BAT AGING BASED ON DENTITION WEAR AND WING SCARRING

by

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AND WING SCARRING

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INTRODUCTION

In order to make conservation efforts for at-risk species, it is important to understand as much as possible about their life history patterns. One important factor to consider is individual age. Individuals exhibit different behaviors depending on their developmental stage. It is commonly acknowledged that young animals, particularly those that are dispersing or migrating for the first time, are naïve and inquisitive, and are therefore more likely to be killed (Gosselink et al. 2007). On the other hand, older and more experienced individuals possess the knowledge and abilities to avoid such fates. Differing energy requirements between juveniles and breeding adults also lead to dissimilar patterns of behavior (Kunz 1973). Gaining knowledge about the habits associated with each age group within a species allows us to create targeted efforts to minimize threats to these individuals.

Bats, in particular, are an ecologically important group of species involved in seed distribution, insect population control, and plant pollination (Blehert et al. 2009). Bat populations are currently at risk due to widespread habitat destruction (Mickleburgh & Hutson 2001). Other factors such as increased incidence of the fungal disease, White-Nose Syndrome, and mortalities associated with increasing number of wind turbines across North America further threaten bat species (Foley et al. 2011, Kunz et al. 2007). Bats are relatively long lived and produce few offspring per year (Wilkinson & South 2002). Due to this slow life history, the high mortality rates of North American bats may have potential population level implications (Baerwald et al. 2009). If mortality rates continue to increase, there is the potential that more bats will die than are born each year. This will leave bat populations unable to sustain themselves, thus increasing their risk of extinction.

To date, very little is known about which species or groups of bats (i.e. gender, age, etc.) are most vulnerable to collisions at wind turbines. One hypothesis is that juvenile bats are more vulnerable than adults. Young bats lack the experience necessary to tell that turbines pose a risk (Cryan & Barclay 2009). If this is the case, a greater number of juvenile carcasses should be found in fatality searches at wind farms than adult carcasses. To determine if a correlation exists between developmental stage and mortality rates, it is necessary for field technicians to age bat carcasses on site.

Several noninvasive techniques exist to determine the age of bats, however, none is definitive. Bats can be readily aged based on epiphyseal growth plate ossification and reproductive status (Kunz & Anthony 1982). In juveniles, the wing joints have cartilaginous ends (epiphyses) which harden and ossify to the bone shaft (diaphysis) as the bat ages (Altringham 1996). Using this method, joints are held to a light source and surveyed for un-ossified growth plates, which manifest as pale bands on each side of the joint. This method is only effective for aging very young bats, however, because the joints fuse after approximately three months (Brunet-Rossinni & Rossinni 2006, Kleiman 1969). Bats with fused growth plates must be evaluated for age based on reproductive status. Bats displaying secondary sexual characteristics are adults, and these traits are missing in juveniles. Where possible (i.e. fresh carcasses with little scavenging), reproductive status of each bat may be determined. Breeding males have descended testicles as well as prominent keratinized penile spines which imply mating readiness (Cryan, et al. 2012). Breeding females can be identified by a bare patch of skin around the nipples which indicates lactation, or by feeling the abdomen for the presence of a fetus. The success of this method, particularly for females, is limited because most adult female bats are not pregnant at the time of collection. As a result, technicians cannot identify juvenile bats at wind facilities, and thus cannot effectively determine whether young bats are at greater risk.

It is possible to age a bat in the laboratory based on the amount of tooth wear incurred. The enamel of bat teeth begins to wear down over time due to mastication (Kunz & Parsons 2009). Bats such as big brown bats (*Eptesicus fuscus*) that feed on invertebrates with hard exoskeletons, like beetles, should exhibit even further wear through time. Teeth can be harvested and sectioned to reveal layers of cementum and dentin. Cementum is the hard, bony surface that covers the root of a tooth, whereas dentin is the hard bulk of the tooth just beneath the outer enamel. These layers are analogous to annual tree rings, with a higher number of layers being indicative of an older bat (Linhart 1973, Fancy 1980). This method is not effective on worn teeth, however, because many of the layers have worn away. We propose another option for aging bats based on dentition wear. We hypothesize that the teeth of young bats will be tall and sharp, and thus can be distinguished from older bats whose teeth are dulled and shorter. Teeth can be harvested from bat carcasses and measured at several locations. Amount of tooth wear can then be analyzed based on these measurements to determine age. These methods are dependent on the ability to remove teeth from bat carcasses, however, and cannot be employed on live bats. Less invasive methods are necessary to determine bat age in living individuals.

We propose that there may be another method of aging live bats. Bats periodically damage their wing membranes during flight. This damage leaves small areas of scar tissue in the wing (Davis 1968). As these scars should accumulate over time, we hypothesize that the number of scars on a bat's wing may be a powerful indicator of age. We aim to determine if there is a relationship between tooth wear and wing scars so that in the future, field researchers will be able to use wing scars to determine bat age. Carcasses collected at Wolf Ridge Wind, LLC, a large scale wind facility in north Texas, will be analyzed for age data based on dentition wear as well as wing scars. We will then attempt to determine if there is a correlation between amount of tooth wear and scarring on the wing membrane. Our findings should be applicable not only to wind turbine fatality monitoring surveys, but to any study in which bat age needs to be considered.

