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Cite as: Knight F, Connor C, Venkataramanan R, Asher RJ. 2020. Body temperatures, life history, and skeletal morphology in the nine-banded armadillo (*Dasypus novemcinctus*). *PCI-Ecology* <https://doi.org/10.17863/CAM.50971>

Posted: 3 April 2020

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Body temperatures, life history, and skeletal morphology in the nine-banded armadillo (*Dasypus novemcinctus*)

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ABSTRACT

The nine banded armadillo (*Dasypus novemcinctus*) is the only xenarthran mammal to have naturally expanded its range into the middle latitudes of the USA. It is not known to hibernate, but has been associated with unusually labile core body temperatures. Like some other xenarthrans (but unlike mammals in general), *Dasypus* also shows relatively frequent departures from species-typical thoracic and lumbar vertebral counts. If and how intrauterine temperature variation in xenarthrans might increase skeletal variation during development, and how xenarthran body temperature varies according to season, housing, and gestational status, are unknown. Here, we report temperatures recorded from 19 female armadillos over the course of three seasons (each for at least one month between November and July), tracking internal body temperatures recorded every 6 to 120 minutes. Average temperatures are similar regardless of housing inside or outside, gravid or nongravid. Nongravid individuals housed indoors show significantly higher daily fluctuations than other treatment groups, despite more stable ambient temperatures indoors than outdoors. Assuming that our estimates for implantation are accurate, gravid individuals show, on average, slightly lower daily fluctuations than nongravid, but the difference is not significant and some of our most extreme fluctuations (6–9°C within 24 hours) took place in gravid animals. Compared to animals housed outdoors, lab-housed individuals less frequently exhibit body temperatures above their overall mean during typically dormant hours (e.g., afternoon), and body temperatures below their overall mean during typically active hours (e.g., early morning). Temperature periodicity as measured by discrete Fourier transforms shows strong 24 and 12 hour cycles in all individuals, reflecting circadian rhythms. CT scans of monozygotic quadruplets from dams with known body temperatures show a surprising range of phenotypic variation. While all offspring had seven cervicals, and most had ten thoracics, five lumbar, and three sacral, three of twelve CT-scanned litters had nine rather than 10 thoracics and some offspring of one dam had two sacral. Dams for two of these exhibited low average temperatures (<35°C) early in their gestation. Two dams had offspring with a polymorphic axial skeleton, despite being genetically identical quadruplets. Our data demonstrate the thermal and phenotypic lability of *D. novemcinctus* but do not decisively link gestational temperatures with particular vertebral phenotypes.

Keywords: Cingulata, Xenarthra, iButton, axial skeleton, vertebral column, variation, temperature regulation, Fourier transform, periodicity, gestation, embryogenesis

Introduction

Endothermic mammals and birds differ from most other organisms by virtue of their relatively high and constant body temperature. Rather than decrease their metabolic rate in cold environments, endotherms do the opposite to utilize the heat released by oxidative respiration to maintain core body temperatures well over ambient. Rates of oxygen consumption and food intake in endotherms are correspondingly higher than those of ectotherms (Bennett 1988). Among both endotherms and ectotherms, some species maintain constant body temperature (homeotherms) and others show substantial variation (poikilotherms) following daily or seasonal cycles (Geiser 2004). Life events such as pregnancy and parturition are known to influence body temperatures in both wild (Gilbert et al. 2007) and domestic (Wrenn et al. 1958) animals. Moreover, temperature plays an important role in

developmental processes such as segmentation (Schröter et al. 2008), which may have downstream effects on skeletal phenotype and intraspecific variation (Hautier et al. 2013), including vertebral counts (Lindsey & Moodie 1967; Tibblin et al. 2016). "Normal" temperatures in humans were defined by Edwards (2006:507-508) as "a range, rather than a tightly defined single figure ... approximately $\pm 0.6-0.8$ " around a mean of 37C. Regarding humans, Edwards (2006) furthermore wrote that "mild exposures during the preimplantation period and more severe exposures during embryonic and fetal development often result in prenatal death and abortion. Hyperthermia also causes a wide range of structural and functional defects." Several non-human mammalian species (including armadillos; see McNab, 1980) seem to withstand much wider temperature fluctuations.

In order to assess how temperature varies during life history events such as gestation, and its potential influences on morphology, we examined variation in body temperatures in 19 wild-caught nine-banded armadillos (*Dasypus novemcinctus*; Table 1). Armadillos in the genus *Dasypus* are unique among vertebrates in their form of polyembryony; i.e., females typically give birth to identical quadruplets derived from a single zygote (Enders 1966; Prodöhl et al. 1996; Loughry & McDonough 2013). *D. novemcinctus* furthermore exhibits delayed implantation. Mating typically occurs in the summer, but the blastocyst does not implant in the uterine wall or begin growth until November-December. Birth of North American *D. novemcinctus* generally occurs in March and April after approximately 4.5 months of gestation (Enders 1966).

Previous work suggests that the armadillo's resting body temperature, around 34C, is low and variable for a placental mammal of its size (Johansen 1961; McNab 1980). Some of this variation may be attributed to cold-induced-fever. When exposed to cold, resting armadillos increase their metabolic rate in excess of that required to maintain the core temperature (at least in laboratory experiments using temperature controlled metabolic chambers) and the core temperature rises in proportion to the cold challenge (Johansen 1961; Mercer & Hammel 1989; Boily and Knight 2004). At the other end of the scale, prolonged exposure to a moderately cold environment (e.g., 24C for 24 hours) may cause depressed metabolism and lower than typical core temperatures (around 32C; McNab 1980). Previous authors have thus observed that *D. novemcinctus* can exhibit substantial individual variation in temperature (e.g., 6C between lows and highs observed by McNab 1980: fig. 1D and Boily & Knight 2004) and tolerance to low body temperatures, such as 25.5C in a non-torpid individual observed by Johansen (1961: 132). Torpor appears to be absent in *Dasypus novemcinctus*, but has been observed in smaller dasypodids such as *Zaedyus pichiy* (Superina & Boily 2007). Evidence that females undergo weight gain during

pregnancy (Lengyel 2011) may lead to increased core temperatures and/or thermal stability while gestating. Reduced temperature variation during incubation exists in at least some vertebrate species (e.g., male emperor penguins; Gilbert et al. 2007) but remains undocumented in gestating *Dasypus*.

Here, we test if previous results are robust to our expanded sample of temperature data and examine two hypotheses concerning mammal physiology and phenotype: (1) animals maintain a narrower range of temperatures during gestation compared to non-gestation, and (2) temperature variability during early gestation exerts a phenotypic effect on skeletal morphology, such as vertebral count. If the first hypothesis is true, we would expect to see lower indices of variation (e.g., daily standard deviation and difference between temperature maximum and minimum) in gestating compared to non-gestating individuals. If the second is true, we would expect to observe offspring with atypical vertebral counts (such as variation in number of ribs and/or position of the sacrum) to have come from pregnant females ("dams") that exhibit different body temperatures during one or more key phases of development relative to dams that give birth to offspring with typical vertebral counts.

Methods

All activities conducted in this study were approved by the University of the Ozarks Institutional Animal Care and Use Committee (protocols 01-2014, 01-2015, 02-2017), and comply with U.S. AWA; the University of the Ozarks USDA registration is 71-R-0104. Field activities on public lands were conducted with permission of the Arkansas Game and Fish Commission (scientific collector's permit #020520153). Laboratory and field activities involving live animals were consistent with the guidelines of the American Society of Mammalogists for the use of wild animals in research and education (Sikes et al. 2016).

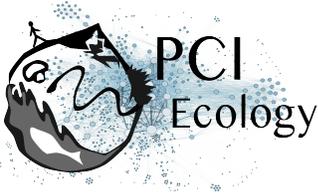
Animal subjects and housing. Wild, adult female armadillos were captured in northwest Arkansas during November and early December—six in 2014, eight in 2015 and eight in 2017 (22 total). Each year some animals were randomly assigned by coin toss to indoor kennels and others to outdoor enclosures. Housing and care for indoor animals were the same as in Power et al. (2018). The ambient indoor temperature was set to 22C and photoperiod was adjusted to approximate the seasonal photoperiod. While our recorded indoor temperatures vary in coverage (e.g., missing before mid-December 2014 and after mid-March 2015), there were no major disruptions to our indoor facilities that would have greatly deviated from the indoor temperatures we did record. Outdoor enclosures were approximately 100m²



in area. Each enclosure contained a large, shallow ca. 8L rubber pan for water and shelter and an L-shaped trench about 1.2m long and 0.2m deep filled with straw or shredded paper. The trenches were covered with 1.5m² of plywood, three bales of straw, and a water resistant tarp. Most armadillos substantially remodeled the trench. Outdoor animals were able to forage, but we provided them with the same chow as those housed indoors once a day near sunset. A weather station (Onset Computer Corp., HOBO data logger, thermistor) recorded ambient air (logger ID #10583323-1) and soil at 15cm depth (logger #533024) temperatures hourly at the site of the enclosures from 2014 through 2016. We did not have soil temperatures after 2016 and retrieved ambient air temperatures for 2017-18 from weatherforyou.com.

Measuring body temperatures. We surgically installed iButton temperature loggers (models DS1921H-F50, DS1922L-F50) within three days of capture. Prior to installation, we programmed the iButtons to log temperature once every 6 to 120 minutes until the memory was filled. iButtons were disinfected with chlorohexidine; sterile suture was bonded to the device with cyanoacrylate and embedded in beeswax. We anesthetized subjects with 2.0-3.5% isoflurane adjusted to effect and conducted the surgery according to standard veterinary practice for small animal abdominal surgery. We inserted the iButton through a ventral incision ca. 2cm in length, to the left of the rectus abdominis, approximately 2 cm caudal to the last rib. The iButton was sutured securely to the visceral peritoneum and abdominal musculature. We closed the incision by suturing the incised muscle and then suturing and gluing (cyanoacrylate) the integument so as to leave no exposed stitching. Before the subject recovered from anesthesia, we administered analgesic, antibiotic and dewormer. The subject was monitored ca. hourly until it recovered from the anesthetic. Subjects assigned to the outdoor treatment remained in indoor cages for one to two weeks after surgery to allow for healing and acclimation to the staple chow diet. Two of the 22 armadillos surgically installed with an iButton escaped the enclosure and the temperature logger of a third failed. Therefore, we report on the data of 19 subjects. Thirteen individuals were gravid during at least part of temperature monitoring, as summarized in Table 1. Original timeseries and temperature data are provided in Appendix S1.

