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Ecological factors affecting reproduction and development in North American cave-hibernating bats

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Project Objectives:

1. Collect baseline data on the relationship between roost temperature, ambient temperature and precipitation and *Myotis lucifugus* (little brown bat) parturition dates, to compare with data in future years when White-nose syndrome enters the state.
2. Study the effect of late parturition on *M. lucifugus* adult female survival.
3. Conduct annual spring *Geomyces destructans* PCR testing on *M. lucifugus* at a leading edge site for White-nose syndrome in Illinois.

Completed Project Description:

1. The earliest *M. lucifugus* parturition dates in 2011 were within the historical range for Illinois, while the 2012 parturition dates were approximately approximately 19 days earlier than in 2011. Spring 2011 experienced greater than average total precipitation, while spring 2012 experienced greater than average mean temperature. Multiple years of data will be needed to conduct statistical analyses, but these early results suggest that spring temperature has a larger effect on *M. lucifugus* parturition date than spring precipitation.
2. The proportion of 2011 *M. lucifugus* adult females recaptured in 2012 was highest for those who gave birth before June 13 and lowest for those who gave birth after June 26. These differences were not statistically significant due to small sample size. Additional data collection over the next two years will if this trend holds when the sample size is large.
3. All individuals PCR tested for *G. destructans* in spring 2011 and 2012 were negative.

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park, less than 500 meters from both a small lake and a tree-lined creek. The surrounding area is agricultural (primarily corn and soybean) with patches of wooded area interspersed. IDNR staff report that *M. septentrionalis* females have been using the picnic pavilion as a maternity roost since the late 1970's, however I found maternal colonies of *M. lucifugus* and *E. fuscus*, and only a few non-reproductive *M. septentrionalis* during the 2011 field season. I had conducted PCR and histological tests for *Gd* on 29 female *M. lucifugus* collected by IDNR from a maternal colony on May 25, 2010. All individuals tested negative, but wing-scarring (WDI = 1-2) was observed on about half of the individuals inspected. All of the collected females were pregnant. IDNR biologist report that over half of the females inspected appeared to be in the late stages of pregnancy, with abdominal bulges about 2.5 - 3 cm in diameter, and no pups were observed yet.

All research was conducted in compliance with collecting and decontamination procedures outlined by the USGS National Wildlife Health Center and U.S. Fish and Wildlife Service,[19,20] handling procedures outlined in American Society of Mammalogists' *Guidelines for Capture, Handling and Care of* Mammals,[21] with approval from the Illinois Department of Natural Resources, and according to protocols reviewed by IACUC and the Division of Research Safety at the University of Illinois. I conducted fieldwork from mid-May through mid-August in 2011 and 2012. In May 2011, I collected wing punch tissue samples from 17 gravid *M. lucifugus* females that I hand-captured from a maternal colony of approximately 125 found roosting in the attic of the pavilion to PCR test for *Gd*, and inspected them for wing damage. I did the same for 12 *M. lucifugus* mist-net in May 2012. I programmed a HOBO temperature loggers (Onset Computer Corporation, Bourne, MA) to record temperature every 10 minutes, and placed it inside the attic near the roosting females. A colony of about 55 *E. fuscus* adults were found in late May 2011 in a roost over 20 feet above ground at one end of the roof of the open-air portion of the pavilion on this date. I began collecting data on this colony since June 2011.

