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Rates of seasonal fuel loading do not differ by sex or overwintering strategy in three species of bats

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For temperate-zone mammals, seasonal changes in weather and food availability often govern energy allocation. In addition, energy allocation strategies usually differ between males and females. Bats are an interesting group in which to evaluate energetic trade-offs as they are highly mobile and lead energetically demanding lives in habitats across a variety of seasonally variable climates. We evaluated year-round changes in body mass and fuel load for three species of bats in northern California: Silver-haired Bat (*Lasionycteris noctivagans*), California Myotis (*Myotis californicus*), and Yuma Myotis (*Myotis yumanensis*). Silver-haired bats are considered migratory species with females likely migrating farther than males. The two species of myotis are considered residents. Body mass of all species peaked in late autumn and was at a minimum during spring. We calculated a fuel load index to normalize size difference between species and sexes. We used sex- and season-specific multiple linear regression models to evaluate rates of change in seasonal fuel loading. Rates of change in fuel load did not differ among species or sexes except for male silver-haired bats that increased fuel loads rapidly during summer. Interspecific comparisons provided valuable insights into the energy allocation and overwintering strategies of these species and are an important initial step toward understanding their ecology over the full annual cycle.

Key words: body condition, body mass, energy allocation, fuel load, full annual cycle, *Lasionycteris noctivagans*, *Myotis californicus*, *Myotis yumanensis*, thrifty female hypothesis

Los cambios estacionales de las condiciones climaticas y de la disponibilidad de alimento a menudo determinan la asignación energética para los mamíferos de zonas templadas. Además, las estrategias de asignación energética suelen diferir entre machos y hembras. El estudio de la asignación energética es desafiante para los murcielagos, en particular para aquellos que no tienen dormideros en áreas conocidas o accesible para el hombre. Los murciélagos constituyen un grupo interesante en el que evaluar las relaciones energéticas coste-beneficio, ya que presentan gran movilidad y llevan vidas de alta demanda energética en una variedad de hábitats con climas variables estacionalmente. Evaluamos los cambios anuales en la masa corporal y carga de grasa de 3 especies de murciélagos en el norte de California: el murciélago canoso (*Lasionycteris noctivagans*), el murciélago orejas de ratón Californiano (*Myotis californicus*) y el Yuma myotis (*Myotis yumanensis*). El murciélago canoso se considera una especie migratoria con las hembras probablemente migrando a distancias mayores que los machos. Las dos especies de myotis se consideran residentes. La masa corporal de todas las especies alcanzó su máximo a finales de otoño y fue mínima durante la primavera. Calculamos un índice de carga de grasa para normalizar la diferencia de tamaño entre especies y sexos. Utilizamos modelos de regresión lineal múltiple específicos por sexo y por estación del año para evaluar las tasas de cambio en la carga de grasa estacional. Las tasas de cambio en la carga de grasa no difirieron entre especies o sexos, excepto para los machos de murciélago canoso, los cuales incrementaron rápidamente la carga de grasa durante el verano. Las comparaciones interespecíficas proporcionaron

información valiosa sobre la alocación energética y las estrategias de invernada de estas especies y representan un paso inicial importante para comprender su ecología durante el ciclo anual completo.

Palabras clave: alocación energética, carga de grasa, ciclo anual completo, condición corporal, *Lasionycteris noctivagans*, masa corporal, *Myotis californicus*, *Myotis yumanensis*

Constrained by seasonal changes in climate and food availability, temperate-zone mammals have evolved a range of reproductive and cold-survival strategies that allow them to balance short- and long-term energetic investments toward growth, survival, maintenance, and reproductive output (Pianka 1976; López-Alfaro et al. 2013). Over the annual cycle, energy allocation differs depending on where a species falls on a continuum of cold-survival strategies including hibernation, migration, and resistance (Auteri 2022). Furthermore, the duration and scale of energetic investment in reproduction can differ greatly between sexes with females typically incurring greater energetic costs over longer time periods than males (Gittleman and Thompson 1988; Buck and Barnes 1999; Beck et al. 2003; López-Alfaro et al. 2013).

Many temperate-zone mammals cope with winter food shortages by increasing fat stores during autumn (Prestrud and Nilssen 1992; Buck and Barnes 1999). Regardless of whether a species migrates, hibernates, or resists winter conditions, fat mass is expected to increase prior to winter, because a better starting body condition improves survival when foraging resources are scarce (Weber et al. 1998; Speakman and Rowland 1999; Willis 2017). Body condition also positively correlates with reproductive success, so the challenges of adding fat stores prior to winter are magnified for mammals that mate during autumn (Bronson 1985; Gittleman and Thompson 1988; Flydal and Reimers 2002). This represents a trade-off period for mammals as the need to increase reproductive output competes with the need to maximize fat stores to survive winter.

Temperate-zone bats avoid the challenges of cold temperatures and low prey density during winter via use of migration, hibernation, or a combination of both (Cryan 2003; Auteri 2022). Although both migration and hibernation require preparatory fattening, we should expect differences in the timing and details of annual mass change based on the sex and overwintering strategy of the individual. Although maximizing body condition prior to winter is important whether a species migrates or hibernates, it is especially critical for strict hibernators because they rely on accumulated fat stores to survive winter. Costs associated with fat accumulation are minimized for hibernating bats because they do not engage in extended periods of flight during winter and risk of predation is low (Speakman and Rowland 1999). However, the quantity and timing of energy storage and use by migratory species is more complicated as they need to balance fat reserves necessary to fuel migration versus increased flight loads (Dechmann et al. 2017). Species overwintering in areas where they remain active may be able to supplement winter energy stores by foraging (Avery 1985; Bernard et al. 2021), which may have a moderating influence on fuel depletion.

