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## Milk composition and lactational output in the greater spear-nosed bat, *Phyllostomus hastatus*

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**Abstract** Growth rates of mammalian young are closely linked to the ability of the mother to provide nutrients; thus, milk composition and yield provide a direct measure of maternal investment during lactation in many mammals. We studied changes in milk composition and output throughout lactation in a free-ranging population of the omnivorous bat, *Phyllostomus hastatus*. Fat and dry matter of milk increased from 9 to 21% and from 21 to 35% of wet mass, respectively, throughout lactation. Energy increased from 6 to 9 kJ·g<sup>-1</sup> wet mass, primarily due to the increase in fat concentration. Total sugar levels decreased slightly but non-significantly. Mean sugar level was 4.0% of wet mass. Protein concentration increased from 6 to 11% of wet mass at peak lactation and then decreased as pups approached weaning age. Total milk energy output until pups began to forage was 3609 kJ. Milk levels of Mg, Fe, Ca, K, and Na averaged 0.55 ± 0.26, 0.23 ± 0.2, 8.75 ± 4.17, 5.42 ± 2.11, and 9.87 ± 4.3 mg·g<sup>-1</sup> dry matter, respectively. Of the minerals studied, calcium appears to be most limiting in this species. The high degree of variability in foraging time, milk composition and milk yield between individuals at the same stage of lactation could

potentially yield high variance in reproductive success among females of this polygynous species.

**Key words** Milk composition · Lactation · Calcium · Bats · Phyllostomatidae

**Abbreviations** *DM* dry matter · *PCE* pulmocutaneous exchange · *BM* body mass · *DLW* doubly labeled water · *Mv* daily milk volume · *Me* daily milk energy output

### Introduction

Lactation defines mammalian reproduction. Although adults of many taxa provision their offspring, only lactation entails extensive morphological and physiological adaptations. Milk, because of its high digestibility (Walker 1979; Robbins et al. 1981), transfers much of the cost of acquiring and assimilating food to the mother, allowing energy ingested by the young to be devoted almost entirely to maintenance and growth (Pond 1977). Because milk composition is physiologically controlled by the mother (and frequently moderated by the suckling behavior of the pup), its nutrient concentration may be correlated with the nutritional needs of the growing young. Duration of lactation varies significantly (Hayssen 1993), from the phenomenally short 4-day lactation period of the hooded seal (Bowen et al. 1985) to the 2-year span of the African elephant (Skinner and Smithers 1990). Growth rates of mammalian young are linked with the ability of the mother to provide nutrients (Robbins et al. 1981); thus, milk composition and output may well represent the best measure of maternal investment during lactation in many mammals.

In general, small mammals have large litter sizes and short lactation periods. Rodents, the most numerous of small mammals, typically produce small litters that are weaned quickly at 30–44% of adult size (Millar 1977); however, bats depart from the typical small-mammal

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lactation strategy. The young are unable to fly or forage independently until they have achieved nearly adult dimensions (Kunz 1987; Kunz and Stern 1995; Barclay 1995). Consequently, near the end of peak lactation mothers must feed young that are nearly of adult size (Kunz et al. 1995). This high nutrient demand not only affects litter size, but may limit growth rate of pups as they approach weaning. As pup growth in bats is dependent on maternal milk production until weaning, milk composition should reflect the nutritional needs of the pup as it develops (Kunz et al. 1995).

## Materials and methods

We studied the omnivorous bat *Phyllostomus hastatus* (Koopman 1993), a primarily cave-dwelling species that roosts in large social groups and exhibits high site fidelity. Study sites in Trinidad included Guanapo Cave, Tamana Cave, and Lopinot-Darceuil Cave (McCracken and Bradbury 1981). Pup growth and water flux were measured at Guanapo Cave, whereas milk samples were taken primarily from bats in Tamana and Lopinot-Darceuil Caves. Age of pups was derived from established growth curves (Stern 1996), thus establishing day of lactation based on age of attached pups. Captured bats were banded with a stainless steel numbered band for identification.

To determine milk intake, pups were captured in their cave roosts soon after adult bats departed at dusk. Since the ceiling of Guanapo Cave is generally less than 3 m, pups were easily captured by hand with the aid of a small stepladder. The population of *P. hastatus* at Guanapo Cave has been the subject of numerous studies (Greenhall 1965; Williams et al. 1966; Williams and Williams 1967, 1970; Williams 1968; James 1977; McCracken and Bradbury 1977, 1981; Kunz et al., in press), and individual potholes and harem roost sites have been identified with painted numbers. We recorded capture location of each pup and returned pups to that location after processing. Pups were injected intraperitoneally with small volumes proportional to body mass ( $\leq 30$  g = 40–50  $\mu$ l; 30–60 g = 70  $\mu$ l; 60 + g = 100  $\mu$ l) of doubly-labeled water (deuterium and oxygen-18). Since milk is the pup's only source of exogenous water, dilution of deuterium over a 24-h period allows calculation of daily milk intake (Ofstedal and Iverson 1987). Hydrogen isotope turnover has been shown to be an accurate measure of milk intake (review: Wright 1982). Dilution of  $^{18}\text{O}$  following injection provided data on total body water. During handling in the field, pups were held in clusters to maintain a euthermic body temperature. After injection and a 60-min equilibration period, approximately 100–150  $\mu$ l of blood were drawn from the anti-brachial vein into two or three heparinized microcapillary tubes. Only 50–100  $\mu$ l were collected from very young pups. Pups were returned to the roost within 2 h of capture, marked with a band covered with red reflective Scotchlite tape to distinguish them from other banded pups. Isotopically labeled bats were recaptured 24 h later and a second blood sample was taken. Blood samples were sealed with critocaps in the field, flame-sealed within 24 h, and kept refrigerated until they could be microdistilled (Nagy 1983). Resultant water was used to determine daily water flux. Methods of isotope analysis are detailed elsewhere (Stern 1996).

