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Research Article

Carcass Age and Searcher Identity Affect Morphological Assessment of Sex of Bats

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ABSTRACT Carcasses provide an important resource for assessing the vulnerability of bat species and sexes to threats, but the reliability of sex data derived from the external morphology (sex_{morph}) of bat carcasses remains uncertain. We used genetic-based assessment of sex (sex_{gen}) to evaluate the effect of carcass age and searcher identity on sex_{morph}-based assessments of eastern red (*Lasiurus borealis*) and hoary (*Lasiurus cinereus*) bat carcasses identified by 15 different searchers at a wind-energy facility. The proportion of carcasses for which sex_{morph} was unknown increased from 0.11 for those recovered within a day of death, to 0.56 within 2–3 days of death, and to $\geq$0.82 at $\geq$4 days after death. The proportion of carcasses for which sex_{morph} was correct decreased from 0.9 for those recovered within a day of death, to 0.65 within 2–3 days of death, and to 0.25 at $\geq$4 days after death. The proportion of sex_{morph} misidentifications of the 108 fresh carcasses (collected within 24 hours of death) varied (0.0–0.43) among searchers. These results suggest that sex_{morph}-based assessments should be limited to fresh carcasses. Furthermore, additional training of people who collect and identify bat carcasses from renewable-energy facilities may improve the accuracy of sex_{morph} data obtained from carcasses.

KEY WORDS bat, carcass, genetics, *Lasiurus borealis*, *Lasiurus cinereus*, morphology, sex, wind energy.

Accurate identification of the sex of animals is critical to the effective management and conservation of many species (Cryan et al. 2012, Paiva et al. 2017). Such information can reveal the relative risk to each sex from various threats, such as habitat loss, climate change, and disease. For example, museum records suggest different range-wide occurrence patterns of female and male tree-roosting bat species during the summer (Ford et al. 2002, Cryan 2003), which could make one sex more vulnerable than another to site- or region-specific risks. Although sex assessment of bats based on morphological identification of external genitalia (sex_{morph}) is generally straightforward (Racey 2009), sex can be difficult or impossible to assess for juveniles and some adults, such as carcasses from fatalities at renewable-energy facilities that are often partly decomposed or scavenged (Johnson et al. 2003, Fiedler 2004, Arnett et al. 2008). Hundreds of thousands of tree-roosting bats are killed annually at renewable-energy facilities (Kunz et al. 2007, Hayes 2013). These species are largely solitary and difficult to observe (Shump and Shump 1982*a*, *b*) and thus such carcasses provide an important resource for scientific study (Pylant et al. 2014, 2016; Sovic et al. 2016), including for assessment of sex ratios (Korstian et al. 2013). For example, there is sex_{morph}-based evidence of more fatalities of adult male than female tree-roosting bats at some wind-energy facilities in North America (Arnett et al. 2008), and one hypothesis for these uneven sex ratios is that they result from lekking behavior in which males congregate during the mating period (Cryan 2008, Cryan et al. 2012).

Determining the sex of bats via molecular detection of sex-specific genes (sex_{gen}) provides a means of obtaining sex data even from juveniles or partly degraded carcasses (Bullejos et al. 2000, Korstian et al. 2013). Korstian et al. (2013) used sex_{gen} data to report that the likelihood of misidentifying sex_{morph} of bat carcasses obtained from a wind-energy facility in Texas, USA, increased with estimated time since death; error rates for sex_{morph} identification were higher (19%) for carcasses that spent >1 day in the field relative to those collected within a day of death (8%). Further insight into the cause of variation in accuracy of sex_{morph} data would be useful

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because it may be possible to control for or reduce such variation. Doing so would be valuable given that such assessments are more rapid and inexpensive than molecular lab-based analyses. Furthermore, sex_{gen} data could help to evaluate sex ratios in fatalities.