We also expect differences in seasonal energy allocation between females and males that follow from differential timing and duration of energy investment into reproduction (Rughetti and Toffoli 2014; Willis 2017; Pretorius et al. 2021). Females have a prolonged period of reproductive energy investment beginning with mating in autumn, sperm storage through winter, gestation during spring, and continuing through lactation in mid- to late summer (Racey and Entwistle 2000). Thus, females must allocate energy to reproduction throughout most of the annual cycle (Zahn et al. 2007). Because energetic demands of mating during autumn are expected to be much less than for males, females may enter winter with greater fuel stores than males (Zahn et al. 2007). Females are also expected to minimize energy consumption during winter to emerge from hibernation with greater fat stores necessary to support gestation in the spring (Czenze et al. 2017). Hence, the thrifty female hypothesis predicts that female bats will accumulate greater fat stores in autumn and deplete them at a slower rate than males during winter (Jonasson and Willis 2011; Czenze et al. 2017). Following winter, females generally increase their body mass faster than males to prepare for gestation and migrations to natal grounds (Dechmann et al. 2014; Rughetti and Toffoli 2014). Lactation is the most energetically expensive portion of the active season (Kurta et al. 1989) and female body mass often decreases during this period in mid-summer (Rughetti and Toffoli 2014; Sommers et al. 2019; Pretorius et al. 2021). However, body condition usually increases soon thereafter to support the high energetic demands of autumn migration or hibernation (Rughetti and Toffoli 2014; Lacki et al. 2015). For example, body mass of migratory *Tadarida brasiliensis* was as high during migration as it was during late pregnancy (Sommers et al. 2019).

For male bats, good body condition is a prerequisite for spermatogenesis (Speakman and Racey 1989; Entwistle et al. 1998). Male body mass is expected to increase gradually through spring and summer before peaking during late summer or early fall prior to mating (Zahn et al. 2007; Gallant and Broders 2015). The energetic costs of mating and migration during autumn sometimes result in a decrease in body mass followed by recovery prior to the onset of winter (Zahn et al. 2007; Rughetti and Toffoli 2014; Kohyt et al. 2016). Because males do not have the same reproductive constraints as females in spring, they can use some of their energy stores to copulate with females during winter and early spring (Czenze et al. 2017; Clerc et al. 2022).

There is growing recognition that the strategies bats use to allocate energy throughout the year will vary among species, sexes, and individuals. Examining this variation can provide insights into the generalities as well as the limits of energy allocation strategies (Auteri 2022). For example, how an

animal that hibernates at northerly latitudes allocates energy throughout the year may differ from one that migrates to areas with more temperate climes for the winter, but some of the same patterns of fat mass deposition and depletion may be observed. Theoretical and empirical studies of temperate bat species life history allow us to generate working hypotheses of relative seasonal energy demands associated with reproductive investment (i.e., males vs. females) and overwintering strategy (i.e., hibernators vs. migrators; Table 1; Rughetti and Toffoli 2014; Willis 2017; Pretorius et al. 2021). We hypothesize that migrant fuel deposition rates will peak in late summer prior to migration, and hibernator fuel deposition rates will peak during autumn, as close to entering hibernation as possible (H 1.1). Further, because migrants are expected to supplement fuel stores by foraging throughout the winter, we expect hibernators to deplete their fuel mass more rapidly than migrators during winter (H 1.2). We also hypothesized that regardless of overwinter strategy, females will increase fuel stores faster than males prior to migration or hibernation (H 2.1) and deplete fuel stores more slowly than males over winter; consistent with the "thrifty female" hypothesis (H 2.2; Jonasson and Willis 2011). Further, we expect that even nonpregnant females will have more variable fuel loads during spring and summer compared to males that will gradually improve body condition during this period in preparation for autumn mating (H 2.3)

To date, few comprehensive data sets have been used to empirically evaluate patterns of annual energy needs in temperate-zone bats. To evaluate our hypotheses, we generated regionally explicit predictions for year-round changes in body mass for three species of bats in northern California that exhibit a range of overwintering behaviors (Table 1). Silver-haired bats (*Lasionycteris noctvagans*) are considered migrants, whereas Yuma Myotis (*Myotis yumanenis*) and California Myotis (*M. californicus*) are considered hibernators. Northern California is a unique study system because its relatively mild climates allow some species to be active year-round. Though silver-haired bats are considered migrants, some individuals may be year-round residents but the majority of the autumn and winter population, particularly females, are expected to be composed of individuals that migrate from elsewhere (Clerc et al. 2017) and both sexes are active during winter (Falxa 2007; Weller and Stricker 2012). Although their winter ecology is not well understood, California Myotis are more active than Yuma Myotis during winter (Falxa 2007; Weller and Stricker 2012). Nevertheless, we expect both myotis species to rely largely on consumption of endogenous energy stores during winter.

Materials and Methods

Between the years 1996 and 2018, we captured bats at 73 sites in five northern California counties spanning an area of ca 43,000 km^2 (Fig. 1). We attempted to capture bats during all months of the year using standard 2.6-m-high mist nets and with three standard mist nets stacked on top of one another. We opened mist nets at sunset and typically tended them until 3.5 h after sunset. We identified species via a combination of morphological measurements and visual inspection. We distinguished *M. yumanensis* from *M. lucifugus* using a combination of forearm length and a recording of echolocation calls upon release (Weller et al. 2007). Categorization to relative age class (adult or young-of-the-year) relied on inspection of degree of ossification of phalangeal epiphyses on the transilluminated outstretched wing (Brunet-Rossini and Wilkinson 2009). We determined the sex of each captured individual based on inspection of external genitalia (Racey 2009). Reproductive condition was observed visually in males (distended scrota, percent filling of caudal epididymes; Encarnação et al. 2004), and visually or by gentle palpation in females (lactation, postlactation, or pregnancy; Racey 2009). We excluded pregnant females and young-of-year bats from analyses. We measured forearm length to the nearest 0.1 mm using dial calipers. We weighed bats to the nearest 0.1 g by wrapping them snugly in a nylon stocking and placing them on a digital scale (Triton T2, HB1 Technologies, Phoenix, Arizona). Beginning in 2007, we held bats in clean, cloth bags between capture and

Table 1.—Hypotheses and predictions associated with annual energy allocation for three species of bats in northern California.