Water flux may be overestimated if metabolic water production is not accounted for. Metabolic water produced from oxidation of 1 g protein, fat, and carbohydrate is 0.39, 1.07, and 0.56 ml, respectively (Schmidt-Nielsen 1990). Complete oxidation of milk with a composition of 72.8% water, 9.3% protein, 13.9% fat, and 4.0% carbohydrate (see below) will yield 20.74 ml water for each 100 g milk ingested. Estimates of milk intake from water flux of pups could therefore result in a 28% overestimate of milk volume if all ingested substrates were completely oxidized (from a water flux of 93.5 ml instead of 72.8 ml). In growing pups, however, some of the

ingested energy is deposited as lean mass or fat. Metabolic water production can be calculated using estimates of total water intake and accretion of protein, fat, and carbohydrate (Ofstedal et al. 1987). We assumed that pups accrued 9.7 g fat and 8.1 g protein (T.H. Kunz and K.A. Neelley, unpubl. obs.) during the first 49 days of growth, and that all ingested carbohydrate was metabolized. We combined estimates of pup body composition just before weaning with total water intake measured by doubly-labeled water (DLW) to obtain an estimate of total milk intake to weaning.

Lactating females were collected in mid-morning for milk sampling. Females were separated from their pups and measurements (forearm and body mass) were taken for both. Although females were initially held separately from their pups for 1–2 h to allow milk buildup, we found after the first few weeks of lactation that milk volume was sufficient without a lengthy separation period. Lactating females were injected intraperitoneally with a solution of oxytocin (2  $\mu$ l  $\cdot$  g $^{-1}$  body mass). After 3–4 min milk was expressed through mammary gland palpation, drawn into a capillary tube with a mouth pipette and expelled into plastic microfuge tubes for storage. Milk was kept cool on frozen gel packs in the field and subsequently stored in liquid N<sub>2</sub> or a –30 °C freezer pending analysis. Lactational stage was determined by the age of the attached pup, which was derived from growth equations (Stern 1996) when date of birth was unknown. Milk was collected at 1-week intervals for a period of 11 weeks, after which time milk volume was too small to warrant collection.

Whether milk collection affects growth rate of pups, either by reducing the milk available to the sucklings or by stressing the mother, is unknown. To avoid potential disturbance, milk was collected from bats in other caves or from females whose pups were not being studied for energetics or post-natal growth. Pups were measured once a week when mother-pup pairs were captured for milking; otherwise, pups were collected in the evening, while adults were foraging, to minimize colony disturbance.

*Phyllostomus hastatus* produces relatively large volumes of milk, and collection of 0.5–1 ml of milk from a single individual is often possible. At least 0.5 ml of milk was necessary for a complete proximate analysis (protein, fat, carbohydrate, dry matter). During the first 1–3 weeks, when milk volume was less than 0.5 ml, samples were pooled from several individuals at the same stage of lactation. Subsampling for dry matter, fat, protein, lactose, and mineral analysis was done at the same time to prevent errors caused by unnecessary thawing and refreezing. In the laboratory, milk samples were warmed to 50 °C and vortexed for 10 s before each subsample was taken to improve homogeneity of the subsamples. Dry matter was determined by measuring mass change of samples dried at 100 °C for 4 h (to constant mass).

Proximate analysis of milk was done using microanalytical techniques summarized in Kunz et al. (1995). Fat was determined in duplicate samples using a modified Roesse-Gottlieb procedure (AOAC 1990) whereby milk fat was separated from water-soluble substances by addition of NH<sub>4</sub>OH, ethyl alcohol, diethyl ether and petroleum ether (see Kunz et al. 1995). Nitrogen was determined through a modified Nessler's procedure (Koch and McMeekin 1924; Koops et al. 1975), and crude protein obtained by multiplying total nitrogen by 6.38. Total sugars were measured by the phenol-sulfuric colorimetric method using lactose monohydrate as the standard (Dubois et al. 1956); they are thus expressed as the monosaccharide equivalent. Energy content was obtained by multiplying the protein, fat, and carbohydrate content by 20.1, 39.7, and 16.7 kJ  $\cdot$  g $^{-1}$ , respectively (Gessaman and Nagy 1988). Milk samples were analyzed for mineral content using atomic absorption (N, Mg, Ca, Fe) or flame emission (Na and K) spectrophotometry on a Varian Spectra AA-20 atomic absorption spectrophotometer according to the protocols described in Studier and Sevick (1992).

