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Wind turbine-associated bat mortality is occurring at unanticipated rates, yet our understanding of the causes of these fatalities is limited. The prominent proximate causes of bat deaths at wind turbines are direct collision (i.e., blunt-force trauma) and barotrauma. The objectives of this study were to use veterinary diagnostic procedures to determine the lesions associated with bats killed by wind turbines and investigate relationships between patterns of injuries and proximate causes of death. A majority of the bats (74%; 29 of 39) examined by radiology had bone fractures; most of these fractures were in the wings and none was in the hind limbs. Visual inspection resulted in 33% fewer detected bone fractures when compared with radiology results. Bats dropped from a turbine nacelle (91.44 m) to determine extent and type(s) of bone fracture did not show signs of significant bone damage. Approximately one-half (52%; 12 of 23) of bats whose ears were examined had mild to severe hemorrhaging in the middle or inner ears (or both). None of the bats found during this study had any pre-existing disease. It is difficult to attribute individual fatalities exclusively to either direct collision or barotrauma. Gross necropsy, histopathology, and radiology complement each others' deficiencies and together give the best insight into cause of death. Delayed lethal effects after nonlethal contact with wind turbines are poorly understood and difficult to quantify by mortality searches alone but can result in underestimating bat mortality caused by wind energy facilities.

Key words: barotrauma, bats, direct collision, fatalities, veterinary diagnostics, wind energy


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Bat mortality at wind energy facilities was an unanticipated phenomenon that has become a prominent concern (Barclay et al. 2007; Cryan and Barclay 2009; Kunz et al. 2007b; Kuvlesky et al. 2007). Wind energy facilities have been associated with unprecedented rates of bat mortality in the United States and Canada (Arnett et al. 2008; Johnson 2005) and Europe (Bach and Rahmel 2004; Dürr and Bach 2004). Although impacts of wind energy facilities on wildlife originally were hypothesized to affect primarily birds, bat fatalities outnumber those of birds at most wind energy facilities studied (Arnett et al. 2008; Kunz et al. 2007a; Kuvlesky et al. 2007). A majority of bat mortality in North America involves migratory, tree-roosting bats, including the hoary bat (*Lasiurus cinereus*), eastern red bat (*Lasiurus borealis*), and silver-haired bat (*Lasionycteris noctivagens*—Arnett et al. 2008). However, relatively high rates of mortality of nonmigratory bats such as the little brown bat (*Myotis lucifugus*) and the big brown bat (*Eptesicus fuscus*) have been observed, specifically in midwestern regions of the United States (Grodsky 2010; Gruver et al. 2009; Jain 2005).

Bat mortality at wind energy facilities is particularly perplexing because bats echolocate and presumably can detect the turbine and individual blades. However, the maximum range at which bats can echolocate is 20 m (Neuweiler 2000); given a turbine blade rotation speed of 75 m/s, bats have approximately 0.25 s to react to spinning turbine blades before being struck or enduring barotrauma (B. D. Dieterle, The University of New Mexico, pers. comm.). Thus, it is unlikely that bats can adjust their flight direction before entering airspace occupied by spinning turbine blades.

Although many hypotheses have been proposed to explain why the behavior of bats makes them susceptible to being killed by wind turbines (Cryan and Barclay 2009; Kunz et al. 2007b), this paper addresses the proximate causes exclusively. Two major proximate causes of bat fatalities occur at wind energy facilities: barotrauma and direct collision. Barotrauma at wind turbines occurs as follows: the moving blades of a wind turbine act as an airfoil, creating a region of low pressure along the top of the blade surface (i.e., most convex) and a spiraling vortex near the blade tips, which has a low pressure core (Green 1995; Milne-Thompson 1958). The drop in atmospheric pressure causes injury in the form of internal mechanical damage of lungs and other organs (Elsayed 1997), killing bats that fly through the sudden pressure change. Necropsy and histopathology results have indicated that dead bats with and without visible external damage show signs of barotrauma (Baerwald et al. 2008; Dürr and Bach 2004). Injuries consistent with barotrauma are internal injuries to the thoracic and abdominal cavity and lesions of the lung, including pulmonary hemorrhage, congestion, edema, lung collapse, and the presence of bullae (Baerwald et al. 2008). The pathology of barotrauma is well described in the literature on blast overpressure (BOP) for the military (i.e., effects of bombs). Pulmonary hemorrhage and ear and other hollow organ damage due to BOP are described in humans, in addition to brain, eye, cardiovascular, and musculoskeletal damage (Hirsch 1968; Mayorga 1997; Ritenour and Baskin 2008); animal models of BOP emphasize pulmonary damage (Bass et al. 1998), which has been documented in sheep (Axelsson and Yelverton 1996; Bass et al. 1998) and rats (Jaffin et al. 1987). Bat deaths due to barotrauma occur without any direct contact with the turbine blades. Barotrauma explains greater mortality in bats compared with birds and could explain why bats are found closer than birds to the base of turbines (Kunz et al. 2007a). Respiratory anatomy and physiology differ between birds (unidirectional airflow) and bats (dead-end airflow), leaving birds relatively exempt from the risk of barotrauma, whereas bats' mammalian lungs cannot sustain the drop in barometric pressure (Baerwald et al. 2008; Maina and King 1984; West et al. 2007).