Hypothesis	Hypothesis details	Predictions
H1: Energy allocation depends on overwinter strategy	H 1.1: Migrants will have greatest fuel deposition rates during summer prior to migration and hibernators will have greater fuel deposition rates during autumn prior to hibernation	P 1.1: Silver-haired bats will have maximum fuel deposition rates, and their greatest foraging activity during summer, whereas these will occur during autumn for California Myotis and Yuma Myotis
	H 1.2: During winter, migrants have lower fuel depletion rates than hibernators because migrants can supplement their fuel supply by foraging	P 1.2: Silver-haired bats will have a lower fuel depletion rate and increased foraging activity during winter relative to California Myotis and Yuma Myotis
H2: Energy allocation depends on sex	H 2.1: Females will accumulate more fuel than males prior to migration/hibernation	P 2.1: Female silver-haired bats will have greater fuel loads, fuel deposition rates, and foraging activity than males prior to migration and female California Myotis and Yuma Myotis will have greater fuel loads and fuel deposition rates than males prior to hibernation
	H 2.2: Females will deplete fuel stores over winter more slowly than males ("thrifty female" hypothesis)	P 2.2: For all three species winter fuel depletion rates will be greater for males than females
	H 2.3: Females will have more variable fuel loads than males as they matriculate from fertilization through lactation during spring and summer while males will gradually improve body condition in preparation for autumn mating	P 2.3: For all three species, daily body mass during summer will be more variable for females, and gradually increase for males; though overall mass change will be similar between the sexes

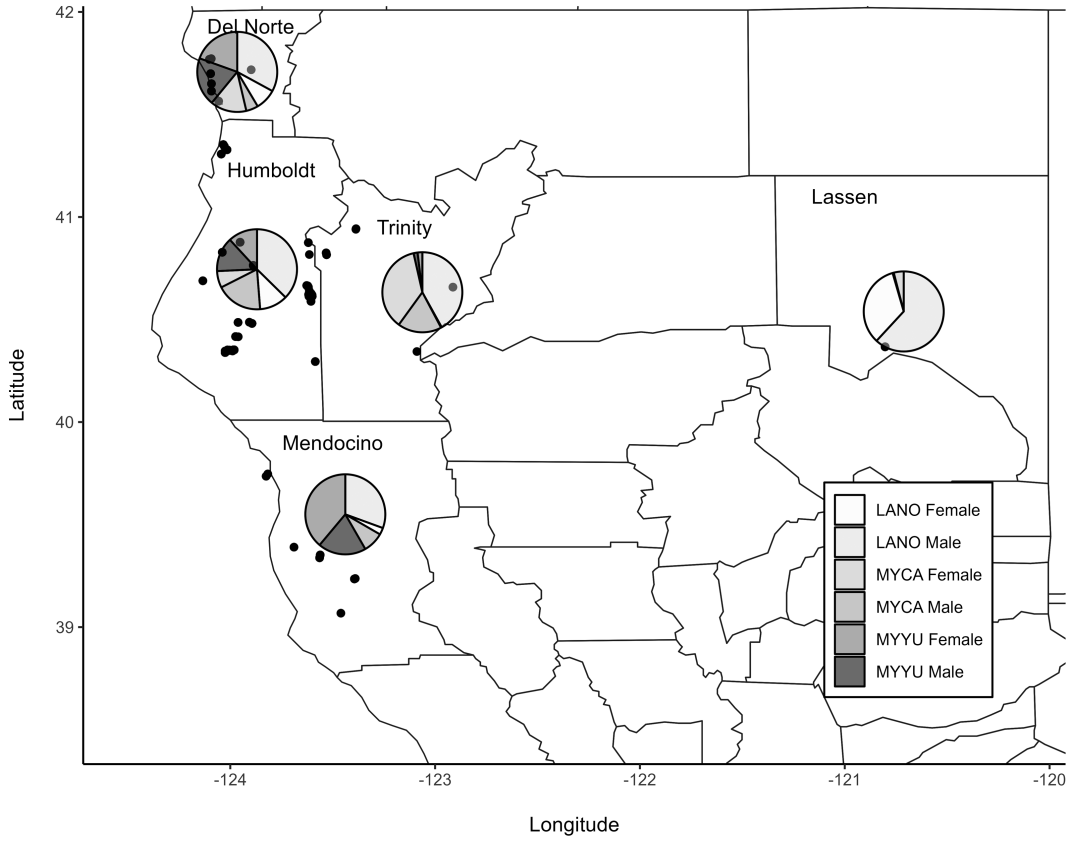

Fig. 1.—Northern California with county borders. Individual capture sites are black dots and pie charts represent the cumulative proportion of three species of bats captured by sex in each county. LANO = Silver-haired Bat; MYCA = California Myotis; MYYU = Yuma Myotis. Sample size of all species by county: Del Norte, $n = 82$; Humboldt, $n = 1{,}369$; Lassen, $n = 223$; Mendocino, $n = 36$; Trinity, $n = 310$.

release to determine whether they produced guano, which would indicate recent feeding (Buchler 1975). We held bats that did not produce guano for a minimum of 1 h (mean ± *SD*, 97 ± 55 min) prior to release.

