

Intrinsic and extrinsic sources of variation in size at birth and rates of postnatal growth in the big brown bat *Eptesicus fuscus* (Chiroptera: Vespertilionidae)

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(Accepted 18 December 2001)

Abstract

We examined the relationship between intrinsic and extrinsic variables and variation in size at birth and rates of postnatal growth in the big brown bat *Eptesicus fuscus* at maternity roosts in central Massachusetts and southern New Hampshire. Body mass and length of forearm at birth were lowest when precipitation in the month preceding births was relatively high. In addition, body mass at birth was significantly lower when ambient temperatures during late pregnancy were relatively low. Pups born to middle age mothers were heavier than pups born to young or old mothers. When evaluated using mark–recapture data (longitudinal method), postnatal growth in length of forearm and body mass was greater for pups born to middle age and older mothers and change in body mass was greater for female pups than for male pups. When growth data were evaluated using the trajectory method, the rate of growth for length of forearm was greatest when ambient temperature was highest. Age prediction equations were improved when maternal age was incorporated into predictive equations. We recommend considering both intrinsic and extrinsic variables in studies where maternal effort is evaluated and age prediction equations are used for estimating postnatal growth rates in free-ranging bats.

Key words: size at birth, postnatal growth, *Eptesicus fuscus*, ambient temperature, maternal age

INTRODUCTION

Climate is an important variable that influences reproduction in temperate insectivorous bats. The annual cycle of cold winters and warm summers in temperate regions restrict pregnancy and lactation in bats to a relatively narrow period in spring and summer following arousal from hibernation and before the pre-hibernation fattening in autumn (Tuttle & Stevenson, 1982; Kunz & Stern, 1995; Kunz, Wrazen & Burnett, 1998). Within this limited period, individual variation in rates of growth and development should largely reflect constraints on a mothers' ability to maximize offspring survival and, ultimately, her reproductive success. Environmental, or extrinsic, conditions may profoundly influence the abundance and quality of food consumed and the costs of thermoregulation. Physiological and genetic, or intrinsic, factors limit the maximum rate of pup growth and patterns of nutrient transfer between a mother and her offspring. Several studies have shown that weather can have a marked effect on growth in bats. Holroyd (1993), Hoying & Kunz (1998) and

Reynolds (1999) found significantly slower rates of postnatal growth in temperate insectivorous bats when summer temperatures were relatively colder and had greater amounts of precipitation than other years examined. Because cool temperatures and rain reduce insect abundance (Williams, 1961; Taylor, 1973), reduced dietary intake should lead to a decrease in milk output by mothers and lowered rates of growth by pups. In addition, inclement weather can increase the use of torpor in both pregnant and lactating females (Audet & Fenton, 1988; Hamilton & Barclay, 1994; Grinevitch, Holroyd & Barclay, 1995; Hoying & Kunz, 1998). Torpor slows metabolism and processes associated with maintaining a developing foetus (Racey, 1973) and production of milk (Wilde, Knight & Racey, 1999), thus further reducing growth rates of bats.

Variation in rates of postnatal growth reflects trade-offs between the costs of maintenance and reproduction in the mother and benefits of maximizing investment in offspring. The costs of reproduction may be higher for younger than for older animals (Clutton-Brock, 1991). Age-related differences in the cost of reproduction in bats are likely to be associated with inefficient foraging, inexperience in suckling young and poor condition following hibernation, as is suggested by lower post-hibernation body mass in young *E. fuscus* females

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(Holroyd, 1993) and low overwinter survival in young bats (Tuttle & Stevenson, 1982). Gender-based differences in body size are also associated with inherent differences in rate or duration of postnatal development (Clutton-Brock, 1991).

Despite direct and indirect evidence suggesting that both intrinsic and extrinsic variables can influence postnatal growth, rarely are these variables considered simultaneously. The goal of this study was to assess the effects of intrinsic and extrinsic variation on size at birth and postnatal growth in the big brown bat *Eptesicus fuscus*, and to evaluate the value of incorporating these variables into age prediction equations. We predict that extrinsic variables are more likely to affect body mass than length of forearm at birth and during postnatal growth. Variation in maternal energy requirements for maintenance can largely be attributed to variation in foraging success and thermoregulation. Maternal demands for calcium and phosphorus, necessary for bone development, should be less variable. Thus, we predicted that body mass and to a lesser extent length of forearm will become larger when ambient temperatures are higher and precipitation is lower. We also predict that mass and length of forearm at birth and during postnatal growth will be greater in female than male pups, resulting in reverse sexual dimorphism found in adults of this species. Finally, because of inexperience, size at birth and postnatal growth rates of pups born to younger mothers should be lower than that of pups born to older mothers.

METHODS

Study species

Postnatal growth of *Eptesicus fuscus* was quantified in 1996, 1997 and 1998 at maternity colonies located in barns in Harvard and Sterling, Massachusetts (42°58'N, 71°58'W, elevation 128 m, 42°43'N, 71°76'W, elevation 153 m, respectively) and at 2 sites in Milford, New Hampshire, U.S.A. (42°83'N, 71°68'W, elevation 80.2 m). In New England, female *E. fuscus* arrive at maternity roosts in April and early May where they give birth to 2 offspring during early to mid June. Females wean and typically abandon maternity roosts after c. 5 weeks of lactation and pups abandon the roost within a few weeks of being weaned. Destinations of *E. fuscus* in autumn and winter are largely unknown, but these bats sometimes hibernate alone or in small groups in both caves and buildings in winter (Kurta & Baker, 1990; Whitaker & Gummer, 2000).