Initial and terminal readings from an implanted iButton were occasionally unstable. In order to ensure that all of our records represented temperatures from fully acclimated iButtons, we deleted the first and last 12 hours of readings from each ibutton; the first followed surgical placement of the iButton and the last were prior to ibutton removal or death of the animal, whichever came first. In addition, in order to have meaningful ranges between daily minima and maxima, we ensured that



deletion of initial/final readings did not lead to a single temperature record for a given day (e.g., deleting one additional reading just past 24:00 if it was the only one on that day).

Implantation. Because it is delayed, we do not know the exact uterine implantation date for any individual. Furthermore, exact dates of parturition were only available for our five gestating, lab-housed individuals (Table 1). Following the literature (Enders 1966), we assumed implantation would have taken place 4.5 months (ca. 135 days) prior to birth, leading to estimated implantation dates for our five individuals with known birth dates from, at the earliest, 4 November (wat3 who gave birth on 19 March 2016) to, at the latest, 2 December (quiche who gave birth on 16 April 2015). The other three individuals with known parturition dates did so on 24, 27 and 28 March (Table 1), suggesting that implantation took place in these individuals during early- to mid-November. While we lacked exact dates of parturition for the females living in burrows (outdoors), we had several indicators for approximating the date of parturition to about 10 days. We were able to observe some females foraging in their enclosures and, when they reared up to scent the air, we could see the state of their teats and the distention of their abdomens. Some females' behavior changed acutely (uniquely not foraging and not eating the chow for one night) around the time we expected birthing, followed by a marked decrease in above ground activity for a week or two. Finally, when we removed the litters from their burrows, we estimated postnatal age from their size (weight, head length, anterior shield length) using regressions of measurements of young whose postnatal ages were known. These observations indicated that all had given birth before 16 April of each season.

Based on these data we estimated the parturition dates within 10 days for the individuals housed in outdoor burrows. We left cells pertaining to gestation status as "unknown" in November when the blastocyst may not yet have implanted, and five days before and five days after (or until 16 April, whichever came first) our parturition estimate, when there was some chance that a litter may have already been born but not yet observed. This increases the number of unknown cells, but reduces the possibility that we have incorrectly categorized some of our temperature recordings during November, March, and early April as "gravid". On both known and estimated dates, we arbitrarily chose 12noon as the hour of parturition. One individual (we1) showed evidence of gestation and birth, but the young had evidently been rejected some time before any behavioral indicators of parturition could be observed. We assumed that individual we1 would have been gestating through at least the end of February, over three weeks prior to the earliest date of parturition

observed in our sample (22 March estimated for yams) and left cells for gestational state in we1 blank until 16 April.

Further information on the timing of implantation is evident from historically observed patterns of birth and from the anatomy of early-stage embryos. Based on a population of *D. novemcinctus* in Texas, Enders (1966:299) wrote

"animals with fetuses approaching full term size (12-13cm crown-rump length) are usually found in late March and early April, although individual pregnant animals are occasionally found at later dates. Of six cases in which parturition occurred in the laboratory, one was in late March, four in the first week of April, and one in the 3rd week in April. ... Parturition can be considered to occur in late March and early April, giving a post-implantation period 4.5 months long."

These observations are consistent with our own, based on individuals captured and examined at several localities in northwestern Arkansas, a region near the species' northern limit. One of us (FK) has housed and studied wild-caught *D. novemcinctus* in south central USA for more than 20 years. Among more than 80 litters born in captivity, none has been born before 1 March and nearly all were born between 15 March and 1 May. Thus, we infer uterine implantation of a blastocyst to take place at some point in November and early December, followed by about 4.5 months of gestation. Fig. 1 shows an embryo we recovered from a gestating dam on 3 December 2012. This embryo shows clear segmentation, a neural tube, limb formation, a paddle-like autopod without fully differentiated digits, and concentrations of cells that radiate laterally from each somite in the thoracic region. This embryo resembles an E11 (Teiler stage 18-19) mouse (<http://www.emouseatlas.org/emap/ema/home.php>), in which somitogenesis and the initiation of axial skeleton differentiation would have begun prior to 3 December.

Assessing embryonic and fetal anatomy. Where possible, we CT scanned offspring of our wild-caught dams. In most cases we used a Sirona Galileos 3D CT scanner at a local dental clinic in Clarksville, Arkansas. This enabled quick (ca. 2 minute) scans for each live pup (sedated with isoflurane to effect) that covered the axial skeleton region, from head to pelvis, in one or two scans. In two cases (we1, we5), offspring did not survive and were scanned at the Cambridge Biotomography Centre (cbc.zoo.cam.ac.uk). We used Drishti 2.6.4 (Limaye 2012) for 3D rendering and anatomical reconstruction of CT data. Boundaries between vertebral regions were defined anatomically, most obviously by the presence of ribs and also including centrum shape, contact with one or both ilia (for sacral vertebrae), and presence of vertebral foramina (for most cervicals). We counted asymmetries (e.g., unilateral ribs or contact with the ilium on just one side) as non-integers; i.e., an individual with

vertebrae showing nine bilateral and one unilateral rib would have 9.5 thoracic vertebrae. We did not consistently recover all of the tail segments in our CT scans, and even if we had, it would be difficult to rule out non-ossified, terminal segments in our fetal specimens. Therefore, we did not include counts of caudal vertebrae.

Calculating periodicity. In order to ascertain the nature and frequency of temperature variations in our dataset, we used the Discrete Fourier Transform (DFT, Proakis and Manolakis 2006). The Fourier transform is widely used to decompose a signal or time series with cyclical properties into its constituent parts. For example, the Fourier transform of an audio signal yields its constituent frequencies and the strength of each frequency component. Applying the DFT to time series data gives a frequency-domain representation, expressing the data as a weighted combination of sine and cosine functions. In our case, the DFT of the temperature time series reveals any periodic components contained in the series, such as circadian rhythms.

We followed Shumway & Stoffer (2017: 168-173) to apply the DFT to our dataset, using R-Base (R Core Team 2016) and the R script shown in Appendix S2. We first subtracted the overall mean temperature for a given animal from each of its individual temperature recordings to obtain a centered temperature time series (R script line 4). We then computed the squared magnitude of the DFT coefficients of the centered time series, and divided by the number of recordings for that individual (R script line 7). This gives a periodogram representing the relative strength of the component sinusoids of our body temperature time series. A given animal with N temperature recordings in the time series has N values in its periodogram. The periodogram values are the relative weights of N sinusoidal components, with periods $(T_0 N)/(L-1)$ hours, for $L=1, \dots, N$, respectively. Here T_0 is the interval between adjacent temperature recordings in hours. The periodogram value at $L=1$ is always zero as the overall mean has been subtracted.

We identified the peaks in the periodogram, defined as values which exceeded 5% of the maximum observed for that individual (R script line 9). Due to the conjugate symmetry of the DFT (Proakis and Manolakis 2006), the periodogram is symmetric around $N/2$ on the x-axis. (I.e., the first half is the mirror image of the last half.) Hence only the peaks in the first half of the sequence (R script line 10) were retained. As explained above, a peak at index L of the periodogram sequence corresponds to a cycle in the animal's temperature data with period $(T_0 N)/(L-1)$ hours (R script line 15). Here T_0 equals the interval between temperature recordings in hours and N equals the total number of recordings for that animal. The highest periodogram values indicate the most pronounced and consistent cycles.

Results

All 19 individuals, regardless of housing (lab vs. outdoor) or gestation status, exhibited substantial daily variation in body temperatures. Our Appendix S3 graphically shows temperatures over the total span of recordings, daily range of temperature fluctuations, and DFT periodograms for each animal. Overall, temperatures ranged from 27.6 to 38.6 with an overall median of 35.56C, mean 35.4, and a slightly longer tail on the left side of the distribution of all 58367 recorded temperatures across our 19 individuals. Figure 2 shows a histogram of all temperature recordings above and below the overall mean temperature of 35.4C, as well as those based on housing and gestational status. Two peaks of body temperatures above the mean occur during the early morning and late evening; one peak below the mean occurs during the afternoon. We defined three time bins centered around each of these peaks (from 00:00 to 09:59, 10:00 to 19:59, and 20:00 to 23:59), and recorded the number of occurrences above and below the overall temperature mean among individuals in each of the two pairs of categories (burrow vs. lab, gestating vs. non-gestating). This yielded two contingency tables, each four rows by three columns, with six degrees of freedom and a critical value of 22.46 at $\alpha = 0.001$ (Rohlf & Sokal 1995: 24). Application of a chi-squared test to the frequencies shown in Fig. 2 demonstrate a highly significant rejection of the null hypothesis that time of day does not affect occurrence of body temperatures above or below the overall mean. The sum of squared deviations from the expected $((\text{observed}-\text{expected})^2/\text{expected})$ yields values of 13424 for lab vs. burrow enclosures and 20122 for gestating vs. nongestating individuals. Both are far in excess of the threshold of significance. At the extremes, we recorded 56 instances (0.00096% of all readings) from eight animals above 37.5C; these were most frequent at night, but were also observed in a few cases during the afternoon and evening. We observed 43 occurrences (0.00074% of all readings) of body temperatures below 32C in eight animals concentrated between 11:00-17:00, with one animal (wat3) around 31C at an unusual time near 23:00 on a few nights from 4-17 April 2016.

Our observations of *D. novemcinctus* indicate that they are warmest during hours of peak activity during the evening and early hours after midnight, and coldest during rest in the late morning and afternoon. The same temperatures and activity patterns hold for each of our treatments: gestating vs. non-gestating and indoor lab vs. outdoor burrow (Fig. 2). The most conspicuous difference among these four groups was the reduced overlap of temperatures recorded on either side of the mean in the lab-housed individuals compared to those housed in an outdoor burrow. In other words, an individual housed indoors was less likely to have a body temperature at or over the mean during what appear to be dormant hours in the afternoon, or have a

temperature under the mean during what appear to be active evening and early morning hours, compared to individuals housed in outdoor burrows. Gravid individuals similarly exhibited less overlap between dormant vs. active hours compared to nongravid individuals, although they did show more overlap in temperatures on either side of the mean during the afternoon compared to lab-housed individuals (Fig. 2). Anecdotal review of our opportunistically taken camera trap photos support this pattern and suggest that they are typically active in late afternoon and early evening during feeding, and again in the early morning, around midnight to 3AM.