From late May through mid-August of 2011 and 2012, I banded, took forearm and weight measurements of all *M. lucifugus* and *E.fuscus* adults and juveniles. Additionally, each time juveniles were captured, I took back-lit high-resolution macro lens photographs of 4th digits of the wings with a reference ruler (in millimeters) on a light box. When mother-pup pairs were found, they were banded, and juveniles were sexed and inspected for the presence of an umbilicus, then returned to their roost location without measurements to minimize disturbance that could lead to pup rejection [22,23]. *M. lucifugus* captured in June 2011 were banded with individually numbered 2.8 mm celluloid split-ring bands (Avinet, Inc., Dryden, NY), while those captured July 2011 or later were banded with Porzana-brand 2.9 mm metal alloy lipped-ring bands (Porzana, Ltd., Icklesham, U.K.) arrived. I generally replaced celluloid bands with Porzana bands when bats were recaptured, except when females found with pups attached. In this case, I recorded mother-pup pairs in field notes and immediately returned them to their original roost location without taking any additional measurements. Since it took about 12 weeks for Porzana bands to arrive, it was impractical to order this type of band for *E. fuscus* when they were first noticed in late May. Therefore, all *E. fuscus* captured in 2011 were banded with individually numbered 4.0 mm celluloid split-ring bands (Avinet, Inc., Dryden, NY). I wrote numbers on the bands with permanent marker, but most of these became illegible by 2012. In addition to hand-capturing pregnant females and non-volant juveniles inside roosts, adults and volant juveniles were captured by mist-netting outside roosts through mid-August.

Wing-punches were stored on ice and transported the same night to the University of Illinois at Urbana-Champaign, where they were stored at -20°C until I extracted DNA and conducted PCR tests for *Gd*. PCR products that yielded a gel band approximately 624-bp in length were purified and sent for sequencing [24]. Only those sequences with a 100% match when BLASTed against the published diagnostic *Gd* sequence posted on GenBank (accession no. FJ231098) were considered *Gd*-positive. I collected wing-punches from 17 *M. lucifugus* females in May 2011, and 12 *M. lucifugus* females in May 2012.

I used ImageJ software to take measurements of the epiphyseal gap lengths of the proximal 4th phalanges using the digital images of pups' wings. These data were combined with juvenile forelimb measurements and compared to previously published data on wing bone growth rates for *M. lucifugus* and *E. fuscus*, so I could fit growth curves to the data and use these equations to estimate birth dates.[23] I estimated any pup with an umbilicus still attached at 1 day old.[23]

Temperature and precipitation are known to affect the duration of the gestational period in vespertilionid bats.[6,22] Local temperature and precipitation data were be recorded daily, which can be included in the analyses comparing the 2011 maternal season to future years or at other sites. Roost temperature has also been found to affect the duration of gestation, since pregnant females may enter torpor to save energy,[25] so these data will be included in analyses to control for their effects.

RESULTS:

All PCR tests of the wing punches collected in May 2011 and May 2012 were negative for *Gd*. Of the 17 females tested in 2011, 16 of these females had Wing Damage Indices (WDI) of 0-1 (no damage or slight damage), and a 17th female had a WDI = 2 (moderate damage). Most bats had a WDI of 0 or 1, and no bats with WDI > 2 were observed during the 2011 field season, so no correlation was detected between WDI and parturition date. I PCR tested 12 individuals for *Gd* in 2012, all of which were negative. Of the 12 tested in 2012, 2 had WDI = 0, 8 had WDI = 1, and 2 had WDI = 2. There was no correlation between WDI and birthrates or parturition dates in 2012. A separate study of wing damage prevalence in the absence of WNS is currently in review with the *Journal of Wildlife Diseases*. The manuscript will be forwarded once it has been accepted for publication.

Between May and August 2011, I banded 5 *M. septentrionalis*, 200 *M. lucifugus,* and 93 *E. fuscus* (Table 1). From May August, 2012, I banded: no (0) *M. septentrionalis*, 226 *M. lucifugus,* 137 *E. fuscus* (Table 1). This is the first time *M. sodalis* has been reported at this site. In addition to these individuals newly-banded in 2012, I recaptured the following numbers of bats banded in 2011: 54 *M. lucifugus* (29 adult females, 18 juvenile females and 7 adult males); 19 *E. fuscus* (17 females, 2 juvenile males). Unlike the metal bands used on *M. lucifugus*, the celluloid bands used on *E. fuscus* in 2011 were not legible after one year. As a result, I was not able to distinguish which returning females were adults in 2011 and which were juveniles in 2011. I know that the 2 recaptured male *E. fuscus* must have been juveniles in 2011, because I did not band any adult males in 2011. None of the *M. septentrionalis* banded in 2011 were recaptured in 2012. Shelly Colatskie of the Missouri Department of Conservation reported that 4 of the *M. sodalis* (2 adult females, 1 juvenile male, 1 adult male) banded in 2012 were found hibernating in a Northeastern

Missouri mine less than 30 miles from Siloam Springs in January 2013. Small numbers of other bat species were captured and released without banding, including: *Lasiurus borealis* (1 in 2011 and 13 in 2012), *Nycticeius humeralis* (1 in 2012), and *Perimyotis subflavus* (1 in 2012).

