Interspecific and Intraspecific Variation in Proximate, Mineral, and Fatty Acid Composition of Milk in Old World Fruit Bats (Chiroptera: Pteropodidae)

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ABSTRACT

We examine the effect of body mass on milk composition among Old World fruit bats, including *Pteropus pumilus* (0.175 kg), *Pteropus rodricensus* (0.265 kg), *Pteropus hypomelanus* (0.571 kg), and *Pteropus vampyrus* (1.133 kg). We describe intra- and interspecific differences in the proximate composition of milk among these four species and the minerals and fatty acids in the milk of the latter two species. There were no differences between species in the concentrations of dry matter, fat, or lactose in milk. However, there were significant, although small, differences in the protein content of milk among species, with protein being significantly greater in *P. rodricensus* than in *P. pumilus* and *P. hypomelanus* and protein being significantly less in *P. hypomelanus* than in *P. rodricensus* and *P. vampyrus*. There were no differences in mineral content between *P. hypomelanus* and *P. vampyrus* in milk minerals, but minor differences were evident in fatty acids 12:0, 14:0, 18:0, 18:1n11, and 18:2n6. Our findings suggest that milk composition is relatively constant across lactation for most proximate, mineral, and fatty acid components. We found a significant increase in dry matter and energy across lactation in the concentration of dry matter and energy in *P. pumilus* and fat in *P. hypomelanus*. In *P. hypomelanus*, we found a significant increase in the concentration of fatty acids 10:0 and 20:1n9 and a significant decrease in Iso15 and 20:1n7. No other differences associated with day of lactation were found. These findings suggest that milk composition is generally similar within the genus *Pteropus*, despite a 6.5-fold difference in body mass between species that we evaluated.

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Introduction

The concentration of nutrients in milk varies greatly among mammals (Jenness and Sloan 1970; Oftedal 1984; Oftedal and Iverson 1995), which may reflect strong selection pressure to optimize energy and nutrient transfer under physiological and life history constraints. The primary goal of many studies in which milk composition is compared among species has been to examine phylogenetic, physiological, and ecological correlates in an attempt to understand the adaptive significance of observed differences (Oftedal and Iverson 1995). Several traits have been suggested to contribute to interspecific differences in milk composition, including body size, pattern of maternal care, thermo- and osmoregulatory needs of the dependent offspring, and phylogeny (Gregory et al. 1965; Martin 1968; Payne and Wheeler 1968; Oftedal et al. 1987; Oftedal and Jenness 1988; Peddemors et al. 1989; Kunz et al. 1995; Oftedal and Iverson 1995; Stern et al. 1997; Tilden and Oftedal 1997).

However, caution is warranted in interpreting observed differences. Intraspecific variation may confound interspecific trends, especially if stage of lactation is not consistent among species (Oftedal and Iverson 1995). Phylogeny may also have confounding effects if not properly accounted for (Garland and Carter 1994). Comparisons based on a limited number of species, or where location is poorly characterized such as Payne and Wheeler (1968) and Ben Shaul (1962), may not hold true when additional or improved data are included.

Our objective was to examine the effect of body size on milk composition in a restricted phylogenetic group. Blaxter (1961) proposed that the young of small-bodied species require milk of high-energy density associated with their greater mass-specific metabolic rate. If the amount of milk consumed is proportional to gastrointestinal capacity, and gastrointestinal ca-

pacity is proportional to body mass (Oftedal et al. 1989), small mammals will not ingest more milk; instead, they will require milk of a greater energy density to satisfy their higher metabolic demand. Payne and Wheeler (1968) reported a significant inverse correlation between the energy content of milk and adult body mass, but this correlation omitted large marine mammals (such as whales and pinnipeds) that produce energy-dense milks and did not take into account potential phylogenetic confounds. Data for restricted phylogenetic groups are inconsistent. For example, among 12 rodent species, Derrickson et al. (1996) found an inverse correlation between body mass and milk dry matter and fat (Derrickson et al. 1996). However, the larger species were in the suborder Hystricognathi, while the smaller species were in the suborder Sciurognathi, so phylogeny may have had a confounding effect. No differences have been found for intrageneric comparisons of milk energy density among equids (1.6-fold variation in body mass; Oftedal and Jenness 1988; Nowak 1999) or among species of the genus *Eulemur* (2.5-fold variation; Terranova and Coffman 1997; Tilden and Oftedal 1997). Thus, interspecific differences may be negligible when possible phylogenetic effects are restricted.

We chose to examine the effect of body mass on milk composition among four species of megachiropteran bats of the genus *Pteropus*. These species ranged in body size from 0.175 to 1.133 g, a 6.5-fold variation in mass. Bats are interesting subjects for study because of their relatively long period of lactation and aerial mode of locomotion. While most mammals wean their young before they reach 40% of adult body mass, bats are weaned at approximately 70% of adult mass and at least 90% of adult wing dimensions (Barclay 1994, 1995). Production and transport of milk may be constrained by aerodynamic and energetic limitations of flight. Thus, it is possible that patterns of milk production reflect trade-offs between costs associated with flight and the energy demands of lactation. Kunz et al. (1995) suggested that the relatively high fat and low water content in the milk of bats could be an adaptation to reduce milk volume and thus reduce the energetic costs of flight for lactating mothers.

Four species of *Pteropus* in this study represent the full range of sizes found within the genus. Despite their large variation in body size, other parameters often correlated with variation in milk composition are similar, including phylogeny, diet, and pattern of maternal care (Pierson and Rainey 1992; Nowak 1994; Kirsch et al. 1995; Jones and Kunz 2000; Kunz and Jones 2000). The diet of *Pteropus* consists of the juices extracted from fruits and leaves (Nowak 1994). Adult *Pteropus* reach reproductive maturity at 1.5–2.0 yr, and females generally reproduce annually, gestating from 140 to 192 d (Pierson and Rainey 1992). All *Pteropus* spp. bear singletons. In *Pteropus vampyrus* and *Pteropus hypomelanus*, and possibly other *Pteropus* spp. where postpartum behavior has not be described, females carry young continuously for the first few weeks of life, but pups are subsequently left behind while their mothers forage (Jones and

Kunz 2000; Kunz and Jones 2000). *Pteropus* pups begin to fly and feed at approximately 3 mo, but weaning does not occur until 3–6 mo (Pierson and Rainey 1992; Jones and Kunz 2000; Kunz and Jones 2000). Thus, if body size is an important selective pressure on milk composition, given the life history similarities, restricted differences in phylogeny, and larger variation in body size among *Pteropus* spp., we would expect greater energy densities among milk for the smaller versus the larger species examined. The results of this study are compared with others that have described milk composition among bats.