Field procedures

Before and during the birthing period, each colony was visited every 2–4 days. Presence of newborn pups was easily determined without disturbing roosting animals, based on their nearly continuous audible calls during

the afternoon and following the evening departure of females. Newborn pups were captured while attached to their mothers in late afternoon and evening. Following the onset of parturition, pups were captured weekly after mothers left the roost to forage and in the early morning immediately after mothers returned to the colony. Mother–pup pairs were captured during the later period. If mother–pup pairs were not captured within the first week post-partum, the probability of determining the identity of the mother of a pup was greatly reduced because the pups became more active and often became separated from their mothers during capture attempts.

Throughout our 3-year study, only 1 marked pup was found attached to a female other than the female it was attached to at first capture. During the holding period, mothers and their pups were allowed to cluster in cloth bags to help reduce stress and to maintain euthermic body temperatures. It was assumed that the umbilical cord dries and falls off within 1 day and eyes open within 2 days (Kunz, 1974), thus pups with closed eyes and an attached umbilicus were categorized as day 1 and those pups without an umbilicus but closed eyes were categorized as day 2. Pups were sexed and marked with numbered, 4.2 mm, lipped forearm bands (Lambournes, Ltd and National Band Company). Body mass, length of forearm and length of epiphyseal gap were measured as described by Kunz & Anthony (1982). Length of the forearm reached an asymptote before the epiphyses of the digits closed and thus the length of the epiphyseal gap is often measured in bats to extend the period of reliable age estimation in pups (Anthony, 1988). An Ohaus portable electronic balance (model CT200) was used to determine body mass (± 0.01 g), dial callipers were used to measure the length of the right forearm (± 0.01 mm) and a Bausch and Lomb dissecting microscope fitted with an ocular micrometer (± 0.05 mm) to measure the length of epiphyseal gaps. For the latter measurement, the wing was transilluminated with a MineSpot miner's lamp (Mine Safety Appliance) and total length of the right fourth metacarpal–phalangeal epiphysis was measured. Lactating females were banded, weighed and length of forearm measured in the same manner. Tooth wear of lactating females was assigned to 1 of 5 categories as an indicator of relative age (adapted from Christian, 1956 and Holroyd, 1993): 1, canines sharp; 1+, canines slightly rounded but tips not flattened; 2, tips of canines worn flat; 2+, canines worn at an angle and > two-thirds of the tooth remained; 3, canines worn at an angle and > two-thirds of the canines were missing as a result of wear. Bats were returned to their roost and all accessible banded individuals were recaptured weekly, weighed and measured for lengths of forearm and epiphyseal gap.

Many individuals were not assigned consistently to the same tooth wear category, with most of the variation occurring between the whole number (e.g. 2) and the more advanced state of wear within the same numerical category (e.g. 2+). In addition, fewer animals were

assigned the '+' categories. Thus, to reduce inconsistencies and for a more balanced design, tooth wear categories were subsequently collapsed into 3 categories, 1, 2 and 3, with 1+ categorized as 1 and 2+ as 2. Following these reassignments, c. 80% of females were scored consistently within a season. Of those pups that were recaptured, the identity of 65% of their mothers were known. For each of these pups, relative maternal age was assigned as 1 (youngest), 2 (middle age), or 3 (oldest) based on the tooth wear of the mother (hereafter maternal age). When tooth wear of the mother increased during the season, the earliest score was used. When the tooth wear score was less than that recorded in previous captures, the most frequently assigned score to that female was used.

Temperature and precipitation data

Daily precipitation and minimum and maximum temperatures were obtained from the National Climatic Data Center (<http://www.ncdc.noaa.gov>) for weather stations occurring closest to each field site (hereafter ambient temperature). Mean daily temperature was determined by adding the minimum and maximum temperatures and dividing by 2. Data were available for Milford, New Hampshire but not for Harvard and Sterling, Massachusetts. The closest weather station to Harvard was in Lowell, Massachusetts (26 km) and the closest to Sterling was in Worcester, MA (19 km).

Roost temperatures were recorded in all colonies in 1998 for the month following peak births. HOBO temperature data loggers (Onset Computer Corp., Pocasset, MA) were placed on wooden beams in the barns near the most frequently used roosting sites. Actual temperature of the microclimate surrounding roosting bats was expected to be higher than that recorded by the instrument, as bats typically cluster in small mortises and crevices. HOBO data loggers were programmed to record temperature every 5 min. We used maximum and minimum temperatures recorded each day for comparison.

Statistical analyses

All statistical analyses were conducted using SAS 6.12 (SAS Institute Inc., Cary, NC). Means are presented with standard deviation. We accepted comparisons as statistically significant at $\alpha=0.05$. For all multivariate statistics, partial-*F* and *P* values are given for significant parameters as an indication of the contribution of each to the overall model.