Direct collision involves bats physically colliding with monopoles or moving or stationary turbine blades. Although potential exists for bats to collide with stationary structures (as seen with migrating birds—Kuvlesky et al. 2007), no bat fatalities have been observed at nonmoving wind turbines (Arnett et al. 2008). Furthermore, a mitigation experiment in Canada that reduced turbine operations saw a consequential reduction in bat fatalities (Baerwald et al. 2009); however, the moving blades of wind turbines can strike flying bats using the same airspace. Thermal infrared imagery has recorded bats being struck by the blades of wind turbines (Horn et al. 2008) and provides hard evidence for direct collision. Direct collisions often are associated with physical injuries such as broken wings, skulls, and vertebral columns and severe lacerations (Baerwald et al. 2008; Johnson et al. 2003).

Although these two causes of bat mortality at wind energy facilities each have supporting data, repeatable methods for differential diagnosis of injuries have not been established. Therefore, the objectives of this study were to use veterinary diagnostic procedures to determine the lesions associated with bats killed by wind turbines, correlate patterns of injuries with causes of death, and create guidelines for future studies. We hypothesized that bat fatalities were not caused predominantly by barotrauma but rather by a combination of barotrauma and direct collision. Information on common injuries in bats killed by wind turbines and the estimated proximate cause of death can help streamline mitigation strategies, make estimates of mortality more accurate (i.e., address delayed lethal effects), and increase our understanding of the ecological consequences of bat mortality at wind energy facilities.

## Materials and Methods

*Mortality searches.*—Bat carcasses were collected 15 July–15 October 2009 as part of a mortality study at the Forward Energy Center (Center) in southeastern Wisconsin. The Center consists of 86 1.5-megawatt wind turbines. Twenty-nine of the 86 (34%) wind turbines (diameter of swept area = 77 m each) at the Center were selected randomly for mortality searches. The total search area was defined identically for all 29 study plots, with each plot consisting of a 160-m by 160-m square (2.6 ha) centered on the wind turbine. Twenty-six of the 29 searched turbines had 19% (0.5 ha) of the total searchable area monitored, using 5 parallel 160-m by 5-m transects. Transects were selected randomly from a grid of 4.6-m by 4.6-m squares superimposed upon the total searchable area. The 5 parallel transects were perpendicular to the turbine access road. The access road plus an extension and the pad of the turbine served as a 6th search transect. At the remaining 3 turbines the entire searchable area was monitored. Three control sites equaling a total of 0.5 ha each were searched outside of the wind energy facility.

Search transects were cleared of vegetation by mowing with a tractor. Each transect was divided into 2 approximate halves longitudinally (each 2.5-m wide). Investigators searched up the 1st half, while scanning ahead and toward the center of the transect, and then searched back down the 2nd half of the transect, while scanning ahead and toward the center of the transect. The 2.6-ha plots were searched by walking parallel transects 5 m apart in a snaking pattern from 1 side of the square plot to the other while the searcher scanned approximately 2.5 m to each side of the search line. For all

29 study plots the gravel and cement areas of the turbine pad also were searched.

When a carcass was found, the animal was assigned a unique carcass identification number, which included the turbine number and date, and placed in a resealable bag. The distance of the carcass from the base of the turbine was estimated using the same grid system in which the search transects were laid. Searchers were provided hard copies of maps for each study plot. Once a carcass was found, the searcher paced off the distance from where the carcass lay to the base of the turbine. Each grid cell was 4.6 m in length, and searchers applied their individual pace to determine distance on the map to the nearest 4.6-m cell. This method of mapping was chosen because the number of searchers and budget limitations precluded equipping searchers with global positioning system units.

Trained technicians searched for bat carcasses within the defined search areas. Searcher efficiency and scavenger removal trials were conducted during mortality searches to account for searcher- or scavenger-related biases. All bat carcasses found were transported to an on-site processing center where SMG identified the bats to species. Bats that could not be identified due to decomposition were sent to the University of Wisconsin Zoology Museum to be identified by skull morphology. Bat carcasses were dipped in a dilute solution of soapy water made with 1 teaspoon of liquid dish detergent per gallon of water to decrease insulation and autolysis and then placed in a refrigerator at the Center's main office. Transport of the bat carcasses from the field to the processing center did not exceed 5 h. All bat carcasses were transported in a cooler from the field site to the Wisconsin Veterinary Diagnostic Laboratory (WVDL) in Madison, Wisconsin on a weekly basis until the end of the study period. Five additional bats collected outside of the fall 2009 study period were included for histological examination. These included frozen bats from study periods in the fall of 2008 ($n = 3$) and spring of 2009 ($n = 2$). The species composition of the bats examined by veterinary diagnostic procedures was representative of that of the mortality searches during a larger study to examine bat mortality at the Center.