Methods

Study Animals

We evaluated the composition of milk from four *Pteropus* spp., including the little golden-mantled flying fox (*Pteropus pumilus*), the Rodrigues flying fox (*Pteropus rodricensus*), the island flying fox (*Pteropus hypomelanus*), and the large flying fox (*Pteropus vampyrus*) housed at the Lubee Foundation, Gainesville, Florida. The means of adult mass for captive bats used in our study were 0.175 kg in *P. pumilus*, 0.265 kg in *P. rodricensus*, 0.571 kg in *P. hypomelanus*, and 1.133 kg in *P. vampyrus* (Kunz and Hood 2000). Captive populations of these four species were maintained on a mixed diet of apples, bananas, grapes, cantaloupe, pears, corn, sweet potatoes, carrots, lettuce, spinach or kale, and dates or figs. Dietary supplements included Zu/Preem Old World Monkey Chow (Premium Nutritional Products, Topeka, Kans.) and Vionate vitamin supplement (Arc Laboratories, Atlanta). Plums, peaches, kiwi, strawberries, nectarines, mangos, papayas, starfruit, oranges, and/or pineapples were also added to the diet when available.

Sample Collection

Lactating bats were separated from their pups for approximately 3 h before milking to allow milk accumulation within the mammary glands. Females were injected with approximately 2 μL/g oxytocin solution (Sigma, 222 IU/mL) 10 min before milk expression. Females were anesthetized with isofluorane, and their nipples were cleaned with isopropyl alcohol before milking. Milk was expressed by manual palpation and collected into capillary tubes with the aid of a filtered mouth pipette. Samples were transferred to Eppendorf tubes and stored at -20°C until analyzed.

Laboratory Analyses

Different sets of samples were used for proximate, mineral, and fatty acid composition analyses. Proximate analysis was completed for all species, with seven to 11 *P. pumilus*, 13–19 *P. rodricensus*, 34–43 *P. hypomelanus*, and 24–27 *P. vampyrus* samples analyzed. All components could not be analyzed for all samples due to limitations of sample volume. Minerals and

fatty acids were only measured in milk collected from *P. hypomelanus* and *P. vampyrus*. Seventeen samples were analyzed for minerals and 28 were analyzed for fatty acids from *P. hypomelanus*. Thirteen samples were analyzed for minerals and 15 were analyzed for fatty acids from *P. vampyrus*.

Proximate components and minerals were analyzed at the Nutrition Laboratory of the National Zoological Park, Washington, D.C. Milk samples were warmed to 37°C and vortexed before subsampling. To determine dry matter and energy content of milk, 100-mg aliquots were delivered onto 10-mg dry, sterile, ether-washed cotton pellets. Dry-matter composition of milk was determined by mass change of samples dried to constant mass in a forced convection oven for 3 h at 100°C. Energy content of the dried pellets was determined by microbomb calorimetry (Phillipson 1964). The mean energy equivalent of cotton that was used as an absorbent, 17.12 J/mg (SD = 1.55), was subtracted from the total energy of the pellet to determine energy content of the milk sample. A micromodification of the Roese-Gottlieb method of fat extraction with ether was used to estimate fat content of 100-mg aliquots of milk (Horwitz 1980). Carbohydrate content of 25-mg aliquots of milk was determined using the phenol-sulfuric calorimetry (Marier and Boulet 1959). Nitrogen content of 100-mg aliquots of milk was determined by the micro-Kjeldahl method (Koch and McMeekin 1924). This value was converted to crude protein by multiplying percent nitrogen by the protein equivalent of 6.38 for milk (Jones 1931). Most samples were run in duplicate, but single samples were run when sample volume was low. Homogenized whole cow's milk was used to estimate error associated with our analyses. Means and standard deviations for the proximate components of bovine milk were as follows: dry matter = 12.31% ± 0.12% ($n = 18$), energy = 2.33% ± 0.24% ($n = 17$), fat = 3.07% ± 0.19% ($n = 19$), protein = 2.97% ± 0.13% ($n = 20$), carbohydrate = 4.84% ± 0.38% ($n = 19$).

Mineral content of sample digests was determined by atomic absorption spectrophotometry. Dried samples were digested on a hot plate with 20 mL of 70% nitric acid and 5 mL of 70% perchloric acid until approximately 2 mL of solution remained. Digests were diluted with distilled deionized water. Modifiers were added to sample aliquots to reduce interference from other elements. Strontium chloride was added to samples analyzed for calcium to create a final concentration of 1,000 ppm Sr. Lanthanum chloride was added to samples analyzed for magnesium to create a final concentration of 1,000 ppm La. Cesium chloride was added to samples for potassium to create a final concentration of 1,000 ppm Cs. Mineral concentration of samples was measured using an acetylene flame. Calcium was measured at 422.7 nm, potassium at 766.5 nm, and magnesium at 202.5 nm using a Smith-Hieftje atomic absorption spectrophotometer. Phosphorous was analyzed using the Association of Official Analytical Chemists–modified Gomorri method (Horwitz 1980). National Institutes of Standards and Technology (formerly the National Bureau of Standards, U.S. Department of Commerce, Gaithersburg, Md.) milk powder was analyzed to determine error associated with mineral analyses. The measured concentration of minerals in the standard were (mean ± SD, $n = 12$ for all minerals) Ca = 12.13 ± 0.32 mg/g, K = 15.58 ± 0.52 mg/g, Mg = 1.20 ± 0.06 mg/g, P = 10.8 ± 0.2 mg/g. Results for magnesium and phosphorus were within the declared range for these elements (Mg = 1.20 ± 0.03 mg/g, P = 10.6 ± 0.2 mg/g). Values for calcium and potassium were slightly lower than the declared values (Ca = 13.05 ± 0.05 mg/g, K = 16.9 ± 0.3 mg/g), with recovery of 93.3% and 92.3%, respectively.

Fatty acid analyses were conducted at Dalhousie University, Halifax, Nova Scotia. Lipid was extracted from milk samples according to the Folch's method (Folch et al. 1957). Fatty acid methyl esters were prepared directly from 100 mg of pure extracted lipid (filtered and dried over anhydrous sodium sulfate), using 1.5-mL 8% boron trifluoride in methanol (w/w) and 1.5-mL hexane, capped under nitrogen, and heated at 100°C for 1 h. Fatty acid methyl esters were extracted with hexane, concentrated, and increased to 50 mg/mL with high-purity hexane. This method of transesterification produced identical results to that using Hilditch reagent (0.5 N H_2SO_4 in methanol).