Data on daily precipitation, minimum and maximum ambient temperatures were compared between years and locations using general linear models procedure (Cody & Smith, 1991) for the period of greatest foetal growth, 30 days before the day of peak births and the lactation period, 30 days after the day of peak births, for known-age pups within each corresponding roost.

Minimum and maximum roost temperature and the deviation of minimum and maximum roost temperature from ambient temperature were also compared for 1998 using general linear models. Data from Milford II were excluded from the analysis because of an instrument malfunction.

To determine which extrinsic (ambient temperature and precipitation) and intrinsic variables (gender and maternal age) made a significant contribution to variation in size at birth, stepwise regression was carried out using the stepwise selection technique and a significance level of $\alpha=0.15$ for entry into the model (Cody & Smith, 1991). Since minimum and maximum daily temperatures are not independent, mean daily temperature was used for all comparisons with postnatal growth. In addition, the time of day that bats were collected may have influenced growth parameters. To control for this variation a 'time collected' variable was added: morning = pups collected between 04:01 and 12:00, after mothers returned from nightly foraging when pups were most likely to suckle from their mothers; afternoon = pups collected between 12:01 and 20:00, when pups were roosting with mothers but vocalizations from the pups suggested that they were less likely to be suckling; night = pups collected between 20:01 and 04:00, when mothers left pups in the roost while foraging. Although mothers returned sporadically to suckle young during the night, for analysis of size at birth, all pups were collected when mothers were absent from the roost. Comparisons for size at birth were made among known-age pups on day 2, because samples sizes were greater and more evenly distributed than on day 1.

To determine which intrinsic and extrinsic variables made a significant contribution to inter-individual variation in rates of postnatal growth, stepwise regression with similar parameters to those described for size at birth was used. Growth data were examined using both the longitudinal (Kunz & Anthony, 1982) and the trajectory methods (McOwat & Andrews, 1995). Longitudinal data are based on changes in mass and lengths of forearm and epiphyseal gap *vs* age for known-age (marked) individuals, i.e. pups must be captured within the first 2 days post-partum so that day of birth is known. Date of birth does not necessarily need to be known for the trajectory method. Growth rates were calculated as described by McOwat & Andrews (1995) and Reynolds (1999), where $R = (L_2 - L_1) / (t_2 - t_1)$, *L* and *t* are the length (*L*) and time (*t*) at capture (1) and (2), thus measurements are based on rate of change, using statistical models based on morphological parameters *vs* rate of growth (*R*). Although the longitudinal method is more accurate and representative than the trajectory method for determining rates of postnatal growth, the results are presented from both methods. Data from 1998 was excluded from the longitudinal analysis because few known-age, marked pups were recaptured between 10 and 25 days of age, although other pups were captured during this period. Thus, the result of a trajectory analysis is also presented so that data from all 3 years could be evaluated.

Table 1. Average daily maximum and minimum temperatures (°C) and precipitation (cm) and total precipitation (T) for 1 month before and following peak births in *Eptesicus fuscus*. Means, standard deviation, and sample sizes are given

	1 month before peak births				1 month following peak births			
	1996	1997	1998	Comparison between colonies ^a	1996	1997	1998	Comparison between colonies ^a
Maximum temperature								
Lowell, MA	23.2 ± 5.8	23.2 ± 5.4	24.1 ± 5.1	A	27.5 ± 3.6	30.1 ± 3.2	26.5 ± 3.6	A
Milford, NH	23.6 ± 4.8	22.6 ± 4.8	23.3 ± 4.1	B	26.8 ± 2.9	29.1 ± 2.5	26.3 ± 3.4	A
Worcester, MA	22.0 ± 5.0	19.7 ± 5.0	21.2 ± 3.9	B	24.0 ± 3.3	26.5 ± 2.4	25.1 ± 2.6	B
Comparison between years	A	A	A		B	A	B	
Minimum Temperature								
Lowell, MA	8.2 ± 4.9	8.7 ± 3.9	8.4 ± 3.2	B	14.6 ± 2.6	15.5 ± 2.4	15.3 ± 2.4	AB
Milford, NH	9.5 ± 5.3	8.3 ± 4.2	9.3 ± 3.3	B	13.9 ± 2.4	14.5 ± 2.6	14.5 ± 2.6	B
Worcester, MA	11.9 ± 4.4	9.1 ± 3.9	11.4 ± 3.6	A	14.8 ± 1.7	16.4 ± 1.8	16.0 ± 2.3	A
Comparison between years	A	A	A		B	A	AB	
Precipitation								
Lowell, MA	0.21 ± 0.49 T = 6.41	0.15 ± 0.63 T = 4.52	0.35 ± 1.33 T = 10.49	A	0.16 ± 0.31 T = 4.85	0.24 ± 0.53 T = 7.13	0.71 ± 1.90 T = 10.01	A
Milford, NH	0.19 ± 0.36 T = 5.55	0.17 ± 0.60 T = 5.16	0.58 ± 1.54 T = 17.33	A	0.12 ± 0.18 T = 5.55	0.25 ± 0.55 T = 7.43	0.34 ± 0.7 T = 10.34	A
Worcester, MA	0.14 ± 0.44 T = 4.10	0.15 ± 0.51 T = 4.41	0.74 ± 1.54 T = 22.20	A	0.38 ± 1.55 T = 4.10	0.15 ± 0.51 T = 4.41	0.23 ± 0.77 T = 6.87	
Comparison between years	B	B	A		A	A	A	

^a Results of Scheffe test; groups that differ are represented by different letters, with A representing the higher values and B representing lower values.