*Radiology.*—Bats were encased in a plastic bag and positioned in ventral recumbency and as flat as possible inside an X-ray cabinet (Hewlett-Packard Faxitron, now Faxitron X-Ray, LLC, Lincolnshire, Illinois). While in the cabinet the bats were placed on top of a sheet of Kodak oncology film (25.4 × 30.5 cm REF 801 5059—Kodak, Rochester, New York) and exposed at a peak kilovoltage of 25 for 80–120 s. The film then was developed in a 100Plus automatic X-ray film processor (All-Pro Imaging Corp., Hicksville, New York) at the University of Wisconsin–Madison School of Veterinary Medicine. Larger bats (i.e., big brown bats, hoary bats) were radiographed 1 at a time, and smaller bats (i.e., eastern red bats, little brown bats, and silver-haired bats) were placed 2 per sheet. All radiographs were randomized and evaluated by a board-certified veterinary radiologist who was unaware of the postmortem findings. Data recorded included the location, number, and type (i.e., fracture classification, dislocations) of skeletal injuries and any abdominal or diaphragmatic hernias. Additionally, the thoracic cavity was examined for radiographic signs of pneumothorax (air in thoracic cavity), hemothorax (blood in thoracic cavity), chest wall trauma, atelectasis, or pleural fluid accumulation. Bats were aged based on the degree of physeal fusion of the wing joints via the examination of radiographs (Brunet-Rossinni and Wilkinson 2009).

In a complementary experiment the question of whether bats can break bones from a freefall (i.e., a barotrauma event without collision with blades) was addressed by dropping bat carcasses off the top of a turbine nacelle (cover housing) 91.44 m above ground level. Because barotrauma events are hypothesized to occur within a zone radiating from the blades of the wind turbine (Baerwald et al. 2008), the nacelle was an appropriate height for the experiment. Little brown bats were obtained postrabies testing from the Wisconsin State Hygiene Laboratory (WSHL) in Madison, Wisconsin. All of these bats had skull damage due to the invasive procedure for the removal of brain tissue for testing; however, wings and bodies were fully intact. Each bat was assigned a number and radiographed before the experiment. Then the bat was dropped from a position atop the nacelle and behind the turbine blades (turbine was nonoperational), collected from the ground underneath the turbine, and radiographed again. The same board-certified veterinary radiologist, without knowledge of the bat drop, reviewed the radiographs. After the examination, any broken bones that were recorded in the predrop radiographs were omitted, because the broken bones were not a result of the drop. New broken bones were detected by comparing predrop and postdrop radiology results.

*Gross necropsy.*—After each bat was radiographed, those carcasses deemed suitable for necropsy (i.e., not fully decomposed based on visual inspection) were placed in dorsal recumbency, and a ventral skin incision was made from rostral mandible to pubis. The abdominal midline was incised, and the abdominal organs were examined. Gonadic development was examined to determine bat age. The liver was retracted to observe the diaphragm. The ribs were incised parallel to the sternum from caudal to cranial, and the thoracic cavity was opened and examined. The lungs and heart were dissected free with gentle traction on the trachea and esophagus and fixed in 10% neutral buffered formalin (4% formaldehyde—LabChem, Inc., Pittsburgh, Pennsylvania). The head was dissected by dislocation of the atlanto-occipital joint, and a complete cross-section of cerebellum and brain stem was sampled. These caudal brain samples were shipped on dry ice to the New York State Rabies Diagnostic Laboratory (NYSRDL) in Slingerlands, New York for rabies testing. Tympanic bullae were palpated and also found by locating the area of the tympanic membrane via the external ear canal. The bullae were dissected and placed in formalin.

*Histopathology.*—Histologic sections of lung and middle ear tissues were made after fixation in formalin for 24 h. The lung lobes were dissected free and placed individually in a cassette for tissue processing. Middle ears were placed in a decalcifying solution for 1 h (chelating agents in dilute HCl—Richard Allen Scientific, Kalmazoo, Michigan) and later cut in half for processing. Tissue processing was done overnight in a Tissue-Tek® VIP® 4 (Sakura Finetek USA, Torrance, California). The lung and middle ear sections were then embedded in paraffin (Richard-Allan Scientific, Kalamazoo, Michigan), sectioned at 5 μm, placed on glass slides (Surgipath Medical Industries, Richmond, Illinois), stained with hematoxylin and eosin (Sigma-Aldrich, St. Louis, Missouri), and coverslipped with mounting medium (Richard-Allan Scientific, Kalamazoo, Michigan). The slides were allowed to dry for 1 h and were examined using light microscopy by a board-certified veterinary pathologist for both pre-existing and acute damage. The lungs were graded by the following scale: 0 = no lesions; 1 = mild hemorrhage in less than approximately 20% of lungs; 2 = moderate hemorrhage in 20–50% of lungs; and 3 = hemorrhage in >50% of lungs. The middle ears were noted to be negative (NEG) if no blood was present, positive (POS) if blood was present, and (POS+) if blood was both in and around the middle ear(s). Bat tissues that were too autolyzed to grade were labeled as such and discounted.

*Rabies testing.*—All brain samples were tested with the direct fluorescent antibody (DFA) procedure at the NYSRDL using the national standard DFA protocol (Centers for Disease Control and Prevention, http://www.cdc.gov, accessed 12 November 2010). Slip-smear preparations of finely minced brain tissue were prepared on microscope slides. Slides were air-dried and fixed in acetone at −20°C for 1 h. Slides were air-dried again and stained with diagnostic conjugates for 30 min. After 2 5-min phosphate-buffered saline washes slides were air-dried and mounted with coverslips before examination at 200× and 400× on a Zeiss Axio-Imager microscope (Carl Zeiss Microimaging Inc., Thornwood, New York) equipped for fluorescent microscopy.

