



# Individual Daily and Seasonal Activity Patterns in Fox Squirrels (*Sciurus niger*) Quantified by Temperature-Sensitive Data Loggers

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Almost all publications on the activity of animals have reported average patterns for the observed group or population and provided little information about the variability of these patterns in individual animals. As natural selection operates mainly on the level of individual animals, detailed data about the everyday life of single individuals and field data of high complexity are needed. This study provides low disturbance continuous recording of the diurnal/circadian activity cycle of individual fox squirrels (*Sciurus niger*) in a natural setting, which allowed us to document the intra-individual and inter-individual pattern variation over the seasons. The daily onsets and offsets of activity were inferred from recordings of skin temperature using iButtons attached to the necks of 14 individual squirrels over almost 4 years resulting in 25 continuous records totalling 1,353 days. All squirrels were clearly diurnal but varied greatly in the number of daily activity bouts showing predominantly unimodal and bimodal patterns. Variations in activity patterns were related mostly to the seasons, followed by the inter-individual variability between squirrels. Fox squirrels spent 66–68% of the day in their nests in spring, summer, and fall, and 77% in winter. In the same season, individual squirrels exited their nests at about the same time every morning. In summer, they left their nests about 3 h earlier in the morning than in winter. The timing of first exits was significantly correlated with the number of daily nest exits and total out-of-nest time (ONT), and both variables were significantly correlated with both photoperiod and daily maximum temperature. Squirrels studied simultaneously showed substantial day-to-day variability in both number of daily exits and total ONT that either matched or exceeded the inter-individual variability. Many squirrels left their nests occasionally during the night, while about half occasionally spent entire nights outside an insulated nest. The timing of nest entries and exits, the modality of the activity pattern, the daily proportion of rest, and the occurrence of activity during the typically inactive time of the activity cycle are all aspects of an animal's activity pattern. They should therefore receive more attention for the characterization of species-specific behavior.

**Keywords:** activity patterns, seasonality, environmental conditions, personality, chronotype, sciuridae, circadian rhythm, foraging behavior

## INTRODUCTION

The appropriateness of an animal's activity level and pattern over the cycle of day and night and over the time course of the seasons is essential for its survival (DeCoursey et al., 2000; Larivée et al., 2010; Zhang et al., 2017) and reproductive success (Schmidt et al., 2008; Speakman, 2008; Zhao et al., 2013). The selective pressures to forage efficiently while avoiding injury and death lead to trade-offs in an animal's strategies between starvation and predation (Dall and Boyd, 2002; Higginson et al., 2012; Quinn et al., 2012; McNamara et al., 2016) that will affect both the daily proportions of time to be active and rest and the timing and regularity of rest and activity. Research on both aspects of activity is therefore essential to understand the biology of animal species. Locomotor activity has been shown to follow a reproducible daily pattern in numerous animal species, including tree squirrels (Tester, 1978; Adams, 1984; Koprowski and Corse, 2005; Wassmer and Refinetti, 2016). More than half a century ago, Aschoff (1966) observed that many, if not most, studies of locomotor activity had evinced a bimodal pattern with increased activity at dawn and dusk rather than a consolidated bout during the day or a consolidated bout during the night. Fox squirrels (*Sciurus niger*) are believed to be diurnal with bimodal activity pattern consisting of activity peaks in the early morning and right before sunset (Koprowski, 1994). However, most published data on tree squirrel species have reported average activity patterns for the observed group of animals or population and provided no indications about the variability of these activity patterns in individual animals (Hicks, 1949; Thompson, 1977; Wauters and Dhondt, 1987; Jodice and Humphrey, 1992; Wauters et al., 1992; Bordignon and Monteiro-Filho, 2000; Johnson et al., 2003; Koprowski and Corse, 2005; Ditgen et al., 2007). Only four studies so far have provided data on individual animals in tree squirrels (Zwahlen, 1975; Adams, 1984; Wassmer and Refinetti, 2016; Anders et al., 2017).

It has been suggested that researchers should move away from the "tyranny of the golden mean" (Bennett, 1987) and acknowledge individual behavioral differences as being more than just statistical noise around the group or population mean (Careau et al., 2008; Williams, 2008). This might help to shed light on how selection acts on the inter-individual variation in behavioral and physiological traits as evolution operates at the level of the individual (Roche et al., 2016). In studies on animals' activity cycles and patterns, it is the inter-individual variation in temporal strategies that provides the raw material for natural selection. The "golden mean" is averaging out this variation and obscures our understanding of the fitness impacts of frequency-dependent alternative temporal strategies. Therefore, detailed data about the everyday life of single individuals and field data of high complexity are needed (Halle and Stenseth, 2000).

Although not currently fully included into the concept of animal personalities (Gosling, 2001; Sih et al., 2004; Stamps, 2007; Wolf and Weissing, 2012), an animal's activity cycle and pattern is of essential ecological and evolutionary importance (Hertel et al., 2017) as it influences, e.g., its temporal selection/avoidance of open vs. protective habitats (Boon et al., 2008; Lone et al., 2015), movement rates (Ciuti et al., 2012), and dispersal (Cooper

et al., 2017). Individual variation of activity cycles and patterns is also important at the population level because the degree of variation in temporal strategies renders populations to be more or less resilient to changing conditions (Wolf and Weissing, 2012; Dingemanse and Wolf, 2013). A related concept regarding the importance of activity patterns and cycles is the chronotype. Being originally developed as a concept in human psychology, classifying people along a continuum from "morning types" with a preference to develop daily routines during the first half of the day (also called "larks") to "evening types," who prefer daily routines during the second half of the day (also called "owls") (Horne and Ostberg, 1976; Vink et al., 2001), chronotypes are now also used in comparative psychology (Labyak et al., 1997; Masuda and Zhdanova, 2010; Pfeffer et al., 2015; Refinetti et al., 2016). However, in almost all studies, averaged activity patterns are used and only some aspects of the activity pattern such as the activity onset, acrophase, and spread are considered (Refinetti et al., 2016). Despite a current renewal of attempts to combine chronobiology, which is traditionally conducted in laboratories, with field ecology (Helm et al., 2017), the linkage between the related concepts of personality and chronotype is currently underdeveloped. Exceptions are recent studies of human psychology and medicine finding that chronotype, sleep, and personality are linked and appear to have at least partial genetic foundation (Randler et al., 2017) and a study of circadian behavioral variation by the pearly razorfish, *Xyriichthys novacula* (Alós et al., 2017), in which the authors suggested that the observed circadian behavioral variation should be viewed as an independent axis of the fish personality, and that the study of chronotypes and their consequences should be regarded as a novel dimension in understanding within-species fish behavioral diversity.

The current study provides low disturbance continuous recording of several hitherto underrepresented parameters of the diurnal/circadian activity cycle [number of nest exits, in-nest time during daylight, total in-nest time during 24 h, nocturnal out-of-nest time (ONT) of individual fox squirrels (*Sciurus niger*)] in a natural setting. The goal of the present study was to use the recorded patterns of nest entries and exits to study the distribution of unimodal, bimodal, and higher-modal activity patterns and the total ONT during day and night in individual fox squirrels, document the intra-individual (day-to-day) and inter-individual pattern variation over the seasons, and address how the observed variabilities relate to the concepts of animal personalities and chronotype.

## MATERIALS AND METHODS

### Location

The study was conducted on an 80-ha area encompassing the campus of Siena Heights University and the adjacent Motherhouse campus of the Adrian Dominican Sisters, in rural Adrian, Lenawee County, in southeastern Michigan, USA (41°54' N, 84°01' W, 240-m elevation). Long-term climate data for Adrian are shown in **Table 1**. Weather conditions during the study were recorded by the Siena Heights University weather station and uploaded to Weather Underground (<https://>

**TABLE 1** | Long-term climate data for Adrian, MI.

Winter average low temperature	−8°C
Winter average high temperature	0°C
Summer average low temperature	15°C
Summer average high temperature	29°C
Annual average precipitation	863 mm

[www.wunderground.com/dashboard/pws/KMIADRIA4](http://www.wunderground.com/dashboard/pws/KMIADRIA4)).

Daily averages as well as high and low temperatures for the correlation matrix (**Table 2**) were retrieved for the Lenawee County Airport (KADG) also via Weather Underground (<https://www.wunderground.com/history/airportfrompws/KADG/>), while the information on snow fall amounts and snow heights originated from US Climate Data (<https://www.usclimatedata.com/climate/adrian/michigan/united-states/usmi0004>).

## Animals

Fox squirrels were caught with double-door Tomahawk Deluxe Transfer traps (Tomahawk Live Traps, Hazelhurst, WI) permanently attached to the trunk of four tall trees on custom-made platforms (Huggins and Gee, 1995). Squirrels were weighed with Pesola spring scales and ear-tagged with Monel type 5 tags (National Band and Tag Company, Newport, KY). Age was categorized as juvenile, sub-adult, and adult according to weight and fur characteristics and by visual examination of external reproductive organs. Sex of adult and sub-adult squirrels was determined by visual examination of the external reproductive organs (Reighard et al., 2004; Schroeder and Robb, 2005). All work was conducted with approval of the Institutional Animal Care and Use Committee of Siena Heights University and followed appropriate guidelines as outlined by (Schmidt et al., 2008).

