

Original Research

Individual Variation in Parturition Timing within and among Years for a Bat Maternity Colony

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Abstract

Background: In monoestrous species, the timing of reproduction can have important impacts on offspring survival. For heterotherms in temperate areas, parturition timing is constrained by cold weather survival strategies, such as hibernation and torpor. Female bats that are year-round residents of temperate regions, such as little brown myotis (*Myotis lucifugus*), invest significantly in parental care resulting in sharp changes in behavior immediately following parturition. These behavior changes may include increases in nighttime roost revisits, which can be used to identify parturition dates for individual bats that have been passive integrated transponder (PIT) tagged and use monitored roosts. **Methods:** Using a system of tagged bats and monitored roosts in Pynn's Brook and Salmonier Nature Park Newfoundland, Canada, we estimated parturition dates for 426 female *M. lucifugus* in at least one year, based on changes in nighttime roost revisit patterns, and quantified the variation in parturition dates within years among individuals, and within individuals among years. **Results:** Overall, we report on a wide variation in parturition dates within years among individuals as well as year-to-year variations, both across the population and within individuals. Spring weather conditions appeared to be important influences on parturition timing. **Conclusions:** Changes in spring and summer temperature and extreme weather events, as expected due to ongoing climate change, may impact parturition timing, and therefore, offspring survival of temperate bats.

Keywords: behavioral ecology; *Myotis lucifugus*; passive integrated transponder (PIT) tags; reproductive timing

1. Introduction

In species characterized by energetically expensive parental care, individuals must maintain their own energy balance while investing in the care of their offspring. In many mammals that exhibit parental care, particularly in temperate environments, parturition is also timed to maximize the probability of offspring survival. In some species, this may mean that offspring are born before seasons with more frequent extreme weather events [1], at times of plentiful resources [2], and/or are relatively synchronous to reduce predation [3]. The phenology of parturition also may vary spatially as a result of resource availability and climate [4]. Any combination of these non-mutually exclusive factors may explain variation in the timing of the parturition of species, populations, and individuals.

Many bat species that are year-round residents of temperate areas undergo a seasonal reproductive cycle consisting of copulation in the fall, sperm storage over the winter, gestation in the spring, and parturition in the spring/summer months [5]. Following winter hibernation, females build up fat deposits to fuel embryonic development [6,7]. However, because spring conditions can at times still be inhospitable, bats use torpor for energy conservation, particularly during periods of increased precipitation and wind gusts [8]. Previous studies have demonstrated interindividual variation in

the use of torpor, with nonreproductive females typically using longer and deeper bouts than reproductive females [9]. Further, among reproductive females, pregnant bats use torpor less than lactating bats [10], as torpor use may slow reproductive processes, including fetal development, and thus, delay parturition [11,12].

Although offspring born earlier in the season may have a greater chance of survival due to a longer period of fat gain before the winter [13–15], the high energetic cost of lactation and low availability of food early in the season means that females must wait until conditions are suitable to support foraging for both mother and pup [16]. Thus, at certain times of the year, females must balance investment in their own survival with that of their offspring to maximize lifetime fitness. Therefore, factors that may explain variation in parturition timing for temperate bats include the timing of their emergence from hibernation (Fontaine *et al.* [17]) and spring torpor use [11,12,16]. Intraspecific variation among females in decisions on hibernation, torpor use, and other factors leads to parturition being asynchronous within populations. As temperate bats are long-lived [18], parturition timing may also vary among individuals of different ages. Given that many bat species produce one offspring per year and are long-lived, there may be changes in reproductive success and timing from year to year as bats



become more experienced.

Some temperate species, including the little brown myotis (*Myotis lucifugus*), form maternity colonies in the spring and summer, where females gather and benefit from social thermoregulation during pregnancy and lactation while males roost solitarily. In these colonies, roost reuse has been regularly observed as well as fission-fusion dynamics of roosting relationships [19]. In this study, we use a long-term (>10 years) monitoring system of maternity roost use of now over 1700 individually marked little brown myotis (*Myotis lucifugus*) in Newfoundland, Canada, and changes in nightly roost revisit behavior in a subset of the adult females to estimate parturition date. Given the high site fidelity of female little brown myotis (e.g., [20]), and parental care responsibilities during the lactation period [21], it is expected that the frequency of night-time visits to maternal roosts will increase at parturition, and therefore, a change in this behavior can be used to estimate the timing of parturition (Fontaine *et al.* [17]). Using these estimates, our goal was to characterize phenological inter- and intra-individual variation in the parturition of little brown myotis in Newfoundland, Canada, across years, by estimating the parturition dates for individual bats in multiple years. We tested hypotheses that may explain inter- and intra-individual variation in the parturition dates, including spring weather conditions, minimum ages, and locations. Additionally, as our unique system consisted of multiple monitored roosts within a small geographical area, we described intra-individual consistency in the used roosts used for parturition and possible instances of bats moving their pups among roosts, to shed light on further decisions that the bat mothers may be making when caring for their offspring.

2. Materials and Methods

2.1 Study System

Female little brown myotis were captured in and around Salmonier Nature Park ($n = 1604$; Lat: 47.3°, long: -53.3°) and Pynn's Brook ($n = 583$; Lat: 49.1°, long: -57.5°) Newfoundland, Canada, between 15 May and 19 August in most years between 2011 and 2021. Bats were captured using mist nets (Avinet, Dryden, NY, USA) and harp traps (Austbat Research Equipment, Lower Plenty, Victoria, Australia), and for each individual we recorded age, sex, and reproductive status. Their age class, either adult or juvenile, was determined based on ossification of the metacarpal joint [22] and individuals first captured as an adult were assigned a minimum age of one for the year of capture, while individuals first captured as juveniles were assigned an exact age of zero. Reproductive status was designated based on palpating the abdomen to detect pregnancy and expressing the nipples for lactation. An obvious bare patch around the nipple with no lactation was designated as post-lactation [23]. Only adult female bats (≥ 1 -year-old) were included in this study. Bats were subcutaneously im-

planted with passive integrated transponder tags (PIT tags; 0.09 g; EID-ID100 implantable transponders, EIDAP Inc, Brampton, Ontario, Canada, and Trovan Electronic Identification Systems, UK) within the scapulae.