We used generalized additive models (GAMs) to visualize detailed patterns of mass change of bats by calendar date separately for each species and sex (P 2.3). We used a Gaussian link function, and the "mgcv" package in R version 4.1.2 which provides automatic smoothing (Wood 2011; R Core Team 2021). We used body mass, rather than a body condition index because it is an effective predictor of fat content and is easily interpreted and measured in the field (McGuire et al. 2018). We also quantified fuel load, to account for size differences among species, to achieve our primary objective of comparing seasonal rates of change in fat stores among species and sexes. Fuel load is dimensionless and defined as the fuel mass (often synonymous with fat mass; McGuire et al. 2018) an individual carries relative to fuel-free (often called fat-free or lean) body mass (Weber et al. 1998). Fuel load is intended to characterize the fat stores of an individual in anticipation of life cycle events such as reproduction, migration, or overwintering (van Der Meer and Piersma 1994) and is not the same as functional fat reserves that are maintained and metabolized in emergencies prior to starvation. However, in practice, it is difficult to distinguish between fat stores and reserves, so fuel load is most often quantified as the total fat mass relative to total fat-free mass. We generated an approximation of fat-free masses for each species–sex combination, by calculating the mean mass of the lightest 5% of individuals encountered across the annual cycle. We assigned a fuel load to each individual by dividing its body mass by the estimated fat store-free mass for its species–sex group, truncating values at 1.0 assuming that fuel load values of 1.0 represented individuals with exhausted fuel stores and that fuel loads greater than 1.0 represented individuals with available fuel stores. To confirm that our approach was reasonable for our system, we compared our fat-free mass estimates to measurements obtained using quantitative magnetic resonance (QMR) for silver-haired bats in New Mexico (Clerc et al. 2022).

We compared mean values of fuel load between sexes within season using two-sample *t*-tests with the Holm correction for multiple tests (P 2.1). We fit linear models of change in fuel load (i.e., fuel deposition rate) as a function of date separately for each species and season using the "lm" function in R. We divided the year into three seasons based on the phenology of bats in our study area and patterns observed in the GAMs. The summer season was between 1 April and 15 August, autumn between 16 August and 31 October, and winter between 1 November and 31 March. We log-transformed fuel load in the models to address issues of constant variance and back-transformed the predicted values. Linear analysis of fuel deposition rates ignored some of the nuances observed in GAMs of mass change but simplified our primary goal of comparing rates among species, sex, and

