Effects of white-nose syndrome on reproduction and survival in Illinois cave-hibernating bat species

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BACKGROUND/INTRODUCTION

Insectivorous bats are the primary predator of nocturnal flying insects, and therefore provide a valuable ecosystem service in agricultural landscapes. In Illinois, bats save farmers an estimated $1.7 billion annually in pesticide application costs.[1] But the majority of Illinois bat species, including the two most abundant (*Myotis lucifugus* and *Eptesicus fuscus*), as well as two endangered species (*M. sodalis* and *M. grisescens*), are expected to decline rapidly due to a newly emergent disease called white-nose syndrome (WNS). WNS has killed between 5-7 million bats in eastern North America since its discovery in 2006,[2] and population models predict that the once common species *M. lucifugus* will be extinct in this region by 2026.[3] The disease is caused by a cold-loving fungus, *Pseudogymnoascus destructans*, that infects bats during winter hibernation when body temperatures are low and immune function is suppressed,[4] causing mortality rates as high as 99%, and averaging 73%.[3] Attempts to treat bats for the disease have been largely unsuccessful, partly because disturbance of bats in winter rouses them from torpor, which rapidly depletes their energy reserves at a time when insects are not available, resulting in death.[5] This leaves not much hope for the persistence of these species, unless populations can evolve resistance to the fungus before their populations decline to virtually zero. At present, the most viable strategy for preventing widespread extinction is to ensure that bats have optimal spring and summer habitats that will maximize survival and reproduction during the active season, providing partial compensation for winter losses due to WNS. If we can slow population declines, this may enough time for bat populations to develop immunity before going extinct.

Early spring is a precarious time for these insectivorous species, which emerge from hibernation with nearly depleted fat stores to await insect emergence. Female bats will delay parturition by entering torpor if insects are scarce, health is poor or temperatures are low.[6,7] Delayed parturition has been linked with lower survival rates for offspring, presumably because they have less time after weaning to forage for insects and store fat before winter hibernation.[8] Any females that survive winter *P. destructans* infections will be in poor body condition in spring. These females are expected to give birth later in the season, and their offspring are expected to have higher mortality rates over the following winter. This could have profound effects on maternal colonies affected by WNS. WNS-positive bats that survive winter will be in poor condition, so female WNS survivors may be more to fall in this category of late parturition and associated decreased survival.

The purpose of our study was to clarify the relative impact of white-nose syndrome, spring temperature and spring precipitation on survival, fecundity and the timing of parturition in two cave-hibernating bat species native to Illinois: *Myotis lucifugus* (Little Brown Bat) and *Eptesicus fuscus* (Big Brown Bat). Additionally, we attempted to determine the effect of parturition date on adult female survival in these two species.

METHODS

This study of *M. lucifugus* and *Eptesicus fuscus* maternal colonies begun at Siloam Spring State Park was conducted from 2011-2014. We recorded roost temperature using data loggers during spring and summer annually, and regional precipitation and temperature data was be obtained from NOAA.[9,10] We checked for newly arrived females by mist-netting immediately around the roosts every 3-4 weeks from late-April through early June. Each female was wing-banded with lipped metal bands with individual ID numbers (Porzana, Ltd., Icklesham, U.K.) and assessed them for reproductive condition.[11] To assess reproductive condition, we used nipple morphology to determine if a female is pregnant, lactating, post-lactating or non-reproductive, then palpated the abdomen for the presence of a fetus.[12] Females with a discernible fetus were assumed to be in the last half of pregnancy. We combined the reproductive condition data for recaptured females to estimate a range of possible parturition dates. These dates were inexact, but allowed placement of females into early and late parturition date categories: before June 21st, or June 21st or later.