Duplicate analyses of fatty acid methyl esters were performed on samples using temperature-programmed gas liquid chromatography according to Iverson et al. (1997), on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30 m × 0.25-mm-i.d. column coated with 50% cyanopropyl polysiloxane (0.25-μ film thickness; Folsom, Calif.) and linked to a computerized integration system (Turbochrom 4 software, PE Nelson). Identifications of fatty acids and isomers were determined from several sources, including known standard mixtures (Nu Check Prep, Elysian, Minn.) and GC-mass spectrometry (Hewlett-Packard 6890 Gas Chromatograph, 1 : 20 split injection, Micromass Autospec oa-TOF mass spectrometer, operated at 1,000 resolution, scanning masses 120–450). Individual fatty acids are expressed as mass percentage of total fatty acids after employing mass response factors relative to 18:0. Fatty acids are expressed as mass percentage of total fatty acids and are designated by shorthand IUPAC (International Union of Pure and Applied Chemistry) nomenclature of carbon chain length : number of double bonds and location (n-x) of the double bond nearest the terminal methyl group. When milk was divided among two or three vials, fatty acid composition of the sample was based on the mean concentration. Although up to 70 fatty acids (range 4:0–24:1n9) are normally detected in the analyses used in this study, only 20 were present in concentrations within reasonable detection limits of the technique, >0.05%. Thus, only fatty acids generally found at ≥0.05% in milks are reported and included in statistical analyses.
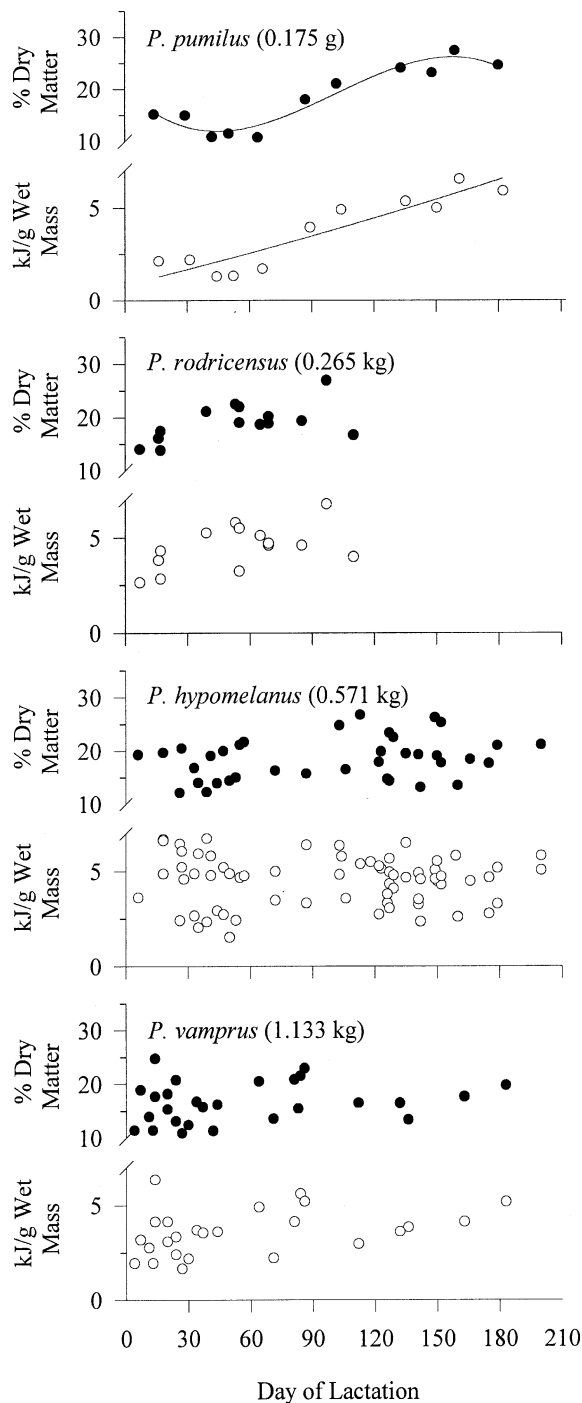
Figure 1. Dry matter and energy content from individual samples of *Pteropus* milk. Symbols in graphs are as follows: *filled circles* = dry matter, *open circles* = energy. There was a significant sigmoidal increase in the dry matter composition (ANOVA, $F_{10} = 39.15$, $P < 0.001$) and curvilinear increase in the energy content (ANOVA, $F_{10} = 57.69$, $P < 0.001$) of *Pteropus pumilus* milk as indicated by the regression lines. No other significant changes in dry matter and energy content of milk during lactation were apparent.

Statistical Analyses and Comparisons

SAS 6.12 (SAS Institute 1996) was used for all statistical analyses. Variation between species was examined by ANCOVA, where milk components were set as the dependent variables, species was the independent variable, and day of lactation was the covariate. When day of lactation was not significant, as for protein and lactose, variation between species was examined with ANOVA. A post hoc Bonferroni procedure was used to adjust for multiple comparisons within proximate, mineral, and fatty acid components. Arcsine transformation was used to normalize proportional data (Sokal and Rohlf 1995).

Variation in the composition of milk across lactation was examined using linear, squared, and cubic regressions for proximate and mineral comparisons, where day of lactation was the independent variable and concentration of each component was the dependent variable. Only linear regressions were completed for fatty acids to prevent inflation of experimentwise error that would occur with the 40 added comparisons necessary to examine squared and cubic functions. Arcsine transformation was used to normalize proportional data (Sokal and Rohlf 1995). A post hoc improved Bonferroni procedure for combining dependent test results was used to adjust for multiple comparison among proximate, mineral, and fatty acid comparisons. This procedure adjusts level of significance for each test performed on the same set of samples, where the level of significance for the test with the largest P value is $\alpha = 0.05$, the second largest P value is $\alpha = 0.05/2$, and the smallest is $\alpha = 0.05/n$ (Haccou and Meelis 1994).

Variation among samples for each proximate component was high for all species. Number of samples collected per individual female ranged from one to 10, and thus, samples per individual were too variable to test for individual differences, as captive animals were milked randomly. Variation associated with body size, collection time, and sample volume was compared in *P. hypomelanus*. Multivariate multiple regression was used to simultaneously compare the variables of proximate composition with body mass, length of forearm, time of day when sample was collected, sample volume, and day of lactation. Samples sizes were too small for the other three species to conduct a multiple regression analysis. Both body mass and length of forearm are functions of body size, with mass serving as an indicator of current nutritional status. Length of forearm is generally less variable and thus served as an index of skeletal size. Sample volume was based on the volume of milk recorded at time of collection.