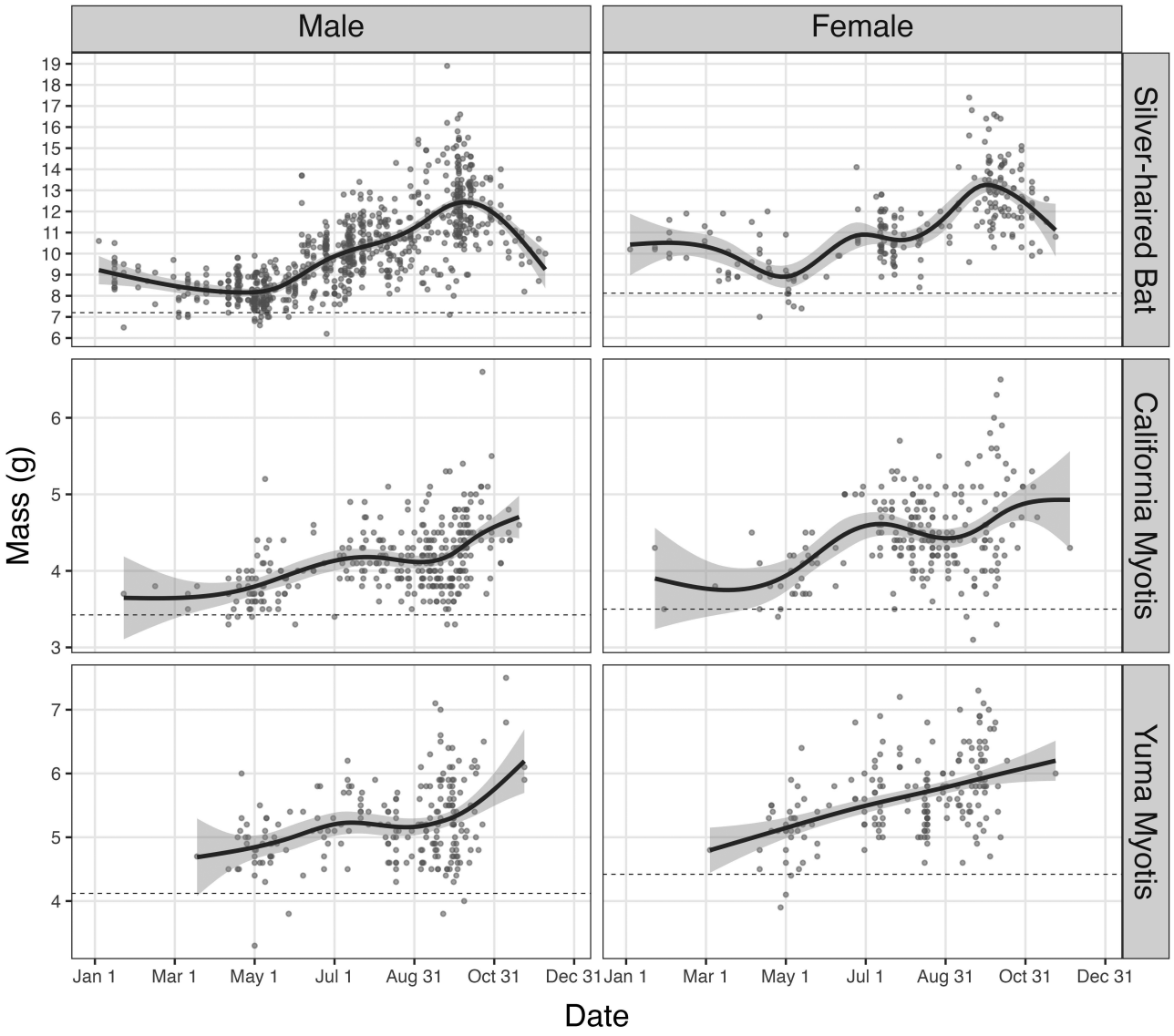

Fig. 2.—General additive models of annual mass change in three species of bats in northern California. Dots represent individual bats. Estimated mean values are solid black lines and gray area is 95% confidence interval. Dashed line represents the estimated fat-free mass for each species–sex combination.

season. We compared seasonal trends in fuel deposition rates with estimated marginal means with the "emmeans" package in R (Lenth 2022). Specifically, we compared rates of fuel load change between: (i) males and females for each species in each season (P 2.1 and P 2.2); (ii) summer and autumn for each species and sex (P 1.1); and (iii) among species within season, matched on sex (P 1.1 and P 1.2). We used the Šidák correction to adjust *P*-values for multiple trend comparisons (Šidák 1967). We compared frequency with which bats produced guano, our proxy for foraging activity, among species and season (P 1.1 and P 1.2) using Chi-squared tests of independence and used Bonferroni-corrected post hoc comparisons.

Results

We obtained mass measurements from a total of 2,019 bats including 817 male and 240 female silver-haired bats, 320 male and 226 female California Myotis, and 218 male and 198 female Yuma Myotis. We captured silver-haired bats between 3 January and 10 December, California Myotis between 22 January and 5 December, and Yuma Myotis between 4 March and 24 November (Fig. 2). We captured 112 silver-haired bats, 20 California Myotis, and 7 Yuma Myotis during winter. Winter capture records support the assertion that silver-haired bats have relatively high activity levels during winter and that, despite intermittent activity, California Myotis and especially Yuma Myotis are largely inactive and presumably hibernating during winter.

Preliminary analyses indicated no biologically meaningful differences in mass among counties, so we pooled data from all counties for subsequent analyses. We estimated fat-free mass silver-haired bats to be 7.77 g for males and 8.13 g for females which is 7% and 2.1% lower than measured with QMR for males and females, respectively, during spring in New Mexico (Clerc et al. 2021). Based on results from silver-haired bats we

inferred that our approach for estimating fat-free mass in other species was also reasonable.

All three species were at maximum body mass in mid- to late October (Fig. 2). Body mass was at a minimum in early April for California Myotis and Yuma Myotis and in late April for silver-haired bats. Female bats of all three species had a short period of mass loss in mid-August followed by resumption of mass gains during autumn (R 2.3; Table 2, Fig. 2). As expected, fuel loads for both sexes of all three species increased during summer and autumn and decreased during winter (Fig. 3). Depending on sex and species, we estimated that, on average, fuel loads increased 0.09–0.26% per day during summer, 0.09–0.25% during autumn, and decreased 0.15–0.29% per day during winter (Supplementary Data SD1). Fuel deposition rates did not differ between summer and autumn for any species–sex combination ($P \geq 0.989$; Fig. 3, Supplementary Data SD2; R 1.1). Male silver-haired bats increased fuel loads during summer at a faster rate than male California Myotis ($t = 5.110$, $P < 0.0001$) and Yuma Myotis ($t = 4.949$, $P = 0.0001$; R 1.1). There were no other within-season differences in fuel deposition rates for any of the other species–sex combinations ($P \geq 0.557$; Supplementary Data SD2).

Mean fuel loads were greater for nonpregnant females than males in California Myotis during summer ($t = 3.73$, $P = 0.001$; R 2.3) and autumn ($t = 6.58$, $P < 0.001$; Fig. 3; R 2.1). However, mean fuel loads did not differ by sex for Yuma Myotis in summer ($t = 0.12$, $P = 0.910$; R 2.3) or autumn ($t = 1.98$, $P = 0.148$; R 2.1), nor for silver-haired bats, during summer ($t = -0.75$, $P = 0.910$; R 2.3). Contrary to our prediction, male silver-haired bats increased fuel loads faster than females during summer ($t = 4.051$, $P = 0.002$; Fig. 3; R 2.1) but not in other seasons (R 2.2; Supplementary Data SD2). The high rate of summer fuel deposition during summer likely contributed to the higher mean fuel load during autumn for male compared to female silver-haired bats ($t = -2.86$, $P = 0.019$; Fig. 3; R 2.1). Males and females of all species had similar rates of fuel depletion during winter (R 2.2), but confidence intervals were wide for the two myotis species because few were measured (Fig. 3).

During spring through autumn, on average 94% of the California Myotis and Yuma Myotis produced guano when captured and proportions did not differ substantially between sexes or among seasons (Fig. 4). Silver-haired bats produced guano less frequently than the myotis in each season. Silver-haired bat guano production was higher during summer than autumn for both males ($\chi^2 = 4.98$, $P = 0.026$) and females ($\chi^2 = 18.09$, $P < 0.001$; R 1.1). But guano production did not differ by sex in silver-haired bats in either summer ($\chi^2 = 2.73$, $P = 0.098$; R 2.1) or autumn ($\chi^2 = 3.26$, $P = 0.071$). During winter, only 2 of 72 male (2.7%) and 2 of 36 (5.3%) female silver-haired bats produced guano (Prediction 1.1). Capture rates for California Myotis and Yuma Myotis were low during winter but at least 50% of individuals in all four myotis species–sex combinations produced guano, including all 13 male California Myotis (Fig. 4).