METHODS

Study Site

The bats used in this study were salvaged at Wolf Ridge Wind, LLC, a utility-scale wind farm located in north Texas (N 33° 43' 53.538" W 97° 24' 18.186") that is owned and operated by NextEra Energy Resources (Fig. 1). This facility began operations in fall 2008 and consists of 75 1.5-MW GE wind turbines extended over 48 km^2 of open cattle pastures, hayfields, winter wheat fields, and shrub forest. Wolf Ridge offers a unique opportunity to investigate the causes of bat mortality at wind turbines, as >900 bat carcasses have been collected since the onset of fatality searches in spring 2009. Carcasses of six bat species have been found at Wolf Ridge: eastern red bat, *Lasiurus borealis*; hoary bat, *Lasiurus cinereus*; silver-haired bat, *Lasionycteris noctivagans*; evening bat, *Nycticeius humeralis*; tri-colored bat, *Perimyotis subflavis*; and Mexican free-tailed bat, *Tadarida brasiliensis* (Fig. 2). Of these, the former three species are known to be migratory, and have been found in large numbers during fatality searches at wind facilities across North America (Arnett et al. 2008).

Carcass Collection

At our study site, teams of two to three technicians used a rope method to systematically search a subset of the wind turbines (14-30 turbines each year) for carcasses from 2009-1012 (Fig. 3a). Briefly, one rope was wrapped around the base of each turbine and secured with a carabiner. Then, a second rope (ranging in length from 35 to 60 m) was attached to the carabiner, extended at a right angle from the tower, and pulled taut. The technicians, spaced from 3 to 7 m apart at the end of the rope, then walked slowly around the turbine, scanning the ground for carcasses (Fig. 3b). When the technicians found a carcass, the location was marked with a flag. The search ends when the entire length of rope was wrapped around the turbine base. After searching the plot, the technicians returned to the marked carcass locations and processed each bat. Since 2009, turbine search intervals have ranged from 1 to 6 days, with the most intensive sampling occurring from July to October each year. This time period coincides with the fall migratory season of North American tree dwelling bats (Baerwald et al. 2007).

The bat carcasses that were found during ongoing fatality searches were each given a unique identification code including the date of collection and turbine at which they were found. Carcasses were then processed on site for a variety of attributes including species, estimated time since death, number of wing scars, and age based on growth plate closure or secondary sexual characteristics. To count the number of wing scars, a field technician extended the wing and held it up to the light. Scars within the membrane appear as white areas of tissue. As some wings may have been damaged or missing due to decomposition or scavenging, we calculated a wing scar index (WSI) as the number of wing scars divided by the proportion of the wing membrane present at the time of carcass collection (Reichard & Kunz 2009). We used a molecular method to reliably determine sex of each carcass (Korstian et al. in press). We analyzed carcasses for epiphyseal plate ossification by holding the wing against a light and checking for light cartilaginous bands on the ends of bones (Fig. 4a). Sexual maturity was determined where possible by the presence of descended testicles or penile spines in males, and lactation or pregnancy in females (Fig. 4b). The carcasses were then placed in a cooler then transported to the lab, and stored in a freezer at 4°C until further processing.

Measuring Tooth Wear

In the laboratory, we examined extracted lower jaws from carcasses for tooth wear. Lower jaws were used as a representative of dentition wear for the entire mouth because lower jaws could be effectively extracted and photographed. We first removed the lower jaw from each bat using forceps and dissection scissors. After each jaw was taken out, we dissected away as much soft tissue as possible. Then, we cut the jaw in half between the small incisors, creating a right and left jaw fragment. Incisors were damaged in this process, and thus were not analyzed for wear. The teeth on each side of the lower jaw consist of one canine, two premolars (except in silver-haired bats, which have three premolars), and three molars (Fig. 5). Both jaw halves were then placed on a page labeled with the bat's unique ID along with a metric ruler, and photographed using a Canon EF 100 mm f/2.8 Macro USM Lens and Canon EOS Rebel SXI 21.1 Megapixel DSLR camera (Fig. 6a).

After photographing each jaw, we analyzed the images using SigmaScan Pro 5 image analysis software. Each image was calibrated for a pixel to centimeter ratio using the metric ruler included in each photograph. To determine patterns of wear, we measured canine, premolars, and molars of right and left jaw fragments at four locations (tooth height, base-width, mid-width, and tip-width) (Fig. 6b). Tooth height was measured from the point the tooth erupted from the gum to the tip of the tooth. Base-width was the horizontal width of the tooth at the point the tooth erupted from the gum, mid-width was taken approximately half way up, and tip-width measured at the apex of the tooth. We also calculated the two dimensional surface area of each tooth by outlining the tooth, and calculating the area of the polygon created. This measurement did not take into account the tooth curvature. The measurements were recorded in an Excel worksheet labeled with the species, age, sex, and wing scarring indices of each jaw's respective bat.

Data Analysis

We examined the height, ratio of mid-width to tip-width, and area of the canines, second premolar, and first peak of each molar (Fig. 6b). Measurements from both sides of the jaw were averaged to give average canine, premolar, and molar dimensions for each bat. In cases where teeth of one side of the mouth were missing, the remaining teeth were was used as representatives for the whole jaw. Our sample size was 323 bats with the following species distribution: 201 eastern red, 72 hoary, 4 silver-haired, 20 evening, 14 tri-colored, and 11 Mexican free-tailed bats. We looked for differences in wear between juveniles and adults for all bats, as well as within a single species (eastern red and hoary) using a two sample T-test to compare means between age groups. We evaluated the difference in WSI between males and females in eastern red and hoary bats for which we had the largest sample sizes. Wing scar indices were also compared to tooth wear data using a correlation approach to try and discern a relationship between the two measurements for all bats and also within the two aforementioned species separated. If there is a correlation between tooth wear and WSI, it would be possible to use one or the other to determine the age of an individual bat.