Additionally, wing tissue punches were taken from 10-30 females in each colony for WNS-PCR testing using a disposable, sterile 3 mm tissue punch, latex gloves (worn over handling gloves that prevent animal bites), disposable cardboard, and forceps.[13] The tissue punches, disposable cardboard and nitrile gloves were used for one bat only to avoid accidental transmission between individuals of *P. destructans*. Forceps were dunked in 95% ethanol and held over the flame of a cigarette lighter to sterilize after picking up wing-punch samples from each individual to avoid tissue sample cross-contamination.[14,15]

Beginning in late May, we visited the sites roughly biweekly at night while mothers are foraging. On these nights, we checked for new pups, then weighed, banded, measured forelimbs and photographed wings. We banded pups with the same size bands as adults to insure that pups don't outgrow bands and suffer forearm injuries.[16,17] Forelimb measurements included overall forearm length. We also took digital photographs of the bats' wings on top of a light box. These digital photographs allowed us to measure the epiphyseal gap length of the 4th metacarpal.[16] Additionally, pups with umbilical cords attached were be estimated at 1 day old.[16] The forelimb data and 1-day estimates were combined to create colony-specific linear regression equations that allowed estimation of each pup's date of birth.[18] These measurements were conducted while mothers are away to forage, according to a previous publication that suggests that handling newborn pups in the mothers' absence may be less stressful for both mothers and pups.[19] When adult females were net with pups attached, we noted mother-pup pairs. This approach maximizes the likelihood that mother-pup pairs will be identified before weaning while minimizing stress on the animals by only handling newborn pups while mothers are absent and not separating nursing pups from mothers.[16,19,20] We mist net outside the roost and recorded recaptures of previously banded bats. These data were used to estimate annual survival rates at maternal colonies using Program MARK.[21]

DNA was extracted from bat wing tissue samples, and PCR was conducted using custom primers and positive control DNA received from the United States Geological Survey-National Wildlife Health Center (NWHC) to test for the presence of *P. destructans*, the fungus associated with WNS.[13] PCR products that produced a gel band roughly 624-bp long were sent for sequencing. The sequences were compared to the published diagnostic *P. destructans* sequence (GenBank accession no. FJ231098) using NCBI's BLAST.[22] Only sequences that were 100% matches to the the GenBank sequence were considered PCR positive for *P. destructans*.[13]

We recorded recapture rates in subsequent years, and when all data have been collected. We used program MARK to estimate apparent survival for adults and juveniles across years, then chose the best models using Akaike Information Criterion (AIC).[21] We compiled composite spring climate data for each year as follows: mean spring temperature (average temperature from 1 March - 31 May), and total spring precipitation (total precipitation from 1 March - 31 May). We tested for differences in fecundity and parturition date between years for both species using chi-square tests. We also tested for a difference in apparent survival rates between early and late parturition for *M. lucifugus* females.

RESULTS

Ecological factors: White-nose syndrome, temperature and precipitation

All PCR test results for the presence of *P. destructans* were negative for both bat species, *M. lucifugus* and *E. fuscus*. Annual weather patterns varied greatly over the study period. The mean spring temperature (March-May) in 2012 was unusually warm (Table 1). Total spring precipitation (March-May) was much lower than average in 2012 and much higher than average in 2013 (Table 1). Roost temperatures varied predictably with ambient temperature, and were excluded from analyses (data not shown).

Reproductive data: parturition dates and fecundity

Median parturition dates in 2011 and 2013 were similar to those expected for *M. lucifugus* and *E. fuscus* in the Midwest, while median dates in 2012 were earlier than expected (Fig. 1, Table 1). The earliest annual parturition date was also earlier in 2012 than in other years for both species (Table 1). The *E. fuscus* median parturition date for 2012 was much earlier than expected for the species than the median parturition date for 2012 in *M. lucifugus* (26 days earlier than average for *E. fuscus*, versus 15 days earlier than average for *M. lucifugus*. Fig. 1A vs. Fig. 1B, Table 1). Annual fecundity was high for *M. lucifugus* (0.81 - 0.96) and *E. fuscus* (0.95 - 1.00) for all years from 2011-2014 (Fig. 2). Fecundity was significantly higher in *E. fuscus* than in *M. lucifugus* (chi-square = 39.8178, $p < 0.001$ - Fig. 2). Fecundity was significantly lower in 2012 for both species (*M. lucifugus*: chi-square = 6.537, $p = 0.011$; *E. fuscus*: chi-square = 16.25, $p < 0.001$. Fig. 2).