Recapture rates for *M. lucifugus* juveniles were low, and I did not find any *E. fuscus* juveniles with an umbilicus attached, so I used published forelimb growth equations for the two species to estimate age in days 1 - 7.[26,27] Since growth rates vary between colonies and years, these dates are inexact, with actual ages possibly being a few days more or less. These approximations for age in days 1 - 7 allowed me to use linear regressions to determine equations that estimate birth dates that could be compared between individuals in *E. fuscus*. I did not have enough recaptures of juvenile *M. lucifugus* to determine age equations by regression, so adjusted published *M. lucifugus* growth equations to fit the data I had from recaptured individuals.

Ages of *M. lucifugus* juveniles were best predicted by the following equations:
If forearm length was less than 33 mm: Age (days) = -8.0 + (0.625 x FL),
where FL = forearm length.
If forearm length was 33 mm or greater: Age (days) = 46.7 - (9.1 x GAP),
where GAP = length of epiphyseal gap of the 4th metacarpal.

Estimated birth dates for *M. lucifugus* juveniles born in 2011 ranged from June 2nd through July 7th, with a median birth date of June 13th, and those born in 2012 ranged from May 13th through July 28th, with a median birth date of June 5th (Table 2). Birth dates for 2011 are similar to documented average dates of birth for *M. lucifugus* in the region,[28] while 2012 are much earlier (Table 3). In 2011, total spring precipitation was 8.6 cm higher than normal and average daily temperature was only 0.3ºC higher than normal; while in 2012, total spring precipitation was only 0.3 cm above normal and average daily temperature was 4.3ºC above normal (Table 3). Reproductive adult females and their female offspring banded in 2011 were assigned to three categories (June 2-13, June 14-25, June 26-July 7) according to approximate date of parturition, and juvenile females banded in 2011 were assigned to three categories according to approximate date of birth (June 2-13, June 14-25, June 26-July 7). For 2011 adult females, June 2-13 n = 21, June 14-25 n = 18, June 26-July 7 n= 6; for 2011 juvenile females, June 2-13 n = 19, June 14-25 n = 14, June 26-July 7 n= 3. For both adult and juvenile females, 2012 recapture percentages were highest for individuals in the early parturition group (June 2-13) and lowest for individuals in the late parturition group (June 26-July 7 - Figure 1). Sample sizes for the groups were too small to yield statistically significant results.

Roost temperature was recorded from late-May through mid-August 2011 and 2012 in the attic where IDNR staff have reported *M. lucifugus* maternal colonies in several years prior to this study. In 2012, I also recorded temperature at a nearby barn where I found a *M. lucifugus* maternal colony, and in the roof of the main shelter house picnic pavilion where I found *Myotis* species night-roosting in May and August. In general, temperatures were most stable (i.e. monthly maximum temperatures where lowest and monthly minimum temperatures were highest) in the night roost and most variable in the barn (Table 4). Reproductive *M. lucifugus* females stopped using the attic roost by early July 2011 and by late May 2012. Roost temperatures hit highs above 37ºC for the first time on June 30, 2011 and on May 26, 2012, respectively.