The longitudinal method was used to determine the influence of mean daily temperature and precipitation for the month following peak births, pup gender, maternal age and collection time on change in mass and lengths of the forearm and epiphyseal gap. Growth in length of forearm was curvilinear, thus the covariate age was entered as a squared value. Comparisons were limited to pup ages 1–24 days because no known-age pups born to old mothers were recaptured beyond this age.

The trajectory method was used to examine the effect of mean daily temperature and precipitation 1 month following peak births, pup gender and maternal age on variation in length of forearm. Since this method is based on the rate of change in length of forearm between 2 dates during the linear phase of growth, data from 1998 was included. However, time of day could not be entered into the analysis because of inconsistencies in the time individuals were collected. Body mass and length of epiphyseal gap were too variable to establish growth trajectories. The trajectory method showed apparent negative rates of growth in length of forearm for some individuals, where time between sampling periods was <4 days, probably due to measurement error exceeding minimal changes in growth. To allow for a greater period of growth and

maintain a consistent range of days between points used to describe growth rates, our comparison is limited to 7 (± 2) days of linear growth.

Growth data from known-age marked individuals from 1996 and 1997 were used to derive age-predictive equations from longitudinal data for estimating the age of pups when date of birth was unknown (Kunz & Anthony, 1982). Because lengths of forearm and epiphyseal gap were more strongly correlated with age than with body mass, lengths of forearm and epiphyseal gap were used for estimating age. Length of forearm was used for predicting age during the linear phase of growth before the asymptote, and the linear decrease in length of epiphyseal gap was used to estimate age of pups when length of forearm was greater than or equal to the length at the forearm at the asymptote.

The best predictive equations were identified by comparing residuals, actual vs estimated age, where length of forearm and epiphyseal gap length were the independent variables and age was the dependent variable, representing all possible ranges of ages. The standard deviation was calculated for the residuals and the proportion of estimates that were ± 3 days of the pup's actual age. Similar calculations were also completed where prediction equations for length of forearm (FA) were divided among the 3 classes of maternal age, the

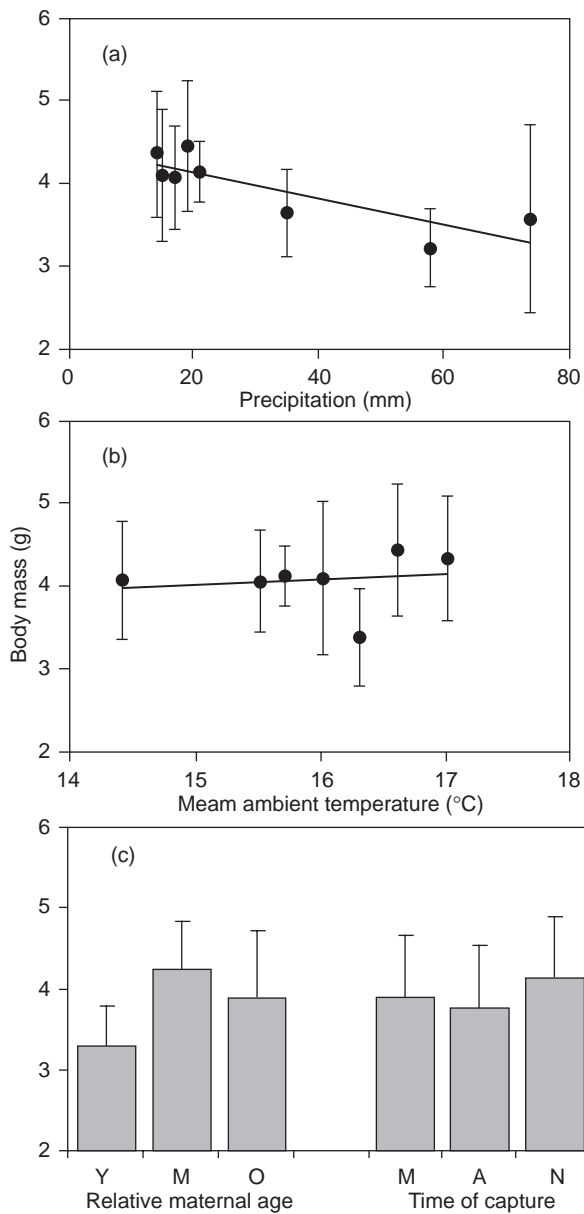


Fig. 1. Variation in body mass of pups at birth in *Eptesicus fuscus* associated with: (a) mean precipitation in the month preceding births; (b) mean ambient temperature; (c) maternal age (Y, youngest; M, middle age; O, oldest) and time collected (M, morning; A, afternoon; N, nights). Error bars, standard deviation.