Data on milk composition are often presented as characteristic of "early," "mid-," or "late" lactation. Although the precise definition of lactation stage tends to be somewhat arbitrary, as lactational changes are gradual rather than discrete, such designations are useful for comparative purposes (Ofstedal and Iverson 1995). We followed the criteria of Kunz et al. (1995) in correlating lactation stage with pup development. We defined early lactation as

the first 4 weeks of lactation. Mid-lactation, from 5–7 weeks, was defined as that phase where rate of pup weight gain had begun to reach an asymptote. Late lactation was defined from the onset of pup foraging at about week 8, coincident with pup achievement of asymptotic mass and when pups began to develop nutritional independence. Definition of lactation length is difficult, as weaning is a process rather than an event (Martin 1984). We used volume collected during milking to determine when lactation ceased ( $x > 0.5$  ml = peak,  $0.5 \geq x > 0.1$  ml = declining,  $x \leq 0.1$  ml = cessation). For analytical purposes, early, mid-, and late lactation were defined as weeks 1–4, 5–7, and 8–11, respectively.

To determine which nutrients might potentially be most limiting to pup growth, we compared milk mineral transfers with mineral accretion in growing pups. The population of *P. hastatus* in our study area, although stable, is small relative to many other bat species. Rather than kill large numbers of *P. hastatus* pups for mineral analysis, thereby disrupting the population and interfering with long-term observations, we extrapolated from published values for mineral accretion in *Myotis velifer* (Studier and Kunz 1995) for our comparison. Mineral concentrations of young *M. velifer* pups at days 1 and 37 were used to estimate the mineral accretion of a *P. hastatus* pup during the same period of development (birth to onset of foraging/weaning). Estimated mineral accretion up to 49 days of a volant *P. hastatus* pup (BM = 63 g) was then divided by estimates of mineral transfers in *P. hastatus* milk to calculate the fractional retention of each mineral studied.

## Results

Length of lactation was variable (Table 1). Some females ceased producing milk ( $\leq 0.1$  ml per collection) at 92 days, whereas others were still producing measurable volumes of milk up to 101 days, when the study ended. Maternal milk production may not be an accurate indicator of weaning, as one female who lost a pup during the eighth week of lactation was still producing milk in measurable quantities 4 weeks later.

Relative proportions of macromolecular components in milk changed throughout lactation (Fig. 1). Dry matter, fat, and energy concentrations increased gradually but significantly. Regression equations describing changes in percent wet mass of macromolecular components are as follows:

$$\text{DM} = 17.7 + 0.2(\text{pup age}) \quad (1)$$

( $F = 27.63$ ,  $df = 1, 31$ ,  $P = 0.0001$ ,  $n = 33$ )

**Table 1** Proportion of captured females at each stage of lactation. Most females had ceased producing measurable quantities of milk by day 100

Lactation Week	Lactation stages		
	mid	late	post
5	1.00	0.00	0.00
6	1.00	0.00	0.00
7	1.00	0.00	0.00
8	0.60	0.20	0.20
9	0.33	0.33	0.33
10	0.20	0.60	0.20
11	0.17	0.50	0.33
12	0.00	0.56	0.44
13–15	0.00	0.29	0.71

$$\text{Fat} = 7.77 + 0.13(\text{pup age}) \quad (2)$$

( $F = 19.82$ ,  $df = 1, 30$ ,  $P = 0.0001$ ,  $n = 32$ )

$$\text{Energy} = 5.28 + 0.06(\text{pup age}) \quad (3)$$

( $F = 15.61$ ,  $df = 1, 21$ ,  $P = 0.0007$ ,  $n = 23$ )

where DM and fat are expressed as percent wet mass, and energy concentration is in  $\text{kJ} \cdot \text{g}^{-1}$ . There was a slight but non-significant decrease in total sugar concentration. Mean total sugar concentration was  $3.96 \pm 1.07\%$  wet mass ( $n = 31$ ). Protein concentration during early, mid-, and late lactation was  $7.8 \pm 1.85$ ,  $9.11 \pm 1.33$ , and  $9.68 \pm 2.54\%$  wet mass, respectively. These values were not significantly different ( $P = 0.2133$ ,  $F = 1.63$ ,  $n = 32$ ).

Mineral composition of milk showed considerable variation (Fig. 2). There were no significant trends over time in Na, Fe, K, or N content. Mean values are given in Table 2. Magnesium and calcium decreased significantly, described by the regressions:

$$\text{Mg} = -0.01(\text{pup age}) + 0.95 \quad (4)$$

( $F = 9.07$ ,  $df = 1, 21$ ,  $P = 0.007$ ,  $n = 23$ )

$$\text{Ca} = -0.11(\text{pup age}) + 13.62 \quad (5)$$

( $F = 7.14$ ,  $df = 1, 21$ ,  $P = 0.01$ ,  $n = 23$ )

where units are in  $\text{mg} \cdot \text{g}^{-1}$  dry matter.

Milk energy and nutrient outputs during lactation can be estimated from milk composition and production. Pups subsisted entirely on milk until 6–7 weeks of age, but lactation continued for 12–13 weeks. Females captured for milk collection produced progressively smaller volumes of milk, however, suggesting a greater degree of nutritional independence in the pups. Water flux increased significantly from birth to week 7 (Fig. 3), expressed as:

$$\text{Daily pup water flux} = 4.34 + 0.24(\text{pup age}) \quad (6)$$

( $F = 18.9$ ,  $df = 1, 19$ ,  $P = 0.0003$ ,  $n = 20$ )

where water flux is in g. Total water intake to day 49, obtained by summation of daily water flux, was 507 g. This amount includes both metabolic water and water contained in the milk. Adjusting for metabolic water (Oftedal et al. 1987), milk intake to day 49 was 552 g. Mean water content of milk to day 49 was 73.4%. Daily milk mass based solely on milk water content,