The composition of milk at peak lactation was compared with other bat species. Peak lactation was defined as the period of maximal lactational output, usually occurring just before the onset of significant consumption of solids by young (Oftedal and Iverson 1995). The period of peak lactation was estimated using data on postnatal growth (W. R. Hood and T. H. Kunz, unpublished data). Peak lactation was defined as the second

half of the linear phase of growth of young (both body mass and forearm length increase linearly during this period), which ranges between 1.5 and 3 mo for all species (W. R. Hood and T. H. Kunz, unpublished data). This agrees with the onset of solid food consumption described for some members of the genus *Pteropus* (Pierson and Rainey 1992; Jones and Kunz 2000; Kunz and Jones 2000) and observations that *P. vampyrus* pups begin feeding on solid food at approximately 3 mo in captivity (J. Seyjagat, personal observation). The period from parturition to 1.5 mo represented early lactation and the period from 3 mo to weaning was considered late lactation.

Results

Proximate Composition

There were no differences between species in the dry matter, energy, fat, and carbohydrate content of milk, and thus, there was no clear effect of body size on proximate milk composition ($P > 0.05$). However, the protein content in the milk did differ between species (ANOVA, $F_{3, 89} = 8.80$, $P < 0.001$). The concentration of protein in *Pteropus rodricensus* milk was significantly greater than that of *Pteropus pumilus* and *Pteropus hypomelanus*, and the concentration of protein in *P. hypomelanus* milk was lower than both *P. rodricensus* and *Pteropus vampyrus*.

The proximate composition of milk produced by the four species of *Pteropus* did not vary significantly with lactation stage for most components when adjusted for multiple comparisons (linear, squared, and cubic regressions, $P > 0.01$; Figs. 1, 2). However, there was a significant increase in the dry matter and energy content of milk from *P. pumilus* (dry matter: sigmoidal regression best fit, $F_{10} = 39.15$, $P < 0.001$; energy: curvilinear best fit, $F_{10} = 57.69$, $P < 0.001$), and the fat content of *P. hypomelanus* milk (fat: linear regression best fit, $F_{43} = 9.78$, $P = 0.003$).

Association between proximate composition of milk and female body mass, length of forearm, time of collection, and milk volume, controlling for day of lactation, was evaluated for *P. hypomelanus* using multivariate multiple regression. The overall model bordered on significance (Wilks's λ: $F_{25} = 1.72$, $P = 0.07$), with milk volume negatively correlated with fat concentration (parameter estimate = -0.04, $P = 0.004$). Body mass, length of forearm, and time of collection did not contribute to variation for any of the proximate components, and milk volume did not contribute to variation in energy, dry matter, protein, or carbohydrate. Day of lactation was not correlated with fat concentration in *P. hypomelanus* when sample volume was included (partial $T = -3.515$, $P = 0.11$).

Means for the proximate composition of milk at peak lactation are summarized in Table 1. Proximate components for other pteropodids are given for comparison.

Mineral Composition

There were no differences in the concentration of calcium, phosphorus, magnesium, or potassium between *P. hypo-*
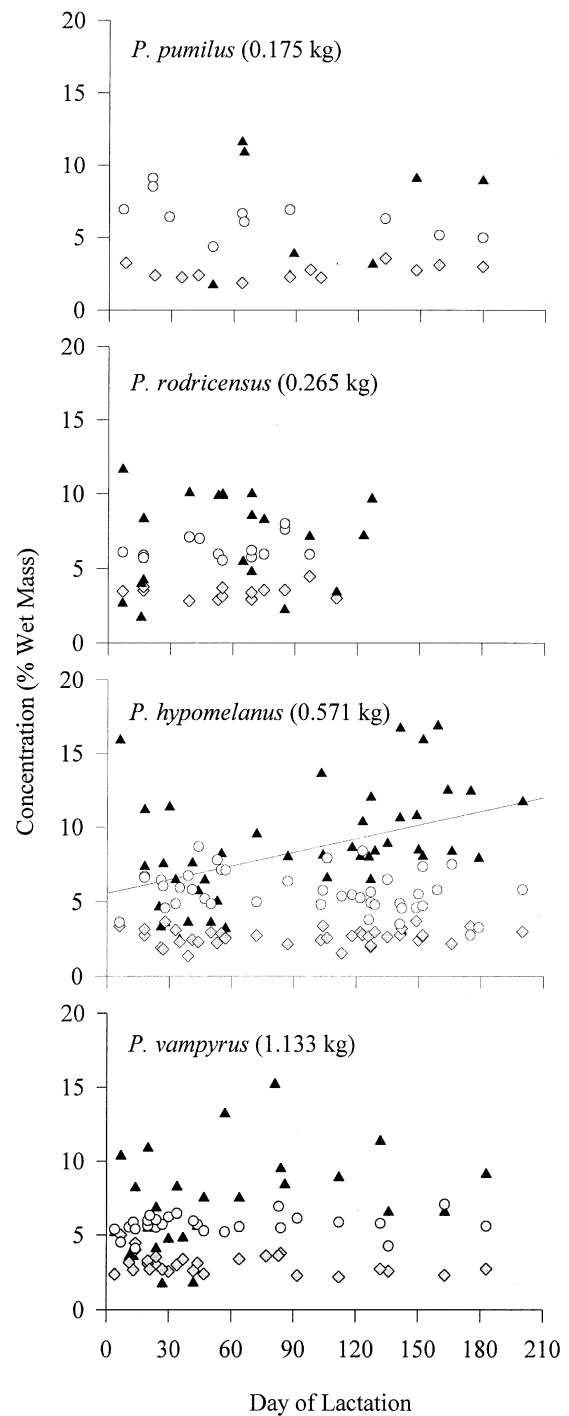

Figure 2. Fat, carbohydrate, and protein composition from individual samples of *Pteropus* milk. Symbols in all graphs are as follows: *filled triangles* = fat, *open circles* = carbohydrate, and *shaded diamonds* = protein. There was a significant increase in the fat composition of *Pteropus hypomelanus* milk as indicated by the linear regression line ($F_{43} = 9.78$, $P = 0.003$). No other significant changes in composition during lactation were apparent.