## Data Collection

Each squirrel was equipped with a data-logger collar containing a temperature-sensitive iButton (DS1922L, Maxim Integrated Products, Inc., San Jose, CA) and released at the site of capture immediately thereafter. Data-logger collars weighed between 8 and 12 g and did not exceed 2% of the animals' body mass. The collar design is described in detail elsewhere (Wassmer, 2017). The monitoring of collar temperature ( $T_c$ ) is particularly suited to provide an index of location (in-nest vs. out-of-nest), as the temperature of the collar quickly rises to core temperature levels after the animals enter their thermally insulated nests and drops rapidly when the animals leave their nests (Lamb, 1995; Kanda et al., 2005; Lazerte and Kramer, 2011; Murray and Smith, 2012; Wassmer and Refinetti, 2016). These assumptions were confirmed by aligning  $T_c$ -records with behavioral logs (Wassmer and Refinetti, 2016) including nest entries. A recent study using a very similar setup with a large population of North American red squirrels (*Tamiasciurus hudsonicus*) confirmed the paradigm with about 700 observations of nest exits and entries (Studd et al., 2016). The iButtons were programmed to record one  $T_c$  reading

every 5, 10, or 30 min with a thermal accuracy of 0.5 or 0.06°C. The limited memory of the iButton allowed data to be recorded for a minimum of 28 days (at 5-min intervals) to a maximum of 6 months (at 30-min intervals). We obtained data from 25 distinct timeseries from 14 individual squirrels, across the four seasons (**Figure 1**). The corresponding ambient temperatures ( $T_a$ ) at the times of  $T_c$  recordings were obtained by the nearby Siena Heights weather station (<500 m away from the squirrel habitat; see the URL above).

Exact times of entry into and exit from the nest were often easily identified by visual inspection of collar temperature records. To avoid potential subjectivity bias, however, the time series was analyzed by a custom-made computer program developed by one of the authors (RR). The computer algorithm defined a nest entry as the data point when the current temperature was higher than the preceding temperature and also higher than or equal to the mean nightly temperature of the individual squirrel. Similarly, the algorithm defined a nest exit as the data point when the current temperature was lower than the preceding temperature and also lower than the mean nightly temperature of the individual squirrel.

Although this simple algorithm worked well for data sets with a temperature gradient of more than 10–15°C between  $T_c$  and  $T_a$ , additional specifications had to be included to ensure successful data analysis during warmer periods that resulted in more noisy data sets (e.g., **Figure 9D**). In addition, some collar preparations showed a lower amplitude of  $T_c$  fluctuations (e.g., **Figure 10D**), and we recognized that the recording interval (5, 10, and 30 min) has an influence on the selection of meaningful criteria. We therefore re-evaluated the algorithm used in Wassmer and Refinetti (2016) and established several sets of criteria for high/low amplitude, short/long intervals, and clear/noisy patterns (**Table S1**) to include the most recording days possible without compromising the event detection and shifting the detection frame, which would result in incorrect detections for many days after falsely determining an event (nest entry or exit).

The first nest exit on each day was defined as the onset of a squirrel's daily activity, whereas the last nest entry on each day was an indication of the daily activity offset. The number of nest exits each day was used to quantify the modality of the activity pattern for each squirrel. Other dependent variables were diurnal nest time (time a squirrel stayed inside of its nest during daylight hours), total nest time (in-nest time per 24 h), and nocturnal ONT (time spent outside of the nest during the night). For the analyses of seasonality, the meteorological definition of seasons was used: Summer starts June 1, lasting until August 31; fall runs from September 1 until November 30, followed by winter from December 1 through February 28, and finally the spring season lasting from March 1 to May 31.

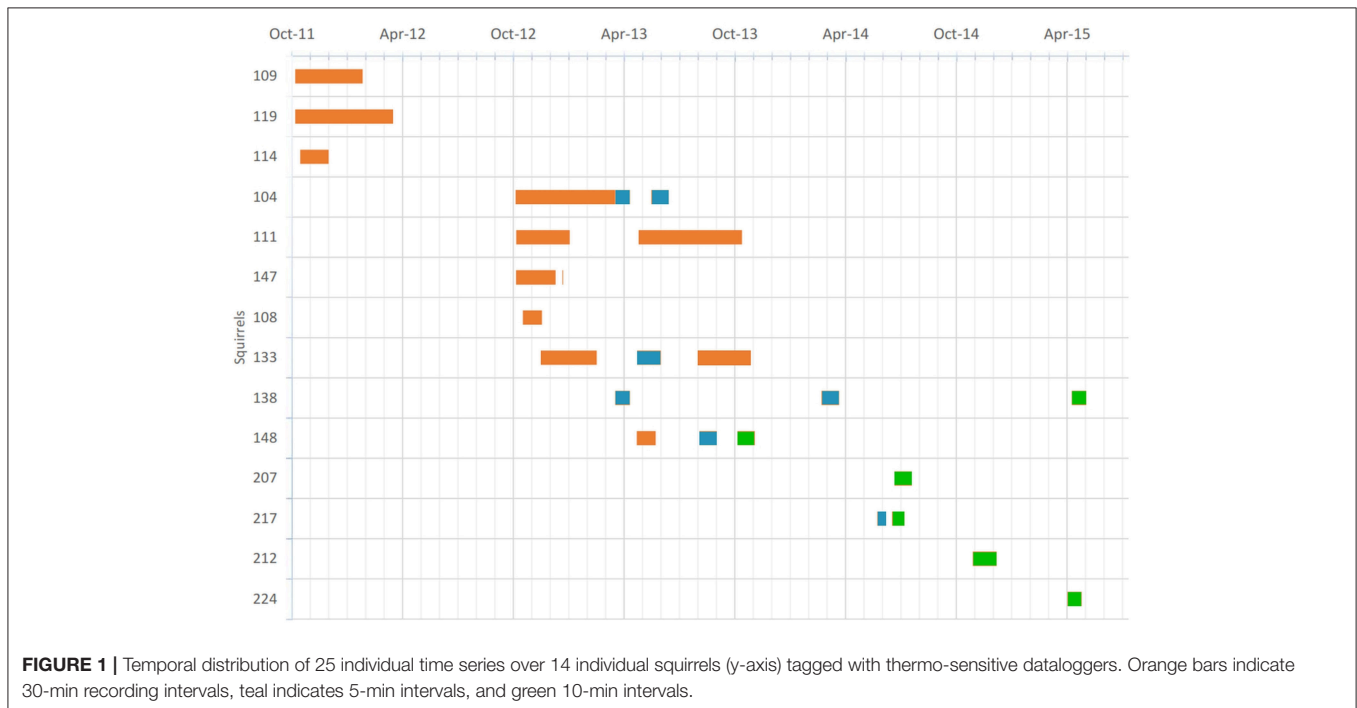
## Data Analysis

Presence of statistically significant 24-h rhythmicity was determined by two methods: chi-square periodogram (Sokolove and Bushell, 1978) and cosinor rhythmometry (Nelson et al., 1979). The daily and seasonal timing of nest exits and entries were analyzed and compared using circular statistics (Batschelet, 1981;

**TABLE 2 |** Correlation matrix between key climatic factors and dependent variables.

	Means	SD	Day-light	Daily highs	Rain	Snow	Snow depth	Wind avg.	First exits	N of exits	ONT totals
Day-light	11.442	2.133	1.000	0.752	0.030	-0.110	-0.189	-0.173	-0.428	0.360	0.275
Daily highs	12.341	10.499		1.000	0.075	-0.216	-0.390	-0.265	-0.449	0.345	0.342
Rain	1.945	5.863			1.000	0.059	-0.034	0.251	-0.010	0.079	-0.024
Snow	0.164	0.983				1.000	0.496	0.188	0.132	-0.080	-0.176
Snow depth	0.647	2.206					1.000	0.103	0.235	-0.146	-0.236
Wind avg.	11.767	6.224						1.000	0.096	-0.045	-0.080
First exits	0.337	0.096							1.000	-0.443	-0.511
N of exits	1.613	0.786								1.000	0.151
ONT totals	7.081	2.924									1.000

Correlations in red are significant at  $p < 0.001$ .



**FIGURE 1 |** Temporal distribution of 25 individual time series over 14 individual squirrels (y-axis) tagged with thermo-sensitive dataloggers. Orange bars indicate 30-min recording intervals, teal indicates 5-min intervals, and green 10-min intervals.

Fisher, 1993; Mardia and Jupp, 2000). Standard statistical tests were used for comparisons of group means, standard deviations, and standard errors (Siegel and Castellan, 1988; Moore et al., 2013; Zar, 2018). Unless specifically stated, means are presented together with their standard deviation (mean ± SD).

We compared the intra-individual (day-to-day) variation to the inter-individual variation contrasting the standard errors of the means of two data sets of equal length assembled either from consecutive or randomly chosen days.