Survival data: annual survival rates and effect of parturition date on survival

Models of apparent survival constructed in Program MARK estimated *E. fuscus* adult survival between 0.65-0.73, and *E. fuscus* juvenile between 0.59-0.63 (Fig. 3). Only 15 *E. fuscus* adult females were captured in 2011, so the standard error for adult survival in that year than in other years (Fig. 3). Captures of *M. lucifugus* were much lower in 2014 than in previous years. Our models of apparent survival for *M. lucifugus* yielded two competitive models according to AIC scores: (1) a model in which survival remained constant across years and encounter probability progressively decreased across years (Fig. 4); and (2) a model in which both survival and encounter probability decreased across years (Fig. 5). In the model with only encounter probability varying, we estimate *M. lucifugus* adult survival between 0.83-0.91, and estimate *M. lucifugus* juvenile between 0.73-0.81 (Fig. 4). In the model with both apparent survival and

encounter probability varying,we estimate *M. lucifugus* adult survival between 0.67-0.84, and estimate *M. lucifugus* juvenile between 0.54-0.80 (Fig. 5). The standard errors for *M. lucifugus* adults and juveniles are greatest in 2014 in both models due to a lower number of captures that year (Figs. 4 & 5). Our model of apparent survival of *M. lucifugus* females according to parturition date found that females who gave birth June 21 or later had lower survival, but the results were not statistically significant (chi-square = 2.1159, p = 0.145, Fig. 6). We did not have sufficient data to conduct a test of the effect of parturition date on survival for *E. fuscus* females.

DISCUSSION

We did not detect the causative agent of WNS, *P. destructans*, in any of the Siloam Springs State Park samples we PCR tested from 2011-2014. Bats that survive winter infection with *P. destructans* can mount an immune response during the spring active season to clear infections.[METEYER] It is therefore possible that some of the bats in these two colonies could have been infected over winter, survived and cleared themselves of the fungal infection prior to PCR testing. We have no evidence that WNS affected either of these populations during the study period from 2011-2014, but we cannot definitively exclude this possibility. Our model of *E. fuscus* survival indicates that survival for this species remained roughly constant across the study period (Fig. 3). Our models of *M. lucifugus* survival give competing support for two different scenarios: either survival remained roughly constant while encounter probability decreased (Fig. 4), or survival and encounter probability both decreased across the four-year study period (Fig. 5).

Either scenario is plausible based on what we know about annual variation in our recapture efforts, trap-shyness of bat species, and the arrival of WNS in the region. Our total annual recapture effort declined in 2013 and 2014 due to the need to coordinate our trapping schedule with IDNR biologists after netting the endangered species *M. sodalis* in 2012. In particular, we tended to stop netting earlier in the evening when the proportion of *E. fuscus* to *Myotis* species tended to be higher. Also, several bat biologists have noted that *Myotis* species tend to avoid areas where they have encountered mist nets previously (Tim Carter, Justin Boyles, Joseph Pettit, personal communication). We observed numbers of *M. lucifugus* and accumulations of fresh guano in the Siloam Springs *M. lucifugus* roost throughout 2014 that were consistent with previous years, despite a lower number of captures by netting.These observations are all consistent with the constant survival model for *M. lucifugus* (Fig. 4). However, *P. destructans* was detected in hibernacula in eastern Missouri in winter 2013. It is possible that members of the *M. lucifugus* colony hibernate at these locations, and some individuals died before returning to the summer colony in 2014. If this is true, then the *M. lucifugus* model with decreasing survival and decreasing recapture probability over time is more accurate. Thus, we know that encounter probability decreased over time, but cannot be certain whether survival also decreased (Figs 4 & 5, respectively). We could resolve this uncertainty with additional years of recapture data. It should be noted that even if survival did decrease for *M. lucifugus* in 2014, our survival estimates are still within the ranges observed in robust, pre-WNS populations for this species.[8] Therefore, even the more pessimistic of the two *M. lucifugus* survival models that we present is not evidence that this population has been affected by WNS.

Our model of apparent survival of *M. lucifugus* females according to parturition date found that females who gave birth June 21 or later had lower survival (Fig. 6). However, the number of females in our sample that gave birth June 21 or later was low (n = 30), so the difference in apparent survival rates for those who gave birth after June 21 was not statistically significant (chi-square = 2.1159, p = 0.145, Fig. 6). Many females were excluded from the parturition date model because we did not have sufficient reproductive data. (i.e. We could not determine approximate parturition dates for females captured in early spring when fetuses were too small to be palpated, nor could we determine dates parturition dates for females that were only captured in the post-lactation stage in late summer.) We could increase the sample size for this study by including additional years of field data. Also, we could conduct parentage analyses using DNA extracted from tissue samples taken for *P. destructans* testing, then determine a mother's parturition date from her pup's date of birth.