DISCUSSION:

All individuals tested in 2011 and 2012 for *G. destructans* were negative, so the data collected in these first two years of my field study will be used to establish the effects of ambient temperature, precipitation and roost temperature on parturition dates of *M. lucifugus.* After I have collected data in future years when individuals test positive for *G. destructans*, I will construct generalized linear models of parturition date, using regional temperature, regional precipitation, roost temperature and the presence of *G. destructans* as predictors. I will then use Akaike Information Critirion to determine which model best predicts parturition date. This will allow me to determine will allow me to determine which of these four predictors affect parturition date. Later parturition dates are linked to decreased survival of offspring in *M. lucifugus* vespertilonid bat species, presumably because they have less time to forage in fall to store the fat needed to survive winter.[8,29] Theoretically, this should also result in lower survival for adult females that give birth later, since they will have less time to replenish fat stores after weaning pups, but this has not yet been tested. My results from the first two years of this study show a decrease in 2012 recapture percentages for 2011 adult females and their juvenile female offspring as parturition date increases (Figure 1). The numbers of recaptured individuals from the 2012 season are too small to give statistically significant results, but they suggest that both mothers that give birth later and daughters that are born later in the season have reduced survival. Once I have collected 4 years of field data, I will calculate apparent survival from recapture data using Program MARK,[30] and test for effects of parturition date on both maternal and juvenile survival.

Parturition dates in 2012 were 7-14 days earlier than expected for the region, while 2011 parturition dates fell within the normal range (Table 2). 2012 was the hottest year on record in Illinois and spring precipitation was within the normal range for the region (Table 2).[31] In 2011, the region experienced temperatures within the normal range, with higher than average spring precipitation (Table 2). These early results suggest that spring temperature has a stronger effect on parturition date than spring precipitation. A full analysis at the end of this four year study will determine if that trend holds.

The reason for the relocation of the *M. lucifugus* maternal colony from the attic to the barn is unclear at this point, but may be related to roost temperature. Temperature fluctuates within a much narrower range in the attic relative to the barn (Table 4), but the barn is a much larger, more open structure with a variety of roosting locations (roof rafters, lumber piles, bales of hay, etc.) relative to the attic. Based on comparison of low temperatures (Table 4), the attic provides superior insulation on the coldest nights, but may not offer refuge from high temperatures because it is a closed space with little ventilation. The *M. lucifugus* maternal colonies stopped using the attic as a roost in late June 2011 and late May 2012, which coincides with the first point in each season when the attic temperature exceeded 37ºC. Additional field studies may help clarify the if represents a maximum temperature that triggers bats to relocate.

I plan to complete this study by May 2015. The results of statistical tests associated with this field data will help determine the extent to which *G. destructans* infection, temperature and precipitation can effect *M. lucifugus* survival in the coming year by altering parturition date. In species with slow life histories like bats, maternal survival is usually the single most important factor in determining population growth rates.[32] Therefore, if late parturition reduces survival in adult female *M. lucifugus*, this will likely have a large negative impact on *M. lucifugus* populations. A clear understanding of the effects of *G. destructans* and environmental conditions on parturition date, and also the effects of parturition date on adult female and juvenile survival, will help improve bat population models used by conservationists, and may hold the key to determining the characteristics of maternal roosts that will maximize population growth as WNS takes its toll.

Table 1. Newly banded bats at Siloam Springs State Park, 2011 and 2012. EPFU = *E. fuscus*, MYLU = *M. lucifugus*, MYSE = *M. septentrionalis*, and MYSO = *M. sodalis*. Ad F = adult females, Ad M = adult males, Juv F = juvenile females, Juv M = juvenile males. These numbers include only individuals that were first banded that year. See Results section for rates of 2012 recapture of individuals banded in 2011.

	2011				2012			
	EPFU	MYLU	MYSE	MYSO	EPFU	MYLU	MYSE	MYSO
Ad F	15	79	3	0	42	88	0	2
Ad M	0	41	1	0	4	31	0	3
Juv F	48	47	1	0	51	67	0	3
Juv M	30	34	0	0	42	40	0	6

Table 2. Estimated range of birth dates for *M. lucifugus* juveniles in 2011 and 2012. ΔDOB = difference in birth dates in 2012 vs. 2011 by Julian date. Note: 2012 was a leap-year, so Julian dates in 2012 = 2011 dates + 1 day for all calendar days after February 29.