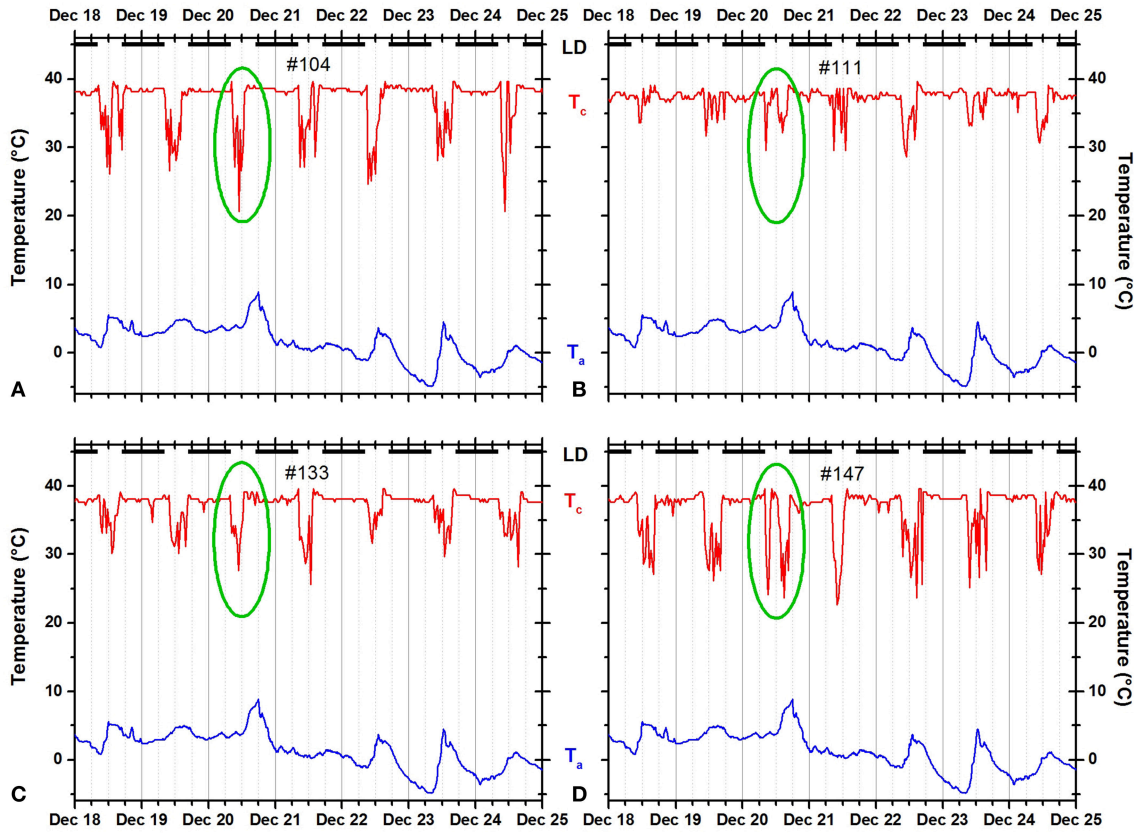
## RESULTS

### General Results

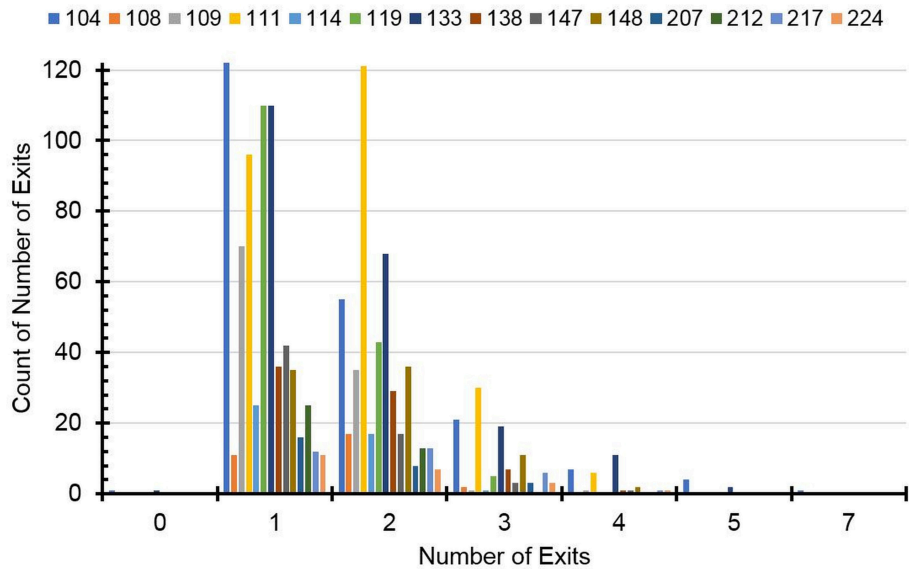
In this study, we deployed 27 data-logger collars on 16 individual fox squirrels over almost 4 years. One of the collared animals was never seen again while the data-logger collars of four

squirrels that were believed to have been lost were found a year later after the leaf nests of these squirrels deteriorated and the collars fell to the ground. Only the data of one of these collars were lost due to an exhausted iButton battery. In summary, we were able to analyze 25 individual time series of 14 individual animals (Figure 1, Table S2). All fox squirrels exhibited statistically significant 24-h rhythmicity ( $p < 0.0001$ ) as determined by the chi-square periodogram procedure and by cosinor rhythmometry. Representative records for four fox squirrels are shown in Figure 2. For all 14 animals, neck temperature was consistently high (37–38°C) during the night, when the animals remained curled up inside their nests, but oscillated noticeably during the day, when the animals exited the nest one or more times a day. Although  $T_a$  oscillated daily, the daily variations in  $T_c$  were independent from variations in  $T_a$  (Figures 2, 9, 10). When correlating 2 weeks of recordings of  $T_c$

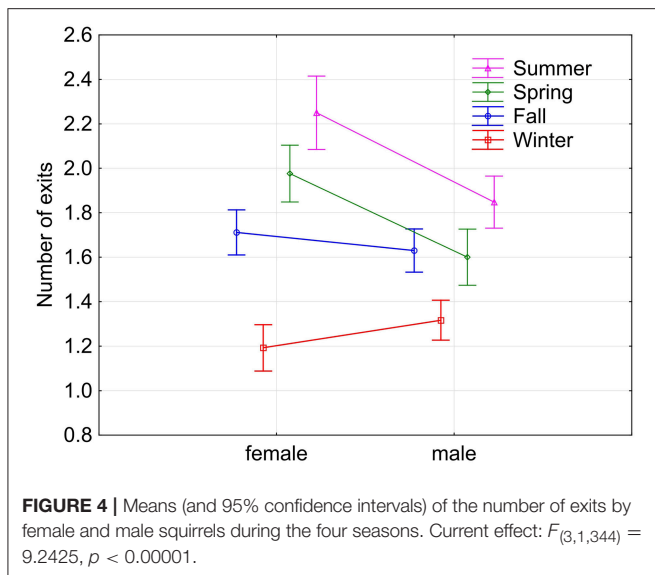




**FIGURE 2** | Records of ambient temperature ( $T_a$ , blue) and neck skin temperature ( $T_c$ , red) for four fox squirrels 104 (A), 111 (B), 133 (C), and 147 (D) living outdoors during the winter. The dark horizontal bars at the top of each panel indicate the duration of the dark phase of the natural light–dark cycle. The green ovals mark the time course of body temperature at the same day illustrating individual variability under the same weather conditions. Squirrels 104 and 133 showed a unimodal pattern, whereas squirrels 111 and 147 showed a bimodal pattern.



**FIGURE 3** | Distribution of the number of exits of individual squirrels.



at a temporal resolution of 0.5 h (672 data points) to simultaneous recordings of  $T_a$  of equal duration and resolution, we found low negative correlations in winter (highest  $r = -0.061$ ,  $p > 0.1$ ) and to some extent higher negative correlations in summer ( $r = -0.139$ ,  $p < 0.05$ ). This indicates that  $T_c$ 's were slightly higher when  $T_a$ 's were lower.

In the combined first-order effects on our dependent variables (number of daily nest exits, in-nest time during daylight hours, total daily in-nest time and ONT during night hours), season had the strongest effect [Wilks lambda = 0.287,  $F_{(21,3,808)} = 98.9$ ,  $p < 0.0001$ ] followed by differences between individual squirrels [Wilks lambda = 0.565,  $F_{(84,8,129)} = 9.4$ ,  $p < 0.0001$ ], age [Wilks lambda = 0.846,  $F_{(7,1,326)} = 34.629$ ,  $p < 0.0001$ ], and recording interval [5, 10, or 30 min, Wilks lambda = 0.908,  $F_{(14,2,652)} = 9.417$ ,  $p < 0.00001$ ]. Although a MANOVA revealed no main effect of sex (Wilks lambda = 1.0), it showed an interaction of sex and season [ $F_{(3,1,344)} = 9.243$ ,  $p < 0.0005$ ].

## Number of Nest Exits

In the pooled data, the average number of nest exits per day was  $1.610 \pm 0.788$  suggesting a slightly higher proportion of bimodal and higher-modal days with two or more daily activity periods separated by one or more rest period in their nest (e.g., squirrels 111 and 147 in **Figure 2**). However, a histogram of the distribution of unimodal, bimodal, and higher-modal days for all 14 individual squirrels (**Figure 3**) shows that most squirrels had more unimodal than bimodal days, with the exceptions of squirrels 111 and 108. There were much fewer days during which the animals exited three or more times a day indicating fewer trimodal and even less polymodal activity patterns. Individual squirrels differed significantly in the mean number of exits ranging from  $1.335 \pm 0.537$  to  $1.875 \pm 0.833$ .