only variable that made a significant contribution to variation in length of forearm. The standard deviation for the residuals considered all 3 equations, 1 for each maternal age class, simultaneously. No significant intrinsic or extrinsic variable was found that made a significant contribution to length of the epiphyseal gap. The age-predictive equations were chosen to maximize overall accuracy of age prediction that could be applied to all pups up to an estimated age of 39 days. Errors associated with estimating age increased when forearm data with increasing length were added. However, the standard deviation of residuals for length for epiphyseal

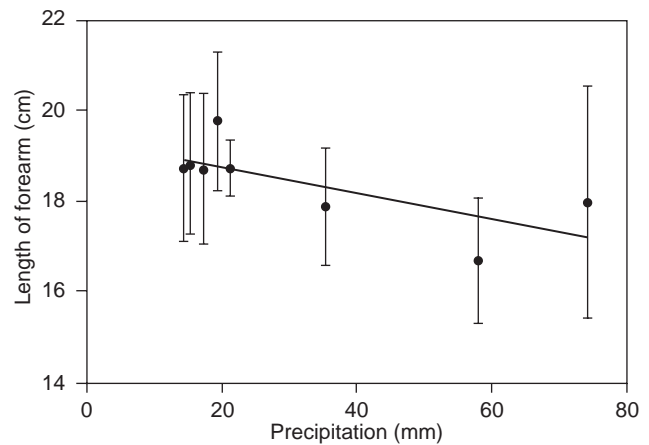


Fig. 2. Variation in length of forearm at birth in *Eptesicus fuscus* associated with variation in precipitation in the month preceding births. Error bars, standard deviation.

gap suggested that the best-fit equation for estimating age from this variable was ≤ 4.8 mm (estimated age 17–39 days). The standard deviations of the residuals for all equations where length of forearm was ≤ 40.4 mm (estimated age 1–17 days), were lower than the standard deviations for epiphyseal gap residuals where the closing epiphysis is > 4.8 mm. Thus, we used a linear regression for length of forearm < 40.4 mm and the linear regression derived from lengths of epiphyseal gaps for pups with forearms > 40.4 mm.

RESULTS

Temperature and precipitation

Precipitation, maximum and minimum ambient temperatures between years and colonies were compared 1 month before and 1 month after the day of peak births at each colony. A significant difference was found in precipitation between years with more rain falling in 1998 before births ($F_{4,264} = 2.66$, $P < 0.034$, year: partial $F_2 = 5.02$, $P < 0.008$; Table 1), but no difference between years for the month after births ($F_{4,264} = 0.73$, $P > 0.571.560$, year: partial $F_2 = 1.06$, $P > 0.346$, Table 1). There was no difference in precipitation between locations. Daily maximum and minimum temperature before births did not differ between years, however, Lowell (nearest Harvard) had significantly higher maximum temperatures than other locations and Worcester (nearest Sterling) had significantly higher minimum temperatures than other locations (maximum $F_{2,264} = 4.25$, $P < 0.003$, location: partial $F_2 = 7.09$, $P < 0.002$; minimum $F_{2,264} = 4.81$, $P < 0.001$, location: partial $F_2 = 7.68$, $P < 0.001$; Table 1). Maximum temperatures were highest in 1997 and Lowell and Milford were significantly warmer than Worcester for the month following births ($F_{2,264} = 20.1$, $P < 0.001$, year: partial $F_2 = 20.9$, $P < 0.001$, location: partial $F_2 = 19.3$, $P < 0.001$; Table 1). Daily minimum temperatures also

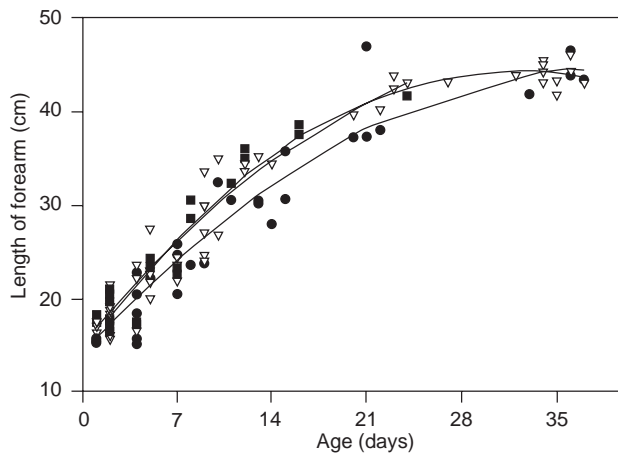


Fig. 3. Variation in postnatal growth in length of forearm associated with relative maternal age in *Eptesicus fuscus*. Relative age of pup's mother: ●, youngest; ▽, middle age; ■, oldest.

varied between years and locations following births with no difference occurring between 1997 and 1998, but with higher minimum temperatures and no difference occurring between 1998 and 1996. No difference was found in minimum temperatures between Worcester and Lowell and Lowell and Milford, with the former being slightly higher than the latter ($F_{2,264} = 6.88$, $P < 0.001$, year: partial $F_2 = 4.75$, $P < 0.010$, location: partial $F_2 = 90.1$, $P < 0.001$; Table 1).