Discussion

We found that seasonal fuel deposition rates did not differ markedly among three species of bats that exhibit a range of overwintering strategies. As is typical for temperate-zone bats, all three species were at their minimum mass in spring and reached maximum mass in late October (Encarnação et al. 2004; Zahn et al. 2007; Rughetti and Toffoli 2014). We expected that fuel deposition rates would be highest prior to migration or hibernation as bats attempted to maximize fat deposition prior to winter (H 1.1). However, fuel deposition rates did not differ significantly between autumn and summer for any species–sex combination. Nevertheless, daily rates of fuel loading were numerically higher in autumn than summer

Table 2.—Hypotheses, predictions, and results for annual changes in fuel loads in three species of bats in northern California.

Hypothesis	Prediction	Result
H1: Energy allocation depends on overwinter strategy	P 1.1: Silver-haired bats will have maximum fuel deposition rates, and their greatest foraging activity, during summer, whereas these will occur during autumn for California Myotis and Yuma Myotis	R 1.1: Fuel deposition rates did not differ between summer and autumn. Male silver-haired bats had higher rates of fuel deposition in summer than male myotis. Silver-haired bats had higher rates of foraging in summer than autumn, but rates were lower than for myotis species
	P 1.2: Silver-haired bats will have a lower fuel depletion rate, and increased foraging activity during winter relative to California Myotis and Yuma Myotis	R 1.2 We found no evidence supporting that fuel is depleted at different rates between silver-haired bats, California Myotis, or Yuma Myotis. Silver-haired bats produced guano less frequently than the two myotis species during winter
H2: Energy allocation depends on sex	P 2.1: Female silver-haired bats will have greater fuel loads, fuel deposition rates, and foraging activity than males prior to migration and female California Myotis and Yuma Myotis will have greater fuel loads and fuel deposition rates than males prior to hibernation	R 2.1: Contrary to prediction male silver-haired bats increased fuel loads faster than females during summer and had higher mean fuel loads in autumn. Rates of fuel deposition did not differ by sex in the myotis species, but mean fuel loads were greater for California Myotis females than males in summer
	P 2.2: For all three species winter fuel depletion rates will be greater for males than females	R 2.2: No observed differences in mean fuel load or fuel depletion rates across sexes for any species during the winter
	P 2.3: For all three species, daily body mass during summer will be more variable for females, and gradually increase for males; though overall mass change will be similar between the sexes	R 2.3: Male silver-haired bats increased fuel loads faster than females during summer but did not differ by sex in myotis species. Females had more variability in fuel loading than males during summer

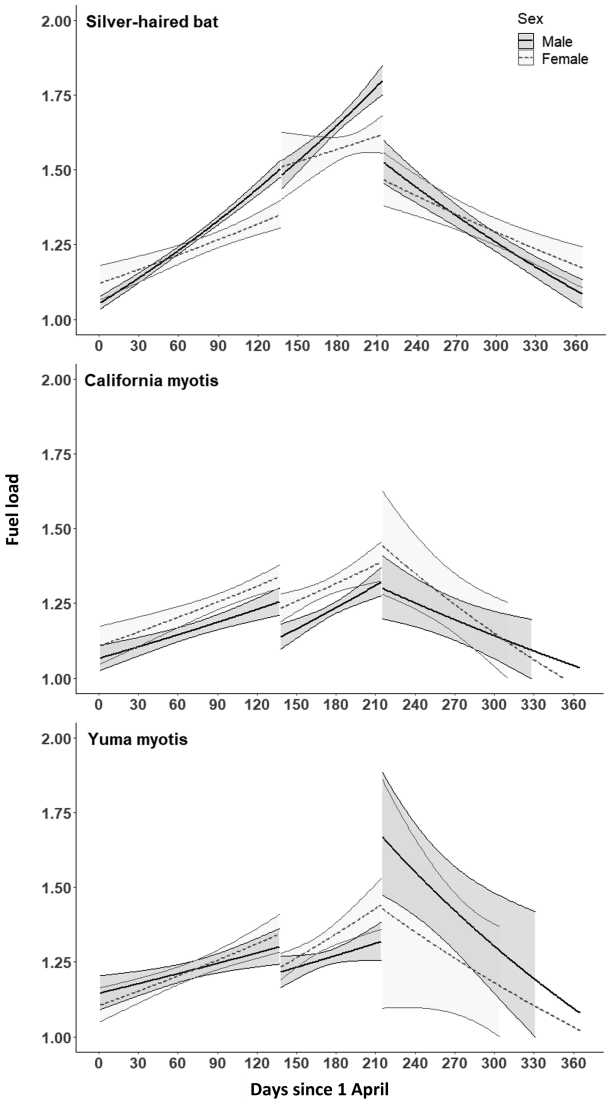

Fig. 3.—Estimated trends in fuel load for male (solid line, dark gray confidence limit) and female (dashed line and light gray) bats in three seasons: summer (1 April to 15 August), autumn (16 August to 31 October), and winter (1 November to 31 March). Fuel loads are proportional changes in mass relative to the lean mass of a bat for each sex–species combination.

for both sexes of both myotis species (Fig. 3). Studies of hibernating bats frequently report rapid gains of mass during early autumn (Kunz et al. 1998; Gallant and Broders 2015; Lacki et al. 2015). We calculated fuel load increases of 7–19% during autumn among our six species–sex combinations which was less than the 27% increase in body mass of male little brown bats that occurred over just a 4-week period during autumn in Nova Scotia (Gallant and Broders 2015). The slower rates of autumn mass gain in northern California may reflect a flexibility in fat storage and overwinter phenology, relative to sites with earlier, harsher winters. For example, a comparative study found that prolonged warm periods during autumn allowed juvenile *M. myotis* in Portugal to achieve a better body condition than those in Germany (Zahn et al. 2007). Fuel deposition by bats in northern California may be a more gradual process if prey remain available and harsh weather conditions, necessitating hibernation, occur later in the autumn relative to higher latitude or mid-continental sites.