Table 1: Proximate composition (mean concentration) of milk in Old World fruit bats (Pteropodidae)

	n^a	Adult Mass (g)[b]	Day of Lactation	Dry Matter (% wet mass)	Energy (kJ/g)	Fat (% wet mass)	Protein (% wet mass)	Carbohydrate (% wet mass)
This study:								
Across lactation:								
Pteropus pumilus	7–11	174.8	7–159	16.17 ± 4.80	3.44 ± 1.93	6.10 ± 4.26	2.62 ± .51	6.42 ± 1.24
Pteropus rodricensus	13–19	264.7	7–127	19.10 ± 3.48	4.52 ± 1.16	6.70 ± 2.99	3.42 ± .45	6.38 ± .78
Pteropus hypomelanus	35–43	570.7	6–179	18.45 ± 3.94	3.98 ± 1.47	8.50 ± 3.76	2.65 ± .54	5.69 ± 1.39
Pteropus vampyrus	24–27	1,132.6	4–163	16.45 ± 3.83	3.53 ± 1.20	7.17 ± 3.36	3.10 ± .69	5.69 ± .69
Peak lactation:								
P. pumilus	2–5	174.8	45–90	12.75 ± 3.21	2.33 ± 1.42	6.09 ± 4.77	2.07 ± .30	6.02 ± 1.16
P. rodricensus	7–9	264.7	45–90	20.14 ± 1.56	4.80 ± .83	7.66 ± 2.83	3.33 ± .32	6.45 ± .96
P. hypomelanus	7–8	570.7	45–90	17.76 ± 3.08	3.28 ± 1.17	5.90 ± 2.51	2.59 ± .30	6.22 ± 1.19
P. vampyrus	5–6	1,132.6	45–90	19.14 ± 3.73	4.42 ± 1.34	10.20 ± 3.23	3.36 ± .56	5.69 ± .71
Previously reported values:								
Peak lactation:								
Epomophorus walhbergi[c]	1 (13)	129.6	NG	12.0 ± .99	…	3.5 ± .85	4.2	4.0
Pteropus poliocephalus:[d]								
Captive	31[e]	899.0	51–99	10.80[e]	2.29[e]	1.74	2.42 ± .48	6.28 ± .37
Wild caught	10–14	…	40–70 est.	12.70 ± .75	2.74 ± .30[e]	2.16 ± .86	3.64 ± .64	6.44 ± .37
Rousettus aegyptiacus[f]	2 (23)	135.0	14–49	…	4.27 ± .38	7.05 ± 1.06	2.33 ± .04	6.05 ± .07

Note. Means and standard deviations are given for the full lactation period at peak lactation. Peak lactation for samples analyzed in this study is defined in "Methods." Definition of peak lactation varies slightly for other studies. Values for range of lactation most similar to definition of peak lactation given here are presented for previously published data. Stage of lactation was not given (NG) for *E. walhbergi*. Comparisons between *Pteropus* species are based on the full lactation period. Although it appears that differences occur between values at peak lactation for some proximate components, these differences are lost when the full lactation period is considered; thus, means for full lactation values may be more appropriate for milk replacers and interspecific comparisons.

[a] Number of milk samples analyzed. Individual samples collected from *Epomophorus* and *Rousettus* were pooled for analysis. Values in parentheses indicate the total number of bats milked, if different from sample size. All analyses were not performed for some samples. In this case, sample size is presented as the range of sample sizes for the milk components.

[b] Adult mass taken from Kunz and Hood (2000), except *Rousettus*, which is taken from Korine and Arad (1999).

[c] Quicke et al. 1984.

[d] Messer and Parry-Jones 1997.

[e] Equal aliquots from individual sample pooled for analysis.

[f] Korine and Arad 1999.

melanus and *P. vampyrus* milk ($P > 0.05$). We found no changes across lactation in calcium, phosphorus, magnesium, or potassium composition following adjustment for multiple comparisons (linear, squared, and cubic regressions, $P > 0.013$; Fig. 3). Means standard deviations for the mineral composition of milk at peak lactation are summarized in Table 2.

Fatty Acid Composition

The concentration of fatty acids 12:0 (ANCOVA, partial $F_{1,41} = 63.57$, $P < 0.001$), 14:0 (ANOVA, $F_{1,41} = 21.63$, $P < 0.001$), and 18:1n11 (ANOVA, $F_{1,41} = 19.45$, $P < 0.001$) were greater in *P. vampyrus* than in *P. hypomelanus*. The concentrations of 18:0 (ANOVA, $F_{1,41} = 14.21$, $P < 0.001$) and 18:2n6 (ANOVA, $F_{1,41} = 11.41$, $P = 0.002$) were greater in *P. hypomelanus* than in *P. vampyrus* (Table 3). Several significant trends in fatty acid composition and day of lactation

were evident based on linear regression for milk samples from *P. hypomelanus*, including a gradual increase in 10:0 ($F_{27} = 17.77$, $P < 0.001$) and 20:1n9 ($F_{27} = 8.27$, $P = 0.008$) and a gradual decrease in Iso15 ($F_{27} = 8.83$, $P = 0.006$) and 20:1n7 ($F_{27} = 21.29$, $P < 0.001$; $P > 0.01$ for all other comparisons). However, these fatty acids were all minor components, each accounting for less than 1% of the milk fat. There were no significant changes in the fatty acid composition in *P. vampyrus* milk (linear regressions, $P > 0.05$; Table 3). After adjusting for multiple comparisons, four fatty acids differed between species.