Besides carcass age, another factor that may affect the accuracy of sex_{morph}-based assessment of bat carcasses is the time of year in which the carcasses were collected. For example, mammary glands visible on nursing females may make them less prone to misidentification during the summer; similarly, enlarged testes on reproductively active males may make them easier to identify during the autumn. Scavenger activity or rates of decomposition of carcasses may also vary through time and thus affect the accuracy of sex_{morph} data. Accuracy of sex_{morph} data from bat carcasses may also vary by observer. For example, although Korstian et al. (2013) report an overall error rate of 8% for fresh carcasses, they did not report error rates for individual searchers, and it is possible that accuracy rates differ among individual searchers. People who search for, and identify, bat carcasses are typically employed by environmental consulting firms, universities, or non-profit organizations, and they receive basic training to morphologically identify the species and sex of bat carcasses. However, such individuals are not typically professional biologists and the accuracy of the data they collect may vary.

The main objective of our study was to assess the effect of carcass age, collection period, and searcher identity on the sex of carcasses of 2 species of Lasiurine tree-roosting bats collected and identified by 15 different searchers at a wind-energy facility. Our first hypothesis was that sex_{morph} accuracy was related to age of carcass and we predicted the proportion of bat carcasses whose sex_{morph} was determined to be unknown and the proportion of individual bats whose sex_{morph} was incorrectly determined, would increase with increasing estimated time since death. Our second hypothesis was that the period of year in which the carcasses were collected would influence the accuracy of sex_{morph} data. Our third hypothesis was that there would be differences among searchers in accuracy of sex_{morph} observations of fresh carcasses. We expected searchers who made relatively more observations or determined that a relatively greater proportion of carcasses were of unknown sex to exhibit higher accuracy. A secondary objective was to determine if there was evidence that sex_{morph} data from carcasses of these 2 species of Lasiurine tree-roosting bats overestimate the proportion of males.

STUDY AREA

We obtained the samples for this study from a wind-energy facility located in Benton County, Indiana, USA. The area received an average of 985 mm of precipitation annually. The annual average temperature was 9.7°C, with an average of 22.5°C in July and −4.9°C in January. The elevation in this facility varied between 213 m and 244 m above sea level, and the topography was flat to slightly rolling. Land use in the study area was predominantly (93%) tilled agriculture for corn and soybeans. Rural developed areas and forest compromised 5% and <1% of the study area, respectively (United States Geological Survey 2001). The utility-scale wind turbines in the study area ranged from 1.5–2.5 MW. The most common bat species killed at this facility included eastern red (*Lasiurus borealis*), hoary (*Lasiurus cinereus*), silver-haired (*Lasionycteris noctivagans*), and big brown (*Eptesicus fuscus*) bats.

METHODS

We obtained tissue samples from carcasses of eastern red and hoary bats during July–October from 2010 to 2012. Fifteen different technicians participated in the collection and identification of carcasses. Each searcher determined the species of each carcass by morphology using identification guides, and these identifications were verified by a biologist certified with an United States Fish and Wildlife Service Native Endangered and Threatened Species Recovery permit. Based on external morphology, each searcher identified the sex (male, female, or unknown) of each carcass that he or she collected (Racey 2009). Searchers recorded the collection date, estimated time since mortality, and an identifier for the searcher. Based on the frequency at which searchers performed carcass searches, and the condition of each carcass at the time it was collected, searchers estimated the time since death to be within the past 24 hours, 2–3 days, 4–7 days, 1–2 weeks, or >2 weeks. Criteria considered when evaluating the age of each carcass included those in Korstian et al. (2013), such as condition of eyes and body, presence of tissues and organs, infestation by insects, degradation of hair and skin, and apparent weathering of bones. Searchers froze the carcasses after collection. In summer 2015 we thawed the carcasses used in this study; we obtained a 1-cm^2 piece of wing tissue from each carcass, placed samples in a 1.5-ml centrifuge tube filled with 95% ethanol, and stored them at −80°C until DNA extraction.