Average temperature across our 19 individuals was 35.4C and did not differ greatly among our four treatment groups (Figs. 3, 4). Gravid individuals exhibited a slightly lower mean temperature than nongravid individuals; lab-housed individuals had a slightly lower mean than those in an outdoor enclosure (Fig. 3A). Middle quartiles of all treatment categories overlapped around 35.45 degrees. Using averages for each individual in each of its possible treatment categories (lab vs. burrow, gravid vs. nongravid) as independent data points in a two-way Anova, none of these mean temperatures differs significantly between our treatment categories of gestation ($p = 0.52$) or enclosure ($p = 0.45$).

Variation. As shown in Fig. 5, animals housed in outdoor burrows experienced ambient air temperatures from a low of -16C (morning of 17 January 2018) to a high of nearly 33C (afternoons of 7-9 June 2015), with an overall standard deviation of 8.4. Outdoor soil temperatures (not available from 2017-18 season) were more stable (stdev = 5.5), particularly compared to the 24-hour cycles evident in outdoor air temperatures (Fig. 5). However, soil temperatures varied seasonally from extremes of 3C (mornings of 19 and 24 January 2016) to 27C (evenings of 8-9 June 2015) and never dropped below freezing (Fig. 5), consistent with the interpretation that extended periods of frozen ground limit the range of *D. novemcinctus* to (largely) the southern areas of North America. In contrast, air temperatures inside animal rooms typically ranged between 22-26C, with peaks up to 29.5 and troughs down to 20.1, with a mean of 24 and standard deviation of 1.04. As expected, ambient temperature variation inside was much lower than outside (Fig. 5).

Regardless of ambient temperatures, all individuals exhibited daily fluctuations of body temperatures. Difference between the minimum and maximum daily temperatures for each animal exceeded 2C in at least some individuals in all treatment groups. Despite the relatively narrow range of ambient temperature fluctuations indoors, nongravid individuals housed indoors exhibited the highest mean daily fluctuation, with a median range of daily temperature fluctuation of 2.77C

compared to 2.09 among lab-housed, gravid dams and 1.97 and 1.72 among nongravid and gravid (respectively) dams housed in outdoor burrows (Fig. 6). Lab-nongravid individuals showed suggestively but not significantly higher daily variation than lab-gravid individuals (Wilcoxon $p = 0.14$; t-test $p = 0.082$; median 2.1C; see Fig. 6). Extreme fluctuations included a difference between minimum and maximum of 9C for we1 on 11 Dec 2017. This individual was gravid (but likely at an early stage of gestation), housed in an outdoor burrow, and continued to provide temperature readings during and after parturition, until 28 May 2018. Its mean daily temperature fluctuation while gravid was 2.33C, and while nongravid was 2.1C. Despite this outlier, daily temperature fluctuations among lab-nongravid animals were significantly larger (median 2.77C) than animals housed outdoors (Wilcoxon and t-test $p < 0.02$; medians 1.97C nongravid, 1.72C gravid). As reported in previous literature, and although we observed instances of substantial daily temperature fluctuations, we did not observe any instance of torpor in *Dasypus*.

For seven individuals in our sample, we had body temperatures both during and after gestation. Exact dates of parturition were known for five of these, of which three had continuous recordings from December beyond the day of birth (Fig. 7). Two of these dams (wat2, le4) exhibited a conspicuous increase of body temperature on and after the day of birth; another (wat3) also showed an increase on the days immediately following birth but a decrease in the following month, such that (unlike the other two dams) its mean nongravid body temperature was slightly below its mean gravid temperature. With a difference of 5.5C between max and min observed on 29 March 2016 (10 days after parturition), wat3 did not show quite as extreme daily range as we2 noted above, but it too is an outlier in terms of its variation, and shows the highest standard deviation in body temperature (1.15) of any animal (Fig. 7 and Appendix S3). However, its overall mean body temperature is unremarkable at 35.1C (35.2C mean during gestation and 34.9C thereafter).

Periodicity. The Discrete Fourier Transform of the temperature data exhibited clear periodicities at 24- and 12-hour intervals (Fig. 8), reflecting the obvious circadian activity patterns evident in our temperature fluctuations (Fig. 7, Appendices S2, S3). In 18 of our 19 individuals with temperature data, the highest DFT value corresponded to a period between 23.9 and 24.4 hours; the one exception (butternut) yielded a highest DFT value (31.24) corresponding to a 12-hour period; its next highest value (29.47) was a close second and corresponded to a 24-hour period. The other 18 individuals also had DFT values indicating a 12-hour period at or over 5% of the observed maximum DFT (Fig. 8). DFT values indicative of longer periods were also evident and usually indicated one-off, abrupt changes in body temperatures that reflect individual variation, or in some cases were artefacts of the

total number of temperature readings; i.e., the first and last readings appeared to define a recurring waveform. The only relatively strong DFT values indicative of cycles with periods substantially below 12 hours were again observed in butternut. This gravid dam was housed outdoors and showed one cycle at 8 hours (DFT = 10.38 or 33% of observed max) and another at 6 (DFT = 3.67 or 12% of observed max). To test if the number of cycles at or near 12- and 24-hours evident in Fig. 8 fit the null hypothesis that peaks occur with similar frequency across all bins, we placed all DFT values in excess of 5% of each individual's highest observed value into 15 gap-coded bins, containing all of the 170 DFT hourly peaks shown in Fig. 8. The concentration of 30 peaks centered in the 12-hour bin, and 81 peaks in the 24-hour bin, greatly exceed the expectation under the null hypothesis, i.e., that each of the 15 bins would contain 11.33 peaks (170/15). The sum of squared deviations from the expected ($(\text{observed} - \text{expected})^2 / \text{expected}$) across all bins is 517, far in excess of the critical value (36.1 for 14 degrees of freedom at $\alpha = 0.001$; Rohlf & Sokal 1995:24).

Beyond the two obvious, circadian sinusoids of 12- and 24-hours, and discounting the cycles with periods that are multiples of the number of temperature readings (evident between 5-33% of the maximum DFT in wat2, le4, butternut, we2, and we1; evident over 50% of the maximum DFT in carrots, yams, we5, and we3), there were relatively few strong peaks. Individual we5 exhibited several DFT values over 15% of the maximum (Supplementary Table S1), which corresponded to neither 12 nor 24 hour cycles. These were (in decreasing strength from 86% to 15% of its strongest sinusoid at 24 hours) hourly cycles of 1462, 292, 731, 91, 97, 209, and 366. All were stronger than this animal's 12-hour cycle which was converted from a DFT unit of 8.9 and comprised just 8% of the peak DFT value (106.3, corresponding to a cycle of 24 hours). This animal was housed in an outdoor burrow between 20 Dec 2017 and 25 Jan 2018 (864 hours) and was gestating during all of its temperature recordings, until its death on 5 Feb 2018. Lunar cycles of 29-30 days correspond to periods of roughly 710 hours between Dec-Feb, during which time we have recordings for this animal. Time elapsed between temperature minima in this animal (32C on 20 Dec 2017 and 31-32C on 22 and 25 Jan 2018) amounts to roughly 792-864 hours. None of these fits into the periods over 24 hours exhibited by we5, so we are unsure as to the biological cause (if any) behind them and attribute its unusual periodicities to ill health. (It was moved indoors on 25 Jan and died on 5 Feb 2018.) In addition, unusual periodicities may result from the magnitude of the maximum DFT value being small, as is the case for butternut, carrots, puddin, we2, and yams (all below 84), as opposed to individuals such as le1 or bertha with maximum DFT values of 1820 and 894, respectively (Supplementary Table S1). These individuals therefore had a lower threshold to exceed 5% of the maximum DFT.

Vertebral counts. We recovered CT scans from litters of all 13 gravid dams (Supplementary Table S2). *Dasyurus novemcinctus* shows a species median vertebral count of 7 cervicals, 10 thoracics, and 5 lumbar, based on 25 individuals reported by Asher et al. (2011), who also noted that *Dasyurus* showed the highest coefficient of variation (4.6) of the summed thoracolumbar count among 42 monotreme, marsupial and placental genera quantified in that study (Asher et al. 2011: fig. 1). In our current sample, we also observed 10 thoracics and 5 lumbar as the most frequent count in newborn *Dasyurus*. In addition, all of our subjects had seven cervicals and usually three sacral. Nine litters exhibited the median count of 10 thoracics and five lumbar and all but four litters observed here had three sacral. Offspring of we1 and we5 exhibited a first sacral but were otherwise insufficiently ossified in the pelvic region to establish a full count. Of the three remaining sets of quadruplets (and abbreviating vertebral counts as 10-5-3 for thoracic-lumbar-sacral), all four offspring of dam quiche exhibited 9-5-3. Three offspring of wat2 also exhibited 9-5-3 and one showed an asymmetric caudal-most rib (Fig. 9) yielding a count of 9.5-4.5-3. Two of the four offspring of we2 showed 9-6-2 (we2a, we2d); we2b showed 9-5-3 and we2c showed an asymmetric first sacral for a count of 9-5.5-2.5 (Fig. 10). Hence, despite the fact that litters of *D. novemcinctus* are almost always genetically identical quadruplets (Enders 1966; Prodohl et al. 1996), two of the 12 litters for which we have data on vertebral phenotype (wat2, we2) were not phenotypically identical (Figs. 9, 10).

The three individuals that had offspring with nine rib-bearing vertebrae were wat2, quiche, and we2. We2 was housed outdoors in a semi-captive enclosure and lacked an exact date of parturition. Based on the behavioral observations outlined above, we estimate a parturition date near 6 April 2018 and, correspondingly, an implantation date around 22 Nov 2017 for we2 assuming 135 days of gestation. Wat2 and quiche were lab-housed individuals with known dates of parturition: 28 March and 16 April 2016, respectively. This indicates an implantation date of 14 November for wat2 and 3 Dec 2015 for quiche. The earliest, average body temperatures for the dams (quiche, wat2) of two of these three litters with an atypical vertebral count (9 thoracics) were unusually low, dipping below 35C in both cases (Fig. 11). We2 exhibited more a typical daily average between 35.5 and 36C throughout its first full month of recorded body temperatures (starting 2 Dec 2017). However, all three individuals likely exhibited implantation prior to the initiation of stable temperature recordings (Figs 7, 11), and we do not know at what points during embryogenesis *Dasyurus* patterns its axial skeleton and establishes vertebral identity.