Within Salmonier Nature Park, 11 artificial 4-chamber roost boxes, within a 1.1 km² area, were outfitted with Radio Frequency Identification (RFID) transponder antennas (LID650, Dorset Identification, MS Aalten, Netherlands). These antennas continuously monitored the passage of tagged bats into and out of the roost and recorded data on individual ID, time, and date. In 2021, three of the monitored roost boxes were no longer available for bats to use due to degradation. More details on reader deployment and potentially missed detections in Salmonier Nature Park can be found in Sunga *et al.* [19].

Within Pynn's Brook, there were 4 artificial 4-chamber roost boxes paired on 2 trees approximately 20 meters apart. These roost boxes were also outfitted with transponder antennas. These antennas continuously monitored roost use but due to malfunctions and technical issues, there was no data collected in 2018 or after 6 July in 2015. No data was collected in either location in 2020 due to the COVID-19 pandemic. Outside of the noted exceptions, the roost boxes in both Pynn's Brook and Salmonier Nature Park were monitored from approximately May until August. However, across both locations, it is expected that some observations were not recorded due to missed detections or malfunctions at individual boxes. Beyond the technical challenges, not all bats in this location were PIT tagged and the bats also used unmonitored, natural roosts in the surrounding landscape. Thus, for this analysis, we focused on female bats that regularly re-used the monitored, artificial roost boxes to ensure that we obtained sufficient information on their roosting patterns.

All data cleaning and statistical analyses were completed in R version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>) [24].

2.2 Parturition Date Assessment

At the group level, Mundinger *et al.* (2021) [25] estimated the parturition timing based on a "persistent increase" in the nightly arrivals of adult females to a roost, as expected by nursing behavior. Breaking this down to the level of the individual allows an assessment of the factors that may influence the parturition timing in species that reproduce asynchronously. Thus, (Fontaine *et al.* [17]) identified parturition dates by seeking a sequence of three or more nights with a single return to a roost followed by three or more nights with at least two returns. As our system contained multiple roosts, individual bats in our system often revisited roosts at night, even early in the maternity season (Fig. 1), although the revisit behavior was highly variable among individual bats. Thus, we instead estimated parturition dates based on a change in nighttime roost revisit patterns relative to each individual rather than by a prede-

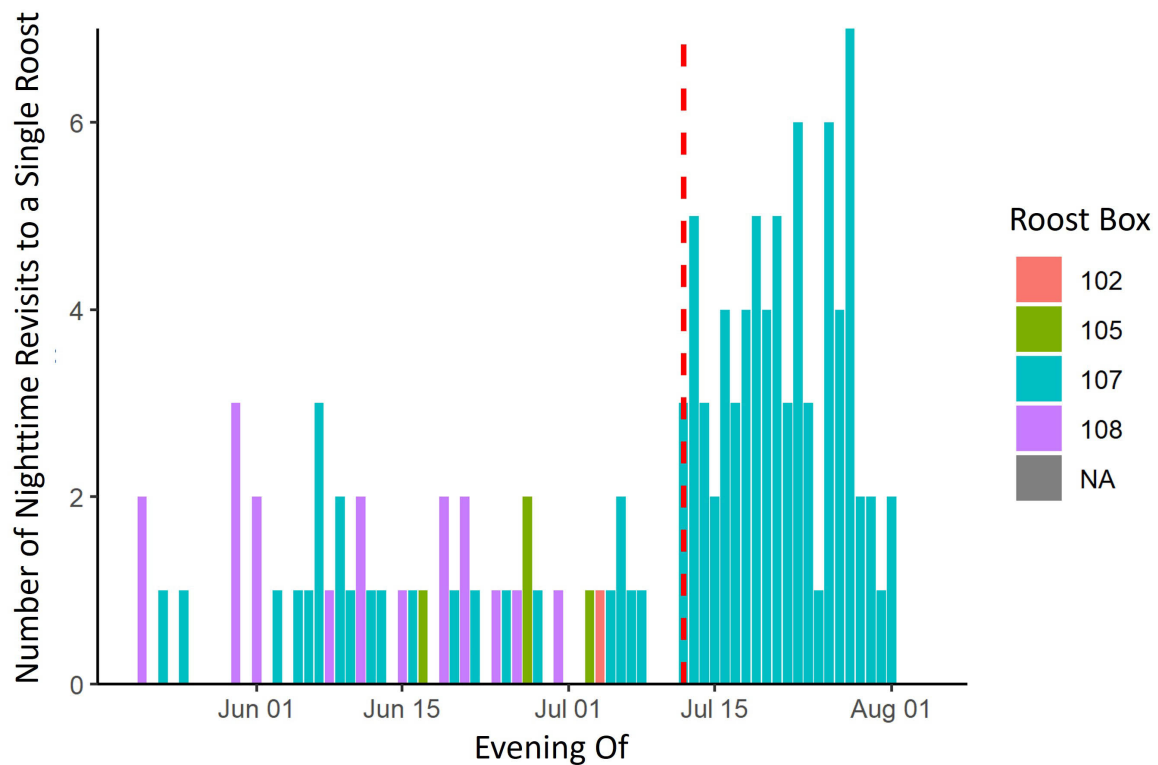


Fig. 1. Example histogram showing the number of nighttime revisits to different roost boxes by a single female *Myotis lucifugus* (PIT ID: 00074EEFAB in 2014) throughout the maternity period in Salmonier Nature Park, Newfoundland, Canada. The red dashed line indicates the detected changepoint, and therefore, the estimated parturition date based on a breakpoint of the mean. Colors represent the different monitored roost boxes.

terminated threshold. We filtered our database within each year to select the individual bats for which we had at least 1 recording on a minimum of 40 days between 1 June and 31 July in any year ($n = 426$ unique individuals). These requirements were selected as the ‘window’, to indicate when we generally expected the parturition to occur based on observations of volant juveniles, and to ensure that our analysis focused on bats that were consistently present in the monitored system.

