Original Article

Revisiting the Importance of Accounting for Seasonal and Diel Rhythms in Fecal Stress Hormone Studies

DAVID S. JACHOWSKI,[1,2] *Department of Forestry and Environmental Conservation, Clemson University, 258 Lehotsky Hall, Clemson, SC 29634, USA*

BRIAN E. WASHBURN, *United States Department of Agriculture, Wildlife Services, National Wildlife Research Center, 6100 Columbus Avenue, Sandusky, OH 44870, USA*

JOSHUA J. MILLSPAUGH, *Department of Fisheries and Wildlife Sciences, University of Missouri, 302 Natural Resources Building, Columbia, MO 65211, USA*

ABSTRACT Measurement of fecal glucocorticoid metabolites (FGMs) has been used to quantify stress responses by a wide variety of species; yet, few studies attempt to conduct baseline research prior to investigating effects of disturbances on FGM levels. We evaluated the potential confounding effect of seasonal and diel rhythms on the interpretation of FGM values, by studying long-term patterns of FGMs in white-tailed deer (*Odocoileus virginianus*) and mourning doves (*Zenaida macroura*) between 2001 and 2002 in a controlled environment at facilities located at the Charles W. Green Conservation Area, near Ashland, Missouri, USA. For 1 year, we collected fresh fecal samples (<1 hr old) on a weekly basis from 17 captive deer and 8 captive doves. We also conducted hourly sampling over discrete 48-hour periods to evaluate diel rhythms. In deer, we observed that FGMs were nearly 2 times higher during spring months of April and May compared with late winter (i.e., Jan and Feb) and late summer (i.e., Jul and Aug). Dove FGMs were 50% higher during the late spring (i.e., May and Jun) compared with early spring and winter months (Jan–Apr), and twice as high as during late summer, autumn, and early winter months of August through December. In both species, we observed considerable variation in diel rhythms that was inconsistent across individuals. Collectively, our findings emphasize the need for researchers to design sampling schemes that account for high levels of individual and temporal variability in baseline FGMs—and consistently ask: What are “normal” FGM values in the context of the animal’s environment and prevailing stressors. © 2015 The Wildlife Society.

KEY WORDS fecal glucocorticoid metabolites, mourning dove, *Odocoileus virginianus*, physiology, stress hormones, white-tailed deer, *Zenaida macroura*.

Noninvasive fecal stress hormone sampling is an increasingly common method used to monitor the physiological state of wild animals and inform conservation decision-making (von der Ohe and Servheen 2002, Millspaugh and Washburn 2004). Measurement of fecal glucocorticoid metabolites (FGMs) has been used to quantify stress responses and fitness of sampled individuals and populations of a wide variety of species (Busch and Hayward 2009). Further, sampling of FGMs is an increasingly common tool used in long-term monitoring and conservation programs for wildlife. For example, long-term monitoring of FGMs in combination with remote tracking of elephants (*Loxodonta africana*) is being used to mitigate human–elephant conflicts (Jachowski et al. 2012).

In addition to potentially indicating exposure to acute stressors, glucocorticoid metabolite levels often vary predictably over time as a result of life-history stages (Touma and Palme 2005). Corticosterone plays an important role in facilitating the availability of energy for metabolic functions (Romero 2002); thus, levels should be highest in certain times of year such as the breeding season (Boonstra et al. 2001, Mooring et al. 2006). Similarly, exposure to stressors associated with seasonal environmental changes can cause predictable changes in corticosterone levels (Millspaugh et al. 2001, Romero 2002, Owen et al. 2005, Khonmee et al. 2014). Consequently, in an effort to maintain allostasis, energy requirements and corticosterone levels can vary based on predictable diel, seasonal or annual rhythms within life-history strategies (McEwen and Wingfield 2003).

Despite the increasing wide-scale use of FGM measurements and evidence of inherent temporal variation in

Received: 8 September 2014; Accepted: 29 April 2015
Published: 10 September 2015

[1]*E-mail: djachowski@gmail.com*
[2]*Joint appointment: School of Life Sciences, University of KwaZulu-Natal, Westville Campus, Private Bag X54001, Durban, South Africa.*

FGMs, few studies attempt to conduct baseline research prior to investigating experimental or observational effects of disturbances on FGM levels. Baseline FGM concentrations provide a critical benchmark of what constitutes "normal" FGM concentration or "unstressed" conditions (Sheriff et al. 2011). However, because monitoring typically occurs only after disturbance of a wildlife population, establishing a pretreatment baseline is often difficult or even impossible in some instances (e.g., Creel et al. 2002). In addition, FGM baselines often differ among populations, making comparisons difficult (Jachowski et al. 2012). Few studies have involved consistent sampling over a long enough duration to account for potential diel or seasonal rhythms that influence hormone concentrations (Millspaugh and Washburn 2004). This is of particular concern for studies in the field of conservation physiology because failure to account for diel or seasonal rhythms could bias comparative study results and impair conservation decision making (Millspaugh and Washburn 2004, Touma and Palme 2005).