There was no difference between colonies in minimum roost temperature ($F_{2,78} = 0.45$, $P > 0.642$) or deviation of minimum roost temperatures from ambient ($F_{2,78} = 0.74$, $P > 0.480$), with each colony maintaining a minimum roost temperature of *c.* 3 °C above ambient. There was also no difference between colonies in maximum roost temperature ($F_{2,78} = 1.56$, $P > 0.217$). Deviation of maximum roost temperatures from maximum ambient temperatures varied by location ($F_{2,78} = 19.4$, $P < 0.001$), with Sterling deviating from ambient by 6.0 °C and Harvard and Milford I deviating by 3.4 °C and 2.3 °C, respectively. Thus, any differences in thermoregulatory costs between colonies should be attributed to differences in ambient temperature.

Body mass and lengths of forearm at birth

Precipitation, mean daily temperature, maternal age and time collected all contributed to inter-individual variation in body mass (overall regression $F_{5,56} = 13.34$, $P < 0.001$). Average mass of pups at birth was as much as 38% greater when precipitation in the month before parturition averaged < 0.2 cm/day than when average daily precipitation was > 0.55 cm/day (partial $F = 55.5$, $P < 0.001$; Fig. 1a). There was also a trend toward pups at birth being heavier when temperatures were warmer in the month before parturition (partial $F = 10.6$, $P < 0.002$; Fig. 1b). Pups born to middle age mothers were largest, averaging 28% larger than pups born to

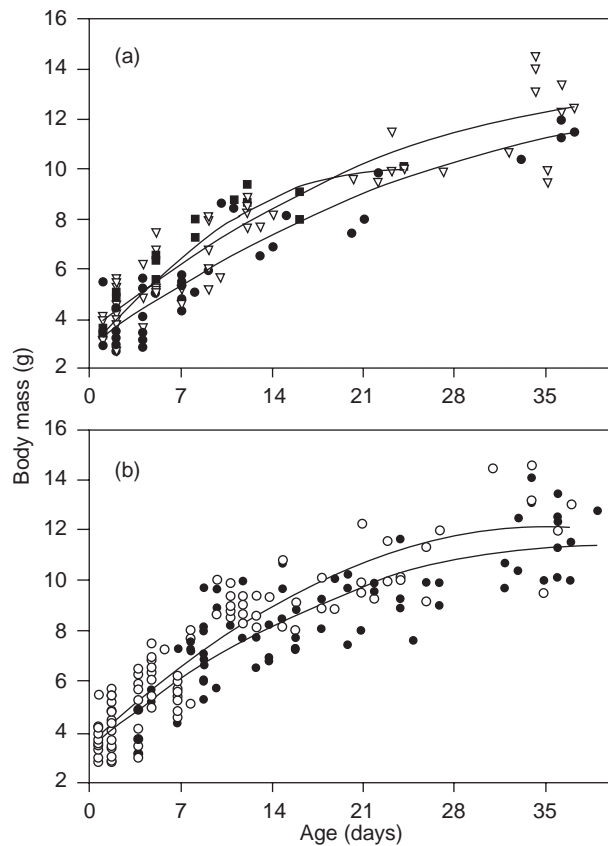


Fig. 4. Variation in postnatal growth in body mass in *Eptesicus fuscus* associated with: (a) relative maternal age (●, youngest; ○, middle age; ▽, oldest); (b) pup gender (●, male; ○, female).

young mothers and 9% larger than pups born to older mothers (partial $F = 7.53$, $P < 0.009$; Fig. 1c). The effect of collection time was also significant, with pups collected at night being heaviest (partial $F = 6.38$, $P < 0.015$; Fig. 1c). Precipitation also had a significant effect on length of forearm at birth but other variables made no significant contribution to variation in length of forearm (overall regression: $F_{5,56} = 35.2$, $P < 0.001$; Fig. 2).

Body mass and lengths of forearm and epiphyseal gap during postnatal growth

Stepwise regression was used to determine if mean temperature and precipitation during lactation, pup gender, maternal age and time collected made a significant contribution to postnatal growth in lengths of forearm and epiphyseal gap and body mass. Maternal age made a significant contribution to variation in length of forearm (overall regression: $F_{2,79} = 520$, $P < 0.001$; maternal age: partial $F = 15.8$, $P < 0.001$; Fig. 3). Both maternal age and pup gender made significant contributions to variation in growth in mass (overall regression: $F_{3,78} = 109$, $P < 0.001$), with pups born to the youngest mothers gaining mass more slowly

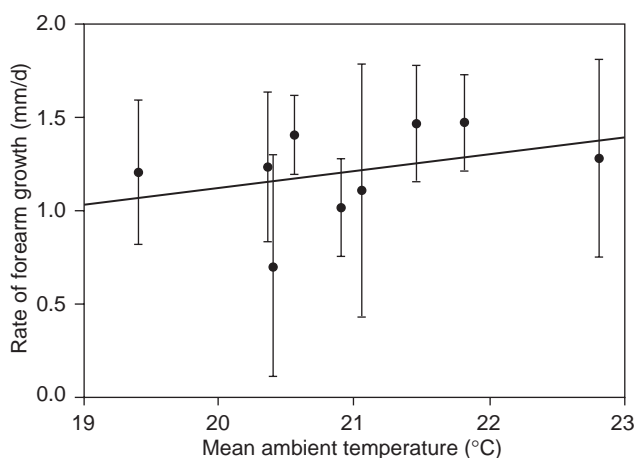


Fig. 5. Variation in rates of postnatal growth in length of forearm associated with mean daily temperatures recorded during lactation in *Eptesicus fuscus* (data for all years combined). Error bars, standard deviation.

than those pups born to middle age and older mothers (partial $F=19.4$, $P<0.001$; Fig. 4a) and female pups gaining mass faster than male pups (partial $F=4.85$, $P<0.031$, Fig. 4b). None of the other intrinsic or extrinsic variables examined made a significant contribution to inter-individual variation in rate of change in length of the epiphyseal gap (overall regression: $F_{1,80} = 27.5$, $P<0.001$).