We identified the parturition date based on roost revisits, where the parturition was estimated based on an abrupt increase in the number of nighttime roost revisits (Fig. 1). We calculated the maximum number of nighttime (23:00–03:00) revisits to a single roost on each night for each individual across all days. To ensure reads at each box were independent and best represented revisit behavior, we further limited our data, whereby the reads at the same roost by an individual were at least 5 minutes apart unless another roost box was visited in between. We used observations up until 4 August, approximately one week prior to when most bats departed from the maternity site. Observations after this date were removed as some little brown myotis displayed a change in roosting habits, often using the monitored roosts infrequently, as they prepared to depart towards swarming locations, which interfered with the changepoint

detection methods described below. Then, we calculated a single change point of the mean using the AMOC (at most one changepoint) method with the `cpt.mean` function in the package “changepoint”, and with an asymptotic penalty value of 0.05 [26]. As this method iteratively assesses for a single changepoint in the mean, occasionally a single night with a large number of revisits could lead to estimates of the parturition date that were likely spurious. Thus, we followed this analysis with a visual inspection of the data and manually corrected estimates that seemed influenced by a single night to either a new parturition date, where a “clear” increase in nighttime revisits was present, or removed in incidents where a changepoint was not clearly visible. Additionally, if the changepoint was influenced by the departure of the bat from the system for a long time (i.e., the changepoint occurred right before a long series of zero nighttime revisits), the estimated parturition date was corrected if a clear increase in nighttime revisits could be detected earlier or later in the season. A “clear” increase in nighttime revisits for visually correcting parturition date was defined by displaying at least three consecutive nights with revisit frequencies all greater than the three preceding nights, a method similar to that of (Fontaine *et al.* [17]) but one that accounted for the possibility of multiple nighttime revisits prior to parturition as a regular behavior of the individual.

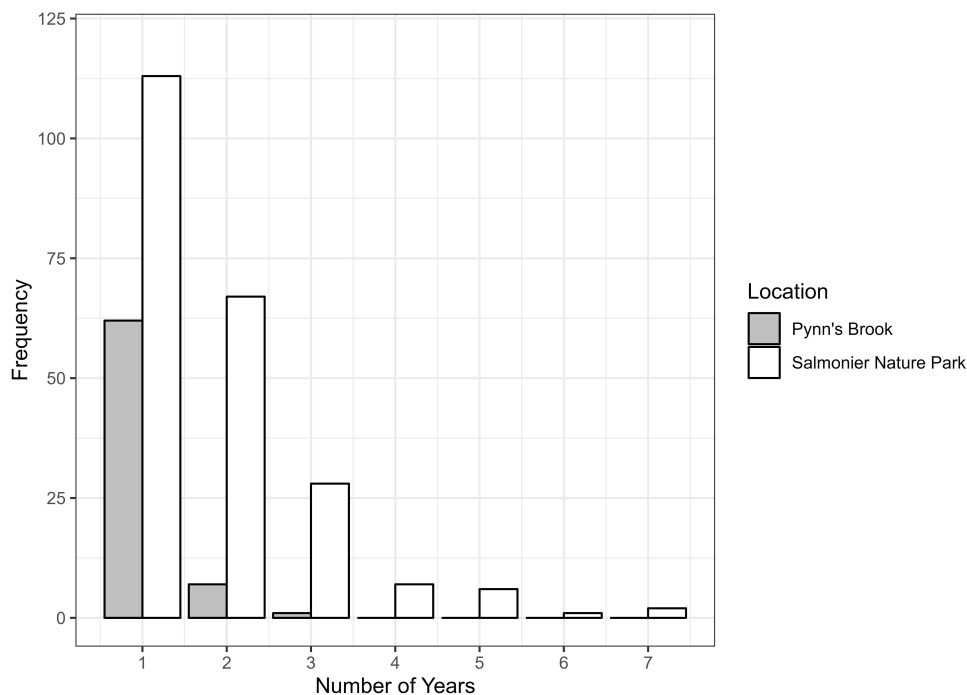


Fig. 2. Histogram of the number of little brown myotis (*Myotis lucifugus*) that were included in multiple years of analysis based on having estimable parturition dates. Individuals were included if they had at least 40-day roost observations between 1 June and 31 July in 1 season and if a parturition date could be estimated based on changes in nighttime roost revisit behavior. Numbers of individuals are separated by the study location in Newfoundland, Canada.

If these criteria were not met and the changepoint analysis was deemed unreliable due to the influence of a single night or potential departure from the system, no parturition date was identified.

As it is not known what time of day little brown myotis give birth to their pups, our estimates of the parturition date may be biased by one day before or after the true parturition date. Given that the bias would be consistent across all individuals it was not expected that this bias would have any effect on our overall conclusions.

2.3 Validation of Reproductive Status

We validated our reproductive status assignments by comparing the change point results to in-hand reproductive assessments for bats opportunistically recaptured within 5, 10, and 20 days of the estimated parturition date. For bats whose pattern of nightly revisits did not indicate a parturition event within our system of monitored roosts, we compared this assessment to available recaptures between 5 July and 30 July, the period when bats were expected to be noticeably pregnant or subsequently lactating if reproductive.

2.4 Parturition Location and Movement

For bats that had a parturition date detected in multiple years (Fig. 2), we also noted the box where the parturition was suspected to have occurred in each year, based on the most frequently revisited box at the detected changepoint in

revisited box behavior.

Within years, we also qualitatively assessed whether there was evidence that the mother had moved the pup to a different roost during the lactation period. To do this, we surmised the location of each pup based on the most revisited box by the mother each night for up to 3 weeks following parturition, the expected length of time for pups to reach volancy [27]. If there was a change in the box that was visited most frequently within a night, we detailed the number of times that this occurred and whether movements were to a box on the same pole, or a box on a different pole in the same area. For Salmonier Nature Park, we also noted whether the movement was between the two closer areas, the visitor's center (VC) and headquarters (HQ), or to the more distant private property (HB). We reported on the minimum number of movements possible, whereby if a bat revisited two roosts the same number of times in a night, and at least one of those roost boxes matched the box used most frequently the night before, no movement was counted. Similarly, in instances where pup movement between different distances may have been observed due to equal revisit counts at multiple roosts, the shortest possible movement distance was recorded.

2.5 Annual and Individual Variation in Parturition Dates

To assess the factors that may influence the parturition timing, we used individuals for which a parturition date could be estimated and conducted a multi-model inference

Table 1. Summary of parturition date detection success and median parturition dates in Newfoundland as measured at Pynn’s Brook and Salmonier Nature Park based on changepoint analysis of nighttime roost revisit behavior.