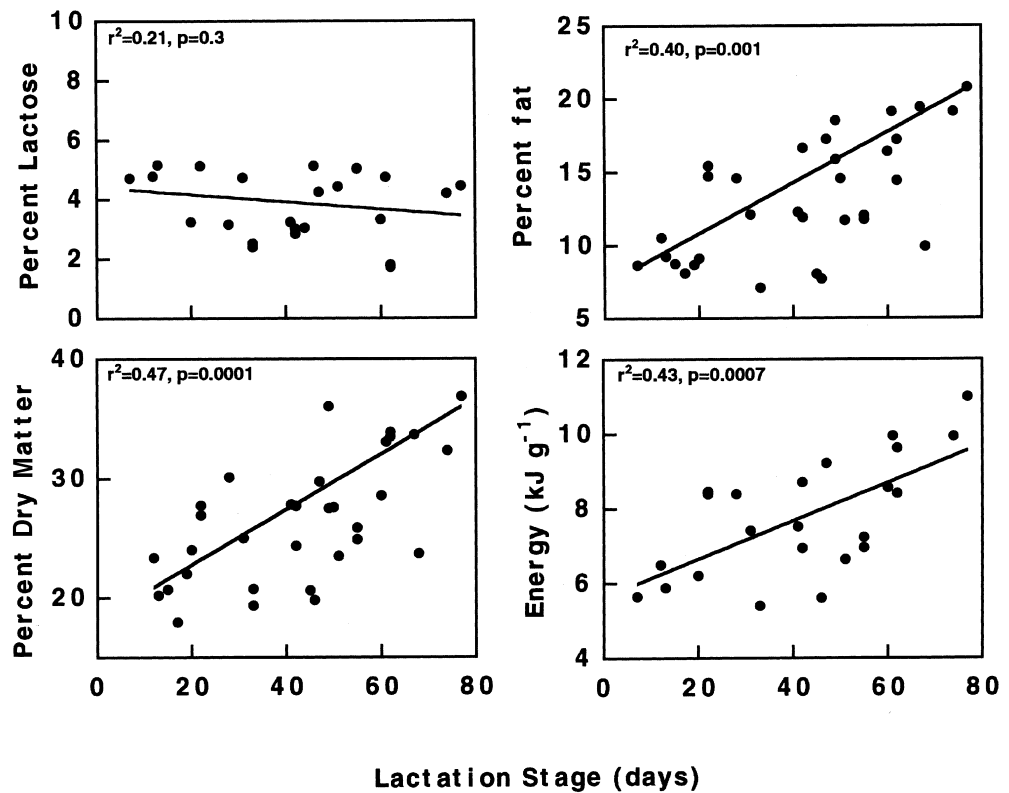
$$M_m = \frac{4.35 + 0.24(\text{pup age})}{0.734} \quad (7)$$

( $F = 18.9$ ,  $df = 1, 19$ ,  $P = 0.0003$ ,  $n = 20$ )

where  $M_m$  = milk mass in g, yields a total milk intake through day 49 of 683 g. Thus failure to account for metabolic water may result in a 24% overestimate of milk production.

If the contribution of metabolic water remains constant, daily milk volume can be expressed as a function of daily water flux. Correcting for metabolic water,

**Fig. 1** Milk composition changes through lactation in *Phyllostomus hastatus* milk. Lactation stage determined by age of attached pup. Sugar decreases slightly but non-significantly. Percent fat, percent dry matter and energy content increase significantly. Regression equations given in text



$$M_m = \frac{4.35 + 0.24(\text{pup age})}{(0.734)(1.24)} \quad (8)$$

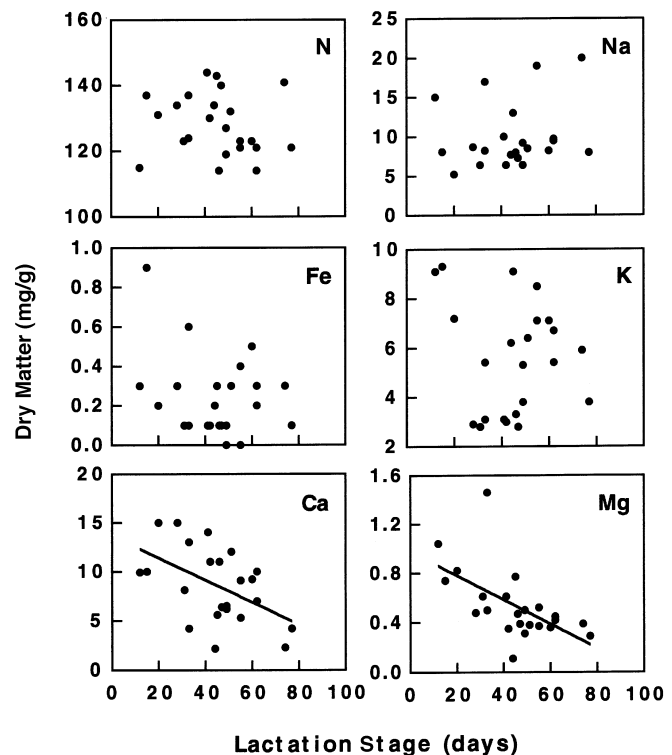
( $F = 18.9$ ,  $df = 1, 19$ ,  $P = 0.0003$ ,  $n = 20$ )

We calculated daily milk energy output ( $M_e$ ) as the product of daily milk mass (Eq. 8) and energy content (Eq. 3):

$$M_e = 25.29 + 1.68(\text{pup age}) + 0.015(\text{pup age})^2 \quad (9)$$

where  $M_e$  = daily milk energy output in  $\text{kJ} \cdot \text{day}^{-1}$ . At the end of week 1,  $M_e = 38 \text{ kJ}$ . At the end of week 6, when pup growth began to reach an asymptote and pups were first initiating flight, estimated daily milk energy output was 122 kJ. Total milk energy output from parturition to day 49 in *P. hastatus* was 3609 kJ.