Discussion

Proximate Composition

Our results suggest that there are no interspecific differences in the concentrations of dry matter, fat, or carbohydrate content in the milk of *Pteropus pumilus*, *Pteropus rodricensus*, *Pteropus*
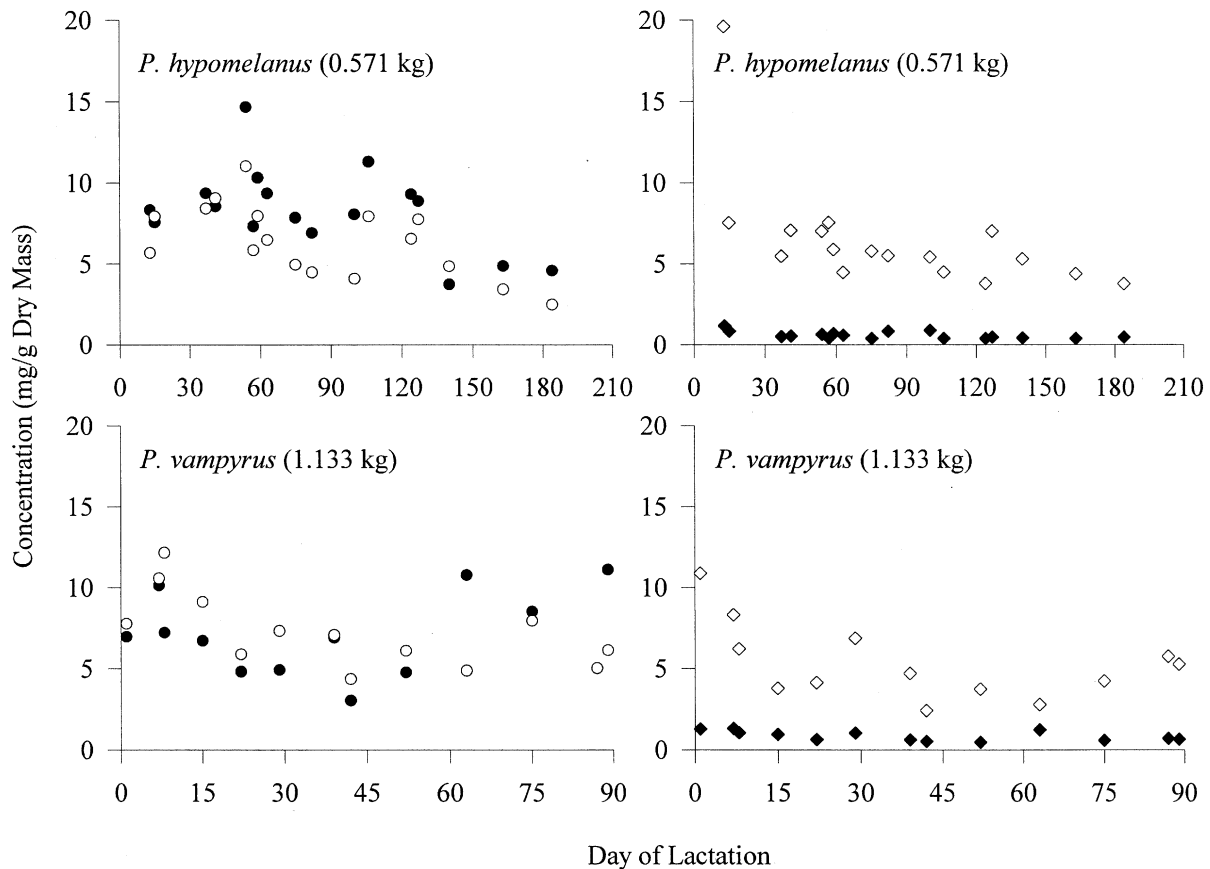
Figure 3. Calcium, phosphorus, magnesium, and potassium content of milk from *Pteropus hypomelanus* and *Pteropus vampyrus*. Symbols in all graphs are as follows: *open circles* = calcium, *filled circles* = phosphorus, *open diamonds* = potassium, *filled diamonds* = magnesium. No significant changes in concentration across lactation were apparent.

hypomelanus, and *Pteropus vampyrus*, despite a 6.5-fold variation in body size. However, we did find a small, but significant, difference in protein concentration. This difference appears not to be associated with species-specific differences in body size, with the protein content of milk being lowest in *P. pumilus* (0.18 kg) and *P. hypomelanus* (0.57 kg), the smallest and the second largest of the species evaluated in this study, respectively. It is more likely that this variation is based on sampling error, with the greatest variation between species less than 2%. The similarity in proximate composition in all four *Pteropus* spp. is consistent with studies where other congeners were compared, as in *Equus* (Oftedal and Jenness 1988) and *Eulemur* (Tilden and Oftedal 1997). Diet composition has little to no affect on the gross composition of milk for healthy animals (Davies et al. 1983), and the effects of diet and captivity on the composition of milk are expected to be minimal. Therefore, we conclude that phylogenetic inertia contributes to the similarity of milk composition within genera.

The composition of milk varies over the course of lactation in most mammals, with the concentration of fat increasing during lactation in domestic rabbits, most rodents, some carnivores, some bats, elephants, giraffes, most pinnipeds, and ruminants, but declining in pigs and horses (Oftedal 1984; Oftedal et al. 1987; Kunz et al. 1995). A rise in fat is often accompanied by rising protein, while carbohydrate often declines (Oftedal 1984). Our study suggests that milk composition is relatively constant during lactation among the four species of *Pteropus*. Dry matter and energy content of milk increased in *P. pumilus* and fat increased in *P. hypomelanus* during the 6-mo lactation period, but no other changes in proximate components were found for these species or in *P. rodricensis* and *P. vampyrus*. Milk composition has been reported for two other megachiropterans (Messer and Parry-Jones 1997; Korine and Arad 1999). The trends in the proximate composition of milk in *P. pumilus* and *P. hypomelanus* are similar to that of *Rousettus aegyptiacus* with increasing fat and energy (Korine and Arad 1999). By contrast, *Pteropus poliocephalus* showed decreasing protein and carbohydrate in milk over the course of lactation

Table 2: Mineral composition of milk from *Pteropus hypomelanus* and *Pteropus vampyrus* milk at peak lactation

	n	Calcium (mg/g dry mass)	Phosphorus (mg/g dry mass)	Magnesium (mg/g dry mass)	Potassium (mg/g dry mass)
P. hypomelanus	6	8.44 ± 4.46	6.22 ± 3.33	.75 ± .51	5.31 ± 2.34
P. vampyrus	5	9.24 ± 4.98	6.02 ± 1.23	.72 ± .29	6.48 ± 3.60

Note. Means and standard deviations are given. Definitions of early, peak, and late lactation for samples analyzed in this study are described in "Methods."

(Messer and Parry-Jones 1997). Among three species of insectivorous bats, milk increased in dry matter, energy, and fat in *Tadarida brasiliensis* but milk only increased in energy and fat in *Myotis lucifugus*. There was a trend toward increased dry matter and energy but not fat in *Myotis velifer* (Kunz et al. 1995). Similarly, the milk of an omnivorous phyllostomid (*Phyllostomus hastatus*) increased in dry matter, fat, and energy (Stern et al. 1997). It is unclear why milk composition is relatively similar across lactation when patterns of composition vary across lactation for many other species. Constant milk composition does not appear to be associated with reduced rates of postnatal development. Microchiropterans typically show an increase in the concentrations of fat and energy during lactation (Kunz and Hood 2000), but their rates of mass deposition adjusted for maternal body mass are similar to that of the megachiropterans (including the pteropodids; Kunz and Stern 1995). It is more likely that constant milk composition is associated with the pattern of maternal care. Pteropodid bats are often suckled long after young first begin to consume solid food (Pierson and Rainey 1992). Whether this behavior is associated with long-term familial bonds is unknown.