Year	Number of bats with estimated parturition date	# of bats with no estimated parturition date	Median estimated parturition
2012	76	10	8 July
2013	58	22	12 July
2014	67	98	12 July
2015	29	27	14 July
2016	46	35	20 July
2017	65	57	20 July
2018	35	55	23 July
2019	79	42	18 July
2021	33	40	13 July

analysis using the conditional Akaike’s information criterion (AIC) to rank models. We applied linear mixed effects models with a numeric response for the parturition day of the year to assess how the environmental conditions could have impacted the timing of the parturition. We included individuals as a random effect in all models to account for the fact that individuals may be included in multiple years and to assess the inter-annual variation in intra-individual parturition dates. We also included the year as a random effect in all models to account for additional year-to-year variation that had not been accounted for in our candidate models. To assess the hypothesis on individual experience influencing the parturition date, we created a model with minimum age as a fixed effect, where minimum age was the number of years since the individual was first captured as an adult. We also tested a hypothesis with the study location (Pynn’s Brook or Salmonier Nature Park) as the only fixed effect to determine whether differences in parturition date may be related to the location of the maternity.

As torpor has been observed to be used more frequently in poor conditions [8], while also possibly delaying fetal development [12], we tested a hypothesis on torpor use pressure during pregnancy being the main determinant of parturition timing. The torpor use pressure candidate model included the effects of the mean minimum temperature from 20 April, the earliest detection of individuals in our monitored system, until the first estimated parturition date across all years, and for the mean daily precipitation for the same time period. Daily measurements of parturition and temperature were used rather than hourly due to data availability constraints. These dates were selected as they represent how pregnancy may be influenced by local weather conditions and a wide timespan was used since it was not known exactly when the individual bats would be returning from hibernacula, and variation among individuals was likely. An interaction between temperature and precipitation was also included, where the temperature was calculated as the differences in the degrees from 25 °C, whereby the highest values of this interaction term would correspond to cold and wet days, which were expected to have the most influence on bat behavior [8]. The mean of this interaction

term, from 20 April until the first estimated parturition date (15 June in 2012), was recorded for each year and location. We also tested a more general hypothesis for spring-time weather conditions, which included the above factors in the torpor hypothesis model plus the proportion of days with wind gusts over 30 km/h. No interaction terms were added for this model as our data source did not include wind speeds for days where gusts were less than 30 km/h. We checked for the collinearity of variables using the function `check_collinearity` in the package “performance” [28]. Our variables of days with wind >30 km/h and precipitation were considered to have a low or “tolerable” correlation. The temperature was also correlated with location, although this is expected given that there were only two levels for the factor.

For both locations, weather data were collected from Environment Canada weather stations available through “weathercan” [29]. For Salmonier Nature Park this weather station was at St. John’s International Airport (47.6212°, –52.7424°), approximately 57 km northeast of Salmonier Nature Park. For Pynn’s Brook, weather data were obtained from the weather station at Deer Lake Regional Airport (49.2128°, –57.3943°), approximately 15 km northeast of Pynn’s Brook. All models were generated using the function `lmer` in package “lme4” [30] and AIC scores were obtained using the function `AIC` in package “MuMIn” [31].

3. Results

There were 78 and 348 unique bats that met the minimum criteria of at least one read on 40 days between 1 June and 31 July in at least one year and for which the parturition date could be estimated at Pynn’s Brook and Salmonier Nature Park, respectively. There were no bats that met the minimum criteria in Pynn’s Brook in 2016 or 2017. Within a site, in any given year, the proportion of bats for which a parturition date could be estimated ranged from 39% to 88% (Table 1). The median parturition date was 10 July in Pynn’s Brook (range: 25 June to 27 July) and 15 July in Salmonier Nature Park (range: 15 June to 3 August). This variation was not unexpected since at Pynn’s Brook, pregnant individuals were captured as late as 21 July, while in

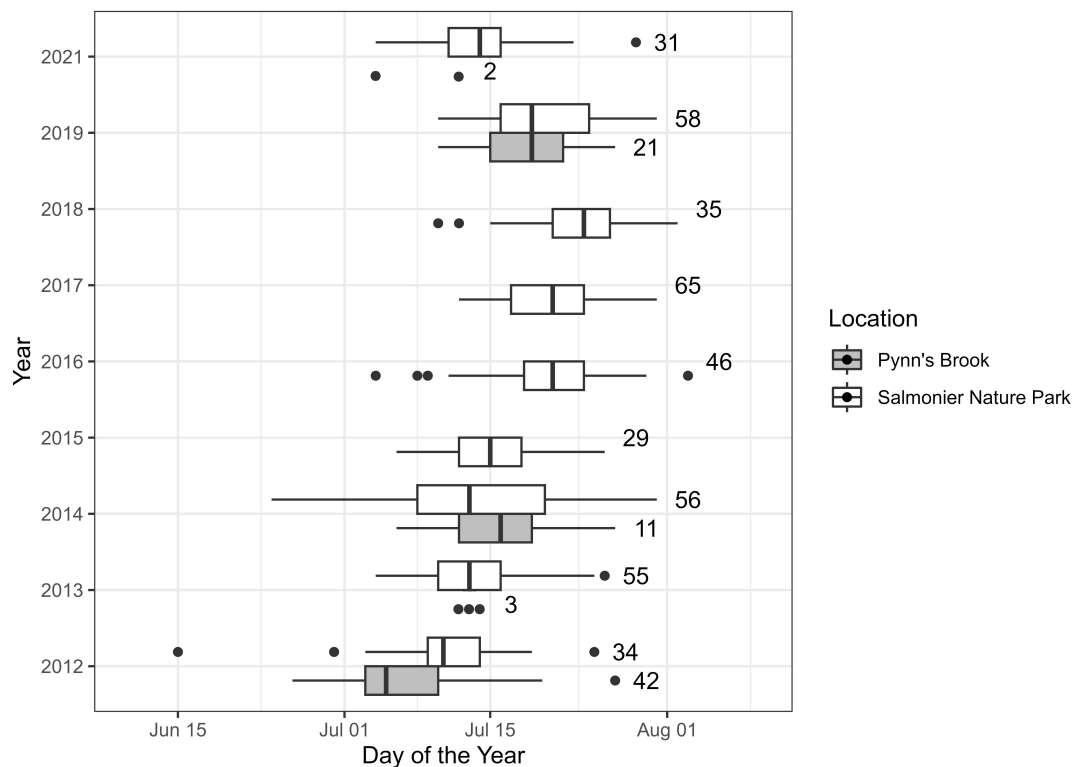


Fig. 3. Distribution of little brown myotis (*Myotis lucifugus*) parturition dates for each year in Newfoundland, Canada, as measured at Pynn's Brook and Salmonier Nature Park. Boxes represent the 50% quantile range and whiskers represent the 95% quantile range. There were no individuals that met the minimum criteria at Pynn's Brook in 2015, 2016, 2017, and 2018.