The only pup recovered from we1 showed 10 rib-bearing vertebrae; this dam also showed unusually low body temperatures early in gestation, including the lowest

daily average (33.48C on 11 Dec 2017) recorded during December of any of our three seasons (2014, 2015, 2017). The relatively low body temperatures of the two dams (quiche and wat2) during early- and mid-December (Fig. 11) may have influenced axial patterning of their offspring and resulted in nine rather than 10 thoracic vertebrae (Fig. 9). However, our average daily temperature of the third dam (we2) with offspring showing a reduced number of precaudal vertebrae (with 9 thoracics) is generally higher during December (>35.4C) and not essentially different from dams that had offspring with 10 thoracic vertebrae (Fig. 11).

Discussion

Gestating dams of *Dasyus novemcinctus* show a similar overall range of body temperatures as non-gravid individuals, whether they are housed outdoors or an indoor lab (Figs. 3,4). There are some differences in daily temperature fluctuations, with nongravid, lab-housed individuals showing a significantly greater magnitude of daily maxima vs. minima than dams housed outdoors (Fig. 6), despite the fact that their ambient thermal environment is relatively stable (Fig. 5). Based on laboratory studies that document cold-induced fever (Johansen 1961, Mercer & Hammel 1989, Boily & Knight 2004), we would expect to see higher body temperatures among outdoor animals, with more variation, compared to indoor animals. Instead, we observed similar overall means (Fig. 3A) and more daily variation (Fig. 6) among our indoor animals. In particular, nongravid, indoor animals exhibited a median daily max-min body temperature of 2.7C, compared to values below 2C among individuals housed outdoors (Fig. 6). Outdoor animals likely minimized their time above ground to that required to find the food dish, eat, and retreat to their thermally more stable burrow on the coldest nights. The two major differences among indoor compared to outdoor animals are the former's much lower exposure to cold, and the spatial confines of their cages. The lack of any substantial 24-hour cyclicity for indoor ambient temperatures (Fig. 5), along with the reduced space in which indoor animals can move, may both have contributed to the greater differences between daily maxima and minima we observe among our indoor, lab-housed animals (Fig. 6).

Gestation is also associated with a reduction of daily temperature fluctuations, but primarily among lab-housed individuals; the median daily fluctuation for animals housed in outdoor burrows is not significantly different between 1.72C (gravid) and 1.97C (non-gravid) individuals. Indoors, the difference is greater but still not significant at 2.09C for gravid and 2.77C for nongravid (Figs. 3B, 6). Indeed, some of the most extreme daily fluctuations we observed took place among individuals we

infer to have been gravid, such as nearly 9C for we1 (from 27.6 to 36.5) on 11 Dec 2017 (noted above) and over 6C for yams (from 30.6 to 36.7) on 27 Dec 2015. However (and assuming our estimates of implantation are correct), these values were observed relatively early in gestation, and other gravid dams show much less variation (e.g., we2, butternut). Overall, and unlike (for example) male emperor penguins, which exhibit lower body temperature fluctuations among incubating relative to non-incubating males (Gilbert et al. 2007: fig. 1), gestation in armadillos results in a slight but not significant reduction in daily variation of the temperature in the developing embryo's thermal environment.

Vertebral Conservatism in Mammals. Nearly all mammals, from armadillo to giraffe, possess exactly seven ribless vertebrae in the neck (Buchholtz & Stepien 2009; Hautier et al. 2010; Buchholtz et al. 2012). One proposed explanation for this conservatism relates to stabilizing selection. Galis et al. (2006) noted high frequencies of vertebral anomalies among radiographs of stillborn/aborted humans from hospital pathology departments, often in excess of 50% (Galis et al. 2006). They correspondingly argued that mammals do in fact generate the morphological novelty behind greater skeletal variation, but such novelties are weeded out by stabilizing selection due to negative, pleiotropic effects of mutations to patterning genes related to the cervical skeleton. Mammals that deviate from the rule of seven (the sloths *Bradypus*, *Choloepus* and manatee *Trichechus*) do so, according to Galis et al. (2006), based on a relatively low metabolic rate and correspondingly lower susceptibility to pathologies such as cancer. Another, non-mutually exclusive hypothesis to explain the mammalian "rule of seven" focuses on the cervical origins of the mammalian diaphragm (Buchholz et al. 2012, Buchholtz 2014). Mammals are unique among vertebrates in having a muscular piston to ventilate their lungs, i.e., a diaphragm separating pleural from peritoneal cavities in the thorax. The cells that ultimately contribute to the diaphragm and its innervation derive from cervical somites, and must descend during ontogeny to the mid-thoracic region. This leads to a developmental constraint on the potential for evolutionary innovations of cervical somites; because of this constraint, mammals exhibit correspondingly less anatomical variation in their cervical spine. Species that escape this constraint, and show more cervical variation (e.g., *Bradypus*) may do so by shifting primaxial vs. abaxial embryonic tissues relative to each other (Buchholtz & Stepien 2009), given that a cervical-thoracic "boundary" remains evident in *Bradypus* (which can have up to ten ribless neck vertebrae) between the late-ossifying centrum of the seventh neck vertebra relative to the early-ossifying centrum of the eighth (Hautier et al. 2010).

If the stabilizing selection hypothesis were the primary driver behind the conservatism of the mammalian cervical spine, we would expect to find more skeletal variation among neonates than adults. This has been documented among cases of mortality among human fetuses represented by radiographs from hospital pathology departments (Galis et al. 2006), but this high frequency is not evident from samples of the general human population. Ultrasound data from human prenatal health checks show a much lower frequency of rib anomalies (6.3%; Hershkovitz 2008) than the hospital stillborns sampled by Galis et al. (2006). Similarly, non-human, mammalian embryos and fetuses do not show a higher rate of vertebral anomalies than adult specimens (Asher et al. 2011: fig. 3). In their sample of 25 museum-collected individuals of *Dasyurus novemcinctus*, including 14 pre- or near-term fetuses and 11 postnatal individuals, Asher et al. (2011) found presacral vertebral anomalies (such as asymmetric ribs) in four individuals or 16% of their sample. Three of those were from postnatal, well-ossified skeletal specimens (3/11 or 27%) and one was a pre-term fetus (1/14 or 7%).

Our current sample of CT scans from 46 newborn *D. novemcinctus* specimens, comprised of multiple sets of genetically-identical littermates, shows anomalies (Figs. 9, 10) in just two individuals or 4% of our sample. This slightly lower rate is unsurprising given our much more genetically, geographically, and temporally homogeneous sample, relative to the sample discussed in Asher et al. (2011). We do not know if the individuals in our sample with vertebral anomalies would have been any different in terms of their capacity to contribute to future generations than those without anomalies, but we can say that the frequency of vertebral anomalies recovered in this study reflect those of previous studies of vertebral variation in humans sampled from the general population (Hershkovitz 2008) and in mammals generally (Asher et al. 2011), not the high rate of anomalies predicted if stabilizing selection were the primary factor behind mammalian vertebral conservatism.

Influence of temperatures on skeletal anatomy. As noted previously, *Dasyurus* is known to have a relatively high level of variation in vertebral counts (Asher et al. 2011: fig. 1). Variation in thoracolumbar count is lower among the offspring of our study animals (CV of 2.7; Supplementary Table S2) compared to *D. novemcinctus* observed by Asher et al. (2011). However, as noted above, this is not surprising given that our study animals are sets of identical quadruplets from a small number of closely situated localities in northwestern Arkansas. Unlike the sample in Asher et al. (2011), these were not museum specimens collected over the course of many decades from localities throughout the Americas. Nonetheless, even for our geographically and temporally restricted sample, skeletal variation is still evident (Figs. 9, 10).

Temperature has been shown to influence vertebral count in many animals, including teleost fish (Tibblin et al. 2016), amphibians (Peabody & Brodie 1975), and birds (Lindsey & Moodie 1967), many of which exhibit substantial levels of intraspecific variability (op cit.). Mammals exhibit relatively less variation in their vertebral column, but gestational temperatures may influence their vertebral counts as well (Lecyk 1965), and xenarthran mammals have some of the most variable vertebral counts among mammals (Jenkins 1970; Asher et al. 2011; Buchholtz 2014). In some teleost and amphibian populations, vertebral count decreases with lower embryonic temperatures as a result of development at colder, higher latitudes (or "Jordan's Rule"; see McDowall 2008; Tibblin et al. 2016). In contrast, data from birds (Lindsey & Moodie 1967) and mammals (Lecyk 1965) show an association between increased vertebral count and higher developmental temperatures.

There are a number of potential explanations for the varying effects of temperature in these groups. Schröter et al. (2008) outlined one mechanism that helps to explain axial morphology in zebrafish. Somites in this group are longest in the mid-trunk and shortest in the tail. Most somites form under a constant somitogenesis period; this increases slightly in the tail where somites are shorter. Schröter et al. (2008) find a "strong temperature dependence on somitogenesis period", in which higher temperatures result in faster somitogenesis; at 20C they reported a mean somitogenesis period of 55.4 minutes, compared to 18.7 minutes at 30C (Schröter et al. 2008: table 1). Given the clock-and-wavefront model of Cooke & Zeeman (1976), they note that "an increase in somitogenesis period should result in longer rather than shorter somites", yet their model organism (zebrafish) exhibits short tail somites. They explain this paradoxical observation by postulating that "the wavefront velocity ... slows strikingly in the posterior half of the embryo". Hence, at least in zebrafish, higher gestational temperatures lead to more somites forming in less time (Schröter et al. 2008: fig. 3), but are compensated by changes to wavefront velocity.