We extracted DNA from the wing tissue samples using Qiagen DNeasy blood and tissue kits (Qiagen, Valencia, CA, USA). We used primers targeting regions within the *Zfx* (F-ZFXBat and R-ZFXBat) and *Zfy* (F-ZFYBat and R-ZFYBat) zinc-finger genes that produce sex-specific size variants to amplify portions of the X and Y chromosomes using polymerase chain reaction (PCR); PCR conditions followed Korstian et al. (2013). We visualized the amplified regions using gel electrophoresis and identified samples as female or male based on the presence a single band at approximately 245 base pair (bp) or bands at 245 bp and 80 bp, respectively.

We used a χ^2 test of independence to assess the influence of carcass age on the number of bats for which sex_{morph} was classified as unknown and the number of bats for which sex_{morph} was incorrect. Using only data from carcasses collected within 24 hours of death, we used Fisher's exact test to assess if the number of bats for which sex_{morph} was incorrect was influenced by period of collection (with periods representing 10-day intervals of each month; i.e., 1–10 July, 11–20 July, ...) or searcher identity. We used generalized linear models (with a binomial distribution and logit link) to assess potential relationships between the proportion of bats collected within 24 hours of death for which sex_{morph} determined by each searcher was incorrect and the number of bats (collected within 24 hours of death) identified as male or female by each searcher and the proportion of bats (collected within 24 hours of death) that each searcher classified sex_{morph} as unknown. Using only data from searchers who had no incorrect sex identifications of carcasses collected within 24 hours of death, we also used a χ^2 test of independence to reassess the influence of carcass age on the number of bats for which sex_{morph} was classified as unknown and the number of bats for which sex_{morph} was incorrect. We performed statistical analyses in PAST version 3.18 (Hammer et al. 2001).

We calculated sex ratios based on sex_{morph} and sex_{gen} data for each species. The sex_{morph}-based sex ratios included all individuals that were morphologically identified as male or female, whereas sex_{gen}-based sex ratios also included individuals that were morphologically identified as unknown sex.

RESULTS

We used data from 220 carcasses, comprised of eastern red ($n = 103$) and hoary ($n = 117$) bats. Of these carcasses 138 were identified as male or female based on sex_{morph}; sex_{morph} of the remaining 82 individuals was classified as unknown. Because of sample-size limitations, we performed analyses on the carcasses from both species together except where noted. Carcass age influenced the number of bats for which sex_{morph} was classified as unknown ($\chi^2_3 = 86.8$, $P < 0.001$). The proportion of carcasses for which sex_{morph} was unknown increased from 0.11 for carcasses recovered within a day of death, to 0.56 within 2–3 days, to 0.86 within 4–7 days of death, and was 0.82 beyond 1 week of death (Fig. 1). Similar patterns in the proportion of carcasses for which sex_{morph} was unknown occurred when examining the data for each species separately. Carcass age influenced the number of bats for which sex_{morph} was incorrectly determined ($\chi^2_2 = 12.5$, $P = 0.004$). The proportion of carcasses for which sex_{morph} was determined correctly decreased from 0.9 for carcasses recovered within a day of death, to 0.65 within 2–3 days of death, and to 0.25 at $\geq$4 days after death (Fig. 2).

Period of collection did not influence the accuracy of sex_{morph} data when we analyzed data from all fresh carcasses together ($P = 0.86$, df = 9) or when we analyzed only fresh carcasses identified as males based on morphology ($P = 0.56$, df = 9). We did not perform this analysis on data from fresh carcasses identified as female based on morphology because only 1 such carcass was misidentified. Most of the sex_{morph} misidentifications (10 of 11) of carcasses collected within 24 hours of death were females that were morphologically misidentified as male. Searchers differed in the number of these fresh carcasses that were incorrectly sexed ($P = 0.02$, df = 8). The proportion of incorrect sex_{morph} identifications for these bats varied from 0.0 to 0.43 among searchers, with values of 0.43 for 2 searchers, 0.25 for 2 searchers, 0.13 for 1 searcher, and 0.0 for 10 searchers (Fig. 3a). Using only data from fresh carcasses recovered within a day of death, there was no relationship between the number of bats that each searcher determined to be male or female and the proportion of incorrect sex_{morph} identifications ($G = 0.03$, $P = 0.87$, $n = 15$), or between the proportion of bats determined to be of unknown sex and the proportion of incorrect sex_{morph} identifications by each searcher ($G = 0.48$, $P = 0.49$, $n = 15$; Fig. 3).