We expected, but did not observe, differences in fuel deposition rates during autumn and fuel depletion rates during winter between the two hibernating myotis species and the migratory silver-haired bats, which maintain greater winter activity (H1). That we did not observe differences during these periods suggests that energy requirements for mating and overwintering among these species are similar. Although our winter sample sizes were low, especially for Yuma Myotis, we estimated that fuel loads in the three species in our study area decreased by 22–44% over winter, comparable to estimates of mass loss for bats that hibernate in caves and mines in mid-continental areas (Johnson et al. 1998; Kokurewicz 2004; Jonasson and Willis 2011). Few studies have measured overwinter mass loss in bats that maintain regular activity, though Meridional Serotine (*Eptesicus isabellinus*) in Tunisia were found to lose 26–30% of their mass (Dalhoumi et al. 2016).

Opportunistic feeding may motivate winter activity in bats (Avery 1985; Boyles et al. 2006; Hope and Jones 2012). However, few of the silver-haired bats we captured during winter produced guano (R 1.2), suggesting that something other than feeding motivated their winter activity. Silver-haired bats were the only species of 10 that did not produce fecal samples during a winter study in Tennessee (Bernard et al. 2021). By contrast, most of the myotis we captured showed evidence of feeding, which suggests that feeding, at least opportunistically, may be an impetus for winter flight in these species. Nevertheless, it is unclear how large a contribution winter foraging makes to winter energy budgets, given the high cost of flight in cold temperatures (Speakman and Racey 1989; Klüg-Baerwald et al. 2016). We speculate that mating, or searching for mates, may be the primary motivation for winter activity by silver-haired bats, an activity that should be energetically expensive, especially for males (Kokurewicz 2004; Ignaczak et al. 2019).

We expected fuel deposition rates during summer in silver-haired bats to be greater than other species because they need to acquire sufficient energy to both recover from winter food shortages and travel longer distances between winter and summer ranges, but we only observed that pattern in male silver-haired bats (R 1.1). GAMs indicated that a linear model for summer mass change that began on 1 April was a reasonable choice for California Myotis and Yuma Myotis (Fig. 2), so we used that date for consistency among species even though silver-haired bats appeared to reach their mass minima in late April. The loss of mass we observed for silver-haired bats in April could result if silver-haired bats begin their summer active period later than the other species or if overwintering bats in the best body condition migrate earliest. Alternatively, some silver-haired bats may have migrated to our study area from their overwintering areas elsewhere, depleting their fuel loads in the process. However, migrating common noctules (*Nyctalus noctule*) gained mass between their hibernation sites and their first stopover site in southern Germany (Dechmann et al. 2014). Sharpened resolution on the phenology of Silver-haired bat

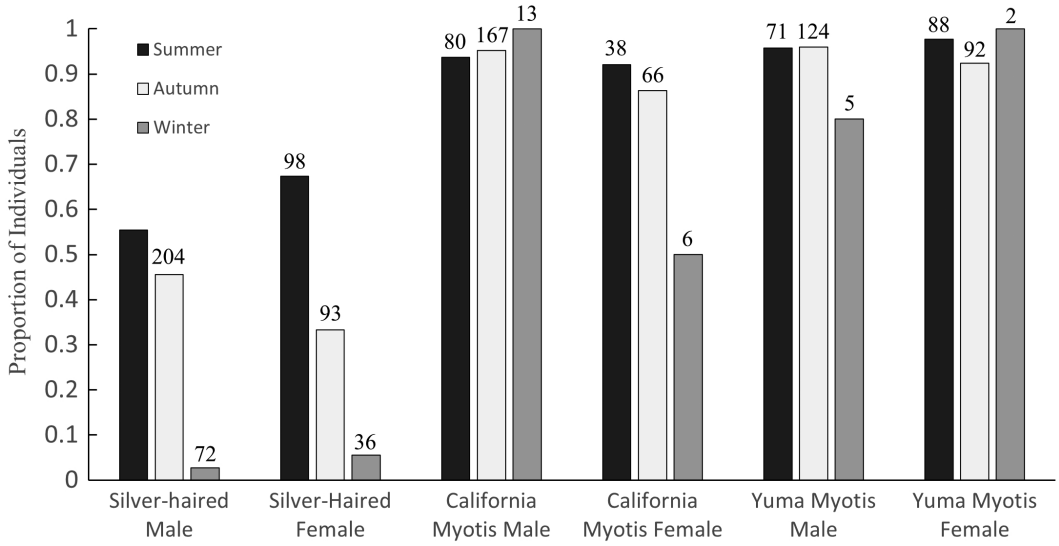

Fig. 4.—Proportion of individuals that produced guano for two sexes of three species of bats in northern California. Numbers above bars indicate sample size of individuals.

activity periods and migratory destinations would help refine understanding of their year-round ecology.

We expected autumn fuel deposition rates to be greater for female bats as we anticipated males would expend excess energy on mating while females maximized acquisition of fat stores prior to winter to facilitate gestation in the spring. Instead, we found similar patterns and rates of mass increase between males and females of all species during autumn (R 2.1). Similarly, a study in Vermont, United States, found that patterns of mass change during autumn were similar between male and female little brown bats (*Myotis lucifigus*; Kunz et al. 1998). If competition for females in our study area was less than others, perhaps because of the later onset of winter relative to other areas, male energy expenditure may be more gradual allowing them to maintain similar rates of mass gain to females (Rughetti and Toffoli 2014).