Figures

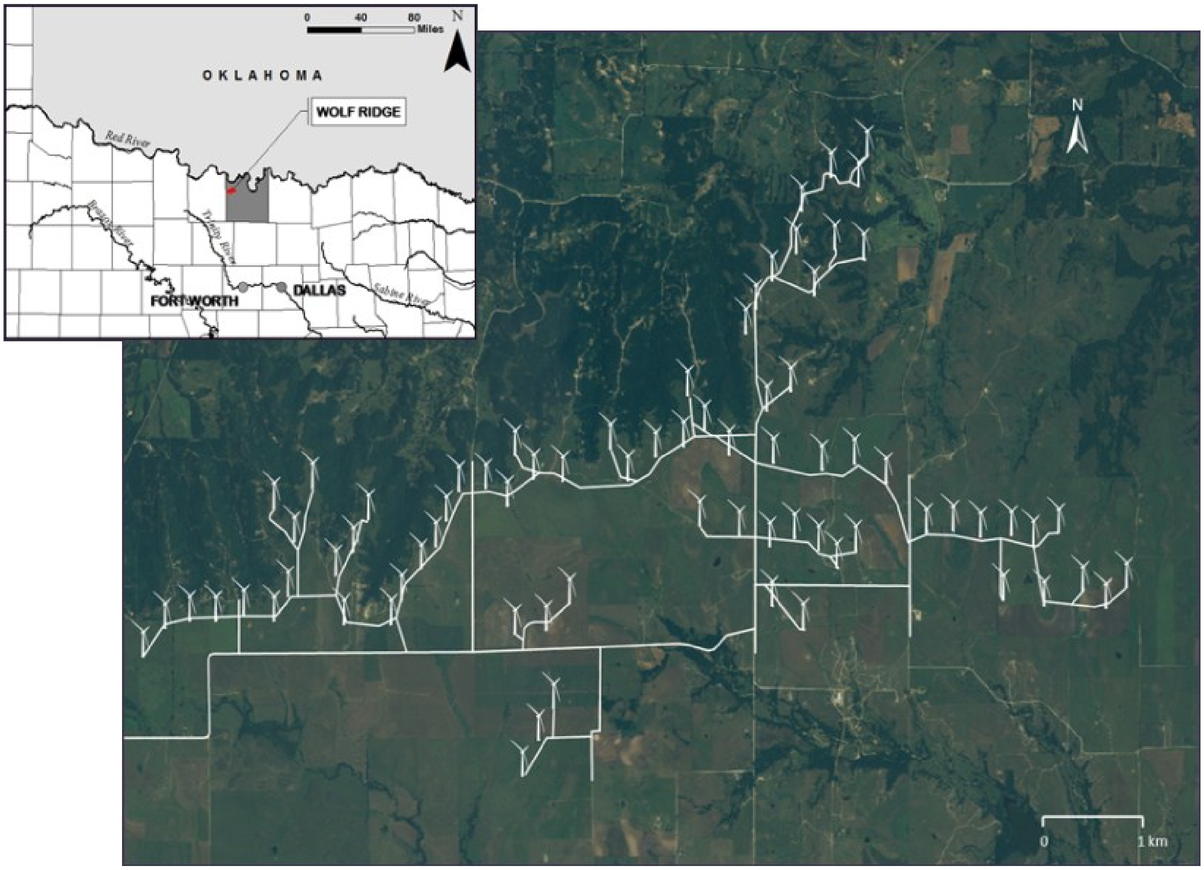


Fig. 1. Map of study site, Wolf Ridge Wind, LLC, located in north-central Texas, USA.

Fig. 2. A field technician expands the wings of a Mexican free-tailed (*Tadarida brasiliensis)* bat carcass.

a)

b)

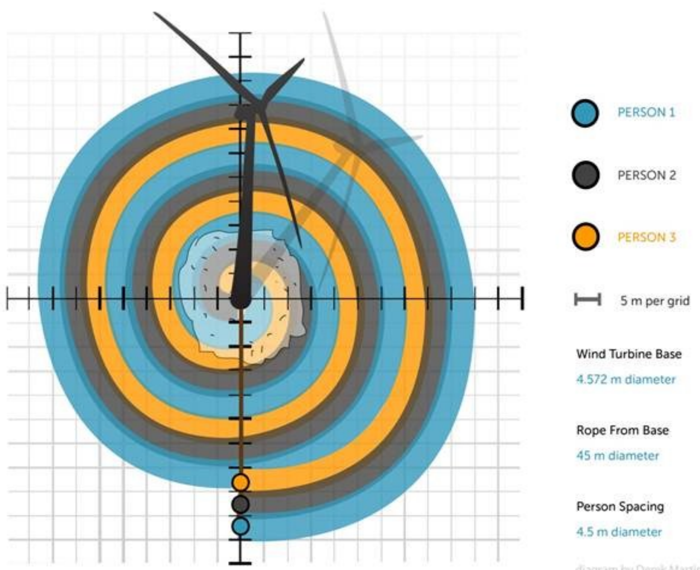
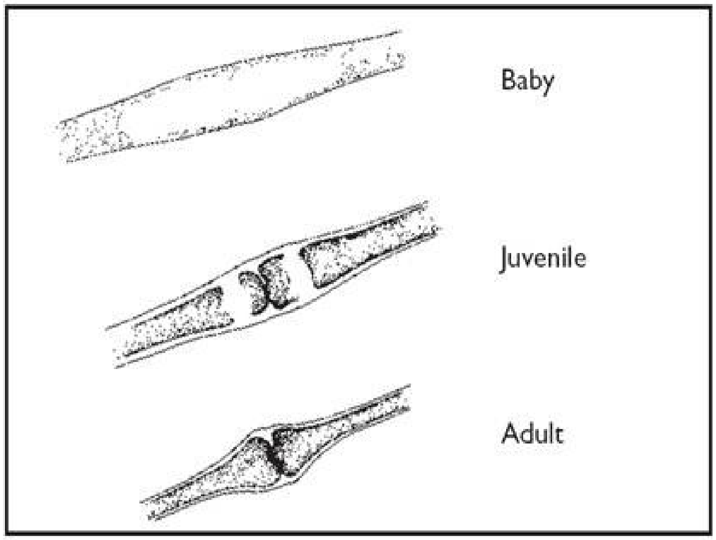