Birds (Lindsey & Moodie 1967) and mammals (Lecyk 1965) show an increase in vertebral number at increased developmental temperatures, in contrast to fewer vertebrae seen after relatively colder developmental temperatures in teleost fish (Lindsey 1988; Tibblin et al. 2016). Following the model of Schröter et al. (2008), this may imply a relatively more stable wavefront velocity along the embryonic axis in mammals and birds which, compared to zebrafish, does less to alter the effect of increased somitogenesis period at high developmental temperatures. If so, we would expect *Dasypus novemcinctus* embryos exposed to relatively low developmental temperatures to show fewer axial segments. Temperatures of two of our three dams

(quiche, wat2) that gave birth to litters with fewer precaudal vertebrae (24 rather than 25 cervical+thoracic+lumbar+sacral, with 9 rather than 10 thoracics; see Fig. 9 and Supplementary Table S2) exhibited low body temperatures within the first month of gestation and are therefore consistent with this hypothesis, but the third dam (we2) that had offspring with 24 precaudal vertebrae (including 9 thoracics) is not, exhibiting daily averages around 35.5C (Fig. 11). Moreover, dam we1 exhibited the lowest daily means for December of any animal, yet the one offspring recovered from we1 exhibited 25 precaudal vertebrae (including 10 thoracics; see Fig. 11).

Precise dates for uterine implantation, and the timing of somitogenesis and axial skeletal differentiation, have never been documented in any species of *Dasyopus*, or indeed in most other species beyond a small number of model organisms. It is therefore possible that our earliest recorded temperatures for armadillo dams during early December all postdate the key developmental phases during which axial skeletal differentiation took place. This possibility is underscored by the relatively advanced state of an embryo collected on 3 December 2012 (Fig. 1), which shows clearly differentiated somites. Further intrauterine temperature fluctuations in this individual (within the limits needed for continued development) after 2 December would presumably have had little influence on precaudal axial segmentation, which had already at least partly differentiated into pre-sacral regions. If the key phases of development that determine axial regionalization are within one or two weeks of an early- or mid-November implantation date, then our temperature data during December are not informative regarding axial regionalization. It is also possible that low (i.e., 35C or below) intrauterine temperatures also occurred in dam we2 during the key phases of axial skeleton differentiation, before our temperature recordings began for that animal on 2 December. Similarly, we might expect some variation in developmental timing such that the <35C temperatures in quiche and wat2 correspond with axial differentiation in their litters, resulting in fewer precaudal vertebrae. At present, our data are not conclusive in determining if any of these scenarios are correct.

More decisively, our data show that despite their genetic identity, quadruplets of *Dasyopus novemcinctus* are not always phenotypically identical. Armadillo dams furthermore show a surprising degree of thermal tolerance in a species of placental mammal which, consistent with our observations, has never been reported to exhibit torpor. Not only does the nine-banded armadillo survive with typical daily temperature fluctuations of 2-3 degrees, and extremes well over 6C and a record of 9C in our study, it can also successfully bring to term litters of identical quadruplets despite temperature extremes within 24 hours that would kill or seriously compromise the offspring of other species, such as humans (Edwards 2006).



Data accessibility

available at <https://osf.io/693kq/> (see "Appendices", below)

Supplementary material

available at <https://osf.io/693kq/> (see "Appendices", below)

Acknowledgements

Amanda Withnell was our invaluable head animal technician and provided observations and insights on armadillo behavior. We are grateful to Ashlee Saunders (2017-18), Sheridan Barajas (2014-15), Ashley Clayton, and James McClellan (2015-16) who performed procedures, collected data and provided animal care, and to many other students at the University of the Ozarks who also helped with field- and lab-work. We also thank Stephen Gaunt and Caroline Willich for discussion and general advice. Funding was provided by grants from the University of the Ozarks Academic Enrichment Fund for students and the McElree professional development fund for faculty, the Arkansas Space Grant Consortium (ASGC), and Trinity Hall and the Department of Zoology, University of Cambridge. Sabbatical leave in 2015-16 provided by the University of Cambridge enabled Asher to participate in fieldwork relevant to this study. The Arkansas Game and Fish Commission awarded F.K. a Scientific Collection Permit and gave permission for armadillos to be collected on ASGC management areas. We thank Darrin Croft, Alexandra Panyutina, and Mar Sobral for their constructive critiques of our manuscript, and the Center for Open Science (<https://osf.io>) for hosting early versions thereof. This manuscript has been peer-reviewed and recommended by *Peer Community In Ecology* (<https://doi.org/10.24072/pci.ecology.100050>).

Conflict of interest disclosure

The authors declare that they have no financial conflict of interest with the content of this article. None of the authors is a recommender for *PCI-Ecology* (but Asher is a recommender for *PCI-Paleontology*).

Tables

Table 1. Summary of *Dasypus novemcinctus* specimens, all female. "CT" indicates if CT scans were taken of that individual's litter following birth. Body temperatures beyond the "dates in outdoor burrow" range were taken in an indoor lab. Parturition dates are known for some lab-housed individuals ("X") and estimated within 10 days for others ("E"). Cells for gestational state in our supplementary csv file (Appendix S1) are blank for five days before and after estimated dates of parturition.

filename w/ original data	animal name	parturition E=estimate, X=exact	CT	dates in outdoor burrow	body temp start	body temp end
Levee 2 Bertha.xlsx	bertha	27 Mar 18 (X)	yes	all lab	26-Nov-17 07:01	30-Dec-17 08:01
Harmony 1 Butternut BodyTE3.xlsx	butternut	12 Apr 15 (E)	yes	12 Dec 15 - 25 May 16	08-Dec-15 08:01	25-May-16 23:01
Levee 1 body T Carrots E 2.xlsx	carrots	29 Mar 16 (E)	yes	18 Dec 15 - 25 May 16	08-Dec-15 08:01	25-May-16 23:01
S3 Body dillo 4.xlsx	dillo4	nongravid	-	all lab	14-Dec-14 06:22	15-Mar-15 23:22
A-S1question body dillo 5.csv	dillo5	nongravid	-	all lab	15-Dec-14 05:10	30-Mar-15 19:55
Levee 1.xlsx	le1	nongravid	-	all lab	26-Nov-17 07:00	30-Dec-17 08:00
-	le3	17 Apr 16 (E)	yes		ibutton failed	
Levee 4 Body TAQ 4.xlsx	le4	24 Mar 16 (X)	yes	all lab	15-Dec-15 05:56	01-Jun-16 20:56
A-E2 body dillo 3 Noodles.csv	noodles	nongravid	-	25 Dec 14 - 29 Mar 15	14-Dec-14 06:24	29-Mar-15 21:09

<i>filename w/ original data</i>	<i>animal name</i>	<i>parturition E= estimate, X = exact</i>	<i>CT</i>	<i>dates in outdoor burrow</i>	<i>body temp start</i>	<i>body temp end</i>
A-E3 body dillo 6 Olive.csv	olive	12 Apr 15 (E)	yes	25 Dec 14 - 30 Mar 15	15-Dec-14 04:40	30-Mar-15 19:55
A-E1 body dillo 1 Puddin.csv	puddin	2 Apr 15 (E)	yes	21 Dec 14 - 1 Jan 15	12-Dec-14 06:00	01-Jan-15 13:45
A-S2 body dillo 2 Quiche.csv	quiche	16 Apr 15 (X)	yes	all lab	12-Dec-14 05:58	01-Jan-15 12:43
Wat 2 Body T AQ 2.xlsx	wat2	28 Mar 16 (X)	yes	all lab	18-Dec-15 03:56	04-Jun-16 18:56
Wat 3 Body Temp AQ 1.xlsx	wat3	19 Mar 16 (X)	no	all lab	18-Dec-15 03:58	20-Apr-16 00:58
Weathers 1.xlsx	we1	?	yes	10 Dec 17 - 26 May 18	02-Dec-17 05:59	26-May-18 23:59
Weathers 2.xlsx	we2	6 Apr 18 (E)	yes	6 Dec 17 - 19 Jul 18	02-Dec-17 06:01	19-Jul-18 08:01
Weather 3.xlsx	we3	nongravid	-	7 Dec 17 - 26 Jun 18	03-Dec-17 06:59	26-Jun-18 06:59
Weathers 5.xlsx	we5	died 5 Feb 2018	yes	20 Dec 17 - 25 Jan 18	05-Dec-17 06:03	4-Feb-18 03:03
Weathers 6.xlsx	we6	nongravid	-	all lab	09-Dec-17 05:55	12-Apr-18 13:55
Wat 1 Yams Body T E 1.xlsx	yams	22 Mar 16 (E)	yes	22 Dec 15 - 30 May 16	13-Dec-15 03:02	30-May-16 18:02

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Appendix

Supplementary data available at <https://osf.io/693kq/>

Appendix S1 ("appendS1.csv"): csv file with original timeseries and temperatures for each individual.

Appendix S2 ("appendS2.txt"): R script to calculate DFTs from temperature data. Raw data are available in supplementary appendix S1 and are represented as data frame "temps".

Appendix S3 ("appendS2.pdf"): graphical summary of recorded temperatures for each individual, daily range of temperature fluctuations, and DFT periodograms.

Table S1 ("knight-asher_supplementaryTables.doc"): Summary of temperature data for 19 *D. novemcinctus* individuals (original data in csv format in Appendix S1)

Table S2 ("knight-asher_supplementaryTables.doc"): Vertebral counts of *D. novemcinctus* offspring.