Salmonier Nature Park, pregnant individuals were captured as late as 26 July.

3.1 Parturition Date Assessment

The number of individuals for which the parturition date could be estimated, and the median parturition date varied from year-to-year (Table 1). The magnitude of intra-year variation in the parturition date also varied among years (Fig. 3). At Pynn's Brook, 8 bats had parturition dates that were detected in multiple years, while in Salmonier Nature Park, 111 bats had estimates of parturition dates in multiple years, while 2 individuals with a detected parturition date in 7 of the 9 study years (Fig. 2). In each year, between 3 and 14 parturition dates were manually corrected due to suspected spurious conclusions based on single nights with a high number of roost revisits or a potential absence from the system. Upon qualitative inspection, increases in the number of nighttime revisits by individual bats were not synchronous within a year or location, and thus, did not noticeably correlate with any extreme precipitation or wind events.

3.2 Validation of Reproductive Status

We report that the parturition estimates were generally consistent with the in-hand reproductive status assignment of the same individuals for reproductive individuals.

For the seven individuals for which in-hand reproductive assessments occurred within five days of an estimated parturition date, six were appropriately assigned as either pregnant or lactating based on when the in-hand assessment occurred relative to the estimated parturition (85.7%). When a recapturing occurred, within 10 days before or after the estimated parturition date, there was an agreement between the reproductive status assigned at recapture and an estimated parturition date in 15 of 16 bats (93.8% correct). In both cases, there was only one bat that was designated as pregnant during the recapturing process, three days after the estimated parturition date. Expansion to 20 days (Table 2) between the recapture and parturition dates caused the recapture reproductive status assignment to agree with the estimated parturition date in 35 of 37 bats (94.6%).

For bats where no parturition date could be estimated, we were not able to reliably designate these bats as nonreproductive. Of the twenty bats where no parturition date was detected and where the bat was recaptured between 5 July and 30 July of that year, only six of these bats were suspected to be nonreproductive during in-hand processing (Table 2).

3.3 Parturition Location and Movement

Based on the most visited box on the estimated day of parturition, individual bats used up to five different boxes

Table 2. Summary of agreement between expected reproductive status based on in-hand assessment and estimated parturition date using changes in nighttime roost revisit patterns of little brown myotis (*Myotis lucifugus*) in Salmonier Nature Park and Pynn's Brook Newfoundland, Canada.

Agreement with in-hand assessment	Expected reproductive status based on estimated parturition date				
	Pregnant		Lactating		Nonreproductive
	5 days	20 days	5 days	20 days	
Correct	5	13	1	22	6
Incorrect	1	1	0	1	14

For expected pregnant and expected lactating bats, an agreement was assessed based on in-hand assessments within 5 and 20 days of the estimated parturition date, while for expected nonreproductive bats, an agreement was based on in-hand assessments between 5 July and 30 July.

Table 3. Conditional Akaike's information criterion (AIC) values and the difference from the top-ranked models (ΔAIC), Akaike's weights (w_i) for all models, and the sum of Akaike's weights (Σw_i) for models comprising $\geq 95\%$ of Akaike's weights, which explain the parturition date of little brown myotis (*Myotis lucifugus*) in Pynn's Brook and Salmonier Nature Park Newfoundland, Canada, between 2012 and 2021. All models, except the null model, also included individual and year as a random effect.

Hypothesis	K	AICc	ΔAIC	w_i	Σw_i
Global (Temperature + Precipitation + Temperature*Precipitation + Wind + Experience + Location)	9	2909.017	0	0.694	0.694
Weather (Temperature + Precipitation + Temperature*Precipitation + Wind)	7	2910.875	1.86	0.274	0.969
Torpor (Temperature + Precipitation + Temperature*Precipitation)	6	2915.272	-17.34	0.030	
Location (Capture area)	4	2922.377	-10.23	8.72×10^{-4}	
Experience (Minimum Age)	4	2930.358	-2.25	1.61×10^{-5}	
Null	2	3133.31	224.29	1.37×10^{-49}	

* The asterisk denotes an interaction term between two variables.

for parturition across all of the years that they were studied. Between years, a bat used at least 5 different boxes for parturition, a bat used at least 4 different boxes, 11 bats used at least 3 different locations, and 65 bats used at least 2 different locations. These numbers represent a minimum estimate, while it is possible that more locations were used, which is why the parturition was not detected at our monitored roost boxes.

Across all the bats and years where the parturition was estimated, in only 17.0% of bats was there no evidence of the bats moving their pups at all in the three weeks following the estimated parturition, based on nighttime roost activity recorded via passive monitoring. Many bats did appear to move their pups, with 54.6% of the bats suspected to have moved a pup between boxes on the same pole at least once, and 46.6% of bats suspected to have moved a pup among poles within a small zone of the study area at least once. Surprisingly, potential pup movement between poles was more frequent than pup movement between boxes on the same pole in Pynn's Brook (Fig. 4). The same pattern was not seen at Salmonier Nature Park. In Salmonier Nature Park, pup movement between boxes on the same pole was the most frequent by total count, followed by pup movement between poles within the same zone, then, pup movement between the closer zones of the headquarters

(HQ) and visitor's center (VC; Fig. 4). Potential pup movement to the private property (HB) after parturition occurred at least once in only 3.4% of individuals (14 of 408), while potential pup movement among the headquarters (HQ) and visitor's center (VC) occurred at least once in 31.4% of individuals.