## Results

*Mortality searches.*—We found 41 bat carcasses during mortality searches. No bat mortality was recorded at the 3 control sites. Mortality included bats from 5 species. Most bat mortality consisted of hoary bats (*n* = 17; 41%) and silver-haired bats (*n* = 12; 29%). The remaining mortality was composed of eastern red bats (*n* = 6; 15%), big brown bats (*n* = 4; 10%), and little brown bats (*n* = 2; 5%). A majority of the bat fatalities was females (*n* = 20; 49%); males (*n* = 11; 27%) and unidentified gender (*n* = 10; 24%) accounted for the remainder of the mortality. All bats that could be aged by gonadic development were found to be adults (*n* = 32). Nine bats could not be aged due to internal decomposition.

*Radiology.*—A total of 39 bat carcasses was radiographed. At least 1 broken bone was detected in 74% (*n* = 29) of the bats radiographed. Fractures to the wing bones—specifically the humerus (*n* = 15; 38%) and the radius (*n* = 10; 26%)—were the most common, and a majority of these fractures was comminuted (i.e., shattered or crushed into many pieces; Fig. 1a, d). Fractures of the skull (*n* = 8; 21%; Fig. 1b) and the scapula, lumbar vertebrae, ribs/sternum, and pelvis were the next most common injuries (*n* = 6; 15%; Fig 2). The highest number of broken bones found in 1 bat was 6 (Fig. 1a). Forelimb fractures were approximately equally divided between the left (*n* = 15) and right (*n* = 13). Additionally, 4 bilateral fractures were observed in the forelimbs. Five appendicular fractures of the wings were noted but not recorded relative to the side of the bat. Fractures of the axial (midline) skeleton numbered 25. We observed no hind limb fractures. Inguinal and diaphragmatic hernias were recorded in 12 bats (31%; Fig. 1c). All bats had closed physes at all metacarpal-phalangeal joints, indicating that those bones had finished growing (i.e., all bats were adults). Two female hoary bats still had a few open growth plates. One of these bats had partially open distal ulnar physes; the other had open distal ulnar and distal femoral physes. All other epiphyseal growth plates of wing and other bones were closed in these 2 bats, indicating that they were nearing the end of their growth but were still subadults.

In the complementary ''bat drop'' experiment 18 bats were dropped. The site of the bat drop experiment had corn underneath the east side of the turbine, and consequently 3 of the 18 bats fell into thick vegetation and were not recovered. No new bones were fractured in most of bats postdrop (*n* = 12; 80%). The 3 bats that broke a bone as a result of the drop had 1 new fracture of the lumbar spine, ulna, and sacrum, respectively. On average, bat carcasses took 10–12 s to fall the 91.44 m to the ground.

*Gross necropsy.*—A total of 33 bat carcasses was necropsied during this study. Of the bats necropsied, the most common lesions found were pneumothorax (*n* = 14; 42%) and hemothorax (*n* = 13; 39%). Pneumothorax and hemothorax were not exclusive injuries and often occurred together. An equal mix of inguinal and diaphragmatic hernias (*n* = 10; 30%) was the next most common injury. A majority of the hernias contained stomach contents; however, hernias also contained liver and intestinal tissues. Some bats (*n* = 8; 24%) had full stomachs, as demonstrated by dissection of intact stomachs or by the contents of hernias. Other lesions of interest found during necropsy, although recorded infrequently, included subcutaneous hemorrhage, hemorrhage in the pectoral muscles and abdominal muscles, and imbibition of blood. Bats with advanced levels of decomposition characterized by the presence of maggots or slippage of the skin constituted 27% of the sample size. The integrity of the carcass and ease of diagnosis varied between bats in this subsample. Upon visual inspection before necropsy, skeletal damage was detected only in 13 of the bats (39%). All of the bats necropsied were in good nutritional condition, and none of the bats was found to have any pre-existing disease.

*Histopathology.*—Lungs from 28 bats were processed for histopathology. Four sets of lungs were too autolyzed to interpret. Three (13%) bats had prominent bronchus-associated lymphoid tissue—evidence of past immune stimulation in