Fox squirrels showed the highest number of nest exits in summer ( $1.982 \pm 0.968$ ) and least in winter ( $1.263 \pm 0.489$ ). All seasons were significantly different from each other except fall and spring (Tukey HSD test,  $p < 0.05$ ). Individual squirrels

were predominantly unimodal in winter with average numbers of nest exits between 1.157 and 2.000 and predominantly bimodal in summer with values between 1.519 and 3.115. Some animals showed more exits in fall and/or spring than in summer. Female fox squirrels showed significantly more nest exits in spring and summer ( $1.976 \pm 0.886$ ,  $2.250 \pm 1.223$ ) than male squirrels [ $1.600 \pm 0.722$ ,  $1.848 \pm 0.781$ ,  $F_{(1,255)} = 13.969$ ,  $p < 0.0005$ , **Figure 4**]. On the contrary, there was no sex difference in nest exits in either fall or winter nor during the pooled data for these two seasons [ $F_{(1,418)} = 1.166$ ,  $p = 0.281$ ]. In addition to the seasonal and individual variability, the number of nest exits also showed considerable day-to-day fluctuations in individual squirrels recorded during the same days (**Figure 5A**).

The number of nest exits showed comparable significant correlations to the daily hours of daylight (Pearson's  $r = 0.360$ ,  $p < 0.001$ ) and daily high temperatures ( $r = 0.345$ ,  $p < 0.001$ ). The correlation between the number of exits and snow depth was less pronounced but still significant, while average wind speed, precipitation, and snowfall were not significantly correlated (**Table 2**).

## In-nest and Out-of-nest Times

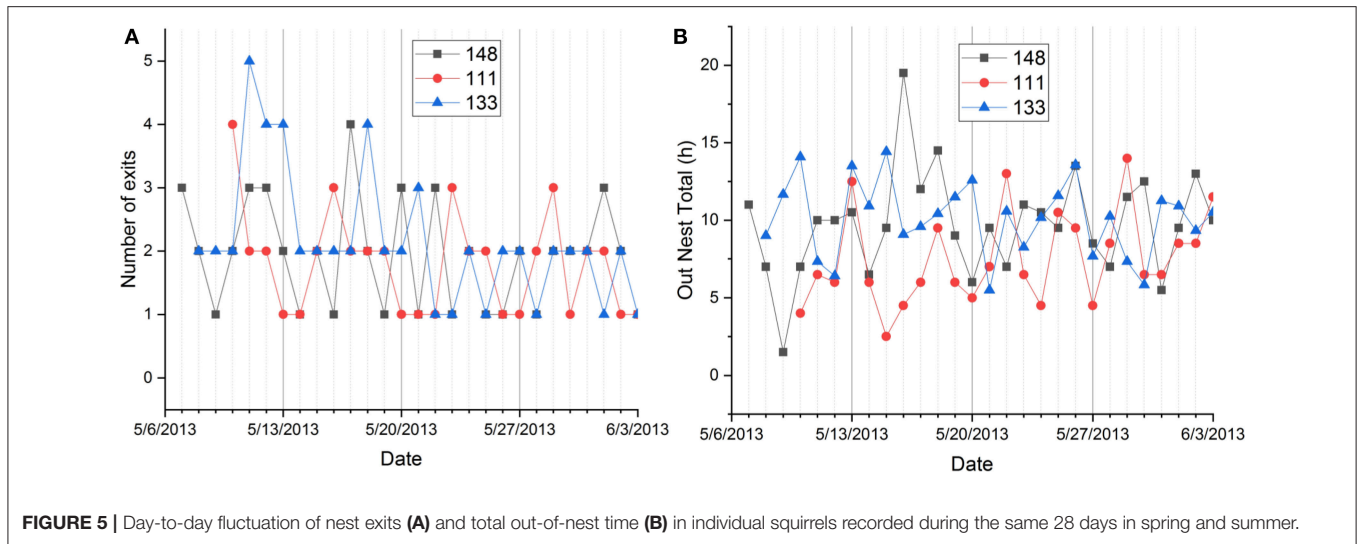
From fall through summer, the animals spent increasingly longer periods inside of their nests during daytime, starting at  $3.318 \pm 2.485$  h in fall to  $7.430 \pm 2.943$  h in summer (**Figure 6A**). All seasons were significantly different from each other regarding time spent in the nest during light hours (Tukey HSD test,  $p < 0.05$ ). Total daily time spent in the nest was highest in winter ( $18.466 \pm 2.129$  h) and lowest in spring ( $15.936 \pm 3.060$  h); however, only winter was significantly different from every other season (Tukey HSD test,  $p < 0.05$ , **Figure 6B**). The daily proportion of ONT during civil daylight hours ranged from 0% (observed only once) to 96% with an average of  $43 \pm 23\%$ . Total nest time allows a rough estimate of the proportion of rest per 24 h. In the pooled data, squirrels spend about 66–68% of 24 h in their nests in spring, summer, and fall, and 77% in winter. This difference was significant (one-way ANOVA and Tukey post-test,  $p < 0.00001$ ).

There were also significant differences between individual squirrels in nest time during daylight hours ranging from  $1.698 \pm 1.213$  to  $9.05 \pm 2.981$  h, total nest time ranging from  $12.523 \pm 1.899$  to  $18.488 \pm 2.581$  h, and nocturnal out of nest time ranging from  $0.0285 \pm 0.181$  to  $1.106 \pm 2.035$  h (Tukey HSD tests,  $p < 0.05$ ). As nest exits, total ONT showed considerable day-to-day fluctuations in individual squirrels recorded during the same days (**Figure 5B**).

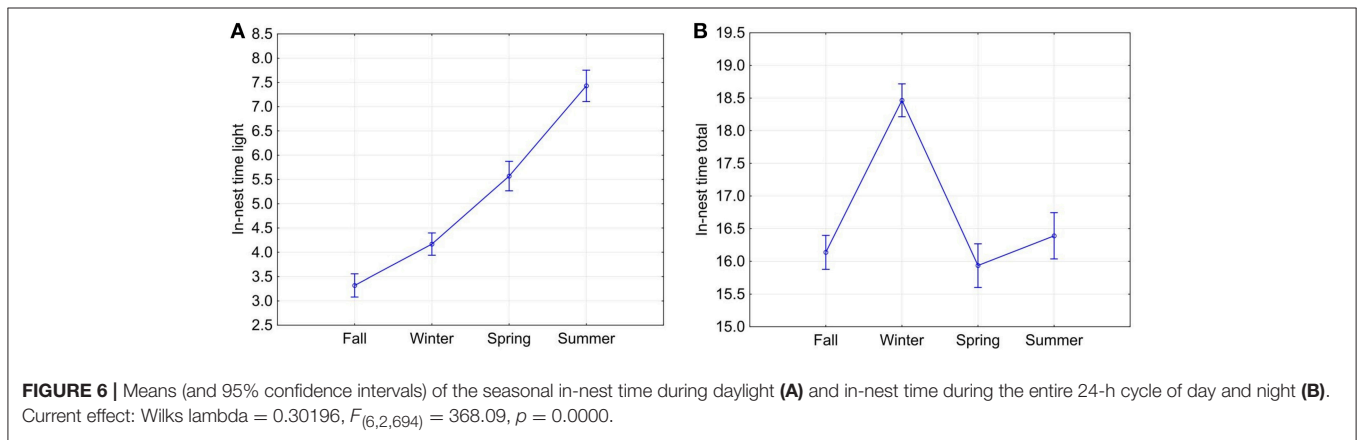
Total ONT was best correlated with the daily high temperatures (Pearson's  $r = 0.342$ ) and less to the hours of daylight ( $r = 0.275$ ). In addition, snow depth ( $r = -0.236$ ) and snowfall ( $r = -0.176$ ) were also significantly but less strongly correlated (all probabilities,  $p < 0.001$ ), whereas average wind speed and precipitation did not seem to influence total ONT (**Table 2**).

## Timing of First Exits

In all seasons, individual squirrels showed highly significant mean vectors for the timing of their first exits with mean vector



**FIGURE 5** | Day-to-day fluctuation of nest exits (A) and total out-of-nest time (B) in individual squirrels recorded during the same 28 days in spring and summer.



**FIGURE 6** | Means (and 95% confidence intervals) of the seasonal in-nest time during daylight (A) and in-nest time during the entire 24-h cycle of day and night (B). Current effect: Wilks lambda = 0.30196,  $F_{(6,2,694)} = 368.09$ ,  $p = 0.0000$ .

lengths ( $r$ ) between 0.76 and 0.99. Individual squirrels differed significantly in the timing of their first exits (Figure 7, Table 3). None of the animals was consistently an early or late riser throughout all seasons. In summer, fox squirrels left their nests 3 h earlier in the morning than in winter (Mardia–Watson–Wheeler test on the pooled data of all 14 animals,  $p < 0.05$ ). The range between early and late risers was highest in summer (4.8 h) and lowest in winter (1.83 h). In summer, first exits of individual squirrels were, on average, 1.72 h apart from each other—in winter, the average spacing was only 37 min. Three individual fox squirrels (#104, 111, 133) were recorded throughout all seasons and could be compared to the pooled data. While #104 and #111 showed similar results to the pooled data with first exits in summer occurring about 4:37 and 3:42 h earlier than in winter, respectively, #133 showed different results with first exits earliest in fall (6:48 EST) and spring (7:36 EST) and late exits in summer (9:57 EST) and winter (10:11 EST) only about 0:14 h apart.