Figures on following pages

F1

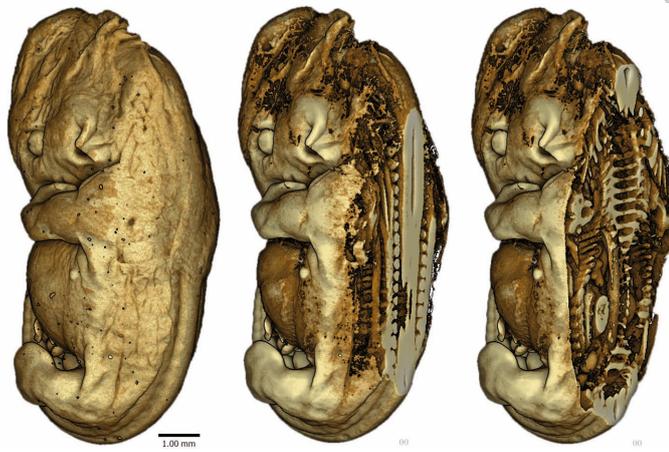


Figure 1. Embryo of *D. novemcinctus* collected 3 Dec 2010.
Scale bar = 1mm

F2

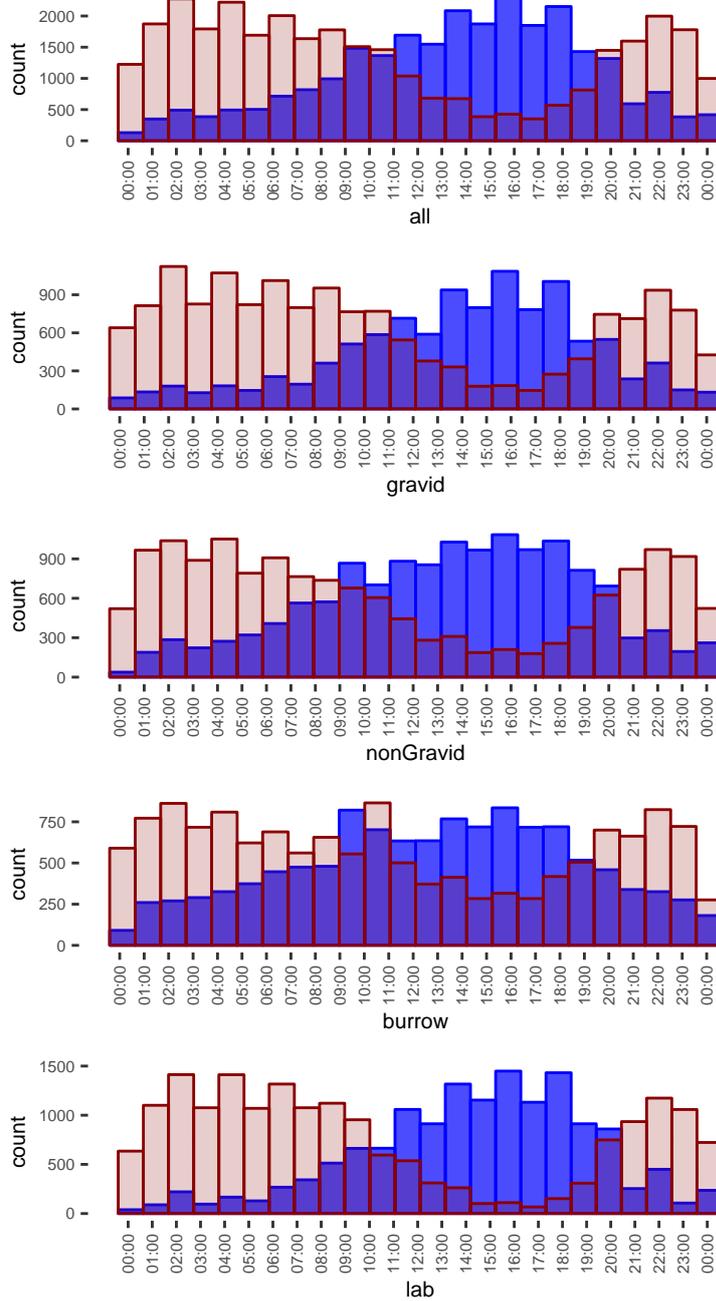


Figure 2. Histogram showing number of occurrences on 24-hour clock of body temperatures below (blue) and above (red) mean of 35.4C across 19 *Dasyus novemcinctus* individuals with temperatures recorded in an outdoor burrow, an indoor lab, and during ("gravid") and outside of ("nongravid") pregnancy. Any individual may have been in multiple categories during temperature recordings.

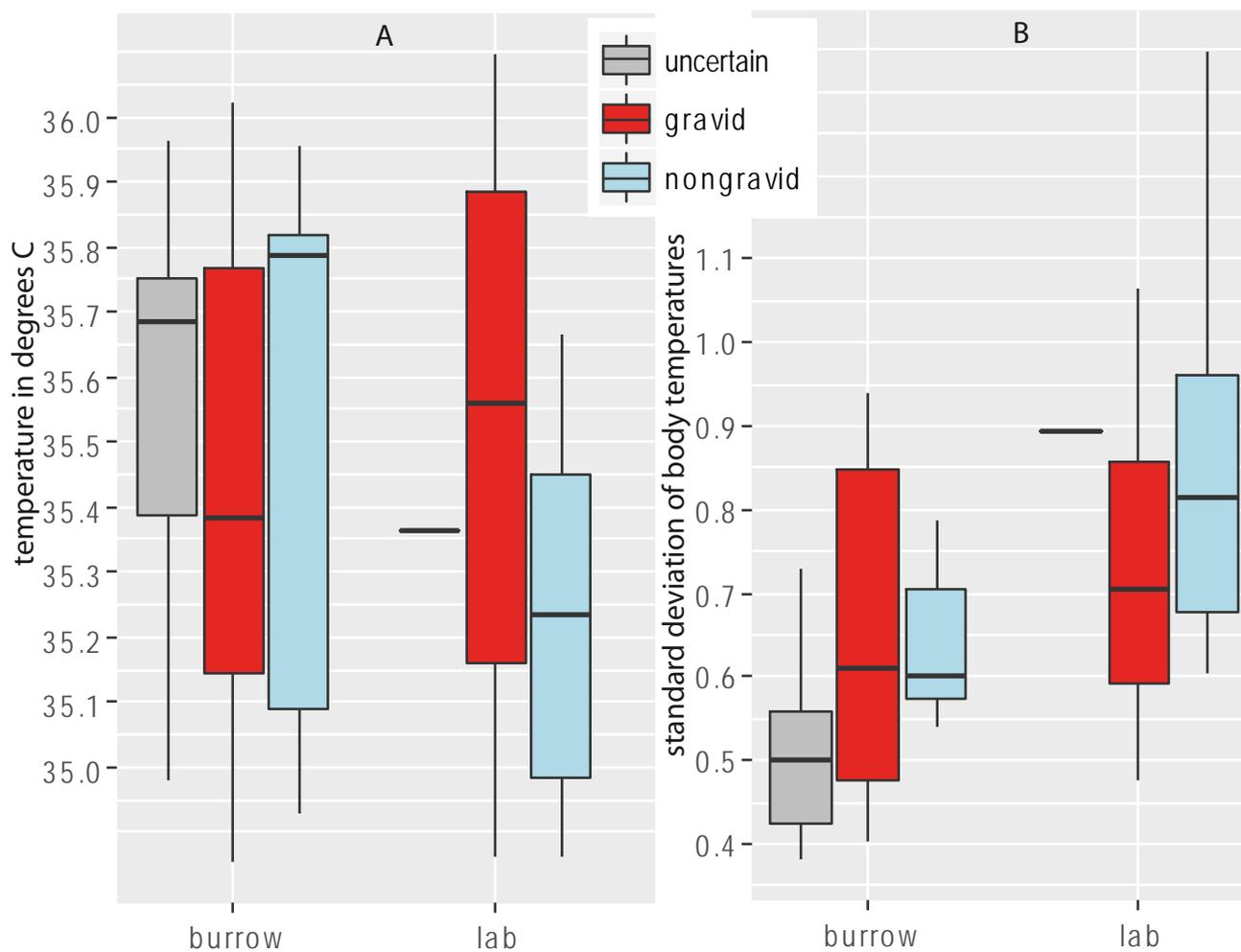


Figure 3. Average body temperatures (A) and standard deviations thereof (B) among gravid, nongravid, indoor (lab) and outdoor (burrow) armadillos. Thick horizontal lines represent medians, vertical rectangles middle quartiles, whiskers range.

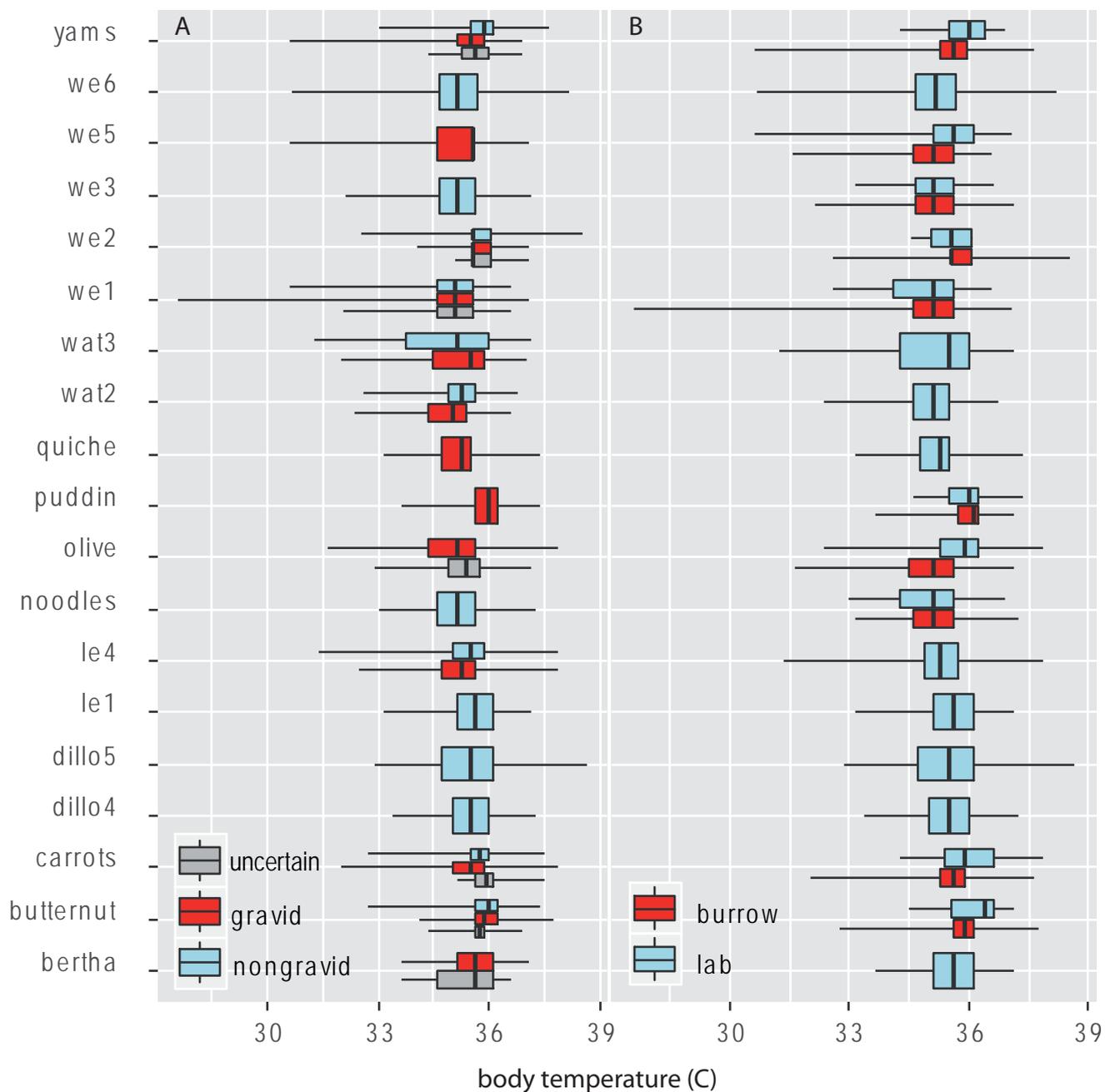


Figure 4. Overview of mean (thick vertical lines), middle quartiles (rectangles), and range (horizontal lines) of body temperatures across 19 armadillos (names at left) recorded over three seasons between 2014-2018 from one to six months (see Table 1).