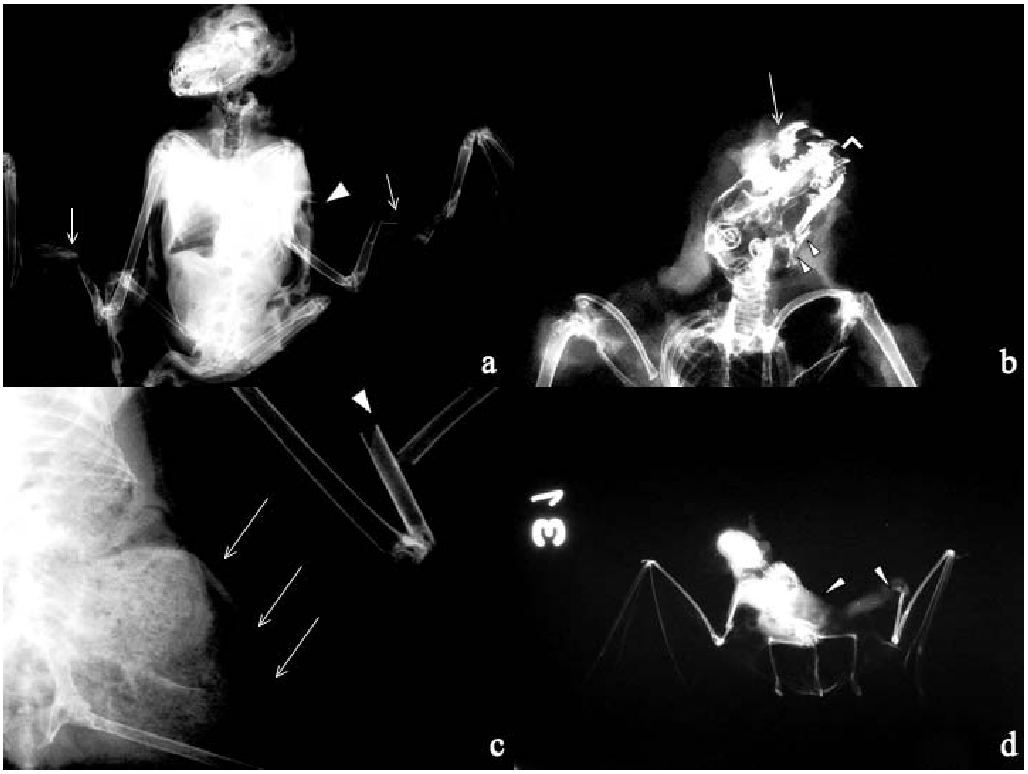


FIG. 1.—Dorsoventral radiographs of bats killed by wind turbines at a southeastern Wisconsin wind energy facility during fall 2009. a) Whole body image of a hoary bat. Note the comminuted fractures of the left and right radius (thin arrows) and the right humeral fracture (arrowhead). b) Caudal aspect of the right mandible of a hoary bat contains a comminuted fracture (small arrowheads), and the mandibular symphysis is separated (^). The left maxilla has an oblique fracture with mild dorsal displacement (thin arrow). c) Part of the gastrointestinal tract of a hoary bat, containing granular ingesta, extends beyond the boundaries of the abdomen (thin white arrows), consistent with body wall herniation. A short oblique fracture of the left radius is also present (arrowhead). d) Whole body image of a silver-haired bat with bilateral humerus fractures. The proximal right humerus has an oblique fracture, and the entire limb is displaced from the rest of the bat (arrowheads).

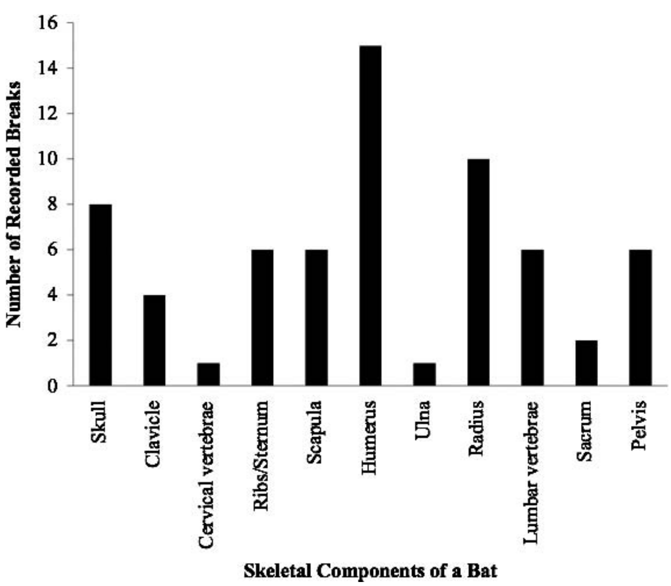


FIG. 2.—Number of broken bones recorded in 39 radiographed bats killed by wind turbines at a southeastern Wisconsin wind energy facility during fall 2009. Note the frequency at which different broken bones were recorded from the analysis of radiographs. Bones in the wing were the most common fractures. No fractures of the hind limbs (i.e., femur, fibula, tibia) were recorded.

the lungs but not currently pathologic. No active lung disease was found. Five (21%) bats had no pulmonary hemorrhage, 3 (13%) had mild hemorrhage, and 8 (33%) had moderate and severe hemorrhage, respectively (Figs. 3a and 3b). Three (13%) animals with pulmonary hemorrhage also had alveolar emphysema.

Middle ears from 24 bats were sampled, and the sections of middle ears from 1 bat were unsatisfactory. The 23 middle ear sections each contained at least 1 section (and usually 2) of tympanic bulla, sometimes with a section of inner ear. We found no pre-existing lesions in the middle ears or surrounding structures. Eleven (48%) bats had no hemorrhage in middle ears or surrounding structures, 4 (17%) bats had middle ear hemorrhage, and 8 (35%) bats had hemorrhage in and around the middle or inner ears (Figs. 3c and 3d). One bat had a ruptured tympanic membrane (Fig. 3d); however, tympanic membranes were not visible in every section of the ear.