Climate records demonstrate that 2012 was one of the hottest, driest springs on record in Illinois,[9] giving us an opportunity to observe the effects of a warmer, drier climate on the survival and fecundity of *M. lucifugus* and *E. fuscus*. We did not find any noteable changes in adult survival in 2012 for either species (Figs. 3-5). Our models do show modest increases in juvenile survival from 2011-2012 to 2012-2013 for *M. lucifugus* (from 0.77 to 0.80) and *E. fuscus* (from 0.59 to 0.63). These increases are well within the standard errors of the other years, and do not demonstrate clear evidence of an effect of temperature on survival. However, we did find substantial differences in reproduction for both species in 2012, both in terms of fecundity, and in terms of the timing of parturition. Fecundity was significantly lower in 2012 for both species (*M. lucifugus*: chi-square = 6.537, p = 0.011; *E. fuscus*: chi-square = 16.25, p < 0.001. Fig. 2). Also, we found that parturition dates were earlier on average in 2012 for both species, especially for *E. fuscus* (Fig. 1, Table 1). The early parturition dates that we observed agree with other researchers' observations that warmer temperatures result in earlier dates of birth in temperate zone hibernating bat species.[6, 19] A previous study by Frick and colleagues found that *M. lucifugus* juveniles born earlier are more likely to survive their first winter.[8] The slight increases that we observed in juvenile survival for the two species in 2012-2013 are consistent with their results. Our study also found that adult female *M. lucifugus* that give birth earlier are more likely to survive to the next year, but the difference was too small to be statistically significant with our sample size. The combined results of these studies support the hypothesis that warmer roosts could increase juvenile survival, and suggest that they could possibly also increase adult survival.

Overall, our data describe a complex interaction of effects of increasing temperature on survival and fecundity for *M. lucifugus* and *E. fuscus*. We observed earlier parturition dates with warmer spring temperature, which may increase survival for juveniles and possibly also adult females. However, we also observed reduced fecundity with increased spring temperature. Thus, there appears to be a trade-off during warm years, where population growth is positively affected by small increases in survival and negatively affected by decreases in fecundity. The reason for the decrease in fecundity is not readily apparent from our data, but could plausibly be related to reduced availability of high-quality insect prey or availability of water needed to support pregnancy. From the perspective of wildlife management, it would be likely be difficult to find a feasible strategy for improving prey quality and abundance during drought years, and small increases in fecundity tend to result in very little impact on population growth for long-lived

animals like bats.[24] Variation in parturition dates may provide a more effective target, since warmer temperatures are associated with earlier parturition, and early parturition is associated with increased juvenile survival. Survival rates could be boosted by providing warmer roost habitat (via insulation and/or heating). The effect of this strategy on population growth rates would be especially strong if we could determine that there is correlation between early parturition and increased female survival, because population growth in hibernating bat species is strongly affected by adult female survival.[3]