Using only data from the 10 searchers who correctly identified all carcasses collected within 24 hours of death, we found that carcass age continued to influence the number of

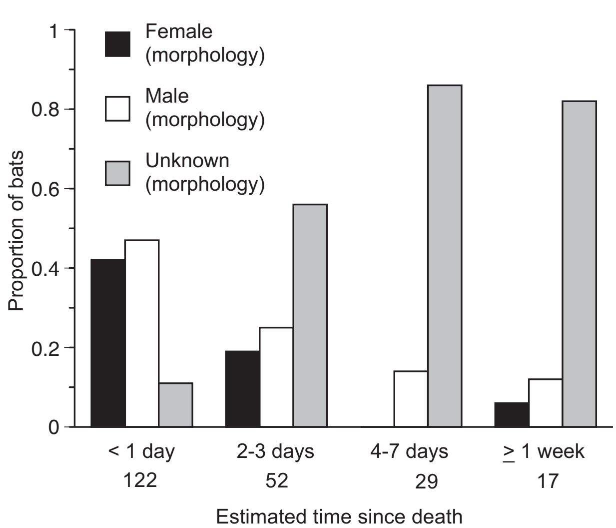

Figure 1. Proportion of eastern red and hoary bat carcasses collected during fatality searches in July–October between 2010 and 2012 at a wind-energy facility in Indiana, USA, that were classified as female, male, or unknown sex (based on external morphology) in relation to estimated time since death. The numbers below the *x*-axis labels indicate the total number of carcasses in each time-period category.

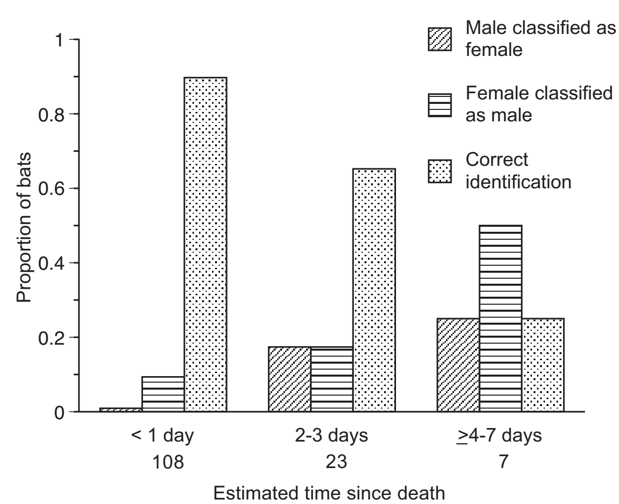

Figure 2. Proportion of the 138 eastern red and hoary bat carcasses collected during fatality searches in July–October between 2010 and 2012 at a wind-energy facility in Indiana, USA, that were incorrectly classified as female or male (based on external morphology) and those that were correctly classified (based on external morphology) in relation to estimated time since death. The numbers below the *x*-axis labels indicate the number of bats that were classified as female or male in each time-period category.