Fig. 3. The rope method of carcass searching. A) Shows a photograph of technicians employing the rope method in search of carcasses. B) Each spiral color represents one technician.

a)



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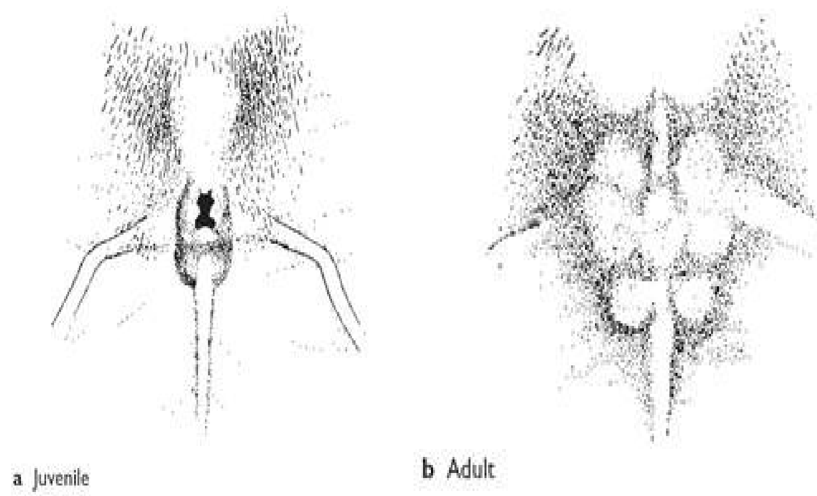

Fig. 4. Illustrations of methods used to age bats. A) The epiphyseal growth plates of juveniles are light, cartilaginous band (top) that solidify as the bat ages (bottom) (Altringham 1996). B) Differences between juvenile and adult male bat genitalia (Altringham 1996).

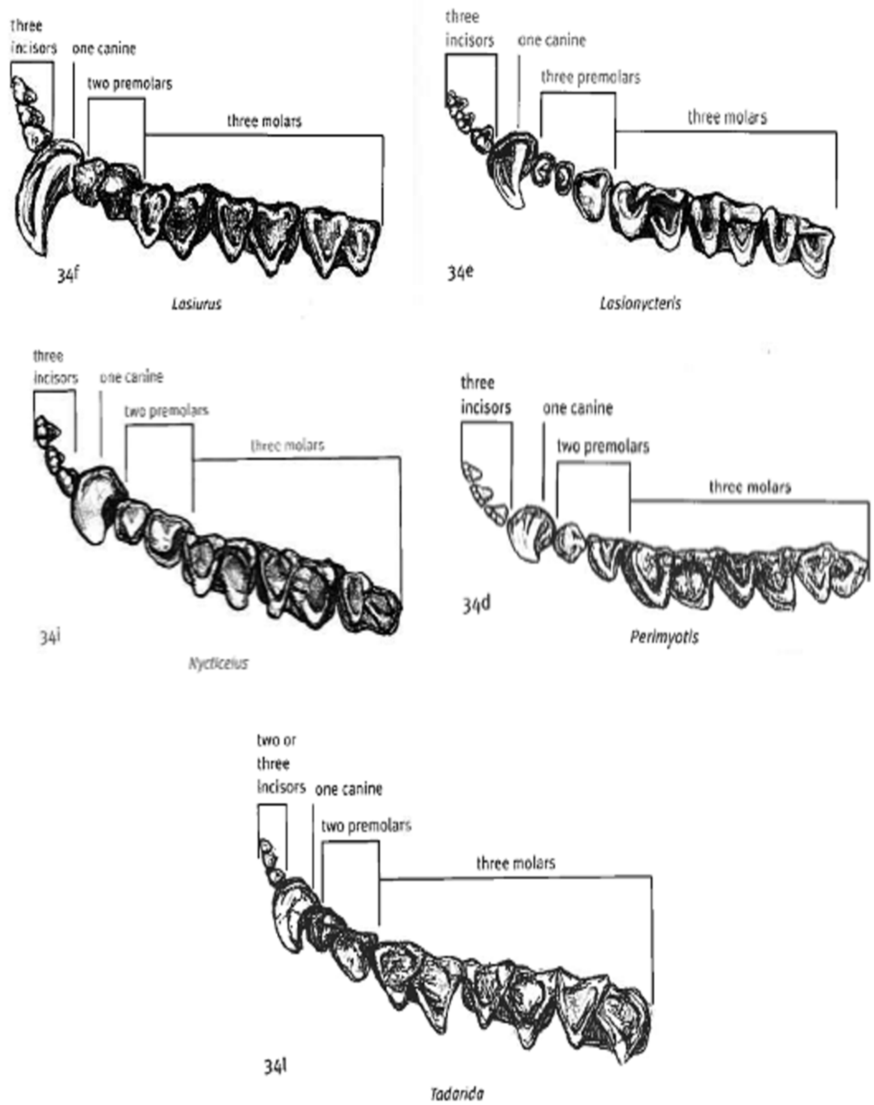

Fig. 5.This figure illustrates examples of lower jaw dentition by genus (Ammerman 2012).