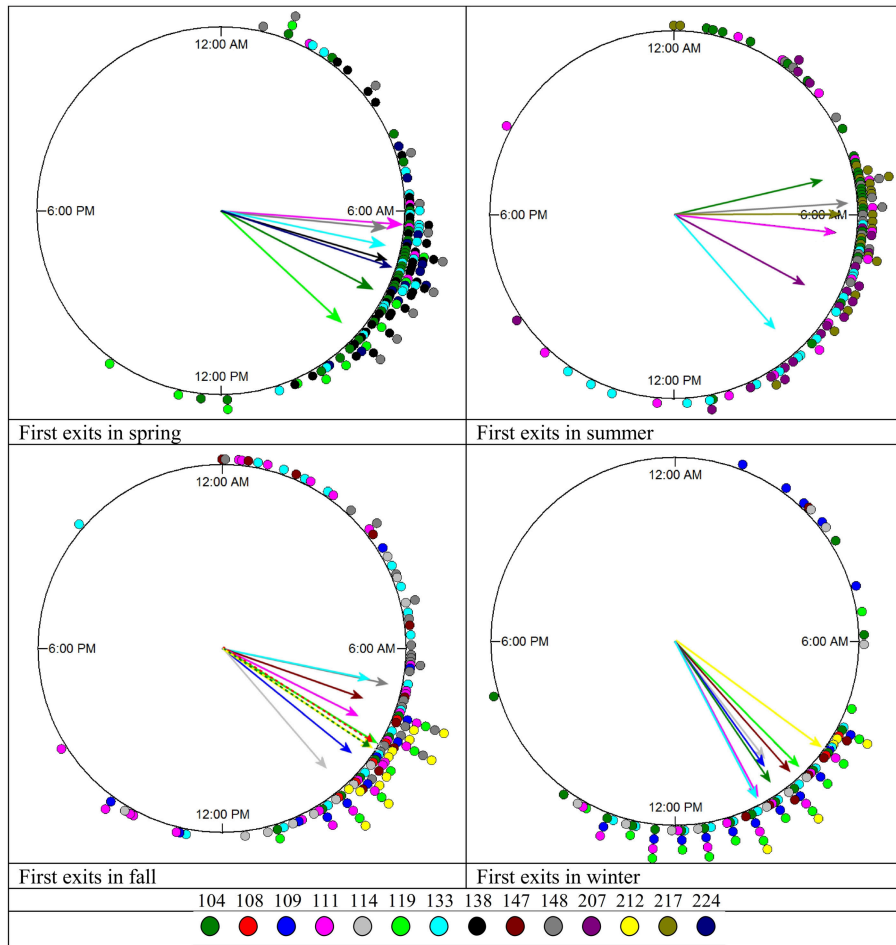
In the pooled data for all seasons, the timing of first exits was inversely correlated with the number of daily exits (Pearson’s  $r = -0.708$ ,  $p = 0.005$ ) and total time outside nests (Pearson’s  $r = -0.685$ ,  $p = 0.007$ ), meaning that squirrels that exited the

nest earlier also exited the nest more times each day and spent longer times outside the nest (Table 4, Figure 8). In addition to the results for the pooled seasons, total time spent outside the nest in fall was also significantly inversely correlated to the timing of first exits in fall.

### Continuous Nest Occupancy for More Than 24 h and Nocturnal Out-of-nest Time

Some squirrels occasionally remained in the nest for more than 24 h and/or occasionally failed to return to the nest at night (Figures 9, 10). The green oval in Figure 9B documents that squirrel #104 did not leave the insulated nest for more than 36 h. In Figure 10B, the green oval marks a 35-h time period during which the animal was not resting in an insulated shelter.

Nocturnal ONT occurred occasionally in many fox squirrels. Seven of 14 squirrels spent at least one entire night outside their insulated nests—mostly during warm nights but exceptionally also during winter (for example, Figure 10B). The pooled data showed little evidence for seasonality of nocturnal ONT. Only fall ( $0.556 \pm 1.306$  h) and winter ( $0.171 \pm 0.761$  h) were significantly different from each other (Tukey HSD test,  $p < 0.00001$ ). In



**FIGURE 7 |** Timing of first exits in individual squirrels during each season. The mean vectors point at the average timing of first entries for each squirrel and are in the same color as the data points plotted on the periphery of the circle (see the color key on the bottom). The length of the mean vector indicates how concentrated or spread the first exits are around this mean time. The exact values can be found in **Table 3**.

contrary to the pooled data for all animals, two of the only three individual animals recorded over all seasons showed significant seasonality in the duration of nocturnal ONT. Squirrel 104 spent the least amount outside of its nest in winter ( $0.072 \pm 0.296$  h) and the most amount in summer ( $0.526 \pm 1.002$  h). A Tukey HSD test reached significance ( $p < 0.05$ ) for the differences between fall and summer and winter and summer. Squirrel 133 spent also the least amount of nocturnal ONT in winter ( $0.225 \pm 1.105$  h) and the most in fall ( $1.612 \pm 2.017$  h). Fall vs. winter and fall vs. spring as well as winter vs. summer were significantly different (Tukey HSD,  $p < 0.05$ ). Contrary to the frequent occurrence of ONT during night, the example of continuous in-nest time for more than 24 h shown in **Figure 9B** was the only occurrence of this type in our dataset.

### Comparison Between the Intra- and Inter-individual Data Variability

Indices of intra- and inter-individual variabilities were calculated by analysis of consecutive data segments of individual animals

and simultaneous (or randomized) segments of different individuals. Inter-individual and intra-individual variabilities were generally comparable to each other regarding time of first exit from the nest, number of exits per day, and time spent outside the nest per day in all four seasons (**Table 5**). For the four seasons combined, inter-individual variability exceeded intra-individual variability for total time outside the nest, and this difference was caused by a difference in the spring (**Table 5**).

## DISCUSSION

### General Results

Individual squirrels differed significantly in the number of exits (and by this in the number of activity periods), diurnal nest time, total nest time, and nocturnal ONT. All recorded variables also showed significant seasonal variation. To a much lesser degree, age and recording interval contributed to the variance in the data, while the sex of squirrels only showed a significant effect in the interaction of sex and season (**Figure 4**). We also detected



**TABLE 3 |** Circular statistics of the seasonal timing of first exits in individual squirrels.

<b>First exits in spring</b>										
Squirrel	104	111	119	133	138	148	224			
N	55	22	35	25	73	25	22			
Mean vector (EST)	07:48	06:17	08:51	06:48	07:05	06:23	07:13			
Mean vector length (r)	0.902	0.962	0.879	0.892	0.936	0.888	0.967			
CSD (h)	01:43	01:03	01:56	01:49	01:23	01:51	00:59			
Rayleigh test (Z)	44.787	20.351	27.046	19.899	63.976	19.695	20.567			
Rayleigh test (p)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001			
MWW test:	104	111	119	133	138	148	224			
104	—	<0.001	0.103	<0.001	0.055	0.054	0.006			
111	22.528	—	<0.001	<0.001	0.034	0.237	0.003			
119	4.551	38.979	—	<0.001	<0.001	<0.001	<0.001			
133	15.807	17.432	15.213	—	0.002	0.04	<0.001			
138	5.814	6.749	18.774	12.739	—	0.316	0.033			
148	5.82	2.877	15.303	6.431	2.304	—	0.007			
224	10.187	11.787	19.426	18.372	6.811	9.79	—			
<b>First exits in summer</b>										
Squirrel	104	111	133	148	207	217				
N	26	92	45	20	27	32				
Mean vector (EST)	05:06	06:25	09:56	05:44	07:52	06:00				
Mean vector length (r)	0.823	0.883	0.846	0.947	0.806	0.901				
CSD (h)	02:23	01:54	02:12	01:15	02:30	01:44				
Rayleigh test (Z)	17.615	71.709	32.213	17.953	17.556	25.961				
Rayleigh test (p)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001				
MWW test	104	111	119	133	138	148				
104	—	0.015	<0.001	0.029	<0.001	0.055				
111	8.417	—	<0.001	0.513	<0.001	0.594				
133	32.017	45.973	—	<0.001	0.008	<0.001				
148	7.106	1.333	32.164	—	<0.001	0.326				
207	19.022	25.976	9.747	23.097	—	<0.001				
217	5.82	2.877	15.303	6.431	2.304	—				
<b>First exits in fall</b>										
Squirrel	104	108	109	111	114	119	133	147	148	212
N	40	28	32	92	24	32	82	39	39	27
MV (EST)	08:13	08:06	08:34	07:46	09:16	08:04	07:36	07:17	06:49	08:13
MVL (r)	0.981	0.988	0.902	0.82	0.858	0.981	0.76	0.812	0.916	0.988
CSD (h)	00:44	00:35	01:43	02:24	02:06	00:44	02:49	02:27	01:35	00:35
Rayl. (Z)	38.509	27.321	26.055	61.935	17.68	30.819	47.399	25.73	32.747	26.375
Rayl. (p)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MWW	104	108	109	111	114	119	133	147	148	212
104	—	0.256	0.007	<0.001	<0.001	0.066	<0.001	0.057	0.004	0.785
108	2.725	—	0.003	<0.001	<0.001	0.581	<0.001	0.006	0.038	0.934
109	9.892	11.752	—	0.06	0.032	<0.001	<0.001	0.574	0.003	0.01
111	26.316	18.023	5.633	—	<0.001	<0.001	0.005	0.079	0.16	<0.001
114	28.633	28.357	6.877	30.032	—	<0.001	<0.001	<0.001	<0.001	0.001
119	5.444	1.085	14.471	28.609	28.735	—	<0.001	0.001	0.003	0.546
133	57.441	45.861	29.37	10.663	22.345	50.189	—	<0.001	0.848	<0.001
147	5.719	10.355	1.111	5.084	27.145	13.385	14.05	—	0.002	0.428
148	11.042	6.537	11.637	3.662	39.979	11.611	0.329	12.833	—	<0.001
212	0.484	0.137	9.263	14.68	13.368	1.21	43.207	1.696	16.002	—