When a similar comparison was made between intrinsic and extrinsic variables and length of forearm using the trajectory method, mean temperature during the month of lactation made a significant contribution to length of forearm, with growth being greater when average daily temperature was greatest (overall regression: $F_{1,55} = 14.4$, $P<0.001$; Fig. 5).

Age estimation

Growth equations were derived from lengths of forearm (FA) and epiphyseal gap (GAP) of known-age individuals (Fig. 6). The most accurate equations for age prediction were as follows:

When maternal age estimates were available:

if FA < 40.4 mm and maternal age = 1:

$$\text{age} = (0.815 * \text{FA}) - 12.3 \text{ mm};$$

if FA < 40.4 mm and maternal age = 2:

$$\text{age} = (0.606 * \text{FA}) - 9.3 \text{ mm};$$

if FA < 40.4 mm and maternal age = 3:

$$\text{age} = (0.719 * \text{FA}) - 12.5 \text{ mm};$$

if FA 40.4 mm: $\text{age} = (-6.62 * \text{GAP}) + 46.8 \text{ mm}$.

When maternal age estimates were not available:

if FA < 40.4 mm: $\text{age} = (0.666 * \text{FA}) - 10.4 \text{ mm}$;

if FA 40.4 mm: $\text{age} = (-6.62 * \text{GAP}) + 46.8 \text{ mm}$.

When data from known-age individuals were placed into these equations, the standard deviation of the residuals (actual age – estimated age) was 1.17, with 88.5% of estimated values within 3 days of the actual

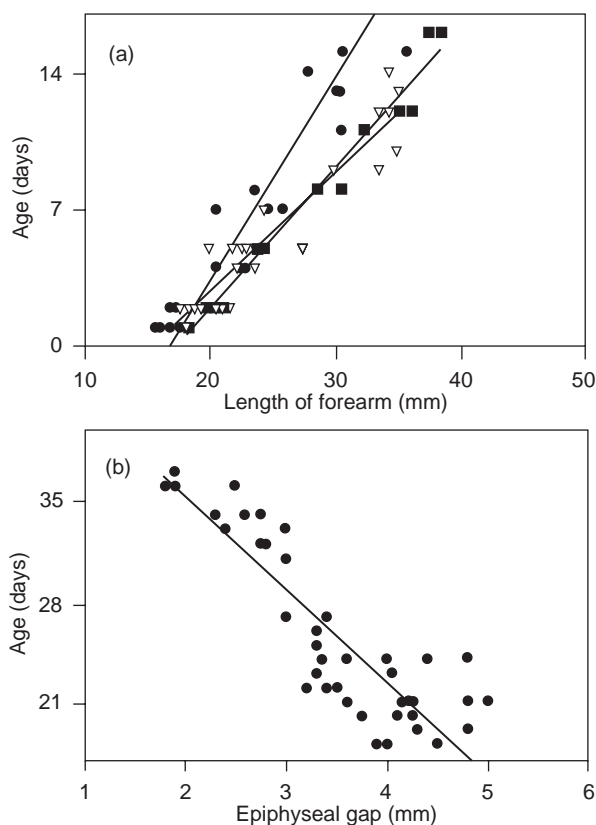


Fig. 6. Age predictive growth curves for *Eptesicus fuscus*: (a) if forearm is < 40.4 cm, length of forearm is applied with best fit prediction equations accounting for maternal age (relative age of a pup’s mother: ●, youngest; ▽, middle age; ■, oldest); (b) if forearm is 40.4 cm, total epiphyseal gap is used for age prediction.

values when maternal age was accounted for. When maternal age was not considered, the standard deviation of residuals was 1.62 with 83.0% of estimated values within 3 days of the actual values. All other possible combinations of equations gave higher residual standard deviations and lower proportions of values within 3 days of the mean.

DISCUSSION

Variation in the size at birth and rates of postnatal growth

Mass at birth in *Eptesicus fuscus* was greater with lower levels of precipitation and higher mean daily temperatures in the month preceding births. Greater rainfall during the second half of pregnancy may have prevented females from foraging and lower temperatures may have reduced insect abundance. Thus, reduced food intake may have caused a decrease in nutrient transfer from mother to the developing foetus. Precipitation had a significant effect on length of forearm at birth, with greater lengths occurring when precipitation was relatively low. Other studies have found no differences

in body mass or length of forearm at birth in temperate bats, except for outliers who were born earlier than others within the colony (Hoying & Kunz, 1998) or died shortly after birth (Holroyd, 1993).