a)

b) \

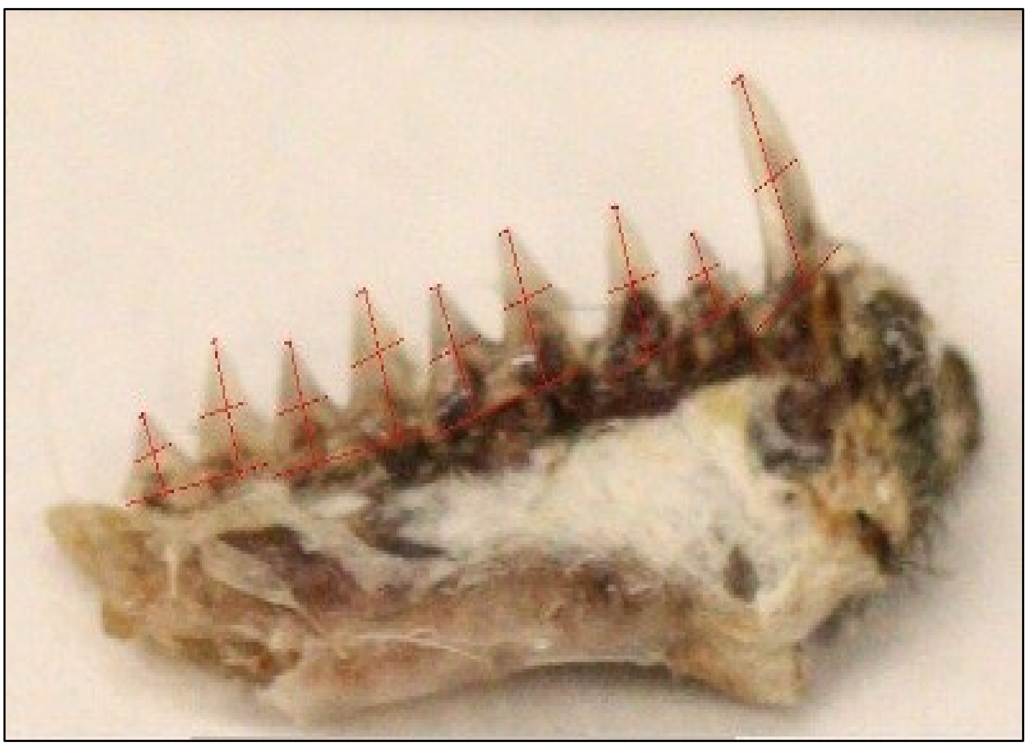

Fig. 6. Laboratory methods for measuring tooth wear. A) Hoary bat lower jaw that was removed in lab and photographed on page labeled with each bat carcass's unique identification code. B) Red lines indicate height, base-width, mid-width, and tip-width measurements. Teeth were also traced for two dimensional surface area.

RESULTS

All Species Analysis

We first looked to see if a correlation exists between WSI and age (adult or juvenile) for the sample of carcasses we had with both age and WSI data (Fig. 7). We found a difference in mean WSI between adults and juveniles ($t = 1.91$, $df = 132$, $p = 0.029$). The mean WSI of adults was significantly higher than for juveniles. Adult mean WSI was 0.0337 and juvenile mean WSI was 0.0169

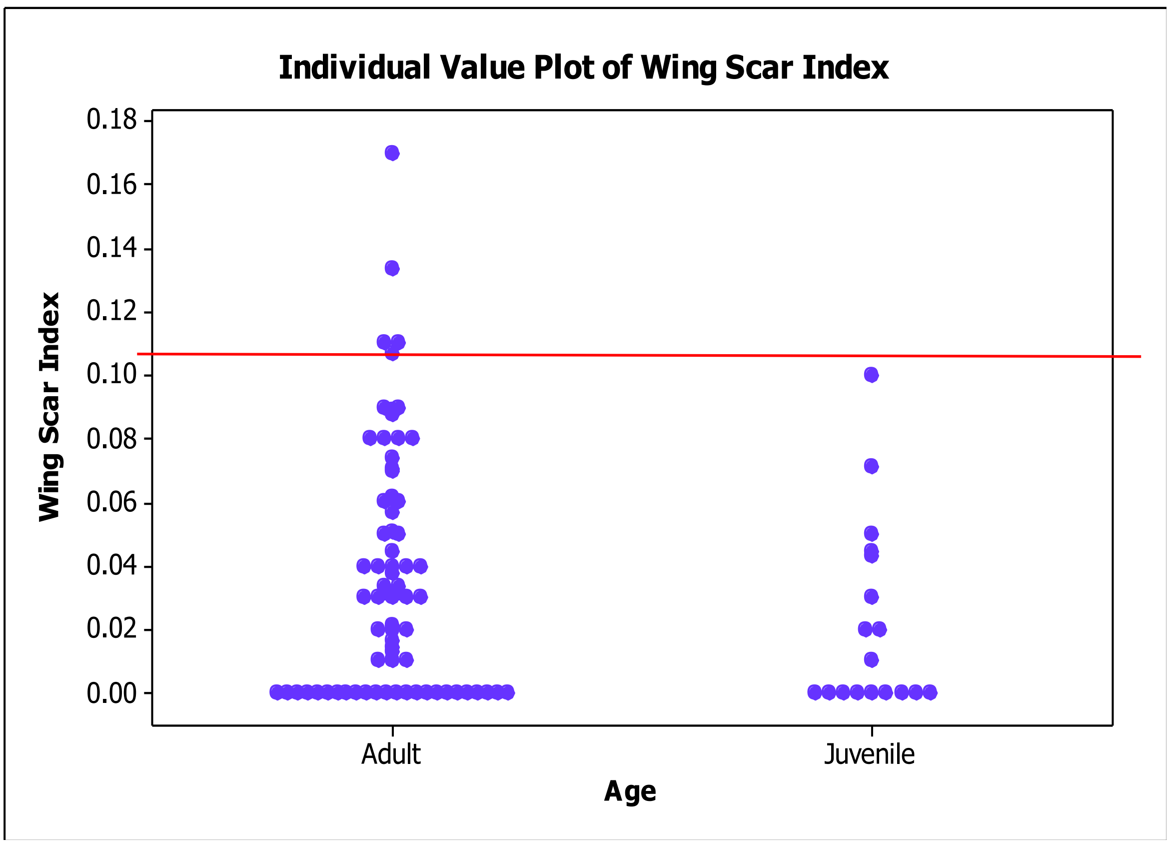

Fig. 7. Individual WSI ratios for adult and juvenile bats of all species.