Once pups began to forage independently, water flux was no longer an accurate indicator of milk intake. We estimated nutrient transfer during lactation, therefore, only to the onset of weaning (day 49) (Table 2). For those components which did not change significantly during lactation (lactose, protein, K, Na, Fe), nutrient transfer was calculated as the product of milk nutrient concentration and mass. Where nutrient density changed (DM, Mg, Ca, fat, energy), nutrient output was expressed as the product of the equations for changes in milk mass and milk nutrient concentration. The summation of each nutrient-output equation was calculated to yield total nutrient transfer for a given lactation stage. (A detailed description of calculations is given in Appendix 1.) These figures are mean values and should be interpreted cautiously, as individual variation in both



**Fig. 2** Mineral content of milk by lactation stage. Calcium and magnesium regression lines are significant at  $P < 0.01$ . Regression equations and means given in text

**Table 2** Minimum milk mass required to supply the mineral and nitrogen needs of a growing *P. hastatus* pup. Milk values are presented as means  $\pm$ SD (SE) for a theoretical 49-day-old pup, BM = 63.0 g, which has just begun to forage independently. Accretion data derived from Studier and Kunz (1995) for *M. velifer* from 1–37 days. Milk mineral production assumes 552 g total milk production (26.6% dry matter) through week 7

Mineral	Concentration in milk (mg $\cdot$ g <sup>-1</sup> DM)	Production in milk to day 49 (mg)	Predicted <i>P. hastatus</i> accretion (mg)	Fraction retained
Ca	8.75 $\pm$ 4.17 (0.8)	1355.76	447.59	0.33
Mg	0.55 $\pm$ 0.26 (0.05)	85.01	16.05	0.19
Fe	0.23 $\pm$ 0.20 (0.04)	33.77	8.22	0.24
K	5.42 $\pm$ 2.11 (0.41)	795.83	117.46	0.15
N	128.41 $\pm$ 9.10 (1.75)	18854.70	2481.12	0.13
Na	9.87 $\pm$ 4.3 (0.83)	1449.23	40.89	0.03

milk composition and mass was high. Of the minerals we examined, calcium exhibited the highest retention rate.

## Discussion

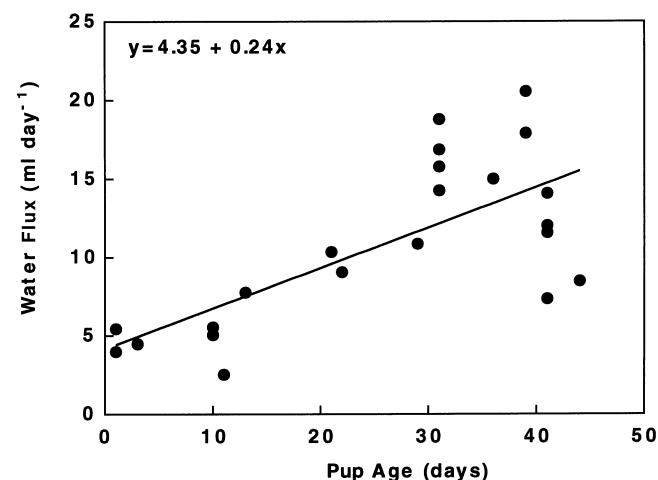
Studies of milk production and composition in bats are few. Jenness and Studier (1976) reviewed available data on milk composition of phyllostomid bats. Huibregtse (1966) examined proteins by electrophoresis and analyzed carbohydrates on a few milk samples from *Lep- tonycteris sanborni* and *Tadarida brasiliensis*. Jenness and Sloan (1970) analyzed one sample from *Myotis thysanodes*. Fatty acid composition was examined by Glass and Jenness (1971) and Stull et al. (1966). Kunz et al. (1983, 1995) measured changes in milk composition (fat, protein, carbohydrates, fatty acids) in four species of insectivorous bats. Studier and Kunz (1995) and Studier et al. (1995) determined nitrogen and mineral levels in milks of one insectivorous and several frugivorous bat species. In many of these studies small sample sizes, pooled samples, and/or lack of knowledge regarding stage of lactation have obscured temporal

changes or individual variation. This is the first study to date which combines data on milk composition from bats of known lactation stage with data on water flux to estimate total milk energy output in a small, free-ranging mammal.