*Rabies testing.*—All 17 bats from this study tested negative for rabies. The species tested included big brown bats ($n = 3$), eastern red bats ($n = 2$), little brown bats ($n = 1$), hoary bats ($n = 8$), and silver-haired bats ($n = 3$). Additionally, 224 bats from an undisclosed wind energy facility in the eastern United States were tested for rabies over a 5-year period. The species tested included big brown bats ($n = 7$), eastern red

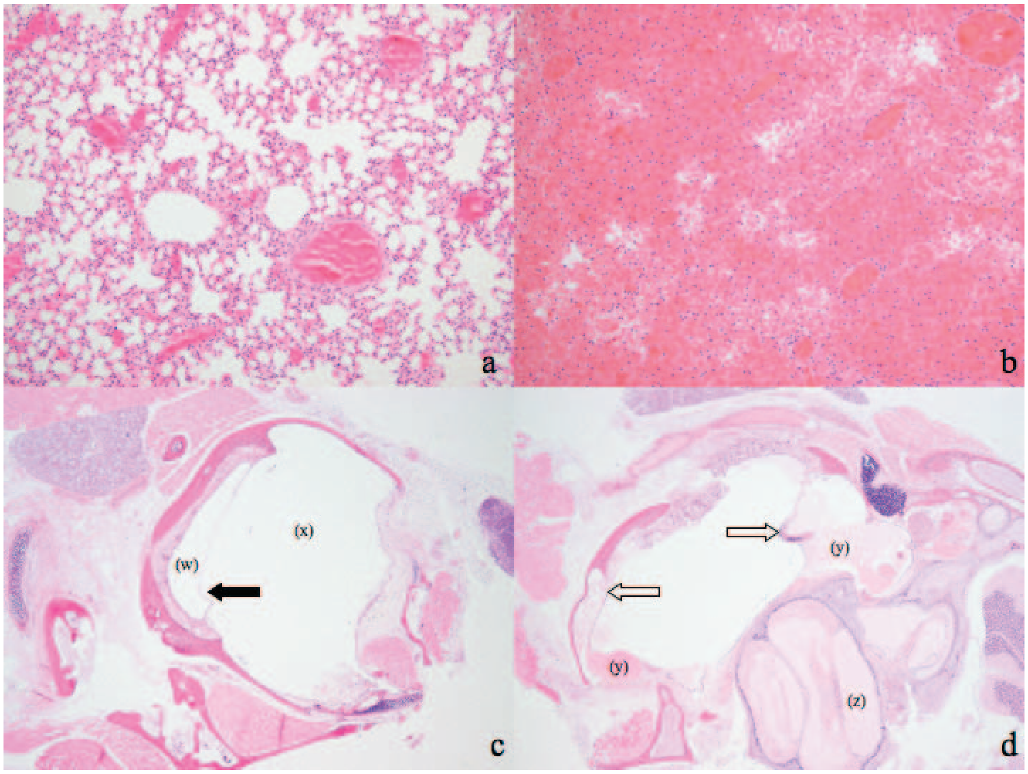


**FIG. 3.**—Histology slides (stained with hematoxylin and eosin) of lung and middle ear tissues from bats killed by wind turbines at a southeastern Wisconsin wind energy facility during fall 2009. a) Normal lung of a hoary bat (200×). A mild atelectasis occurs when the elastic lung retracts from the pleura. It can occur premortem if pneumothorax, hemothorax, or diaphragmatic hernia is present, or postmortem when the diaphragm is punctured at necropsy and negative pressure is consequently lost in the chest. Note pulmonary congestion (i.e., blood in vessels), a common postmortem finding. b) Hemorrhagic lung of a hoary bat (200×). Severe acute blood loss into alveoli has drowned the oxygen exchange surfaces of the lung, in addition to potentially resulting in hypovolemia and acute anemia. This lesion has been described in both barotrauma and blunt-force trauma. c) Normal middle and external ear canal of an eastern red bat (40×). The middle ear [normal; (x)], bordered by the tympanic membrane (black arrow), is filled with air and communicates with the pharynx via the Eustachian tube (not visible). The external ear canal [normal; (w)] is separated from the middle ear by an intact tympanic membrane. d) Hemorrhage in middle ear of a silver-haired bat (40×). This section of the middle ear is indented by the very large inner ear [normal; (z)], thus it lacks the rounded outline seen in c). The severe acute effusion of blood [(y)] in the middle ear is very abnormal. The external ear is blood-free but communicates with the middle ear because the tympanic membrane is ruptured (open arrows show both ends of ruptured tympanic membrane). The attachment of the malleus is visible on the internal surface of the ruptured tympanic membrane. See online version to view figure in color.

bats ($n = 33$), little brown bats ($n = 24$), hoary bats ($n = 51$), eastern pipistrelles (*Perimyotis subflavus*; $n = 2$), silver-haired bats ($n = 72$), and 35 bats of unknown species. Of the 224 bats tested, 1 big brown bat and 1 hoary bat tested rabies positive, 148 bats tested negative for rabies, and 74 samples were unsatisfactory.

*Carcass distance.*—A total of 38 bat carcass locations was measured relative to distance from base of the turbine. A majority of the bats (71%) was found within 30 m of the base of the turbine. The highest percentage of bats (29%) was found within 10 m of the base of the turbine. Bats with ≤1 broken bone recorded by analysis of radiographs comprised 82% of the bats found within 10 m of the base of the turbine. Although bats with limited bone breaks (i.e., 0 or 1) were found throughout the range at which bat carcasses were located from the turbine, a majority of the bats with the fewest broken bones was found within 40 m of the base of the turbine.

## DISCUSSION

Despite our intensive application of various veterinary diagnostic procedures, the exact cause of death (i.e., barotrauma or direct collision) could not be determined in most bats due to the variability of injuries and a lack of exclusively attributable lesions. Our findings suggest that cause of death for bats killed by wind turbines is not exclusively or predominantly barotrauma or direct collision but rather an indiscernible combination of both. However, identifiable patterns in injuries do exist.