(Continued)

**TABLE 3 |** Continued

First exits in winter								
Squirrel	104	109	111	114	119	133	147	212
N	89	75	47	19	91	88	24	11
MV (EST)	09:43	09:36	10:07	09:30	09:01	10:11	09:15	08:21
MVL (r)	0.92	0.837	0.949	0.793	0.947	0.958	0.937	0.98
CSD (h)	01:33	02:16	01:13	02:36	01:15	01:07	01:22	00:45
Rayl. (Z)	75.336	52.583	42.365	11.941	81.541	80.789	21.086	10.57
Rayl. (p)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MWW	104	109	111	114	119	133	147	212
104	—	0.389	0.022	0.809	0.001	0.018	0.661	0.004
109	1.891	—	0.443	0.317	<0.001	0.658	0.516	0.002
111	7.655	1.626	—	0.328	<0.001	0.957	0.532	0.002
114	0.425	2.295	2.231	—	<0.001	0.025	0.686	0.003
119	13.181	28.846	30.276	15.23	—	<0.001	0.042	0.006
133	7.988	0.836	0.088	7.397	36.37	—	0.134	0.002
147	0.827	1.322	1.262	0.753	6.357	4.019	—	0.002
212	11.035	12.691	12.259	11.894	10.331	12.343	12.416	—

The upper unshaded part describes the circular distribution of first exits for each squirrel, whereas the shaded lower area shows the results of pairwise Mardia–Watson–Wheeler tests (MWW). The upper part provides the significance levels—the lower values are the W scores of the tests. EST, Eastern Standard Time; CSD, circular standard deviation.

**TABLE 4 |** Linear correlations between the timing of first exits and modality (number of nest exits per day) and timing of first exits and total time spent outside of the nest per 24 h (total ONT).

Season	Variable pairs	N	Pearson's r	P-value
Spring	First Exit–Modality	7	−0.622	0.136
	First Exit–Total ONT	7	−0.419	0.349
Summer	First Exit–Modality	6	−0.650	0.163
	First Exit–Total ONT	6	−0.336	0.515
Fall	First Exit–Modality	10	−0.481	0.159
	First Exit–Total ONT	10	−0.777	0.008
Winter	First Exit–Modality	9	−0.550	0.125
	First Exit–Total ONT	9	−0.509	0.162
All seasons	First Exit–Modality	14	−0.708	0.005
	First Exit–Total ONT	14	−0.685	0.007

Correlations in red are significant.

substantial day-to-day variations in the dependent variables of individual squirrels (Figure 5) that were partially correlated to weather events (Table 2).

T<sub>c</sub> was barely and insignificantly negatively correlated with T<sub>a</sub> in winter, and only slightly higher but significantly inversely correlated in summer. The inverse nature of these correlations shows that T<sub>c</sub> was not merely following fluctuations of T<sub>a</sub> in any season. Compared to significant correlations between T<sub>a</sub> and other environmental factors such as snow fall (Table 2), the T<sub>c</sub>–T<sub>a</sub> correlations were smaller and, in the majority, insignificant. We are therefore confident that T<sub>c</sub> is a reliable proxy to infer nest entries and exits.

### Number of Nest Exits

Individual squirrels showed slightly more unimodal than bimodal days and only rarely exited more frequently than

twice per day (Figure 3). The number of daily exits showed a distinct seasonality with significantly more exits in summer than in winter. In addition, the number of exits was significantly correlated with the hours of daylight and weather parameters, especially the daily maximum temperature (Table 2). These seasonal trends agree with the results of most studies on fox squirrels (Hicks, 1949; Adams, 1984; Koprowski and Corse, 2005) and other tree squirrels (Thompson, 1977; Wauters et al., 1992), which reported bimodal patterns in summers, and unimodal patterns in winter. However, in the current study, we found gradual and erratic rather than categorical and continuous changes in the seasonal distribution of activity patterns in individual squirrels with the occurrence of bimodal days in winter (Figure 2) and unimodal days in summer (Figure 10), substantial day-to-day fluctuations (Figure 5A), and more nest exits occurring in fall/winter than summer in some squirrels suggest that the seasonal variability in the activity patterns is driven not only by changes in the photoperiod and temperature (Halle and Stenseth, 2000) but also by (stochastic) environmental factors such as unfavorable weather (Table 2 and Williams et al., 2014; Studd et al., 2016), predation (Eriksen et al., 2011; Connolly and Orrock, 2017), social interactions (Schmidt et al., 2008; Agostini et al., 2012), and/or intrinsic individual characteristics such as personality (Boon et al., 2008; Careau et al., 2008) and chronotype (Refinetti et al., 2016; Alós et al., 2017). As in the case shown in Figure 2, on the same day, December 20, 2012, two of four animals (#111 and #147) showed a bimodal pattern, while the other two animals showed a unimodal pattern. It is unlikely that weather conditions influenced the activity patterns of these animals in different ways, but we cannot exclude other possible extrinsic factor such as predation or social interactions, or intrinsic differences between the animals. There are only two studies known to us comparing individual activity

patterns in tree squirrels over the seasons of the year (Studd et al., 2016; Anders et al., 2017). Anders et al. (2017) found unimodal, bimodal, and even trimodal patterns in the Eurasian red squirrel (*Sciurus vulgaris*) in Japan during the months of July to October. We also found trimodal and even polymodal patterns in some seasons of the current study. However, these patterns often occurred during the warmer months when the gradient between  $T_c$  and  $T_a$  was reaching levels below  $10^\circ\text{C}$  and the detection of entries and exits was not always satisfactory (Figures 9, 10). Nevertheless, as Anders et al. (2017) used a data logger capable of recording collar temperature and light intensity simultaneously, their detection of trimodal patterns supports that some of the trimodal or polymodal patterns we detected might not be artifacts. Studd et al. (2016) used an almost identical design to the current study to investigate the activity patterns of lactating female North American red squirrels (*Tamiasciurus hudsonicus*) in southwestern Yukon, Canada, between February and June. The authors found an overall pattern of transition from unimodal daily activity centered at the end of the day on cold days to bimodal daily activity at intermediate temperatures and unimodal daily activity centered at the beginning of the day on warm days. Studd et al. (2016) confirmed the influence of weather conditions previously reported by Williams et al. (2014) also in lactating females from the same population of red squirrels using light sensors. Both studies found trimodal patterns in lactating females. We also found trimodal patterns in the current study (e.g., several days in Figure 10C). The animal shown (#133) is a female and the month of May falls within the predominant lactation period for fox squirrels (Koprowski, 1994); thus, it is possible that this squirrel was lactating and needed to forage more food to cover the higher energy demand (Havera, 1979) while having to return in regular intervals to nurse her offspring leading to a trimodal pattern (Williams et al., 2014; Studd et al., 2016).

### In-nest and Out-of-nest Times

From fall through summer, fox squirrels spent increasingly longer periods inside of their nests during daytime while total time spent in the nest during the full 24-h cycle of day and night was highest in winter (Figure 6). Nocturnal out-of-nest activity was highest in fall and lowest in winter.

There were also significant differences between individual squirrels in nest time during daylight hours, total nest time and nocturnal ONT. While longer in-nest times during the long daylight hours of summer can simply be a consequence of longer days, long in-nest times in winter can be caused by the long nights and colder temperatures of this season. The surprising and seemingly contradictory difference between in-nest time during daylight and during the entire 24-h activity cycle is based on significantly shorter in-nest time during the daylight hours in fall as compared to any other month (Figure 6A). We speculate that this difference makes sense in the light of the fox squirrel's natural history, with fall being the food caching season and with the approaching reproductive season in winter (Koprowski 1994). Nocturnal ONT, which was always short (on average below 37 min in the pooled data) but was more than four times longer in fall than in winter can also not be simply related to the changes in photoperiod. Here we suspect again a joint