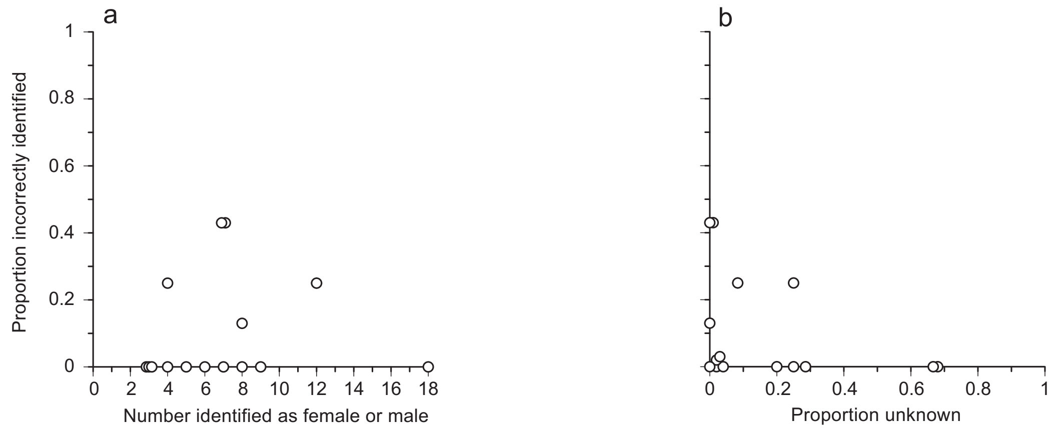

Figure 3. Relationship between a) the number of bats that each searcher identified as male or female and the proportion of incorrect sex identifications by each searcher and b) the proportion of bats that each searcher classified as unknown sex and the proportion of incorrect sex identifications by each searcher for fresh eastern red and hoary bat carcasses collected within 24 hours of death during fatality searches in July–October, 2010–2012, at a wind-energy facility in Indiana, USA. Note that some data points in each panel have equivalent values on the *x* and *y* axes, and those data points were slightly offset from each other in this figure so that they are visible.

bats for which sex_{morph} was classified as unknown ($\chi^2_3 = 54.1$, $P < 0.001$). The proportion of these carcasses for which sex_{morph} was unknown increased from 0.14 for carcasses recovered within a day of death, to 0.59 within 2–3 days of death, and >0.86 beyond 4–7 days after death. Carcass age also continued to influence the number of bats for which sex_{morph} was incorrectly determined ($\chi^2_2 = 45.8$, $P < 0.001$). The proportion of these carcasses for which sex_{morph} was determined correctly was ≤0.55 for carcasses recovered ≥2 days after death.

Sex ratios (female:male) based on sex_{morph} data were 35:65 for eastern red bats and 53:47 for hoary bats. Sex ratios based on sex_{gen} data were 44:56 for eastern red bats and 61:39 for hoary bats.

DISCUSSION

Our results support the hypotheses that carcass age and searcher identity affect the accuracy of sex_{morph} data obtained from carcasses of Lasiurine tree-roosting bats. We observed increased proportions of carcasses of unknown sex and sex misidentifications from bats estimated to have died >1 day prior. These results are similar to those of Korstian et al. (2013) who observed that the proportion of bats whose sex_{morph} was classified as unknown increased from 0.15 for fresh carcasses, to 0.5 for carcasses recovered within 2 days of death, to 0.9 for carcasses recovered within a week or more of death. Also like our results, Korstian et al. (2013) observed an increase in the proportions of sex_{morph} misidentifications as carcass age increased beyond 1 day. The concordance of our results and those of Korstian et al. (2013) occurred despite differences in land cover and climate between the study regions, with ours being primarily row crop agriculture with relatively lower temperatures in eastern Indiana and Korstian et al. (2013) being a mix of pasture, cultivated land, and shrub-woodland with relatively higher temperatures in northern Texas, USA. Furthermore, it is likely that there were differences in searcher training between the studies. Thus, given more misidentifications and greater proportions of carcasses of unknown sex from bats estimated to have died >1 day earlier in both studies, these results suggest that the sex of such carcasses is likely to be most accurately assessed using alternative techniques (e.g., the sex_{gen} approach used herein).

Our results indicate that the most effective way to increase the accuracy of sex_{morph} data is to only perform such identifications on fresh (<1 day old) bat carcasses. However, even sex_{morph} identifications of fresh bat carcasses are not always correct; we found that 10% of such carcasses were misidentified across all searchers. The rate of misidentification of the sex of these carcasses varied considerably (0–43%) among searchers. Ten of the searchers had perfect classification of the sex of fresh carcasses, which suggests that misidentifications of such carcasses in our study were confined to a limited number of searchers rather than there being a consistent level of misidentification across searchers. The extent to which the individual-level variation in error rates of identification of the sex of fresh carcasses that we observed in our study occurs at other renewable-energy facilities and with other searchers is uncertain. Furthermore, the individual-level variation error rates we observed are difficult to compare with other studies (e.g., those using data collected by non-experts, such as citizen scientists) because such studies usually report accuracy in aggregate and do not quantify inter-individual variation despite its presumed occurrence (Kosmala et al. 2016). These results should not discourage sex_{morph} assessment of fresh bat carcasses from renewable-energy facilities but should instead spur efforts to identify and minimize such mistakes.