3.4 Annual and Individual Variation in Parturition Dates

Parturition dates varied greatly among years even within individuals in both Salmonier Nature Park and Pynn's Brook. Our top model set included both the global model (Akaike's weight 69.4% and corrected R-squared 0.63) and the weather hypothesis model (Akaike's weight 27.4% and corrected R-squared 0.65; Table 3). Greater precipitation was related to earlier parturition dates; however, the interaction of precipitation and temperature indicated that years with colder and wetter conditions resulted in later parturition, while warm and rainy conditions may have resulted in earlier parturitions (Table 4). The model-averaged estimates for other variables plus or minus the standard error crossed zero; thus, we cannot conclude the importance of these variables from our data (Table 4).

There was considerable interannual variation in parturition dates within individuals. Parturition in some individuals appeared to be consistently later or earlier than the rest

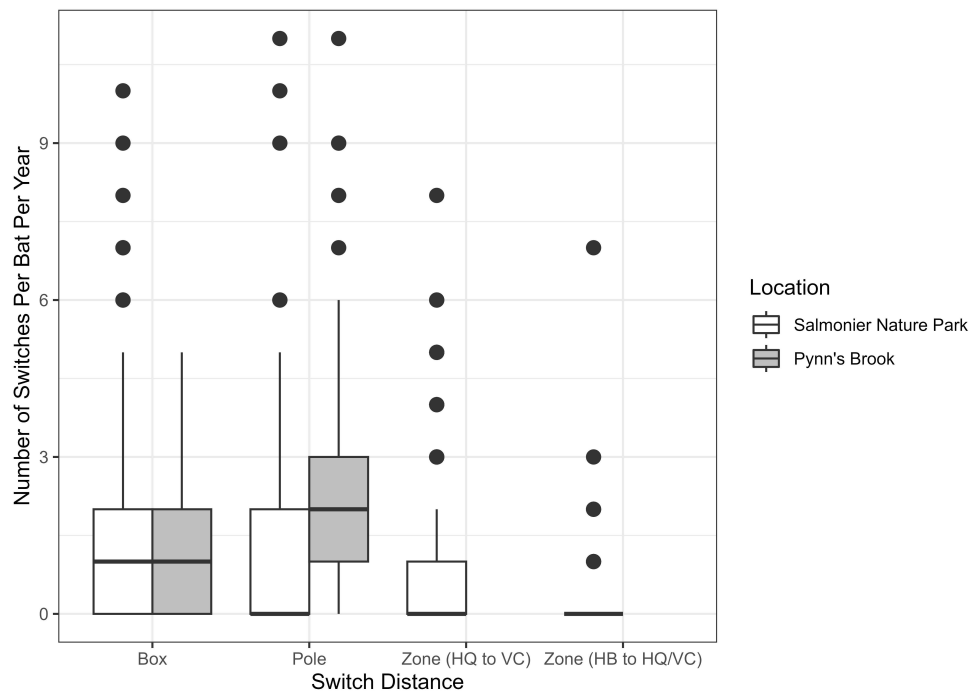


Fig. 4. Observed frequency of suspected pup movement based on changes in the most revisited box by adult female *Myotis lucifugus* in Newfoundland, Canada, between 23:00 and 03:00. Counts are based on 21 nights following estimated parturition to represent when pups were expected to not yet be volant, and thus, had to be carried by their mothers. “Box” movements represent moves between two monitored roost boxes on the same pole, “Pole” movements represent moves between two poles within the same area of the study site (distance <50 m), and the maximum observable movement distance at Pynn’s Brook. For Salmonier Nature Park, “Zone” movements are also included, which are separated into headquarters (HQ) to visitors’ center (VC), representing movement between zones (separated by ≈ 100 m), and movements between HQ or VC and the more distant private property on the study site (HB; max distance ≈ 1 km).

Table 4. Model averaged parameter estimates and standard errors for the 95% confidence set of models explaining the parturition date of little brown myotis (*Myotis lucifugus*) in Pynn’s Brook and Salmonier Nature Park Newfoundland, Canada, between 2012 and 2021.

Parameter	Estimate	Standard error
Precipitation	−26.08	9.76
Temperature*Precipitation	1.12	0.45
Minimum age	0.12	0.21
Temperature	−0.51	1.90
Capture location (Salmonier)	3.12	3.12
Proportion of days with maximum windspeed >30 km/h	−1.85	9.93

* The asterisk denotes an interaction term between two variables.

of the individuals at the same location in multiple years, although this trend was not seen in many individuals in this study population (Fig. 5). The random effect of individuals had a variance of 14.2 ($\pm$ SD 3.4), although this was small relative to the overall residuals of the model 25.8 ($\pm$ SD 5.1).

4. Discussion

By using changes in nighttime roost revisit behavior, we were able to estimate the parturition dates for 426 individuals across two locations and multiple years. Ex-

pectedly, parturition was asynchronous among individuals within sites and varied within individuals between years at both Salmonier Nature Park and Pynn’s Brook, Newfoundland. Among years, spring-time weather conditions explained only some of the variation in parturition timing from year to year. The apparent variation in parturition date in response to spring-time weather conditions, particularly precipitation and its interaction with temperature, means that parturition timing, and thus, reproductive success, may be susceptible to anticipated changes in weather patterns due to global climate change.