Next, we looked for a correlation between levels of wear between premolars, molars, and canines. There was a significant correlation for tip-width to mid-width ratio between premolars, molars, and canines ($r \geq 0.212$ and $p \leq 0.011$ in all cases). Only premolar 2 showed significantly different wear with age ($t = 2.17$, $df = 142$, $p = 0.016$). Adult premolars were more worn than juveniles (Fig. 8). The average tip-width to mid-width ratio in adult premolar 2 was 0.290, and in

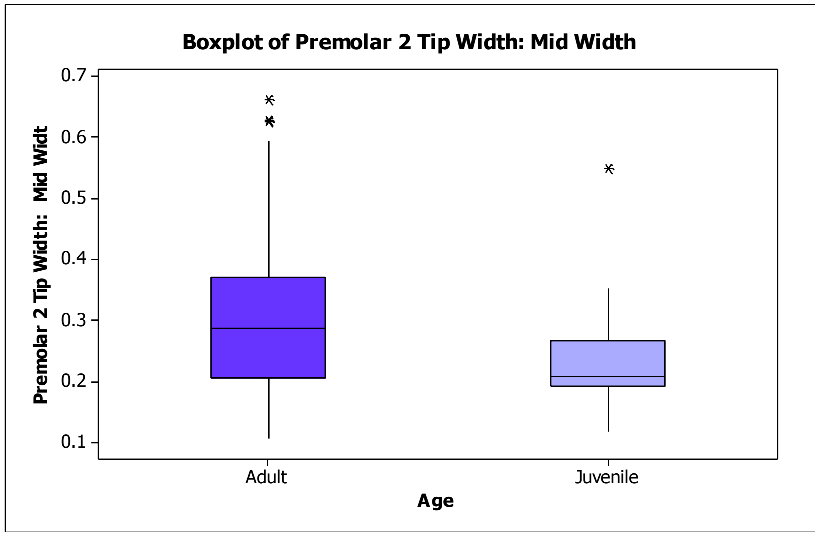

Fig. 8. Median tip-width to mid-width ratio of premolar 2 for adult and juvenile bats.

juveniles the average ratio was 0.236. Canines did not show different amounts of wear between adults and juveniles (Fig. 9). For canines, the average tip-width to mid-width ratio was 0.252 in

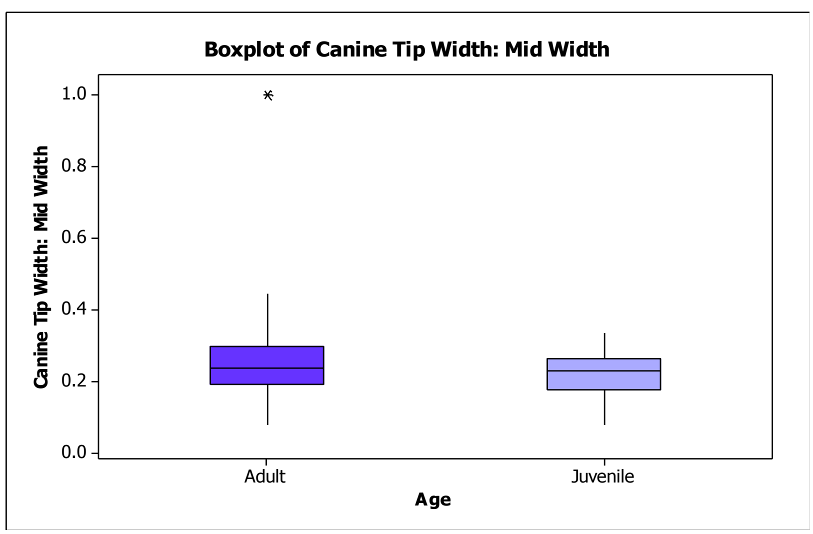

Fig. 9. Median canine tip-width to mid-width ratio for adult and juvenile bats.

adults and 0.221 in juveniles ($t = 1.43$, $df = 141$, $p = 0.077$). There was no significant difference in wear between adults and juveniles for molars 1, 2, and 3 between adults and juveniles ($p > 0.080$ in all cases).

For WSI, we found no significant correlation with tip-width to mid-width ratio for any teeth ($P > 0.180$ in all cases). Even though adults and juveniles showed different levels of premolar wear as well as different WSI, the two measurements showed no correlation with age ($r = 0.119$, $p = 0.17$); (Fig. 10).