We expected females to enter winter with larger fuel loads and to consume fuel more slowly than males to facilitate gestation in the spring, consistent with the thrifty female hypothesis (P 2.1 and 2.2; Kokurewicz 2004; Jonasson and Willis 2011; Czenze et al. 2017). However, winter fuel depletion rates did not differ between sexes in any of the three species, nor were there any consistent numeric patterns between sexes as males depleted fuel faster in silver-haired bats and Yuma Myotis, but females depleted fuel faster in California Myotis (R 2.2; Supplementary Data SD1). This may be due to the relatively mild winters of northern California relative to areas where the thrifty female hypothesis was developed. However, prior observations of other species have also failed to support the thrifty female hypothesis: female Indiana bats (*M. sodalis*) lost 23–33% while males lost 15–22% of their mass while hibernating in a cave in Indiana, United States (Johnson et al. 1998) and male and female common noctules, *N. noctula*, a migratory species, emerged from hibernation in southern Germany with similar body conditions (Dechmann et al. 2014).

Contrary to our prediction, we found that male silver-haired bats increased fuel loads at a faster rate than females during summer (R 2.3). We estimated that on 1 May male silver-haired bats had a fuel load of 1.14 which was 2.3% less than the 1.17 fuel load value we estimated for females. However, by 13 August, at the end of summer, the median fuel load for male silver-haired bats of 1.50 was 11% greater than the median female fuel load of 1.35. The difference in fuel deposition rate that we observed contrasted with those observed from Lake Erie, Ontario, during spring when female silver-haired bats were 11% heavier than males (Jonasson and Gugliemo 2016). If a better body condition in male silver-haired bats allows them to be in a state of mating readiness by late summer, prior to arrival of females, their fuel deposition rates during summer would need to be faster than females, particularly if they start in worse condition in the spring. Thus, our findings are consistent with our prediction (P 2.3) that males should steadily increase fuel loads during summer (Clerc et al. 2021) while rates of fuel loading in females will vary according to their phase of reproduction (Zahn et al. 2007; Rughetti and Toffoli 2014).

Summer feeding rates, as indexed by guano production, did not differ between male and female silver-haired bats which indicates that fuel load changes may be more a result of energy outputs than energy inputs. Female silver-haired bats engage in energetically expensive lactation while males likely use daily torpor during summer to increase fuel stores (Grinevitch et al. 1995; Weller et al. 2009; Jonasson and Gugliemo 2016). Additionally, if females make longer seasonal movements than males (Cryan 2003), they would incur greater energetic expenses relative to more sedentary males. Thus, energetic outputs of male silver-haired bats may be lower than females allowing them to maximize fuel loads prior to mating even if they start from a relatively worse condition than females in spring.

Silver-haired bats produced guano less frequently than either species of myotis in all seasons of the year (R 1.1). Nevertheless, both male and female silver-haired bats produced guano more often in summer than autumn (Fig. 4). Despite the need to gain fat in preparation for winter, male bats, especially, may reduce feeding during mating which occurs primarily during autumn. In addition, silver-haired bats in northern California may be at or near the end of their autumn migration (Cryan 2003). Thus, they may not have a strong need to feed during autumn and, instead, may be able to rely on daily torpor as a more effective means of retaining fat stores at this time of year (McGuire et al. 2014). Presumably, it would also be beneficial for myotis to prioritize mating activities over feeding and maximize use of torpor during autumn. However, we did not observe a similar reduction in guano production during autumn for male myotis. It may be that their overall smaller body size constrains myotis to continue foraging to maximize fat stores prior to winter or that they continue foraging longer into autumn because their favored prey remains active.

Evaluating changes to fuel loads provides important insights into seasonal energy allocation strategies of bats. Our study comports with others showing that preparation for winter is demanding for temperate-zone bats, whether their overwintering strategy involves hibernation or migration (Sommers et al. 2019). It also highlights that, even in areas with relatively mild winters, autumn represents a critical period in the annual cycle for temperate-zone bats. We evaluated changes in fuel loads at the population rather than individual level which inevitably leads to a lack of precision in true patterns of change. However, our work provides a first approximation of year-round patterns of mass change and fuel loading in three species of bats for which such data are difficult to obtain (Zahn et al. 2007; Pretorius et al. 2021). Few studies have quantified mass change in species that do not aggregate in human-accessible locations (McGuire et al. 2012; Jonasson and Gugliemo 2016; Clerc et al. 2021), in part because of the challenges of capturing free-flying individuals during the cold season. Further, we used a regional data set covering a 22-year period to assess intra-annual changes in body mass, which disregards interannual changes and larger geographic patterns in body mass that likely occur (Alston et al. 2023). Assessment at the population level and over multiple years provides a first approximation of seasonal energy budgets in these species that will be a useful point of comparison for other species and other locales.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Author Contributions

TJW: Conceptualization, data curation, formal analysis, funding acquisition, methodology, investigation, visualization, writing—original draft, writing—review and editing; JC: Conceptualization, formal analysis, investigation, visualization, writing—review and editing; MJL: Data curation, formal analysis, writing—review and editing; and NGJ: Formal analysis, visualization, writing—review and editing.

Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Percent daily change in fuel load by sex and season for three species of bats in northern California.

Supplementary Data SD2.—Contrasts among trends in fuel load among three species of bats, both sexes, and three seasons. Trends were calculated from an estimated marginal means analysis using a Šidák correction for multiple comparisons. Degrees of freedom for all contrasts were 1947. Four-letter species codes are *Lasionycteris noctivagans* (LANO), *Myotis californicus* (MYCA), and *M. yumanensis* (MYYU).

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