RELEVANCE OF RESEARCH TO HUMANS AND ECOSYSTEMS

Efforts to understand what we can do to support bat reproduction through protection or creation of suitable roosts will be critical in helping bat populations rebound from declines due to WNS, wind turbines and other causes.[22] WNS mortality affects all cave-hibernating species native to Illinois, including two endangered species (the Grey Bat, *Myotis gricescens*, and the Indiana Bat, *Myotis sodalis*).[5, 22] Bats are the second most diverse order of mammals (after rodents), and are the primary predator of nocturnal flying insects, including major agricultural pests and vectors for human disease.[24] A recent article in *Science* estimates that Illinois farmers will need to spend nearly $1.7 billion per year on additional insecticide application if bat populations become regionally extinct.[1] Current models of WNS-related mortality for *Myotis lucifugus* (one of the most abundant species in Illinois) predict regional extinction in the next 15 years.[3] We investigated the factors of bat ecology and health that affect reproductive success and parturition date in Illinois bats, which effects likelihood of winter survival of offspring. These data provide a valuable reference point for upcoming years as the effects of WNS spread throughout the state, and can be used to construct population growth models and inform land use policies that will help maintain bat populations that are beneficial to humans.
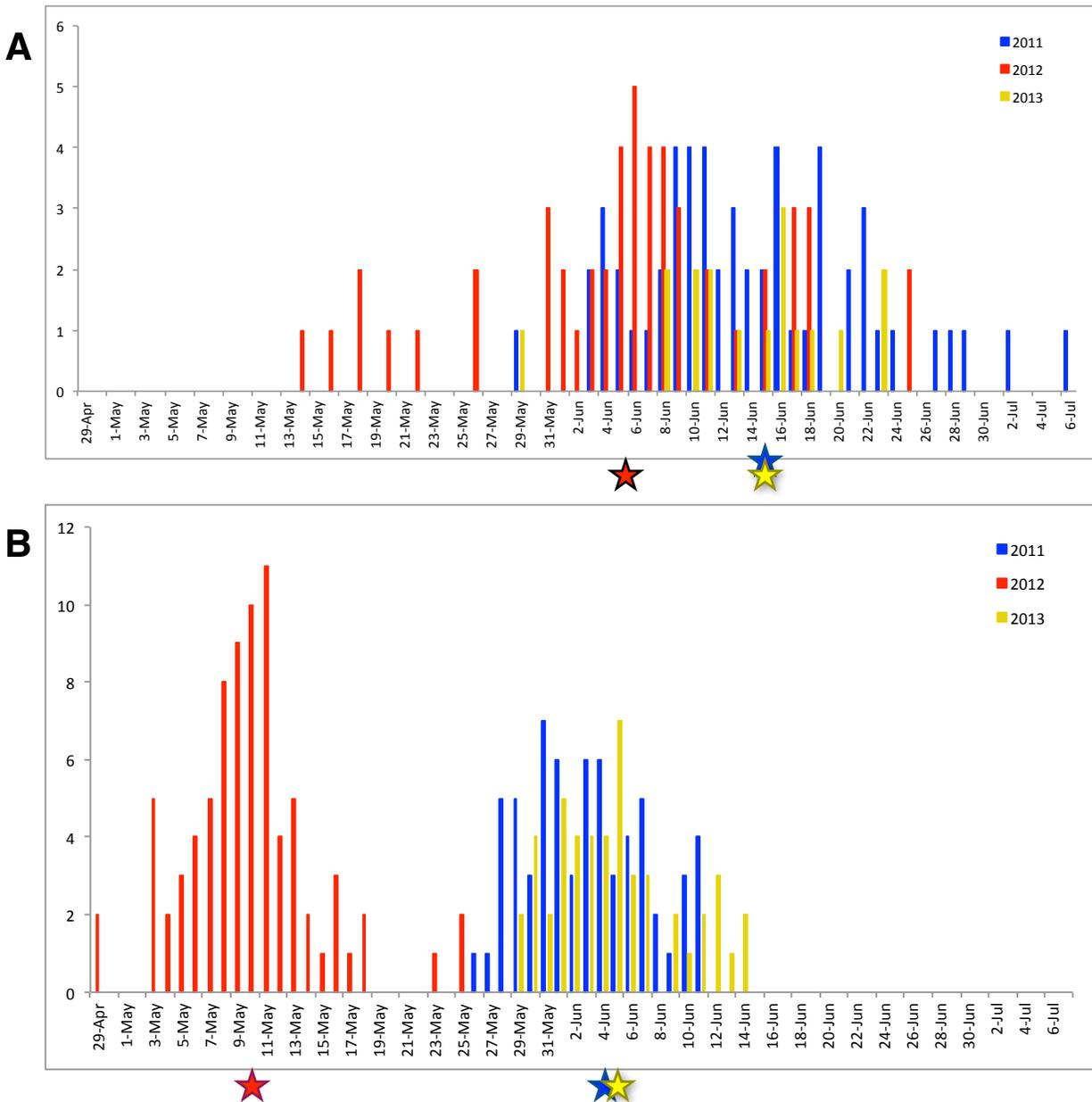
Fig. 1. Distribution of births at bat maternal colonies at Siloam Springs State Park in 2011 (blue), 2012 (red), and 2013 (yellow). Stars mark median annual birth dates. (1A) Distribution of *M. lucifugus* births. (1B) Distribution of *E. fuscus* births.