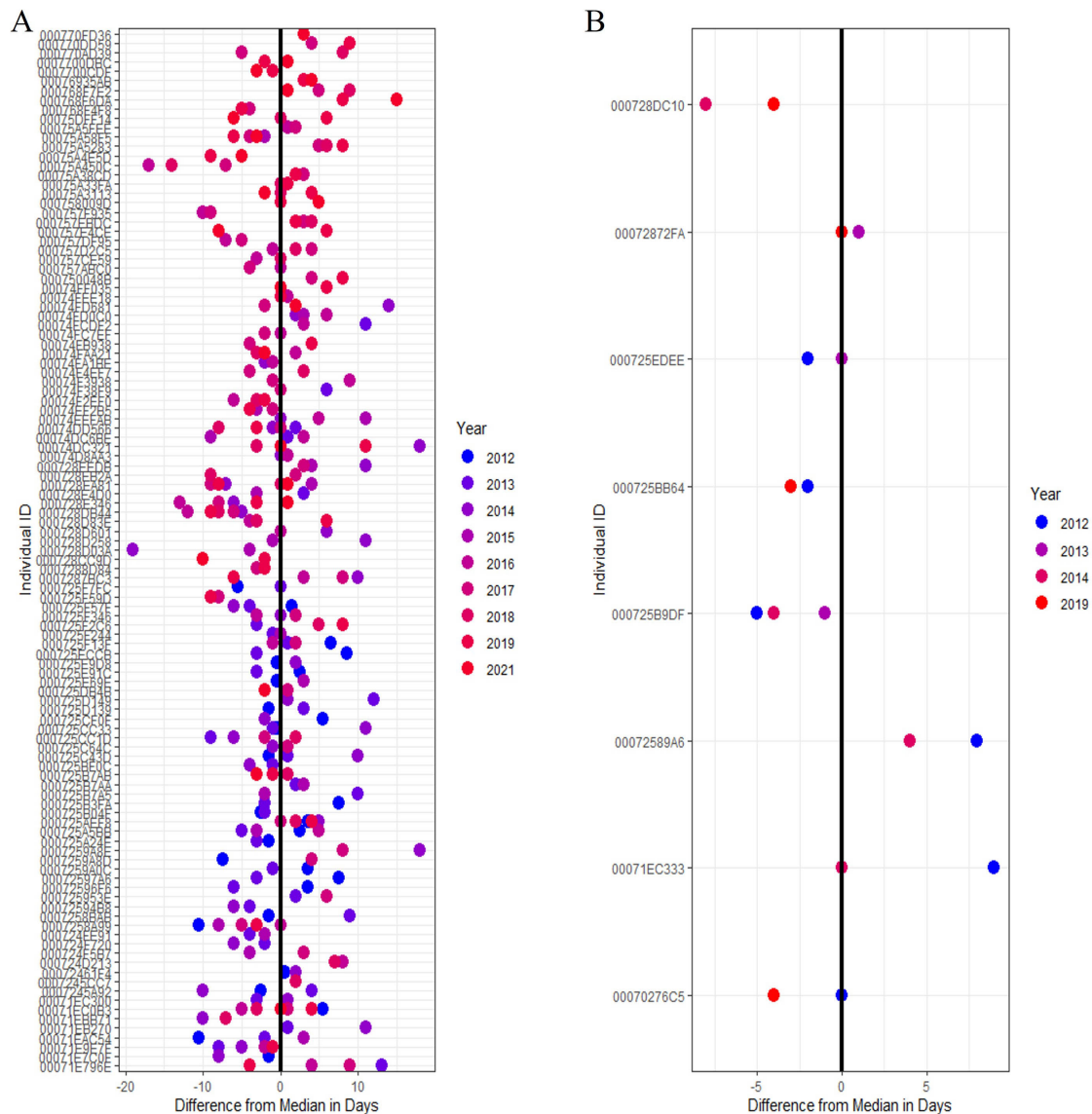


Fig. 5. Difference in individual parturition dates from the population median, represented as a solid black line, in (A) Salmonier Nature Park (N = 111) and (B) Pynn's Brook (N = 8) in each year for little brown myotis (*Myotis lucifugus*), where parturition date was estimated for at least two years using a breakpoint in the mean of nighttime roost revisits. Each point represents the difference in the estimated parturition date from the population median in the corresponding year and the color of the dot represents the year.

Overall, the asynchrony of parturition timing, as estimated by our method, provides reasonable confidence that these patterns are not driven by external factors, such as weather variation; however, it cannot be entirely ruled out that social behavior may, in part, also drive changes in nighttime roost revisit behavior. For individuals where parturition dates were estimated, the parturition date and assignment of the individual agreed in 93.8% of cases, when in-hand assessments occurred within 10 days of the estimated parturition date and 85.7% of cases within 5 days. Although this sample size was small since capture efforts were not scheduled specifically to assess reproductive timing and typically capture efforts aim to avoid the peak of population parturition to prevent potential pup mortality,

this provides us with some confidence that the changepoint method can reliably designate reproductive individuals into the correct reproductive period and estimate parturition timing with an accuracy of at least ± 5 days. Further examples of recaptures closer to the estimated parturition dates and captures of juveniles at the immediate onset of volancy would help to solidify this method. Meanwhile, bats for which a parturition date could not be detected could not be reliably designated as nonreproductive. To make these sorts of conclusions, we would require greater coverage of potential roosting sites within each study location. An evaluation of nighttime roosting behavior in known nonreproductive individuals based on capture assessments would also assist in eventually identifying nonreproductive individuals from

roost use data alone.

For individuals for which a parturition date could be estimated, the parturition dates varied greatly from year to year. The interaction of temperature and precipitation from mid-April until the beginning of parturition had an expected influence, whereby the colder and wetter springs resulted in later parturition, a result consistent with findings in other myotis species [32]. These conditions favor torpor use in bats, thereby delaying parturition [12], as demonstrated in little brown myotis [33], and are consistent with findings in other studies where warmer springs led to earlier parturition dates [17,34]. However, contrastingly, we did not observe any influence on parturition timing by spring temperature, outside of the interaction with precipitation. One potential reason for this is that the spring temperatures at hibernacula may be more influential once the bats arrive at the maternity sites, given that spring conditions also affect departure from hibernacula, and therefore, parturition timing [34]. In our system, greater precipitation appeared to result in earlier parturition, once the interaction between cold and rain was accounted for. This may be due to an increase in insect abundance with greater levels of precipitation, which has also been previously associated with higher adult survival [16]. Cumming and Bernard [35] found that African bat species timed parturition so that juveniles were weaned right before maximum insect abundance. Given that there may be a delay between increased precipitation and peak insect abundance, bats may be timing parturition to improve survival outcomes for their young. However, it has been shown in other studies that increases in precipitation can delay parturition or reduce parturition success as reported by Burles *et al.* [33]. This may be attributable to the reduced ability of little brown myotis to forage in wet conditions due to their aerial hawking foraging strategy [33]. Therefore, it is possible that the increased precipitation in Newfoundland may eventually no longer result in earlier parturition timings.