Relatively few studies pertained to diagnoses of the cause of death for bats killed by wind turbines, and our study represents the first effort to radiograph bats. Past studies have examined carcasses for external damage and broken bones based on visual inspection alone (Johnson et al. 2003), and some have found that many bats examined in this manner did not readily exhibit a cause of death (Baerwald et al. 2008). Our results indicated that visual inspection of bat carcasses was not adequate for conclusively diagnosing fatal injuries, including broken bones. Severe skeletal damage (e.g., decapitation) is an obvious cause of death; however, it usually is more difficult to determine whether fractures were exclusively and immediately fatal. The analysis of radiographs often revealed broken bones that were not detected by visual inspection. Carcasses in this study visually inspected for broken bones and lacerations on the skin recorded 33% fewer broken bones compared with the radiology results. Lesions such as hemothorax and pulmonary congestion have been classified as indicators of barotrauma (Baerwald et al. 2008). However, a hemothorax often occurs from blunt-force trauma (e.g., a dog hit by a car), and pulmonary congestion is a frequent postmortem finding in animals that die of any cause (McGavin and Zachary 2007). Ruptured alveolar walls resulting in emphysema and bullae can be an effect of barotrauma (Baerwald et al. 2008; Elsayed and Gorbunav 2007) but can also be an incidental terminal change that is suggestive of agonal, premortem dyspnea (labored breathing—McGavin and Zachary 2007). Contrary to the study by Baerwald et al. (2008), which suggested that most bat deaths at wind turbines are caused by barotrauma, our results indicated that bone fractures from direct collision with turbine blades contribute to a higher percentage of bat deaths than originally anticipated. These injuries can lead to an underestimation of bat mortality at wind energy facilities.

The process of determining lesions indicative of either barotrauma or direct collision was convoluted by the variation associated with bat fatalities. For instance, a barotrauma event can be followed by direct collision, making the interpretation of skeletal injuries more complicated. The force of the vortices originating from the blade tips of spinning wind turbine blades could dislocate or break the wing bones of bats as they are contorted by the spiraling air currents. These spiraling air currents have a greater reach beyond the blades with increased blade speed (B. D. Dieterle, The University of New Mexico, pers. comm.). However, our radiology results showed no sign of dislocations, and a majority of the fractures were comminuted. Last, autolysis is a major contributor to the variability in diagnoses. Although radiology is useful regardless of the condition of the carcass, decomposition makes necropsy findings and histopathology more difficult to interpret. Autolysis makes both gross and microscopic interpretations more difficult via red blood cell lysis, tissue discoloration due to imbibition of blood, formation of gas bubbles resembling bullae from clostridial (postmortem bacterial) spread to lungs and other organs, and loss of cellular details and cell sloughing (McGavin and Zachary 2007). The level of decomposition in our sample was dictated more by the condition of the carcass when it was found during mortality searches than by storage method (i.e., dipping in soapy water), duration of storage, or transport time. Freezing carcasses did not prevent us from obtaining a histological diagnosis in the bats collected outside of the fall study period, which were frozen rather than refrigerated.

Several interesting patterns arose from our data. The frequency at which broken bones were detected coupled with the extent of the skeletal damage in many of these bats makes direct collision with the blades indisputable as one of the causes of fatalities for bats killed by wind turbines. Meanwhile, a majority (83%) of the bats without broken bones and with correlative histological data ($n = 6$) had moderate to severe pulmonary hemorrhage suggestive of barotrauma. All of these bats were found within 20 m of the base of the turbine, and half of them were found on the turbine pad itself. All bats without broken bones, regardless of the presence of histological findings, were found within 40 m of the base of the turbine. These findings suggest that bats killed primarily by barotrauma are more likely to be found closer to the base of the turbine than bats killed primarily by direct collision. Because some bats were found on the turbine pad during carcass searches and none of the bats dropped during the bat drop experiment hit the pad, the type of fractures, if any, a bat might incur from falling onto the pad are unknown. Approximately 25% of the bats necropsied had full stomachs, and given the accelerated rates of digestion in bats to reduce flight weights (Neuweiler 2000), bats were feeding at an indeterminate but relative proximity to the Center before they were killed. The majority of the skeletal damage observed was dorsally located, which would indicate that many bats with fractures are being struck from above. However, several of the bats without prominent skeletal damage had hernias and broken bones located ventrally (e.g., clavicle). These bats are likely being struck on the upswing of the turbine; the abdominal cavity of these bats could be absorbing the blow of the turbine blade, and consequently they sustain hernias but fewer broken bones.

None of the bats had any detectable disease before being killed by wind turbines. However, $<1\%$ of the bats found under wind turbines in the eastern United States tested positive for rabies. During the same time span hoary bats and big brown bats, whose cause of death was unrelated to wind turbines, recorded rabies positivity rates of 5.1% and 3.2%, respectively, indicating that rabies is observed less frequently in bats killed by wind turbines. A low incidence of rabies characterizes bats submitted for testing, and an even lower incidence of rabies in bats killed by wind turbines, suggesting that rabid bats are not more likely to be killed by wind turbines. However, rabies is a zoonotic disease; thus, proper precautions afforded by working in a biosafety level 2 laboratory were taken in this study and are recommended for future studies. Given the species-specific, high incidence of rabies in hoary bats, the abundance of hoary bats found under wind turbines in most studies, and the potential for encountering injured bats under wind turbines, it is important to acknowledge the potential for rabies transmission to scavenging animals and human searchers not using proper safety precautions.