Table 1. Annual weather and dates of birth (DOB) for two bat species, *E. fuscus* (EPFU) and *M. lucifugus* (MYLU) at Siloam Springs State Park 2011 - 2013. T_spring = mean temperature from 1 March - 31 May; P_spring = total precipitation from 1 March - 31 May. Data in bold represent departures from expected.

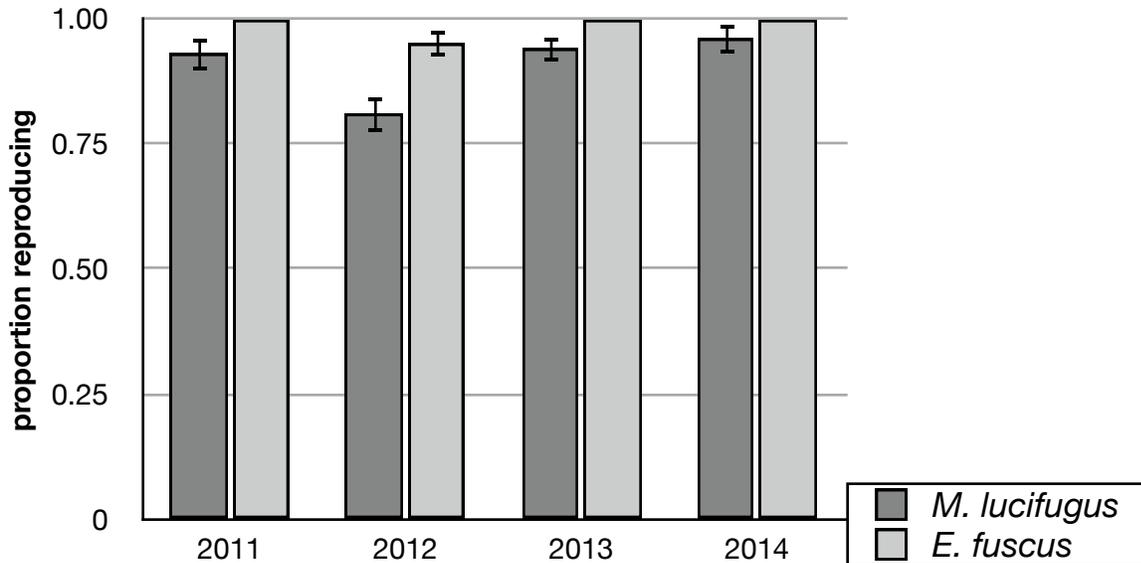|	2011	2012	2013	average
T_spring (C)	12	**16**	10	11.2
P_spring (cm)	23.1	**14.7**	**51.7**	27.8
MYLU Median DOB	15 June	**5 June**	15 June	mid-June
MYLU 1st DOB	29 May	**14 May**	29 May	early June
EPFU Median DOB	4 June	**10 May**	5 June	early June
EPFU 1st DOB	26 May	**29 April**	29 May	late May

Fig. 2. Proportion of female bats reproducing at Siloam Springs State Park from 2011-2014. *M. lucifugus* females (dark gray): 2011 n = 65, 2012 n = 100, 2013 n = 71, 2014 n = 46; *E. fuscus* females (light gray): 2011 n = 15, 2012 n = 60, 2013 n = 68, 2014 n = 77. Bars represent standard errors.
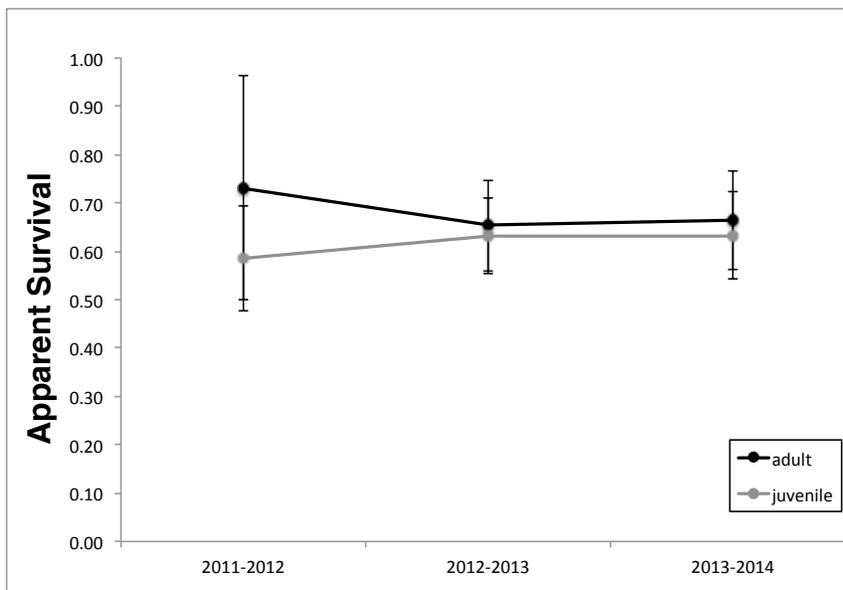