Differences in pre-study background or experience of different searchers could contribute to the variation we observed in their accuracy of sex determinations of fresh carcasses. For example, individual searchers with less experience may have greater rates of misidentification than searchers with more experience. Unfortunately, we lack quantifiable data concerning the background and experience of the searchers in our study. Thus, we conducted an exploratory analysis to assess if there was a negative relationship between the number of sex determinations a searcher made and individual-level accuracy of sex assessments of fresh carcasses, based on the idea that individual searchers with less experience in identifying sex_{morph} of bat carcasses during the course of our study may be more prone to misidentify sex_{morph} than searchers with more experience. We observed no such relationship (Fig. 3a). Furthermore, if some individual searchers are less conservative than other searchers in whether they make an identification of sex_{morph} (vs. identifying the sex as unknown) we might expect a negative relationship between the proportions of carcasses classified as unknown sex and individual-level accuracy of sex assessments of fresh bat carcasses; however, we also observed no such relationship (Fig. 3b). We speculate that variables that we did not quantify and that are challenging to assess, such as differences in natural ability, effort, or commitment among individuals (Kosmala et al. 2016) may have caused variation in accuracy of sex_{morph} assessments among searchers in our study.

One factor that improves the accuracy of data collected by non-experts is training. In particular, the type of training (e.g., in person vs. remote, reading vs. listening or watching) can affect data accuracy (Ratnieks et al. 2016). Thus, besides only performing sex_{morph} assessment on fresh carcasses, an avenue to pursue to attempt to improve the overall accuracy of sex_{morph} data may be to implement consistent, in-person training of personnel that perform sex_{morph}-based assessment, if such training is not already in place. We also recommend sex_{morph} designations by searchers be confirmed by crew leaders or other personnel with significant experience handling and identifying bats. Finally, organizations that collect and identify bat carcasses should collect metadata about searcher background and experience, such as the years that the individual has spent working with bats and the number of bats identified in his or her career because such data could be used to further assess the cause of potential variation in accuracy of sex determinations among searchers.

Reports suggest that there may be more adult male than female tree-roosting bats killed at some wind-energy facilities in North America (Johnson et al. 2003, Fiedler 2004, Arnett et al. 2008). Such results are important because they have implications for identifying the potential reason(s) that large numbers of tree-roosting bats die at renewable-energy facilities. However, these reports were based on sex_{morph} data from carcasses of varying age, and our results suggest that there may be overrepresentation of males in such datasets. This bias appears to exist because relatively more females are classified as unknown and are misidentified from sex_{morph} data. Korstian et al. (2013) observed a similar difference, with sex_{morph}-based sex ratios (female:male) of 39:61 and 40:60 for eastern red and hoary bats, respectively, but with more even sex ratios of 50:50 and 48:52, respectively, based on sex_{gen} data. Thus, we suggest that it may be premature to conclude that male Lasiurine tree-roosting bats are generally killed in relatively greater proportions than females at wind-energy facilities.

MANAGEMENT IMPLICATIONS

Sex_{morph} data obtained from bat carcasses from renewable-energy facilities are useful to decision makers in wildlife management and conservation. Our results suggest that the most straightforward and effective approach for decreasing error rates of sex_{morph}-based assessments is for searchers to attempt to determine sex_{morph} only for fresh (<1 day old) bat carcasses. The sex of older carcasses is likely to be best-assessed using genetic approaches. Additional training and oversight of searchers may further improve the accuracy of sex_{morph} data obtained from fresh carcasses recovered within a day of death.

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