Although there were significant variations during postnatal growth in minimum and maximum temperatures during 1996 and 1997 and between colonies, no effect was found of temperature or precipitation on rates of change in body mass during this period. Differences in temperature were small, all $<6^{\circ}\text{C}$ and there were no significant differences between years or colonies in precipitation. Using the longitudinal method for assessing postnatal growth, no significant effect was found of extrinsic variables on lengths of forearm. However, using the trajectory method, a significant effect was found of mean ambient temperature on rates of forearm growth, with the rate increasing with increasing temperature, when data from 1998 were included in the analysis. This difference may in part reflect the methods that were used to evaluate postnatal growth rates, but it more probably reflects a real effect of temperature on variation in length of forearm when a greater range of temperatures was included in the analysis. No differences were found in roost temperatures between colonies, thus variation in rates of forearm growth are more probably associated with the effects of temperature on insect abundance than the thermoregulatory needs of roosting young. Our data contrast with findings by Hoying & Kunz (1998) who found no variation between years in length of forearm in *Pipistrellus subflavus*, presumably associated with differences in temperature and/or precipitation. They did, however, provide evidence that temperature had a significant effect on growth in length of forearm for pups born outside the range of peak births. Several investigators have suggested that calcium rather than energy maybe an important limiting nutrient during lactation and especially in those species that bear twins (Kwiencinski, Krook & Wimsatt, 1987; Studier, Viele & Sevic, 1991; Barclay, 1994, 1995; Studier, Sevic *et al.*, 1994). It is possible that bats reduce their intake when cooler temperatures reduce insect abundance and thus have lower intakes of calcium, which may influence rates of forearm growth. If energy intake was still sufficient to sustain changes in body mass, body mass may not be affected by ambient temperature whereas growth in length of forearm is more variable. However, since temperature and precipitation did not explain a significant proportion of variation in rates of change epiphyseal gap length, this explanation seems unlikely.

Maternal age had a significant effect on body mass at birth but not on length of forearm, with the pups born to middle age mothers weighing significantly more than pups born to mothers belonging to the youngest and oldest age classes. Low body mass at birth in pups born to young mothers may be influenced by several factors associated with differences in maternal condition and foraging success. Canine wear in older mothers may reduce the extent that food is masticated before swallowing and thus reduce overall digestibility (Holroyd,

1993). This may also account for some of the variation observed between size at birth of pups born to middle age and older mothers. Gender differences in size at birth would imply that mothers are physiologically 'aware' of the gender of their pups before birth and adjust their investment accordingly, or that male and female pups are predisposed to different rates of metabolism and growth *in utero*. Variation in body mass at birth between male and female pups is consistent with findings for *E. fuscus* in Alberta (Holroyd, 1993), although Kunz (1974) found no effect of gender on size at birth for this species in Kansas.

Pup gender and maternal age also had a significant effect on rates of postnatal growth, with body mass and lengths of forearm increasing at reduced rates for pups of young mothers and lengths of forearm growing at a reduced rate in male pups relative to female pups. Although Holroyd (1993) examined the effect of maternal age on size at birth, she did not evaluate the effect of the mother's relative age on the rates of postnatal growth. Our findings suggest that this is an important life-history trait that needs further investigation in studies of postnatal growth in bats. Variation in rates of body mass gain among male and female pups may be associated with differential investment in sons and daughters by mothers or inherent differences in rates of development between sexes. Unfortunately, sample sizes for milk intake (W. R. Hood, pers. obs.) were not sufficient to determine if mothers transferred less milk to sons than to daughters. Reverse sexual dimorphism is exhibited by adults in *E. fuscus* (Kurta & Baker, 1979; Burnett, 1983), thus, it is possible that differences associated with gender are largely influenced by physiological distinctions between the sexes in the regulation of postnatal growth. Forearms of male *E. fuscus* pups grew significantly slower than females during a colder summer in Alberta (Holroyd, 1993). Greater rates of mass gain by female pups corroborate findings for *Myotis lucifugus*, another temperate insectivorous species that exhibits reverse sexual dimorphism (Reynolds, 1999).

Finally, because we found a significant effect of time of day collected on pup mass at birth, with those pups collected at night being greatest, we recommend that investigators collect animals within the same time range or account for time collected in statistical analyses of body mass data.

Age prediction

Our findings suggest that inclusion of maternal age into age prediction equations can significantly improve values for age estimation, although this difference was small, it did increase the number of individuals assigned ages within 3 days their actual age by 5%. We suggest that the effect of both intrinsic and extrinsic variables on age prediction equations be considered in future studies of postnatal growth. Dividing equations among significant sources of variation may only be necessary in

exceptional cases, as in situations with severe deviations in weather from normal conditions.

Acknowledgements

We thank the Ferguson, Frechette, Hoffman and Zahn families for access to their barns for bat collection. Katarina Peterson, Paula Herman, Malory Axtel, Elad Bicer and Bronwyn Williams assisted in the field. This work was supported by grants from the American Museum of Natural History, Sigma Xi, the American Society of Mammalogists to WRH, National Institutes of Child Health and Development (5-T32HD078387) to I. P. Callard and the National Science Foundation (DBI-9605157) to THK.

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