Fig. 3. Apparent survival of *E. fuscus* females at the Siloam Springs State Park maternal colony from 2011-2014. Adult survival estimates (black) were between 0.65-0.73 for all years; juvenile survival estimates (gray) were between 0.59-0.63 for all years. Adults: 2011 n = 15, 2012 n = 63, 2013 n = 71, 2014 n = 80; Juveniles: 2011 n = 48, 2012 n = 49, 2013 n = 40, 2014 n = 70. Bars represent standard errors.
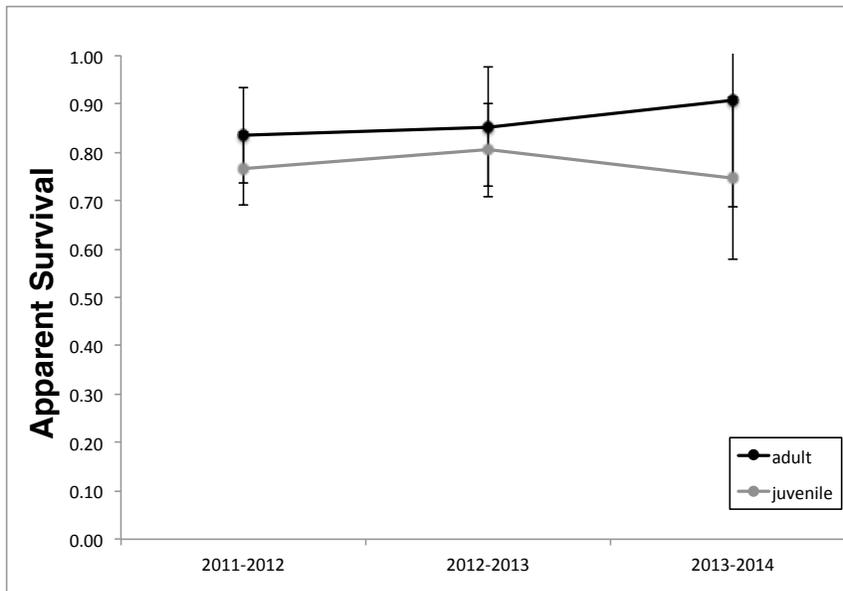
Fig. 4. Apparent survival of *M. lucifugus* females at the Siloam Springs State Park maternal colony from 2011-2014, assuming lower number of captures in 2014 were due to a lower encounter probability. Adult survival estimates (black) were between 0.83-0.91 for all years; juvenile survival estimates (gray) were between 0.75-0.81 for all years. Adults: 2011 n = 62, 2012 n = 134, 2013 n = 96, 2014 n = 45; Juveniles: 2011 n = 47, 2012 n = 79, 2013 n = 34, 2014 n = 8. Bars represent standard errors.
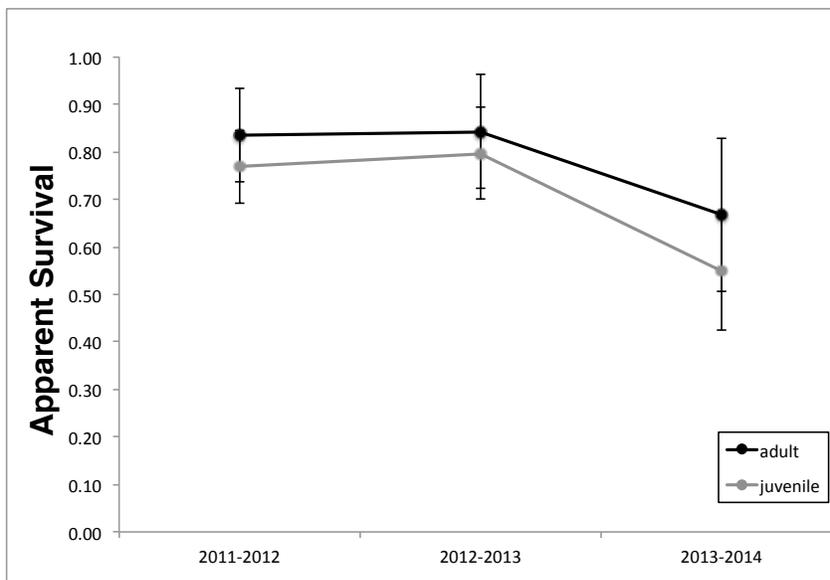