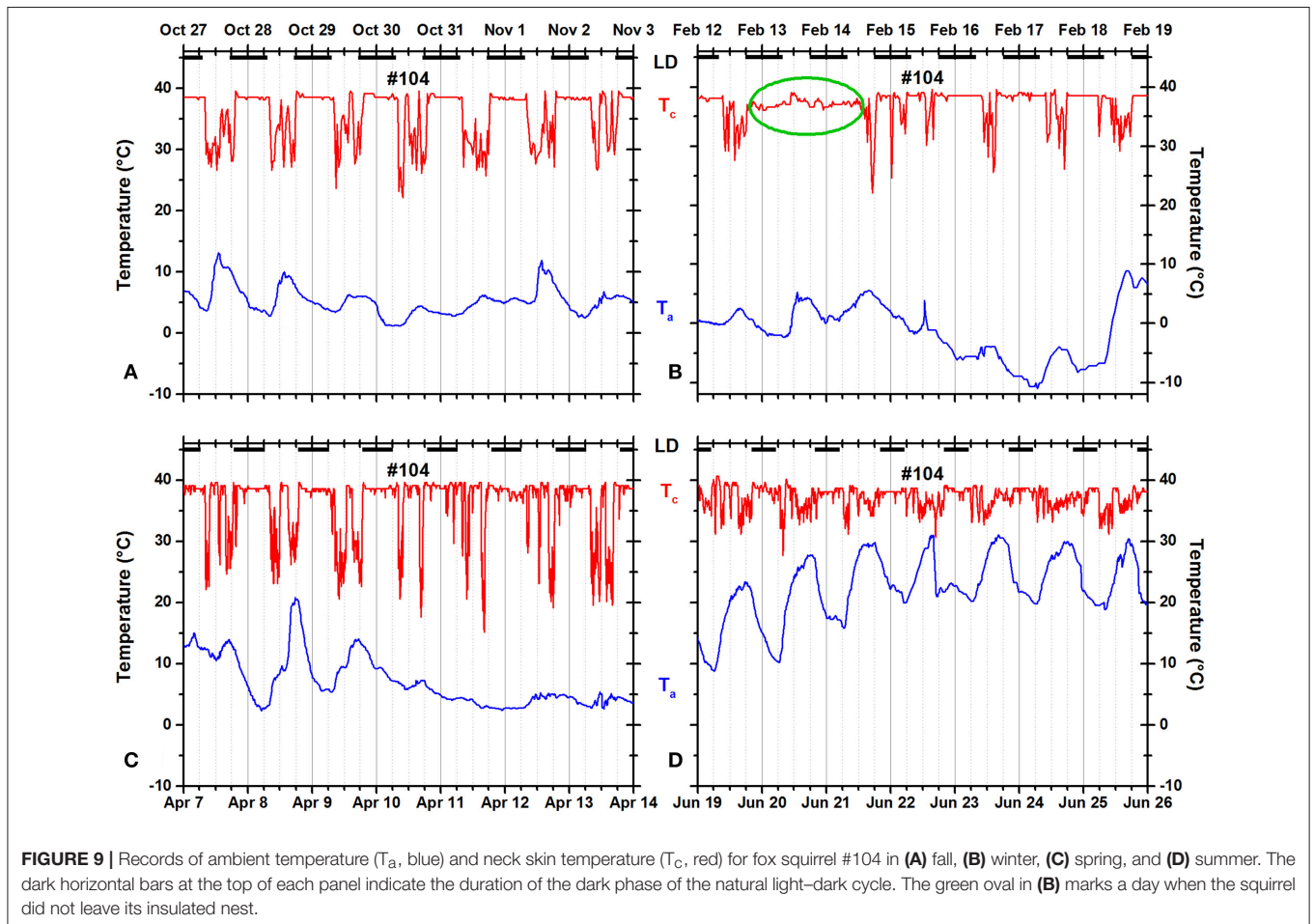
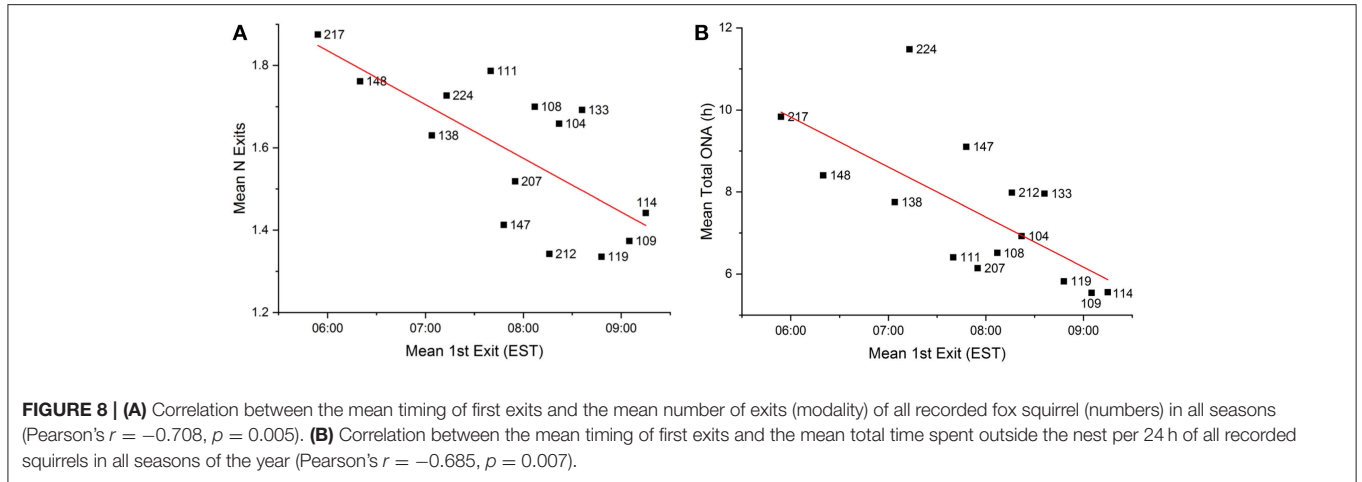
influence of the still mild night temperatures in fall, the end of the food-caching season, and the approaching major mating season causing significantly longer nocturnal ONT in fall compared to winter. These assumptions are supported by our findings that both photoperiod and certain weather conditions especially daily maximum temperatures were significantly correlated to total ONT (Table 2).

In the study by Studd et al. (2016) that we cited above in the discussion of the number of nest exits, temperature also highly affected the proportion of time spent out of the nest during morning hours (from 10% at  $-30^\circ\text{C}$  to approximately 100% at temperatures of  $0^\circ\text{C}$ ). As temperatures increased from  $-20^\circ\text{C}$  to  $15^\circ\text{C}$ , time spent out during afternoon declined from 75% to 10%, and time spent out during the evening declined from 100% to almost 0%. Anders et al. (2017) found that the daily activity time of red squirrels generally increased with increasing temperature and decreased with increased precipitation and average wind speed across both years. We can confirm this trend for temperature but not for precipitation and wind speed (Table 2). Studd et al. (2016) confirmed the influence of weather conditions previously reported by Williams et al. (2014) also in lactating females from the same population of red squirrels using light sensors.

Total nest time allows a rough estimate of the proportion of rest per 24 h. In the pooled data, squirrels spend about 66–68% of 24 h in their nests in spring, summer, and fall, and 77% in winter. As visual focal animal observations (Altmann, 1974) of six squirrels during various hours of the day when the animals were outside of their nests indicated that the animals were sitting or lying quietly, apparently resting, during 45–67% of the time observed (Wassmer and Refinetti, 2016), the total rest time might be considerably longer, probably in the realm of 80–85%. Studd et al. (2016) described nest attendance patterns of lactating red squirrels (*Tamiasciurus hudsonicus*) as highly variable as the daily proportion of time spent out of the nest during civil daylight hours ranged from 5% to 85% with an average of 45%. Diurnal in-nest time of fox squirrels (*Sciurus niger*) in this study was very similar ranging from 0 to 96% with an average of 43%.

### Timing of First Exits

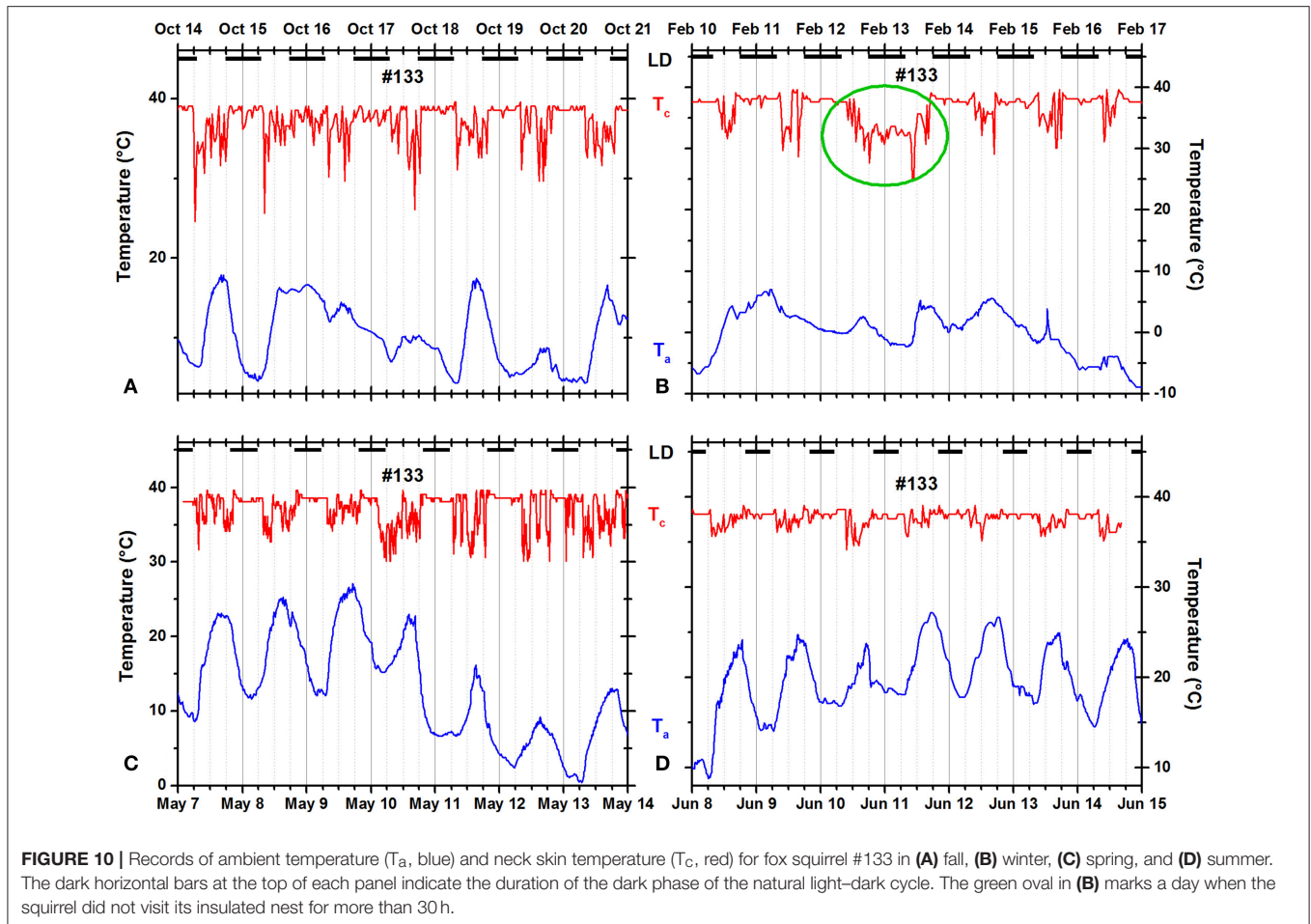
In all seasons, first exits of individual squirrels were significantly concentrated around a narrow window of time in the early to late morning (Figure 7) and were in many cases significantly different from each other (Table 3). However, none of the individual squirrels recorded during multiple seasons was clearly and consistently an early or late riser. In summer, first exits of individual squirrels were more spread out, whereas in winter, individual squirrels exited their nest much closer to each other, which is expected as winter days are substantially shorter than summer days. Interestingly this mimics the distribution of activity onsets in the nocturnal Ord's kangaroo rat (*Dipodomys ordii*) (White and Geluso, 2007), which also shows a wider range of individual activity onsets in summer and a much narrower range in winter. Enright (1966) showed that in house finches kept under controlled conditions in a laboratory, cooler temperature at a constant photoperiod and shorter photoperiods at the same ambient temperature caused delayed activity onsets. Our results