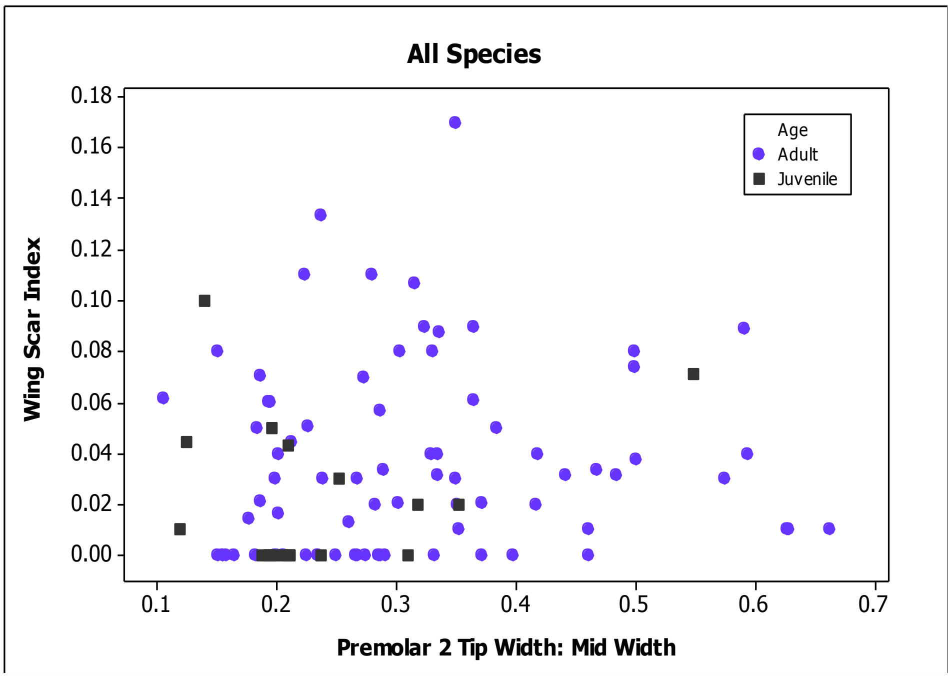

Fig. 10. Relationship between wing scar index and premolar 2 tip-width to mid-width ratio of adults and juveniles for all species.

Single Species Analysis

We looked for differences in WSI between males and females in both eastern red bats and hoary bats as these two species represented our largest sample sizes. Mean WSI did not differ between males and females within eastern red bats, however, the mean WSI of male hoary bats was over twice that of females (Table 1).

	Female		Male	
	n	WSI	n	WSI
Eastern red	55	0.0335	56	0.0336
Hoary	9	0.005	9	0.011

Table 1. Mean WSI in eastern red and hoary males and females.

As eastern red bats were our largest sample size by far, we then used them to evaluate WSI and tooth wear within a single species. Premolar 2 tip-width to mid-width ratio was higher in adults than in juveniles (Fig. 11). Mean adult tip-width to mid-width ratio of premolar 2 was 0.296, and mean juvenile tip-width to mid-width ratio or premolar 2 was 0.230 ($t = 2.58$, $df = 43$,

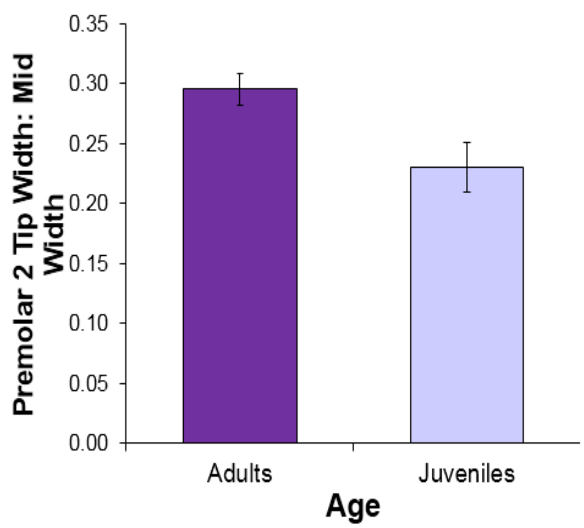

Fig. 11. Eastern red Premolar 2 Tip-Width: Mid-Width mean for adults and juveniles.

p = 0.0097). We found that adult mean WSI was significantly greater than juvenile mean WSI (Fig. 12). Adult mean WSI for eastern red bats was 0.040 and juvenile mean WSI was 0.016

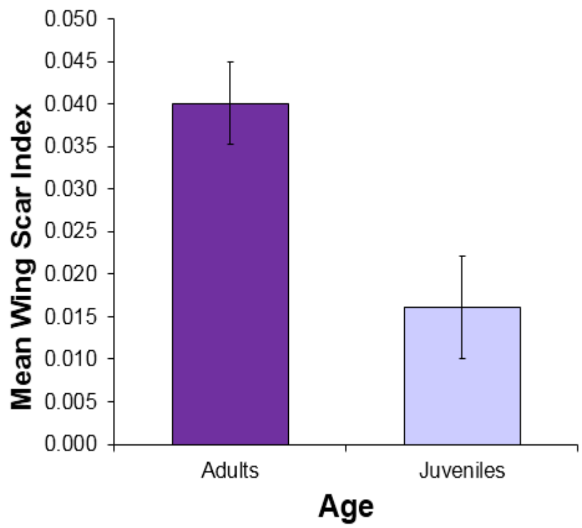

Fig. 12. Differences in wing scar index based on age in eastern red bats.

(t = 2.41, df = 99, p = 0.009). Again plotting premolar 2 tip-width to mid-width ratio along with WSI showed no correlation between adults and juveniles for eastern red bats (r = 0.071, p = 0.48); (Fig. 13).