	2011	2012	ΔDOB
Earliest birthdate	June 2	May 13	-19 days
Latest birthdate	July 7	June 28	-8 days
Median birthdate	June 13	June 5	-7 days

Table 3. Two-year trends in DOB and climate at Siloam Springs *M. lucifugus* colony. Earliest DOB = first-born by Julian date, Spring Temp = mean temperature March 1 - May 31, Spring Precip = total precipitation March 1 - May 31. Aberrant values in bold text.

	2011	2012	average
Earliest birthdate	June 2	**May 13**	early June
Earliest DOB	153	**134**	150-155
Spring Temp (C)	11.7	**15.7**	11.4
Spring Precip (cm)	**11.6**	3.3	3.0

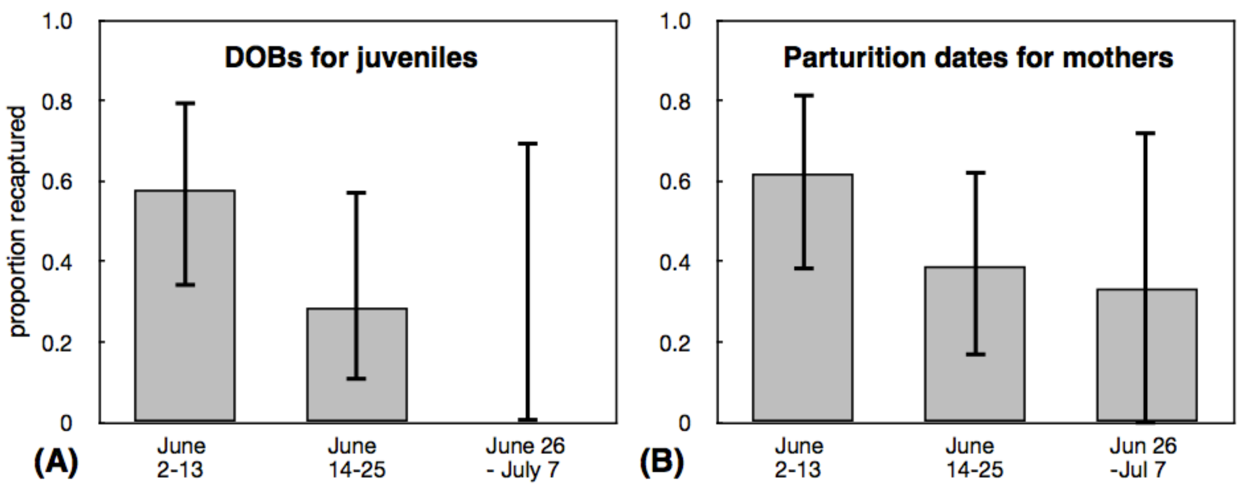

Figure 1. Relationship between 2011 parturition dates and 2012 recaptures of *M. lucifugus* females. A) Proportion of females born in 2011 that were recaptured in 2012, according to approximate dates of birth (DOBs); B) Proportion of reproductive adult females from 2011 recaptured in 2012, according to approximate dates that they gave birth. Error bars represent 95% confidence intervals.

Table 4. Two-year trends in DOB and climate at Siloam Springs *M. lucifugus* colony.
Earliest DOB = first-born by Julian date, Spring Temp = mean temperature March 1 - Ma

	Attic 2011	Attic 2012	Barn 2012	Nt Roost 2012
Lowest May	11.8	15.2	10.7	13.4
Highest May	32.9	38.4	45.6	32.9
Mean May	21.1	27.2	27.5	25.0
Lowest June	17.5	11.3	6.5	10.7
Highest June	38.9	43.2	52.3	38.8
Mean June	25.8	27.7	28.6	25.7
Lowest July	21.9	22.7	18.7	22.5
Highest July	44.7	45.1	54.5	40.0
Mean July	31.9	32.2	33.2	30.4
Lowest Aug	20.4	16.4	12.2	17.4
Highest Aug	43.1	40.4	50.7	36.1
Mean Aug	29.3	26.9	27.9	25.5

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