indicate that both daylength and temperature had simultaneously the same effects on fox squirrels in the wild. As first exits constitute activity onsets, these results are complementary to the (seasonal) changes of the unimodal–polymodal gradient and the total in-nest/out-of-nest time (proportion of activity/rest) and are therefore aspects of the overall activity pattern of fox squirrels.

The number of nest exits was significantly inversely correlated with the timing of the first daily exits in the pooled data for all seasons. This means that the early riser chronotype showed significantly more nest exits than the late riser chronotype (**Table 4**). The larger number of exits might reflect a behavioral trait of early risers or simply the availability of a longer daily





**FIGURE 10 |** Records of ambient temperature ( $T_a$ , blue) and neck skin temperature ( $T_c$ , red) for fox squirrel #133 in (A) fall, (B) winter, (C) spring, and (D) summer. The dark horizontal bars at the top of each panel indicate the duration of the dark phase of the natural light–dark cycle. The green oval in (B) marks a day when the squirrel did not visit its insulated nest for more than 30 h.

activity time. Under controlled laboratory conditions, early-rising antelope ground squirrels were found to have significantly longer daily activity times (Refinetti et al., 2016), and this association may occur also in the fox squirrel, which would contribute to the higher number of nest exits.

### Continuous Nest Occupancy for More Than 24 h and Nocturnal Out-of-nest Time

Nocturnal ONT occurred occasionally in many fox squirrels—mostly during warm nights but exceptionally also during winter (for example, **Figure 10B**). While most nocturnal ONT occurred only for brief periods, some events took the entire night. In the pooled data and in two out of three animals recorded in all seasons, nocturnal ONT was shortest in winter (0.1–0.2 h) and longest in fall or summer (0.5–1.6 h). Except for a recent publication (Anders et al., 2017) using continuously logged temperature data as well, nocturnal ONT was not known to occur in tree squirrels so far. This clearly demonstrates a key advantage of data loggers to discover previously hidden details of animal behavior and natural history. We can only speculate why fox squirrels spent time outside of their nests during some nights. Temperatures outside of the nests might be more comfortable in summer than the insulated nests or invite for

short trips for urination and defecation. In colder nights, such as the documented example in **Figure 10B**, it might be that the squirrel's nest was destroyed or not available.

While nocturnal ONT was detected repeatedly, continued nest occupancy for more than 24 h was only found once in the entire data set (**Figure 9B**). Squirrel 104 is a female, but the middle of February would be relatively late for parturition to happen. However, the date falls well into the lactation period while weather conditions were not specifically unfavorable (no precipitation or snow, relatively warm with highs around 3.5°C and lows at –6°C).

Nocturnal ONT (or more general, activity during the typically inactive time of the activity cycle) and days with unusual patterns are two more dimensions that should be considered to describe the bandwidth of activity patterns more inclusively.

### Comparison Between the Intra- and Inter-individual Data Variability

The inter-individual variabilities of total ONT and the number of exits were either higher than the intra-individual variabilities or comparable to them (**Table 5**). These results underline the importance of studying the activity patterns of individual tree squirrels to complement and contrast pooled data for entire

**TABLE 5** | Comparison between the intra-individual and inter-individual variation in three variables.

Season	Variable	N	Mean SE intra-individual	Mean SE inter-individual	t	p-value (one-tail)
Spring	First exit (h)	7	0.531	0.547	-0.133	0.448
	Number of exits	7	0.271	0.273	-0.021	0.492
	Total ONT (h)	7	0.894	1.4	-2.503	0.015
Summer	First exit	6	0.745	0.998	-1.176	0.135
	Number of exits	6	0.382	0.305	0.691	0.258
	Total ONT	6	0.972	1.285	-1.450	0.091
Fall	First exit	8	0.184	0.188	-0.122	0.452
	Number of exits	8	0.188	0.184	0.122	0.452
	Total ONT	8	0.87	0.67	1.654	0.060
Winter	First exit	8	0.359	0.45	-0.828	0.212
	Number of exits	8	0.173	0.176	-0.210	0.419
	Total ONT	8	0.616	0.753	-1.708	0.055
All seasons	First exit	14	0.477	0.554	-1.013	0.161
	Number of exits	14	0.186	0.185	0.077	0.470
	Total ONT	14	0.654	0.841	-2.607	0.008

Intra-individual variation was calculated as the standard error (SE) for each fox squirrel recorded during a certain season and during an equal amount of consecutive or random days (N). Inter-individual variation was calculated using N consecutive (or random) days during which all squirrels recorded during this season (N) were recorded. ONT, Out-of-nest time. Red denotes significant correlations.

groups and populations but also hint at the astonishing day-to-day variabilities within the same squirrels (Figure 5). Very few researchers have compared intra- and inter-individual variabilities of circadian rhythms. We are aware of only four studies that conducted such comparison for the rhythms of body temperature (Refinetti and Piccione, 2005; Romeijn and Van Someren, 2011), melatonin secretion (Barassin et al., 1999), and cortisol secretion (Selmaoui and Touitou, 2003). In one of these studies (Refinetti and Piccione, 2005), four different species were used: the laboratory rat, the 13-lined ground squirrel, the domestic dog, and the horse. Intra- and inter-individual variabilities were computed for the mean level, range of oscillation, acrophase, and robustness of the rhythm. The variabilities differed in different parameters of the rhythm and in different species but, whenever there was a difference between inter-individual variability and intra-individual variability, the latter was always smaller than the former. That is, the day-to-day variability of an individual's rhythm never exceeded the variability between the rhythms of different individuals, which is the same as we found in this study on fox squirrels.

## CONCLUSIONS

The results of our study illustrate the importance and advantages of collecting activity-related data from individual animals. Such data allow the estimation of how much individual fox squirrels differ from each other and how much they can fluctuate from day to day. We found that the magnitude of inter-individual variability of a number of behavioral variables was similar to the magnitude of intra-individual (day-to-day) variability. Although on most days the squirrels spent the night in their nests and exited the nest one or more times during the day, there was considerable variation (10–20% of the mean) from one squirrel to another and from day to day for the same animal.

We were working with a relatively small sample size and, due to the storage limitations of iButtons, with brief bouts of data. The idiosyncratic behaviors of individual fox squirrels that we documented might therefore not be entirely representative of a larger population of fox squirrels. However, some of the patterns and behaviors described here were not known before and illustrate the value of using data loggers to discover hidden details of the lives of even common species living in close proximity to humans in a suburban to urban setting.

There are possible methodological shortcomings in the use of collar temperature as a proxy of activity. In late spring/summer when higher ambient temperatures occur, the overnight patterns of  $T_c$  of several animals indicated that they did not spend the night in insulated nests (e.g., Figures 9D, 10C). On such days it is much more difficult—and sometimes impossible—to detect and distinguish in-nest time from ONT using our method. We therefore plan to supplement and extend the current study by using combined temperature and light sensors (Anders et al., 2017). We also plan to look at the patterns recorded for female fox squirrels during their expected gestation and lactation periods and compare these results to Studd et al. (2016) and Williams et al. (2014).

## ETHICS STATEMENT

All work was conducted with approval of the Institutional Animal Care and Use Committee of Siena Heights University and followed appropriate guidelines as outlined by Sikes et al. (2012).

## AUTHOR CONTRIBUTIONS

TW conceived, designed, and performed the data collection. TW and RR analyzed the data and wrote/edited the paper. RR designed the custom software used in the data analysis.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2019.00179/full#supplementary-material>

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