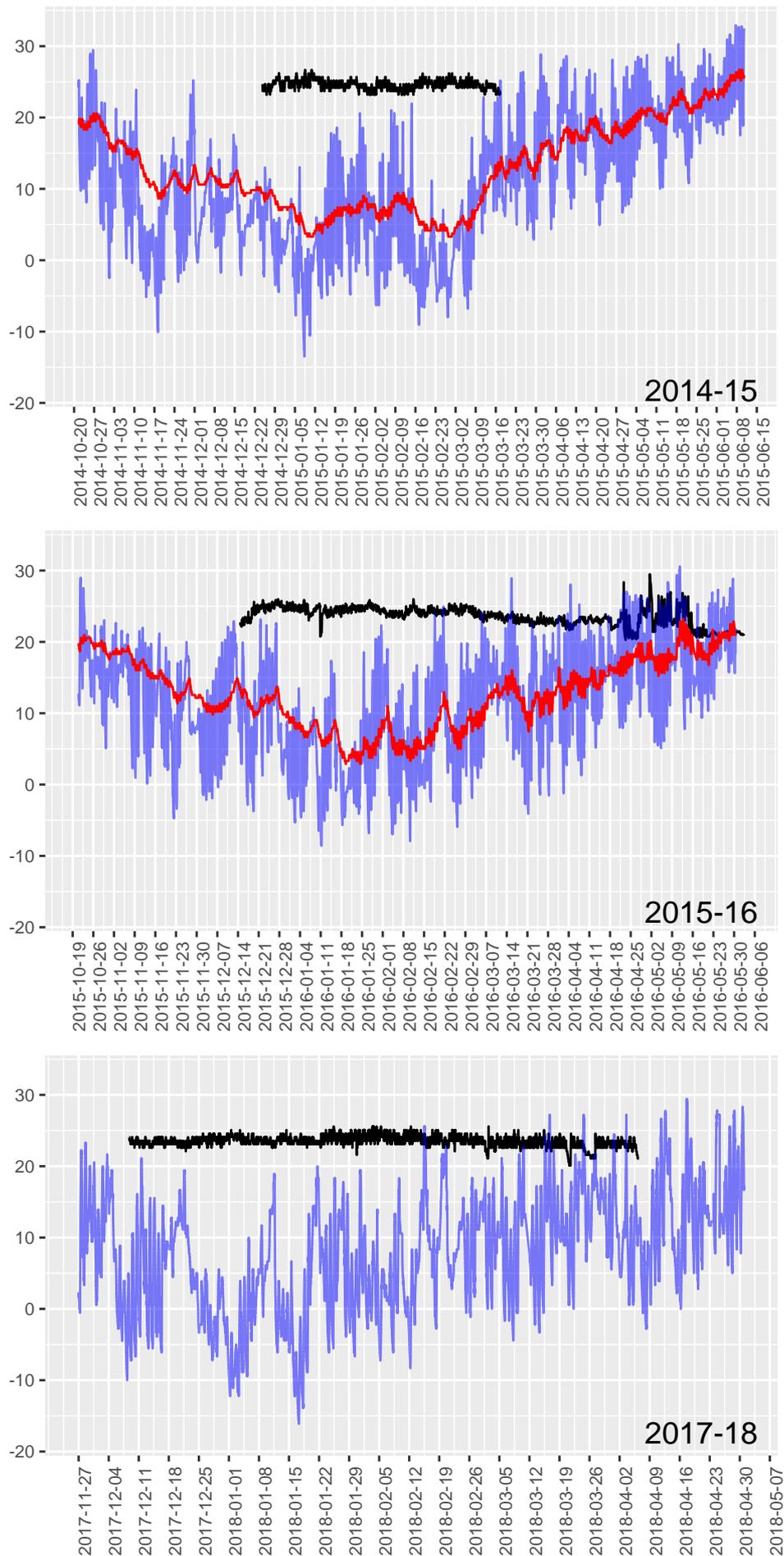


Figure 5. Ambient temperatures (black = indoor lab, red = outdoor soil at 15cm depth, blue = outdoor air) for each season (soil only available from 2014-2016).

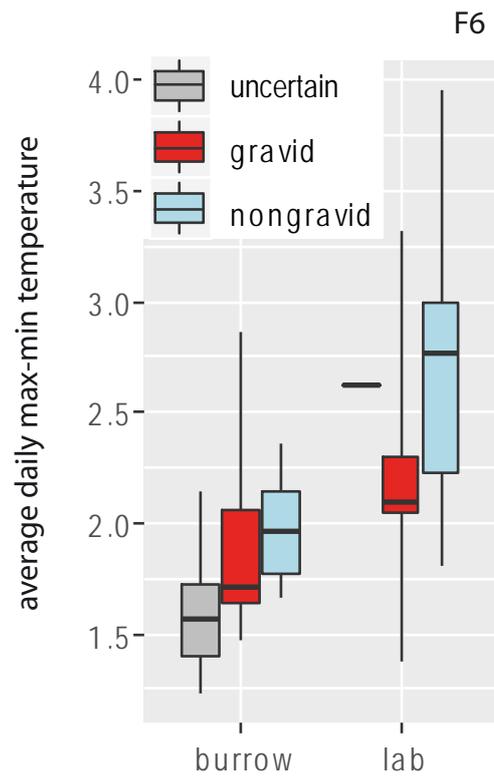


Figure 6. Average daily temperature fluctuations in C across individuals grouped by treatment. Thick horizontal lines represent medians, vertical rectangles middle quartiles, whiskers range.

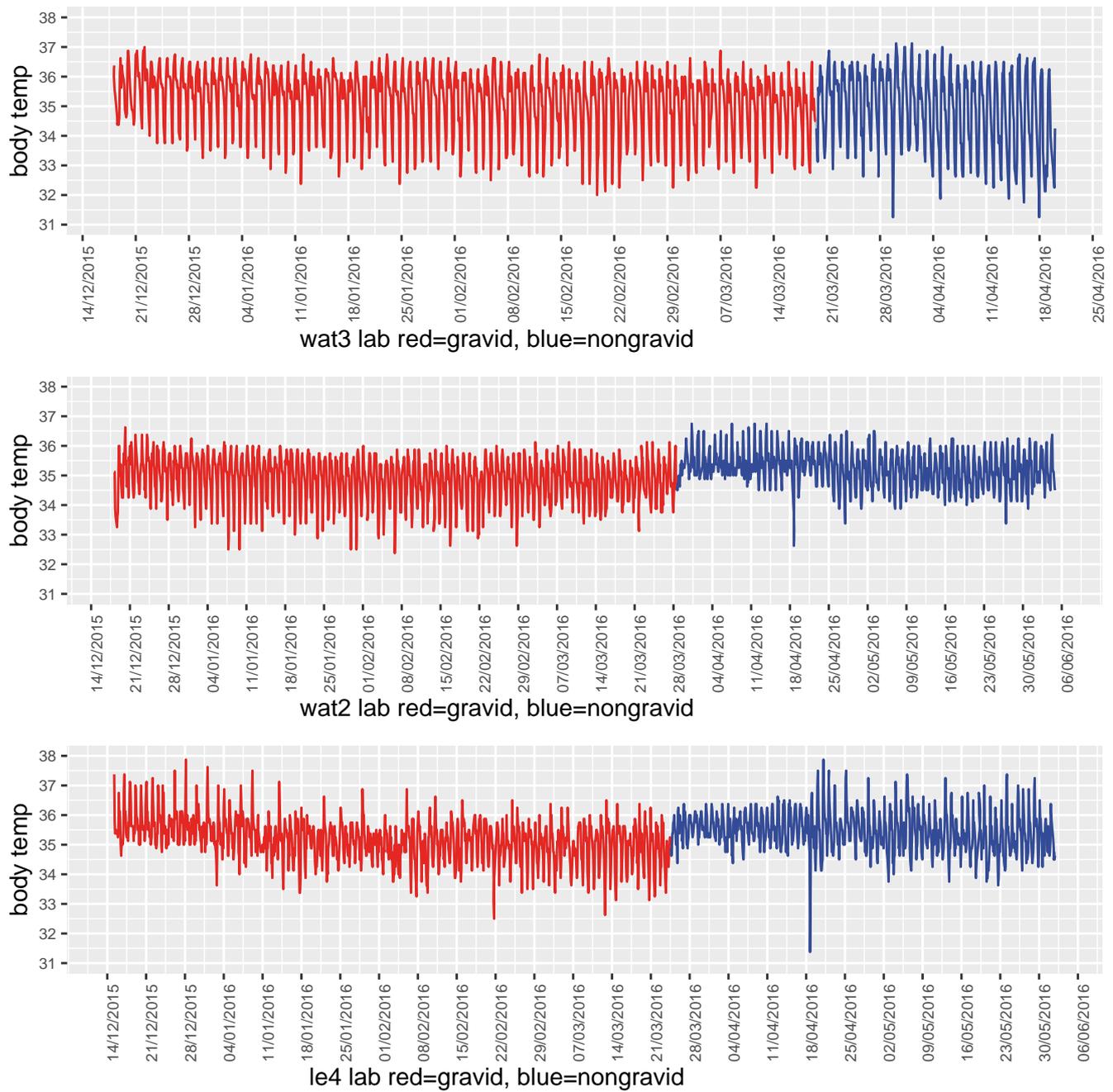


Figure 7. Daily body temperatures recorded for the three dams (wat3, wat2, le4) with known dates of birth and continuous recordings while gravid (red), from December through the weeks following birth (nongravid = blue).