Each diagnostic procedure had pros and cons that should be acknowledged for future studies. Radiology was important for determining skeletal damage and can be used regardless of carcass condition. Decomposition can lead to misdiagnosis of hernias, for instance, when maggots are present in the abdomen; however, these misdiagnoses occurred rather infrequently ($n = 2$). Interpretation of thoracic cavity lesions, such as pulmonary contusions, pleural effusion, or pulmonary atelectasis, was difficult to differentiate on the radiographs based on the size of the bat and the single projections provided. Gross necropsies were suitable for interpreting internal injuries to the abdominal and thoracic cavity of the bats but did not provide enough information to diagnose pulmonary hemorrhage because all bats necropsied had postmortem pulmonary congestion. Thus, histology was useful for analyzing trauma to lung tissue and examining the middle ear. Perhaps trauma to the middle ear could be detected via otoscopic exam, but histological study is a more realistic procedure. The use of all 3 diagnostic procedures is necessary to gain a comprehensive understanding of bat fatalities. If project resources are limited, future studies should prioritize diagnostic goals, using radiology to address skeletal injury or necropsy and histopathology to address internal injury. Procedures in the field are limited to gross necropsy. In this case necropsies should be performed only on bats exhibiting limited to no decomposition, as necropsies on autolyzed bats provide little useful information for the effort. Although field necropsies are useful for diagnosing internal injury, necropsies at a veterinary diagnostic lab, for instance, are recommended due to the expertise a pathologist can offer and the safety precautions available in a laboratory setting, such as those instituted for rabies. Bats found during carcass searches are better evaluated using veterinary diagnostics when decomposition is limited, regardless of where the procedures take place (i.e., field or laboratory).

The results of this study directly pertain to the issue of delayed lethal effects and the potential impact these effects can have on overall bat mortality. The surface and core pressure drops behind the spinning turbine blades are equivalent to sound levels that are 10,000 times higher in energy density than the threshold of pain (130 db—Cmiel et al. 2004) in humans and can cause significant ear damage to bats flying near wind turbines. Histological analysis revealed that 50% of the bats had moderate to severe hemorrhage in the middle ear, which can result from either blunt-force trauma or barotrauma. The auditory system as a whole plays a major role in echolocation and consequently affects bats' ability to feed (Hill and Smith 1984; Neuweiler 2000). Bats crippled by ear damage would have a difficult time navigating, as the inner ear transmits information about the acoustic environment to the brain (Neuweiler 2000). Damage to the inner ear causes ataxia in all domestic animal species (De Lahunta 1983) and is likely to do so in bats. As seen in the radiology results, a majority of the bats radiographed had broken bones as a result of blunt-force trauma and collision with the turbine structure or blades. Many fractures, especially those on the wings, can hinder a bat's ability to fly and consequently prevent a bat from feeding or migrating. During mortality searches for this study 3 bats were found crippled under wind turbines (Grodsky 2010). Additionally, images of bats being hit by turbines and continuing on an altered flight path afterward were recorded with thermal infrared imagery by Horn et al. (2008). During nightly searches conducted by personnel from the Tennessee Valley Authority at the Buffalo Ridge Wind Energy Facility in Tennessee, a bat lying on a turbine pad was observed flying away when touched (J. K. Davenport, DeTect Inc., pers. comm.). Sounds assumed to be bats being hit by turbine blades also were heard during these nightly searches; one of these sounds was followed by the descent of a limp bat and the apparent revival of the bat as it re-established a flight path before hitting the ground (J. K. Davenport, DeTect Inc., pers. comm.). Klug and Baerwald (2010) suggested that injured bats were more likely to be found under turbines searched every day; thus, past studies that searched under turbines less frequently might have encountered and accounted for fewer crippled bats. Depending on the severity of a barotrauma event or the blunt-force trauma caused by direct collision, bats can survive long enough to exit the search area of mortality studies. Therefore, mortality searches and, more important, mortality estimators do not capture delayed lethal effects, thereby possibly providing a lower estimate of actual bat mortality. Knowledge of the proximate causes for bat mortality at wind energy facilities can lead to better mortality estimates, improved management, and more efficient mitigation (e.g., adapting turbine blades to minimize potential for barotrauma or direct collision). Currently, strategies to reduce bat mortality are limited to curtailment of turbine cut-in speeds (Arnett et al. 2011; Baerwald et al. 2009) and acoustic deterrents.

Future studies on wind turbine-associated bat mortalities should focus on acquiring and analyzing the freshest possible bat carcasses and getting them from the field to the lab in the shortest amount of time. The use of all 3 diagnostic procedures mentioned herein should be implemented to identify injuries sustained by bats killed by wind turbines. Knowledge of the physics of blade tip vortices is essential to a comprehensive understanding of bat fatalities at wind turbines and should be considered in veterinary diagnostic studies. Studies that can provide hard evidence for the cause of bat fatalities at wind turbines, such as those that use thermal infrared imagery or night vision, will contribute to the findings of bat injuries using veterinary diagnostics.

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