Fig. 5. Apparent survival of *M. lucifugus* females at the Siloam Springs State Park maternal colony from 2011-2014, assuming lower number of captures in 2014 were due to a combination of lower encounter probability and reduced survival. Adult survival estimates (black) were between 0.67-0.84 for all years; juvenile survival estimates (gray) were between 0.55-0.80 for all years. Adults: 2011 n = 62, 2012 n = 134, 2013 n = 96, 2014 n = 45; Juveniles: 2011 n = 47, 2012 n = 79, 2013 n = 34, 2014 n = 8. Bars represent standard errors.
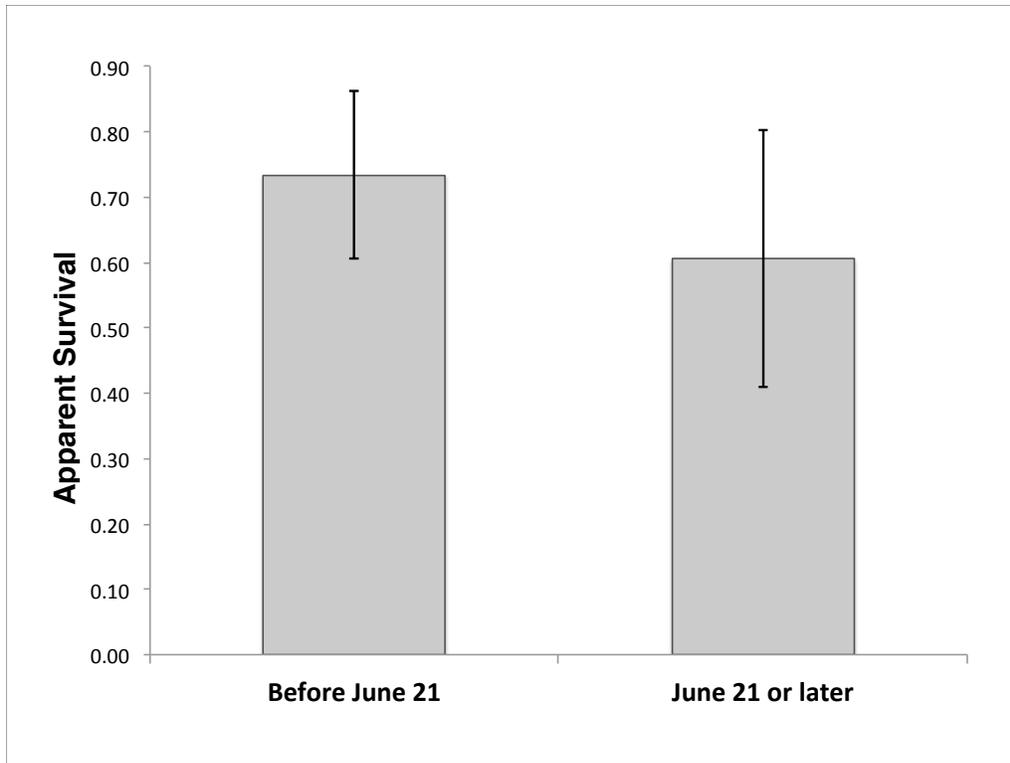
Fig. 6. Apparent survival of *M. lucifugus* reproductive females categorized by parturition date at the Siloam Springs State Park maternal colony from 2011-2014. Before June 21, n = 160; June 21 or later, n = 30. Chi-square = 2.1159, p = 0.145; no significant difference at 0.05. Bars represent standard errors.

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