## Doubly-labeled water technique

Inaccuracies in estimating milk production from pup water flux could be caused by water recycling via the mother (Baverstock and Green 1975), pulmocutaneous water transfer between pups (Friedman and Bruno 1976), or metabolic water production. Evidence of water recycling from transfer of isotopes from pup to mother has been observed in bats (Steele 1991; McLean 1995). Ingestion of urine from pups may contribute up to 39% of the maternal water turnover in xeric rodent species (Oswald et al 1993), and has been calculated to cause underestimates of water flux in the tammar wallaby of up to 4% (Dove et al. 1989). The error is directly proportional to the mass of the litter relative to the mother and the degree of water recycling (Dove et al. 1989), and recycling is higher in xeric than mesic species. Given that our study animals spent all of their non-foraging time in an environment  $>$ 90% relative humidity (Stern 1996), the rate of water recycling, if it occurs, is likely to be closer to the 3.2% of maternal intake found in voles than the  $>$ 40% found in desert rodents (Oswald et al. 1993). Review of many hours of videotape showed no evidence of excreta consumption in this species (J. Boughman, pers. comm.). McLean (1995) found that isotope levels in mothers of injected long-eared bat pups were less than 1% of final pup isotope levels, suggesting that water recycling has a minimal effect on DLW measurements. Potential errors due to water recycling were also minimized in this study by the high rate of water flux of  $>$ 50% of body mass over a 24-h period (Stern 1996) observed in adult females.

Pulmocutaneous exchange (PCE) of isotopic water could have occurred in our study, as pups and mothers roost in tight clusters. If several animals in a group are injected with isotope, PCE may result in underestimates of water flux if injected individuals inhale isotope-labeled water derived from other injected individuals. The



**Fig. 3** Water flux, measured with doubly-labeled water, in free-ranging *Phyllostomus hastatus* pups. Pups are weaned between 7–8 weeks of age

probability of PCE of isotope from other individuals was higher in adult females (since DLW was done on harem groups) than with pups, which were scattered through various harems. The error caused by PCE is probably quite small (Baverstock and Green 1976).

### Milk composition and growth

In most wild and domesticated mammals, milk composition changes over the course of lactation (Jenness 1974; Oftedal 1984; Green and Merchant 1988). The typical growth curve for bats follows a logistic model, characterized by initial linear growth which slows as the young approach weaning (Kunz and Stern 1995). During the linear growth phase, nutrient needs of suckling young presumably increase with the increase in body mass. This increased nutrient demand can be met by increasing nutrient concentrations in milk, increasing overall milk production, or both. In *P. hastatus*, both milk yield and nutrient concentrations increased to accommodate the increased demand. Although an earlier study of milk composition in insectivorous bats (Kunz et al. 1983) found no trends over time, inaccurate estimation of lactation stage and pooling of samples may have obscured differences. A more thorough subsequent study of insectivorous bats (Kunz et al. 1995) showed increases in milk fat, dry matter, and energy concentrations similar to those we observed for *P. hastatus*.

### Fat

Dietary fat serves several roles in growing young, providing energy for daily maintenance, essential fatty acids, and precursors for energy storage. Body fat levels are often higher immediately prior to the onset of flight in young bats than they are in adults, presumably due to the need for energy reserves while foraging skills develop (review: Tuttle and Stevenson 1982). The transition from suckling to foraging can be difficult, and weanlings typically exhibit a lag in growth as pups develop foraging skills (Kunz 1987). A safety margin of a few days in the form of energy reserves as fat could mean the difference between starvation and survival. Although linear growth of pups (as measured by forearm length) may slow as pups approach weaning, body mass of pups continues to increase as milk fat content, and fat deposits in the young, increase. This pattern of an increase in fat content of milk during late lactation is apparent across a wide range of taxa, although the pattern is not universal (Oftedal 1984; Stern 1996). Our data show that, although protein content of milk remained constant, fat content continued to increase, suggesting that mass gain immediately prior to weaning is primarily due to fat deposition. This observation is consistent with data on body composition in small insectivorous bats (Stern 1996; T.H. Kunz et al., unpubl. obs.).

### Carbohydrates

In *P. hastatus*, milk sugar is secondary to fat as an energy source. The reason for the subordinate role of carbohydrate is not dietary, since the primary dietary constituent of lactating *P. hastatus* is *Cecropia* fruit, a food high in carbohydrates but low in fat and protein (Herbst 1983). Dietary carbohydrate is an important source of carbon for fatty acid synthesis in non-ruminants (Folley and McNaught 1961). Carbon skeletons that originated from dietary carbohydrates may appear in milk as sugars (such as lactose), fatty acids, or other constituents. Thus, one would not expect a direct correspondence between the composition of diet and that of milk. It is likely, however, that concentrations of lactose and other sugars are constrained by a role in maintaining osmotic balance in the mammary gland (Allen et al. 1991; Peaker 1977).

### Individual variation

Previous analyses of milk composition in insectivorous bats (Kunz et al. 1983, 1995) utilized pooled samples which obscures individual variation. Some of the observed variation in macromolecular components in this study may be due to individual differences. A study in which humans were monitored throughout lactation found interindividual variation to be much higher than intraindividual variation, suggesting that milk composition may be characteristic of individuals (Allen et al. 1991). Likewise, some breeds of dairy cows consistently produce milk with 20% greater solids, protein, and calcium and significantly less fat than of other breeds (Reinart and Nesbitt 1956).