The fat and dry matter content of milk from the *Pteropus* spp. in our study was highly variable, suggesting that these components could be influenced by several factors. Previous studies have shown that the proximate composition in milk may vary according to individual (Derrickson et al. 1966), body condition (Sampson and Jensen 1984), time of milk collection (Saxena et al. 1997), and degree of gland evacuation (Cross 1977; Daly et al. 1993). Effect of female body mass, length of forearm, time of day, and volume collected on proximate composition was examined in *P. hypomelanus*. Results from the multivariate multiple regression suggest that there is no association between these variables and milk composition. However, milk volume was negatively associated with fat concentration, independent of day of lactation, but this significance was not evident in the multivariate analysis. The relationship between milk fat and volume warrants further investigation. Daly et al. (1993) have shown the first sequential sample collected from full human mammae is lower in fat than the final milk collected. Thus, collecting a greater percentage of milk from a fully engorged gland should reflect a greater concentration of fat than if a lower percentage of the milk was evacuated. If the gland is not filled to capacity, the milk is likely to be higher in fat, than from a gland that is full, reflecting the residual high milk fat left in the gland following the previous milking (Daly et al. 1993). Thus, a negative relationship between milk fat and degree of gland evacuation in *Pteropus* suggests that either most or all of the milk within the gland was not consistently expressed and that the first milk collected from an engorged gland was higher in fat than the residual milk, which contradicts Daly et al. (1993), or alternatively suggest that milk was collected from bats with different degrees of glandular fill. A smaller volume collected may indicate that a pup had suckled shortly before premilking separation, whereas a larger volume, with lower fat concentration, suggests that the mammae of females were more fully engorged at the time of milking.

Mineral Composition

There were no differences between the concentrations of minerals in the milk of *P. hypomelanus* and *P. vampyrus*, and no trends in composition of minerals were associated with day of lactation. Changes in the concentration of minerals during lactation are common in mammals, including swine, bovine, rats, and humans, where samples have spanned the full lactation period (Pond and Maner 1974; Nicholas and Hartmann 1991; Atkinson et al. 1995; Casey et al. 1995). Potassium is generally negatively correlated with decreasing lactose and thus may rise during lactation (Nicholas and Hartmann 1991; Atkinson et al. 1995). Calcium and magnesium, which bind phosphorylation sites on milk caseins, are positively correlated with increasing protein in some species (Pond and Maner 1974) but not in humans (Atkinson et al. 1995) or the phyllostomid bat *P. hastatus* (Stern et al. 1997). In *P. hastatus*, both calcium and magnesium decreased during lactation, whereas sodium, iron, and potassium remained constant (Stern et al. 1997). It is possible that the minerals in milk of *Pteropus* spp. show similar correlations with proximate components as described in other species, but these relationships remain unclear because there were no changes associated with day of lactation for lactose, protein, potassium, calcium, or magnesium.

Fatty Acid Composition

Relatively small differences were found between the concentrations of the fatty acids 12:0, 14:0, 18:1n11, 18:0, and 18:2n6

Table 3: Fatty acid composition of milk (mass %) from *Pteropus hypomelanus* and *Pteropus vampyrus*[a]

Fatty Acid	P. hypomelanus	P. vampyrus	Comparison between Species	Fatty Acid	P. hypomelanus	P. vampyrus	Comparison between Species
10:0	.26 ± .08 I	.23 ± .13	NS	18:1n11	…	.05 ± .04	$P < .001$
12:0	.42 ± .09	.75 ± .21	$P = .0026$	18:1n9	34.53 ± 3.32	33.69 ± 4.25	NS
14:0	10.21 ± 1.02	11.89 ± 1.31	$P = .0025$	18:1n7	2.17 ± .94	1.98 ± .65	NS
14:1n5	.94 ± .48	1.29 ± .38	NS	18:1n5	.36 ± .94	.37 ± .16	NS
Iso15	.15 ± .28 D	.16 ± .21	NS	18:2n6	1.39 ± .36	2.00 ± .81	$P = .002$
16:0	36.69 ± 3.37	35.21 ± 5.62	NS	18:3n3	.17 ± .08	.23 ± .15	NS
16:1n9	.22 ± .10	.27 ± .18	NS	20:1n9	.15 ± .11 I	.24 ± .14	NS
16:1n7	7.69 ± 2.30	8.20 ± 1.80	NS	20:1n7	.08 ± .12 D	…	NS
7Me16:0	.23 ± .15	.32 ± .13	NS	20:3n6	.07 ± .04	.06 ± .06	NS
18:0	3.81 ± 1.05	2.65 ± .79	$P = .0029$	20:4n6	.09 ± .04	.07 ± .06	NS

Note. Means, standard deviations, and comparisons between species for full lactation period are given. "NS" indicates that the comparison between species was not significant. Significant change in fatty acid concentration during lactation occurs for those results marked by an "I" for those fatty acids that increased across lactation and a "D" for those that decreased.

[a] Up to 70 fatty acids (range 4:0–24:1n-9) are normally detected in this analysis, but only fatty acids found at ≥0.05% are reported.

in *P. hypomelanus* and *P. vampyrus* milk. The means of these fatty acids differed by less than 2% and thus were likely to vary as a result of chance or minor dietary differences rather than reflect true differences among species. Only minor changes were apparent in the fatty acid composition in the milk of *P. hypomelanus*, but no changes were apparent in the milk of *P. vampyrus*. By contrast, an increase in the concentration of medium-chain fatty acids occurs over lactation in humans, horses, rats, and rabbits (Hall 1971; Bitman et al. 1985; Jensen 1989; Doreau et al. 1992), whereas changes in long-chain fatty acids have been found during lactation in the red kangaroo and tammar wallaby, with 16:0 decreasing and 18:0 and 18:1 increasing in both species (Griffiths et al. 1972; Green et al. 1983).

Comparison to Other Chiroptera

In this study, the proximate composition of milk at peak lactation in the four species of *Pteropus* are similar to those reported for three other pteropodids that have been described (Quicke et al. 1984; Messer and Parry-Jones 1997; Korine and Arad 1999). The protein and carbohydrate content of milk among these pteropodids adhere to a relatively narrow range of means, 2.07%–3.64% and 5.69%–6.45%, respectively, whereas the fat, dry matter, and energy are more variable (Quicke et al. 1984; Messer and Parry-Jones 1997; Korine and Arad 1999). The fat content of the four species of *Pteropus* in our study had mean peak fat concentrations most similar to milk in free-ranging *R. aegyptiacus* Korine and Arad 1999), at 5.90%–10.20% fat, but were greater than values reported for captive and free-ranging *P. poliocephalus* (Messer and Parry-Jones 1997) and pooled samples of captive and free-ranging *Epomophorus walhbergi* (Quicke et al. 1984). The proximate composition of free-ranging and captive *P. poliocephalus* were

similar (Messer and Parry-Jones 1997), suggesting that milk composition is independent of diet.