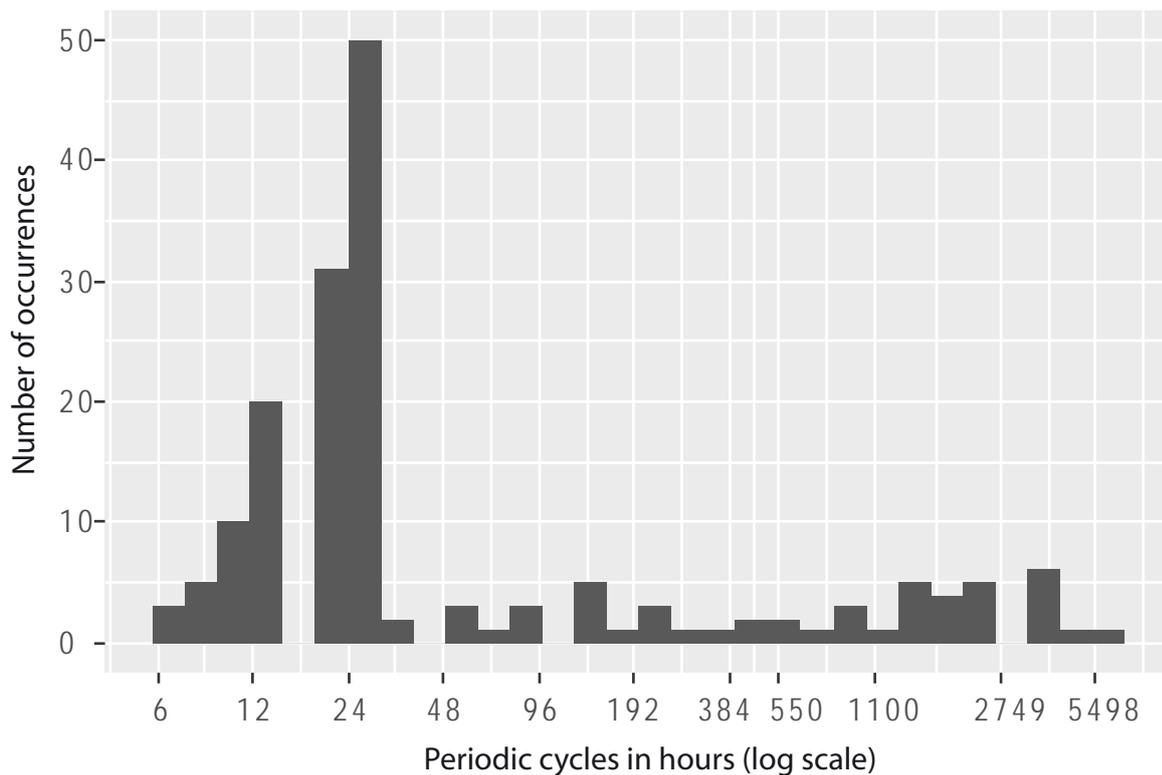


Figure 8. Histogram showing dominant periodic cycles recognized by DFT for all 19 individuals with temperature recordings. Y axis corresponds to number of occurrences across all individuals of a given periodic cycle of at least 5% of each individual's maximum observed DFT in hours (X-axis). Periods indicative of the same number of hours (e.g., 24) may occur multiple times per individual.

F9

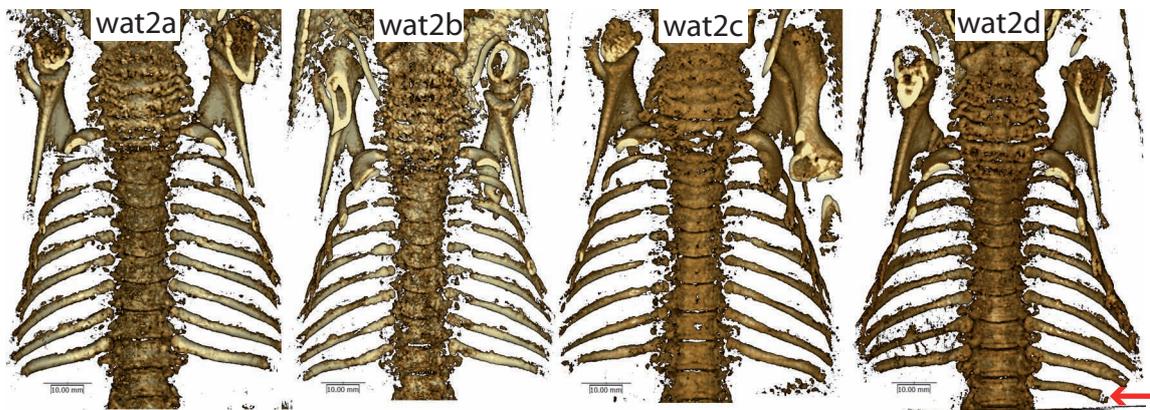


Figure 9. Thoracic region of the four offspring of dam Wat2 showing nine rib-bearing vertebrae in wat2a-c and an asymmetric 10th rib in wat2d (red arrow). Scale bars = 10mm

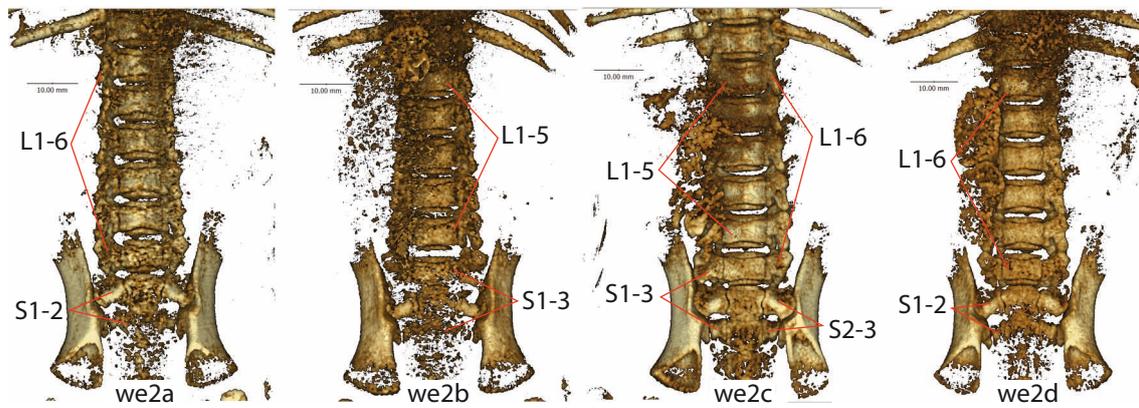


Figure 10. Partial thoracic, lumbar, and sacral regions of the four offspring of dam We2 showing variable lumbar ("L") and sacral ("S") vertebral counts. Note asymmetry of lumbosacral transition in we2c. Scale bars = 10mm

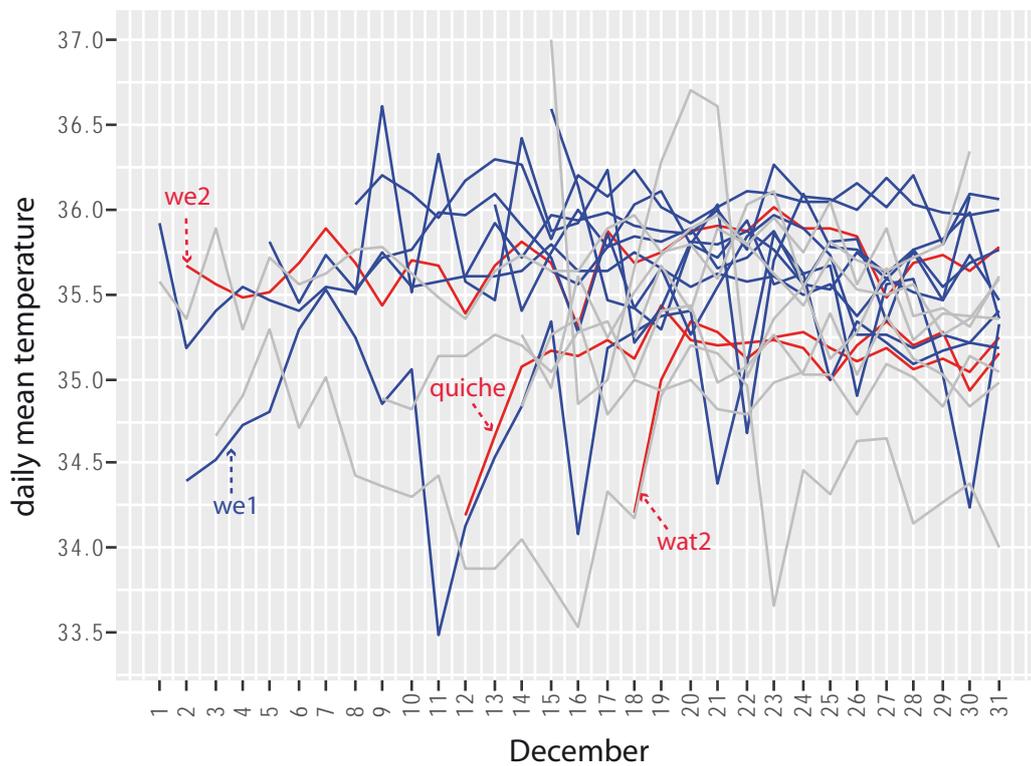


Figure 11. Mean daily temperatures for each dam during December. X-axis shows day of the month; blue lines indicate dams with offspring showing 10 thoracics, red lines dams with offspring showing 9 thoracics; gray lines are from non-gravid individuals. Names & dotted arrows adjacent to lines indicate dams mentioned in the text.