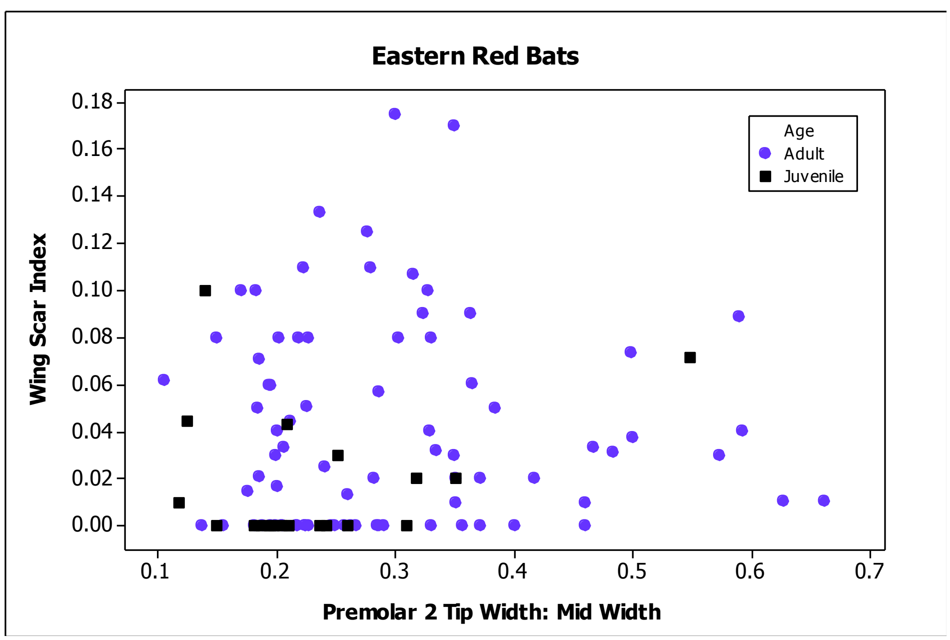

Fig. 13. Relationship between WSI and premolar 2 tip-width to mid-width ratio for adult and juvenile eastern red bats.

DISCUSSION

We conclude that adult bats have higher wing scar index score than juvenile bats. This is true across all species as well as within eastern red bats. Similarly, premolar 2 tip-width to mid-width ratio is greater in adult bats than juvenile bats, indicating higher levels of wear in adults for all species and within a single species. Levels of wear across all teeth were correlated, indicating that in future studies only one tooth may need to be measured as a representative for the entire lower jaw. However, we found no correlation between tooth wear and WSI for adults and juveniles.

While tooth wear and WSI may be reliable indicators of age separately, the two cannot be used together to determine age. There is no correlation between WSI and tooth wear for all bats, or for eastern red bats alone. It is possible that wing scars and tooth wear accumulate at different rates due to temporal differences. Had the two measurements shown a clear correlation based on age, it would have been possible to use tooth wear and WSI jointly to determine the age of an unknown individual. As this was not the case with our data, however, we recommend using one method or the other, and offer suggestions that would lead to greater success using each technique.

WSI may be used readily in the field to determine whether an individual is an adult. As all juvenile bats had WSI ≤ 0.10, we can conclude that any bat with WSI ≥ 0.11 is an adult. No minimum wing scar count indicated an individual to be a juvenile. This method cannot be definitively used to identify juvenile individuals as bats with WSI ≤ 0.10 could be adults or juveniles. It is important to note that many bat carcasses could not be used in this analysis, as there was not enough remaining wing tissue present to calculate an accurate WSI. In future studies using this method, we recommend daily fatality searches in order to locate and process fresh carcasses before wing tissues have been overly decomposed or scavenged.

It seems that eastern red bats show no difference in WSI between males and females, while male hoary bats have more than double the average WSI of female hoary bats. It is possible that male hoary bats partake in some sort of behavior that leads them to accumulate more wing scars than females, however, a larger sample size of bats with useful WSI data is needed to further explore this relationship.

We conclude that measuring tooth wear is an effective and simple lab-based technique for aging bats. The large differences in wear between adults and juveniles indicates that measuring premolar 2 is a powerful method of aging bat carcasses. As wear between adults and juveniles for canines and molars was similar, we conclude that these teeth are not useful for bat aging. Premolars likely show greater wear with age than other teeth due to their function. The premolars are intermediate in shape between the tall, sharp canines and the dull, stout molars. Canines are used to pierce prey and bring it into the mouth, whereas premolars are used to initially crush the hard exoskeleton and molars are used to further masticate the already softened material. This pattern of mastication would result in the most wear accumulating on the premolars. This pattern of tooth use is supported by the work of Freeman (1992).

Due to these results, future studies need only measure the second premolar to provide an estimate of bat age. As it is likely that attempts to remove single teeth would damage them, it will still be necessary to remove the entire lower jaw of the individual. A standard regression curve needs to be developed in which bats of known age have premolars extracted and measured for wear. Future studies could then utilize this standard regression curve to estimate the age of an individual based on its second premolar wear measurements. Obtaining individuals of known age is problematic, as wing banding has been shown to injure and potentially kill individuals (Herreid et al. 1960), and no other reliable method has been shown to accurately track the age of individual bats.

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ABSTRACT

Bats are an ecologically important vertebrate group, and yet many populations are threatened by habitat destruction, emerging diseases like White-Nose Syndrome, and wind energy development. In order to make targeted conservation efforts for these species, we need to have a better understanding of their basic life history patterns and know which individuals are at most risk (e.g. juveniles vs. adults). Current techniques to determine age in live bats, such as checking for growth plate closure or evidence of sexual maturity, are restricted in their accuracy. Alternatively, bats can be more accurately aged based on level of tooth wear, as tooth enamel wears down over time due to mastication. This method, however, can only be used on dead bats as it requires the jaws to be removed in order to accurately measure the teeth. As bats routinely damage their wing membranes during flight, another potential method of aging bats could be based on wing scar accumulation over time. The purpose of this study was to develop a new method of aging bats based on a combination of tooth wear and wing scarring. Using bat carcasses collected from a wind farm in north-central Texas, we removed lower jaws and measured teeth from 323 bats representing six species. We measured tooth height, the ratio of tooth mid-width to tip-width, and two dimensional surface areas of the canines, first premolars, and molars. We then looked for differences in wear based on species, sex, and the number of wing scars. We found there to be a difference in tooth wear between adults and juveniles for premolar 2, as well as a difference in wing scar index based on age. The two measurements show no correlation, however, across all species and also within eastern red bats. Male hoary bats seem to have over twice the number of wing scars as females, though a larger sample size is needed to further explore this relationship.