As climate change predictions call for an increase in global mean temperature, including increases in temperature across the island of Newfoundland [36], there is the potential for a decrease in cold and wet spring conditions, and thus, for parturition timing to become progressively earlier, which may improve survival outcomes for juvenile little brown myotis in Newfoundland. This shift was not seen in our 10-year study, although this time period may simply be too short to demonstrate these effects directly. This expected shift was found over a 40-year period in bats in the Czech Republic, where warming temperatures were correlated with a shift to earlier parturition timing in Daubenton's bats (*Myotis daubentonii*; [34]). These projected future changes in temperature may change the roosts that are the most suitable for having pups. Conversely, climate projections for Newfoundland currently predict an increase in extreme precipitation events over the next 50 years [37]. Given the large amount of precipitation already occurring

on the island of Newfoundland, an increase in precipitation, particularly extreme precipitation events may be detrimental to insect availability and thermoregulation, and therefore, little brown myotis reproduction. A previous study showed a decrease in little brown myotis reproductive success with increased precipitation [33], possibly attributable to fewer insects flying during rain events [38] and interference with echolocation abilities [39,40]. It is unclear why some bats use certain roosts for parturition or move pups between roosting locations, yet it remains that these patterns suggest that a community of roost are advantageous for population viability. Roosting options in the face of changing environmental conditions will allow a behavioral buffer for animals to adjust to variable climate conditions.

Parturition timing at Pynn's Brook, Newfoundland was on average six days earlier than in Salmonier Nature Park; however, based on our analysis, the location did not appear to be an influential factor in predicting parturition timings. Despite that, Pynn's Brook is farther north than Salmonier Nature Park and had lower minimum temperatures, while there was generally also less precipitation in Pynn's Brook than in Salmonier Nature Park. It is uncertain whether differences in climatic conditions between the two locations are biologically meaningful to cause differences in parturition timing and success, and what other environmental differences between the two locations may impact little brown myotis reproductive patterns.

The minimum age did not appear to have an effect on the reproduction timings. Given that we do not know the exact age of the individuals assessed here and the long lifespan of this bat species, it is uncertain whether our study truly reflects a relationship between parturition timing among different aged individuals or if there may be a change in parturition timing within individuals as they age. Further, given that parturition dates were progressively later from 2012 to 2019, environmental changes may be masking potential age effects in our study system. It is additionally possible that there is a nonlinear effect of age on parturition timing and success, such that there is an optimal age range where females are best able to give birth to offspring early in the year after a few years of experience, yet before any detrimental effects from older age, as seen in pinnipeds [41]. We are not able to assess these long-term effects here due to only having a few years of estimated parturition dates for most individuals compared to the decades-long life expectancy of the species [18].

Within years and within locations, parturition was expectedly asynchronous with some years more variable than others. Whether an individual was consistent between years also varied by individual. Many individuals changed their parturition timing greatly between years, although there were a few individuals that were consistently among the earliest or latest parturition dates each year relative to the rest of the population. This variation in the consistency of individual parturition timings was reflected in our mixed

effects model, as although the random effect of the individual had a low standard deviation relative to the predicted value, the variance attributed to the individual was small compared to the remaining overall unexplained variance in our models. Thus, there are likely other factors that better explain why individual parturition timing varies within and between years that we have not included in this study. Additional factors that could influence the timing of parturition on an individual basis include previous reproductive success, the fat stores accumulated in the previous year, winter weather conditions, and spring foraging efficiency, as these would influence the energy stores females have available for pregnancy and lactation [10,21]. Since we do not know where the bats in our study system hibernate, we were unable to assess many of these factors, though some of this may be captured in the random effect of year, which also had a low standard deviation relative to the predicted value. Similarly, variation in the timing of the parturition will influence when bats leave for hibernation as the lactation period cannot be shortened, only extended in response to food scarcity [5].

Although synchronicity of parturition has been shown to correlate with better reproductive success both in bats [33] and other mammal species, such as bison [42], variation in the above factors and parturition timing within the population may assist in ensuring population persistence through changing climate conditions and increasing frequency of extreme weather events. Just as varied individual behaviors in reproductive timing will provide resiliency for the population against changing climatic effects, it is also important to provide variation in habitat, particularly roosts for parturition and lactation as weather conditions continue to change. Our findings of bats using different roosts for parturition over the years emphasize that these habitat options may provide opportunities for behavioral compensation to weather conditions. Thus, on the island of Newfoundland, the continued protection of natural habitats, particularly forests with dead-standing trees and anthropogenic roosting structures, is imperative. Better information is needed on population reproductive success in both study locations, including information on the proportion of reproductive individuals, survival of offspring through winter hibernation, and how offspring survival is related to the parturition timing, and roosting decisions of the mother. These efforts and this information would greatly assist both in understanding the reproductive ecology of little brown myotis in Newfoundland and in best directing efforts to maintain stable populations.

5. Conclusions

Our results show wide variation in parturition date within years among individuals, and within individuals among years. Spring weather conditions were an important influence on parturition timing, and thus, changes in spring and summer conditions may impact parturition timing and

subsequently the survival of *Myotis lucifugus*. We suggest that variation in parturition timing improves population resiliency to changing conditions and emphasize the role that variable habitat may play in accommodating different reproductive timing and strategies within a population.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available as they are part of an ongoing, long-term research program but are available from the corresponding author on reasonable request. Example code used for analysis described in this manuscript is publicly available on GitHub at https://github.com/juliasunga/parturition_timing/. Weather data used in this manuscript are accessible through weathercan in R.

Author Contributions

JS and HB designed the research study, performed the research. JS wrote the manuscript and analyzed the data. JH and HB oversaw initial data collection. All authors contributed to editorial changes in the manuscript. All authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be included as contributors to this work.

Ethics Approval and Consent to Participate

All animal handling protocol was approved by the animal care committee of Saint Mary's University, Halifax, Nova Scotia (AUP number 16-12) and the University of Waterloo, Waterloo, Ontario (AUP number 30066). Wildlife scientific research permits were also obtained from the Government of Newfoundland and Labrador, Department of Fisheries and Land Resources, Forestry and Wildlife Branch for each year of the study.

All handling and PIT tagging was performed by trained project members. Animals were held individually after capture and prior to processing. During PIT tagging, a cloth was placed over the individuals eyes/head to reduce stress during the procedure, and following the tagging, bats were held for 5 min to allow surgical adhesive to dry and to ensure animals were in good health prior to release. Holding and handling times were kept to a minimum and all were released at the point of capture.

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Conflict of Interest

The authors declare no conflict of interest.

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