High variability in some minerals in bat milk may represent fluctuations in diets or physiological states of lactating females. Linzell (1967) found that ionic concentration of goat milk varied depending on time of day and nutritional state of the animal. Fasted goats showed an increase in sodium and chloride concentrations with a concurrent decrease in potassium and lactose. All these ions contribute to maintaining osmotic balance in milk (Allen et al. 1991; Peaker 1977). Given that variation occurs in milk ion and mineral concentrations in domestic mammals and humans, it is not surprising that free-ranging animals may exhibit even greater variability. It may be difficult to discern consistent patterns among mammals or within even closer taxonomic groupings when individual variation is high, sampling protocols are variable and few samples have been assayed for most species.

### Potassium, iron, and magnesium

Values for potassium, iron, and magnesium were rather similar to reported values for temperate insectivorous bats (Studier and Kunz 1995). However, high values of

iron may be due to contamination as it was not feasible to rigorously clean the nipples under field conditions, nor were the collection tubes acid-washed. Iron is a pervasive contaminant in natural conditions. Sodium concentrations in milk were sixfold higher than reported values for temperate insectivorous bats (Studier and Kunz 1995) and approximately double that found in fruit-eating pteropodids (T.H. Kunz et al., unpubl. obs.). In general, ion concentrations are inversely related to lactose concentration because milk is isoosmotic to blood (Jenness 1974). This inverse relation suggests that lactose concentration in *P. hastatus* should be lower than in pteropodids and significantly lower than in insectivorous bats. Such was not the case, however (Studier and Kunz 1995; T.H. Kunz et al., unpubl. obs.).

### Calcium

The previously reported value for calcium in *P. hastatus* milk was approximately one-third the mean calcium level in our study (Studier et al. 1995). The lower value previously reported, based on a single sample, does fall within the range observed in our study and underscores the importance of an adequate database when making extrapolations such as nutritional requirements. Prediction of total calcium accretion is difficult, because ossification of bones continues after nutritional independence is achieved (Papadimitriou et al., in press). As lactating females generally operate under a calcium deficit (Kwiecinski et al. 1987; Barclay 1994; Studier and Kunz 1995), it seems unlikely that milk would provide an excess over the needs of pups. Milk calcium levels probably correspond more closely to pup requirements than do more abundant minerals. Calcium accretion of pups increases on a daily basis, both absolutely and as a proportion of body mass as bones lengthen and ossify (Studier and Kunz 1995). This increase in calcium demand can be met by an increase in either milk yield or milk calcium concentration. Since milk calcium concentration actually decreases through lactation (Fig. 2), our analysis suggests that increased milk yield alone must provide sufficient dietary calcium for bone growth and mineralization. It is tempting to speculate that the decline in calcium concentration is associated with de-

pletion of maternal calcium stores, but as most calcium is probably bound to protein (Jenness 1979) is not clear what the mechanism would be.

### Calcium and growth rates

It has been suggested that calcium availability may influence growth rates in bats (Barclay 1994). Our data indicate that calcium retention rates are higher than the other minerals we examined (Table 2). Interspecific comparison also yields evidence that calcium supply may relate to growth rates (Table 3). Calcium content of *P. hastatus* milk appears to be slightly higher than that of *Tadarida brasiliensis* and substantially higher than that of three *Carollia* species. If dietary calcium is correlated to growth rates, we would expect slow pup growth in *Carollia* species relative to species with milk of higher calcium content. Although growth rate is negatively correlated with asymptotic body mass, *P. hastatus* and *Carollia perspicillata* have very similar growth rates (Kunz and Stern 1995) despite a five fold difference in body mass. Data are needed on additional species to determine the correlations between milk constituents and rate of growth.

### Maternal effort

Bats are unique among mammals in that pups do not begin foraging independently until they have nearly achieved adult dimensions, possibly due to the constraints of flight (Kunz 1987; Barclay 1994; Kunz and Stern 1995). Maternal effort, as measured by lactational output, varies depending on number of young produced, rate of pup growth, and pup size at independence (Ben Shaul 1962; Jenness and Sloan 1970). Oftedal (1984b) examined milk energy output at peak lactation according to reproductive strategy. Primates, which most commonly give birth to single, slow-growing young, typically have low daily milk energy outputs in comparison to species which produce many, fast-growing young that are weaned at an early age. In many respects, reproductive patterns in bats resemble those of their phylogenetically close relatives, the primates. Both

**Table 3** Nitrogen and mineral concentrations in milk of five bat species. Mineral content is in  $\text{g} \cdot \text{mg}^{-1}$  dry matter. Growth constants taken from Kunz and Stern (1995)

Species	BM (g)	Ca	Mg	Fe	K	N	Na	Growth constant	Reference
<i>Phyllostomus hastatus</i>	84.0	8.75	0.55	0.23	5.42	128.41	9.87	0.0620	This study
<i>Tadarida brasiliensis</i>	10.4	6.11	0.51	0.13	3.46	52.25	1.50	0.1680	Studier and Kunz (1995)
<i>Carollia castanea</i>	–	1.86	0.30	–	18.9	28.93	1.14	–	Studier et al. (1995)
<i>C. perspicillata</i>	14.9	1.63	0.26	–	20.8	29.72	1.42	0.0629	Studier et al. (1995)
<i>C. brevicauda</i>	–	2.79	0.47	–	24.6	–	1.88	–	Studier et al. (1995)

taxa are typified by long lifespans, litters of single or few slow-growing young, and extended parental care (Hayssen 1993; Kunz and Pierson 1994). In addition, *P. hastatus* is omnivorous and has a complex social structure comparable to many primate species (McCracken and Bradbury 1977, 1981; J. Boughman, pers. comm.). In body size, however, bats are more like rodents or other small mammals that produce large litters. Oftedal's regression equations yield an estimate for daily milk energy output,  $M_E$ , at peak (= mid) lactation. The prediction for a mammal comparable in size to *P. hastatus* at peak lactation is:

	Predictive equation (in kcal)	Predicted energy output for 77-g female
Primates, single young	$M_E = 49.1 \cdot (\text{body mass in kg})^{0.67}$	36.86 kJ · day <sup>-1</sup>
Species w/many young	$M_E = 175 \cdot (\text{body mass in kg})^{0.812}$	91.30 kJ · day <sup>-1</sup>

Our calculation of 122 kJ · day<sup>-1</sup> (1.58 kJ · g<sup>-1</sup> maternal mass) for daily energy output of *P. hastatus* at peak lactation not only exceeds the predicted value for primates, but also exceeds the value for species with many small young, including rodents.

In searching for correlates to maternal milk energy output, Oftedal (1984a) found that litter metabolic mass (LMM) was a better predictor of energy output than maternal metabolic mass. According to Oftedal's equation ( $M_e = 942 \text{ kJ} \cdot \text{LMM}$ ), at peak lactation, *P. hastatus* young have a mass of 0.063 kg, equivalent to a metabolic mass ( $\text{BM}^{0.83}$ ) of 0.101 kg. The predicted energy output, 95 kJ, is 78% of our observed value. Thus, the large milk output is primarily a function of the large size of the young at peak lactation.

An intriguing aspect of this study is the degree of variability in milk composition and yield between individuals at the same stage of lactation. Significant advances have been made in recent years that consider stage of lactation, time of day, and (in the case of domestic animals) breed, in addition to interspecific differences. In humans, milk composition and yield may vary with time of day, stage of lactation, parity, age, diet, water intake, and throughout a nursing bout (Morrison 1952; Macy and Kelly 1961; Neville et al. 1984; Brown et al. 1986; Viverge et al. 1986). Even so, values for milk composition and yield in humans and other mammals are usually presented as means. While facilitating broad taxonomic comparisons, this statistical treatment obscures the individual variation that is the raw material for natural selection. Reproductive success is the driving force behind evolution, and among mammals, failure to lactate successfully equals failure to reproduce. Inasmuch as the ability of females to provision young successfully has a heritable component, lactational output will be the target of strong selective forces. Given that bat pups in a population generally experience similar roosting conditions (e.g. the relatively uniform temperature and humidity of a cave), the factor

determining pup survival is likely to be the mother's ability to produce an adequate amount and composition of milk. Milk production may be determined primarily by intrinsic factors (e.g. individual physiological make-up), extrinsic factors (e.g. habitat quality), or a combination of the two (e.g. social rank).

We were not able to follow individual bats through lactation, as repeated disturbance would have resulted in abandonment of both roost and young. Moreover, frequent milk collection could potentially alter growth rate of pups. Notwithstanding, the high degree of variability in milk composition at the same stage of lactation implies substantial intraspecific variation. In humans, milk composition appears to be individually determined. A study of free-ranging humans (which tend to be more amenable to experimental treatment than are bats) showed the intraindividual coefficient of variation to be much lower than the interindividual coefficient of variation for most milk components, suggesting that milk composition is a characteristic of individuals (Allen et al. 1991). Future studies should focus on the effect of interindividual differences in milk production on pup growth and mortality rates. We predict that for bats, individual differences in milk yield will influence both pup growth and mortality.

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## Appendix I. Calculation of daily and total nutrient output

Where nutrient density changes (dry matter, fat, energy, Mg, Ca), daily nutrient output can be expressed as the product of the equations for daily changes in milk volume and milk nutrient concentration. For example, the daily concentration of Ca =  $-0.11x + 13.62 \text{ mg} \cdot \text{g}^{-1} \text{ DM}$ , where  $x$  = age in days. Daily milk volume =  $4.79 + 0.26x$ . Because Ca is expressed in units per g DM, the change in %DM (=  $17.7 + 0.02x$ ) must also be included. Thus, daily Ca output in mg is  $(-0.11x + 13.62 \text{ mg} \cdot \text{g}^{-1} \text{ DM})(4.79 + 0.26x \text{ g WM})(17.7 + 0.02x \text{ g DM/g WM})/100$ . At day 49, daily Ca production is thus 39.7 mg. Total Ca production to day 49 can be calculated by summing the daily Ca production. Daily and total output for dry matter, fat, energy content, and Mg can be calculated in like fashion. Since the equations for fat and energy content are given in terms of wet mass, change in % DM can be left out.

Concentration of Fe did not change significantly over lactation. To simplify calculations, we used the mean DM to day 49 of 26.6%. Thus daily Fe output in mg =  $(0.23 \text{ mg} \cdot \text{g}^{-1} \text{ DM})(4.79 + 0.26x)(26.6 \text{ g DM}/100 \text{ g milk}) = 0.29 + 0.016x$ . To calculate total Fe output to day 49, we simply multiplied  $(0.23 \text{ mg} \cdot \text{g}^{-1} \text{ DM})(552 \text{ ml})(26.6 \text{ g DM}/100 \text{ g milk})$  to get a total yield of 33.77 mg Fe. Calculations for daily output of lactose, protein, K, and Na were done the same way.



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