The constraints of flight and mammary capacity may select for more concentrated milk among those bats that forage over longer distances and suckle young less frequently (Kunz et al. 1995). In free-ranging animals, young *Pteropus* appear to be suckled on demand during the day, and possibly at night during the first few weeks postpartum when they are carried by their mothers as they forage. Following this perinatal period, young bats are left at the roost while their mothers forage (Nelson 1965; Pierson and Rainey 1992; Jones and Kunz 2000; Kunz and Jones 2000). Foraging distance and duration have been described for relatively few pteropodids. Foraging distances reported for *P. vampyrus* and *P. poliocephalus* are similar at 32–48 km and 50 km, respectively (Lim 1966; Eby 1991). Assuming that milk composition reflects life history characteristics in the wild, it is unlikely that foraging distance accounts for differences in the milk fat composition of *P. poliocephalus* and *P. vampyrus*.

The composition of pteropodid milk is fairly similar to that of frugivorous phyllostomids (see Kunz and Hood 2000 for a review of milk composition among bats). The composition of milk in *Artibeus jamicensis* at peak lactation (Oftedal and Iverson 1995) is nearly identical to that of *Pteropus* spp. as observed in this study. The dry matter, fat, and energy content of milk in *Pteropus* was lower than that of several of the values presented by Huibregtse (1966); however, his values should not be considered representative because they are based on only one or two samples pooled from several bats in which the stage of lactation was unknown. The carbohydrate content of pteropodid milk is approximately 2% higher than most frugivorous phyllostomids. The milk of an omnivorous phyllostomid, *P. hastatus*, is higher in dry matter, fat, protein, and energy than *Pteropus*, but the carbohydrate content of the milk is lower and

similar to other phyllostomids (Stern et al. 1997). The milk of insectivorous bats of the families Vespertilionidae and Molossidae are generally higher in dry matter, fat, and protein than that of *Pteropus*, but the carbohydrate content of insectivorous bat milk is only about half that of *Pteropus* (Kunz and Hood 2000). These differences between insectivorous and frugivorous bats appear to reflect differences in diet composition, corroborating results from Kunz and Stern (1995). The similarity between the proximate composition in the relatively small phyllostomids and the larger pteropodids suggest that body size may not be a significant factor contributing to milk composition.

Data on the mineral and fatty acid composition of bat milk are limited. Mineral composition of milk has been reported for three other pteropodid species, *Cynopterus horsfieldi* (short-nosed fruit bat), *Dycopterus spadiceus* (Dayak fruit bat), and *Megarops ecaudatus* (tailless fruit bat; Studier et al. 1995). Although the concentration of minerals in *Pteropus* milk from this study is in close agreement with these other species, data are based on single samples, and thus, it is unclear whether they are typical and representative of all stages of lactation. Relationships between milk minerals, diet, and phylogeny were evaluated among two frugivorous pteropodids, *P. hypomelanus* and *P. vampyrus*, an omnivorous and frugivorous phyllostomid, *P. hastatus* and *A. jamaicensis*, respectively, and one insectivorous molossid, *T. brasiliensis* (Studier and Kunz 1995; W. R. Hood, E. H. Studier, and G. G. Kwiecinski, unpublished data). Magnesium and potassium were highest among the frugivores, followed by omnivores, and lowest in the insectivorous species. No correlation was found between calcium content of milk and diet. Significant differences in the mineral composition of milk, for calcium, magnesium, sodium, and potassium, was found between the two families of frugivores, Pteropodidae and Phyllostomidae. The concentration of milk calcium, magnesium, sodium, and potassium appears to be weakly associated with phylogeny, based on a comparison of pteropodids and phyllostomids, with calcium and potassium being highest among the pteropodids and magnesium and potassium in milk most concentrated in phyllostomids (W. R. Hood, E. H. Studier, and G. G. Kwiecinski, unpublished data).

This is the first report of fatty acid composition in pteropodid milk. The result of previous studies that examined the concentration of fatty acids in the milk of bats (Stull et al. 1966; Glass and Jenness 1971; Jenness and Studier 1976; Kunz et al. 1983; Kunz and Hood 2000) should be interpreted with caution. Most species in these studies are represented by a single sample, resolution for long-chain polyunsaturated fatty acids is poor, and high proportions of unidentified fatty acids were included (Iverson and Oftedal 1995). Similar to other bat species, palmitic (16:0) and oleic (18:1n9; location of the double bond is not given in previous studies) are the most prevalent fatty acids in *Pteropus* milk, each accounting for approximately 35% of milk fat, and myristate (14:0) and palmitoleate (16:

1n7) were prominent. The fatty acid profiles of milk from the two pteropodid species in our study are similar to those in other frugivorous phyllostomids that have been described (Stull et al. 1966; Jenness and Studier 1976). Differences in fatty acid profiles among bats appear to reflect differences in diet, with the fatty acids in the milk of frugivores being high in long-chain saturated and monounsaturated fatty acids (Kamel and Kakuda 1992; Peng 1992). The milk of insectivores have high levels of 18:2n6 and 18:3n3; the latter fatty acids are likely to be derived from their insect diet (Iverson and Oftedal 1995). Medium-chain fatty acids (12:0) may result from the de novo synthesis by insectivorous species, *Myotis thysanodes* (Glass and Jenness 1971), *Myotis lucifugus*, and *Eptesicus fuscus* (Kunz et al. 1983; Iverson and Oftedal 1995). Because medium-chain fatty acids do not occur in *Pteropus* and in only trace amounts among other species, they appear to be a derived product within a lineage that includes *Myotis* and *Eptesicus* (Stull et al. 1966; Jenness and Studier 1976).

Conclusions

Our findings suggest that milk composition is generally similar within the genus *Pteropus*, despite a 6.5-fold difference in body mass among species that we evaluated. Minor differences in protein composition and the fatty acids 12:0, 14:0, 18:0, 18:1n11, and 18:2n6 occurred between the species of *Pteropus* described, but all other components were relatively constant between species, despite large variations in body size. These findings agree with other comparisons within genera, where milk composition does not vary between species. Milk composition is relatively constant across lactation for most proximate, mineral, and fatty acid components among the four *Pteropus* species examined in this study. A significant increase was found in the content of dry matter and energy in *Pteropus pumilus* and fat in *Pteropus hypomelanus*. The concentrations of dry matter and milk fat were highly variable across lactation for all species, suggesting that any trends associated with day of lactation in *Pteropus rodricensis* and *Pteropus vampyrus* were obscured by this variability. The weak correlation observed between milk fat and milk volume warrants further investigation.

Physiological and ecological patterns in the density of fatty acids and milk minerals remain tentative due to inadequate representation of species, undefined stage of lactation, and errors associated with methods of analysis. Comprehensive analysis of additional species with different dietary habits and life history traits will contribute to a better understanding of how minerals and fatty acids are influenced by ecological, physiological, and phylogenetic constraints.

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