there are limited groups of individuals that represent each stage. There were several stages for which we did not have representation. This small sample size may not be representative of the species and therefore may not be significant enough from which to draw conclusions. Four specimens were not included due to inadequate contrast uptake. Three of these specimens were from fetal stages and one was a subadult. Had there been a greater number of specimens including more varied stages of development, there may have been more significant data present from which to draw conclusions. Several studies have been attempted to discuss the homologies of bat turbinals using adult specimens. However, this is difficult to establish without a comparison to fetal anatomy (Ito et al., 2021; Bhatnagar et al., 1974). Comparison to an orally emitting species would provide insight into the effect that nasal echolocation emission has on the nasal cavity and help tease apart the combined effects of echolocation and olfaction on nasal and turbinal anatomy.

References

- Bhatnagar, Kunwar P. "Olfaction in *Artibeus jamaicensis* and *Myotis lucifugus* in the context of vision and echolocation." *Experientia* 31.7 (1975): 856-856.
- Bhatnagar, Kunwar P., and Frank C. Kallen. "Morphology of the nasal cavities and associated structures in *Artibeus jamaicensis* and *Myotis lucifugus*." *American Journal of Anatomy* 139.2 (1974): 167-189.
- Carter, R. T., A. Stuckey, and R. A. Adams. "Ontogeny of the hyoid apparatus in Jamaican fruit bats (Chiroptera: Phyllostomidae) in unraveling the evolution of echolocation in bats." *Journal of Zoology* 308.4 (2019): 301-307.
- Cretekos, Chris J., et al. "Embryonic staging system for the short-tailed fruit bat, *Carollia perspicillata*, a model organism for the mammalian order Chiroptera, based upon timed pregnancies in captive-bred animals." *Developmental dynamics: an official publication of the American Association of Anatomists* 233.3 (2005): 721-738.
- Giannini, N. P., Macrini, T. E., Wible, J. R., Rowe, T. B., & Simmons, N. B. (2012). The Internal Nasal Skeleton of the Bat *Pteropus lylei* K.Andersen, 1908 (Chiroptera: Pteropodidae). *Annals of Carnegie Museum*, 81(1), 1–17. doi: 10.2992/007.081.0101
- Ito, Kai, et al. "On the Embryonic Development of the Nasal Turbinals and Their Homology in Bats." *Frontiers in Cell and Developmental Biology*, vol. 9, 23 Mar. 2021, doi:10.3389/fcell.2021.613545.
- Macrini, Thomas E. "Development of the Ethmoid In *Caluromys Philander* (Didelphidae, Marsupialia) With a Discussion on the Homology of the Turbinal Elements in

Marsupials.” *The Anatomical Record*, vol. 297, no. 11, 14 Oct. 2014, pp. 2007–2017.,
doi:10.1002/ar.23024.

Shaw, Jason B. “Evolution and Development of Wing Form, Body Size and Flight in Large- and Small-Bodied Fruit Bats (*Artibeus Jamaicensis* and *Carollia Perspicillata*).” Aug. 2011,

Neuweiler, Gergard. *The Biology of Bats*. Translated by Ellen Covey, Oxford University Press, 2000.

Ortega, Jorge, and Iván Castro-Arellano. “*Artibeus Jamaicensis*.” *Mammalian Species*, no. 662, 5 June 2001, doi:10.2307/0.662.1.

Van Valkenburgh, Blaire, et al. “Aquatic Adaptations in the Nose of Carnivorans: Evidence from the Turbinates.” *Journal of Anatomy*, vol. 218, no. 3, Apr. 2011, pp. 298–310.,
doi:10.1111/j.1469-7580.2010.01329.x.