To evaluate the potential confounding effect of seasonal and diel rhythms on interpretation of FGM values, we studied the long-term patterns of FGMs in 2 relatively well-studied avian and mammalian wildlife species—mourning doves (*Zenaida macroura*) and white-tailed deer (*Odocoileus virginianus*)—in a captive environment. Specifically, we hypothesized that these habituated animals would exhibit distinct diel (Raminelli et al. 2001, Carere et al. 2003) and seasonal patterns (Boonstra et al. 2001, Harper and Austad 2001, Pravosudov et al. 2002) in FGM secretion. We also hypothesized that differences in FGMs would be evident based on the sex of an individual (Schatz and Palme 2001, Touma et al. 2003, Chelini et al. 2010). By relating the diel and seasonal rhythms we observed to previously reported stress responses, we evaluated the potential for bias in the interpretation of results in studies that fail to account for these "natural" rhythms.

METHODS

Study Area and Species Biology

White-tailed deer and mourning doves were housed in an outdoor captive facilities located at the Charles W. Green Conservation Area, near Ashland, Missouri, USA (38°54′N, 92°18′W). The study sites lie within the Ozark Plateau Physiographic Province in central Missouri, an area primarily composed of upland deciduous forests dominated by oak (*Quercus* spp.) and hickory (*Carya* spp.). Mean annual temperature was 12.8° C. Study animals were exposed to distinct spring, summer, autumn, and winter seasons, with average temperatures in January (−1.0° C) and July (25.6° C) being the coldest and warmest months, respectively. Mean annual precipitation was 940 mm.

We used a group of captive, hand-raised adult deer that were maintained by the University of Missouri and accustomed to human activity, but otherwise underwent reproductive cycles and were exposed to external environmental conditions typical for resident deer in the region (Beringer et al. 2004). Annual cycles for male deer physiology and life history in this region are typically identified as a period of peak physical condition and antler growth that occurs through the late spring to autumn (Apr–Sep), with declines in condition during peak reproductive behavior (primarily during the months of Oct–Dec) and overwinter period when foraging resources are diminished (Jan–Mar; Nixon et al. 1991). Female deer gestation lasts 190–210 days, with fawns being born in late May to early June.

Mourning doves in our study area consist of both migratory and nonmigratory individuals that typically undergo a distinct summer breeding period. Breeding typically begins in April. From the time an egg is laid to fledging of young typically takes approximately 30 days, allowing multiple nesting attempts through the summer months and into September (Westmoreland et al. 1986). Migratory individuals typically leave the area by mid-October each year, with some individuals residing in the study area throughout the year.

We captured wild mourning doves near the captive facilities using modified Kniffin traps (Reeves et al. 1968) during December 2000 to June 2001. Upon capture, we banded each bird with an individually numbered metal leg band, and assigned age and gender based on plumage characteristics (Mirarchi 1993, Schulz et al. 1995). We transported mourning doves to the T. S. Baskett Wildlife Research and Education Area, located 15 km south of Columbia, Missouri (38°45′N, 92°11′W). During captivity, doves were kept individually in outdoor 1.8-m × 1.8-m × 1.8-m wooden-framed wire-mesh pens raised 0.6 m above ground (Mong et al. 2002, Jachowski et al. 2014). A wild-bird seed mixture (sorghum grains, wheat, crimped corn, oats, and sunflower seed) and water were provided *ad libitum*. We obtained approval for capturing and transporting animals in this study through U.S. Fish and Wildlife Service scientific collection permit MB040276-0 and Missouri Department of Conservation wildlife collector's permit no. 11790, 11013, 11403. Husbandry of both species was maintained in accordance with University of Missouri animal care and use protocols (Protocol Reference no. 3581 and 3624).

Fecal Sample Collection

Deer—diel rhythms.—We randomly selected 2 uniquely identifiable female deer that were similar in terms of social status, age, body condition, and personality (i.e., response to humans and a penned environment) as subjects for 8 diel rhythm trials. We conducted trials on both habituated deer during 4 seasons: autumn (4–5 Nov 2001 and 11–13 Nov 2001), winter (25–27 Feb 2002 and 3–5 Mar 2002), spring (28–30 Apr 2002 and 5–7 May 2002), and summer (14–16 Jul 2002 and 21–23 Jul 2002). Both adults entered into late-stage gestation during the spring trial. During each individual trial, a single deer was housed in an outdoor wooden holding pen (8 × 22 m) for 72 hr. We provided deer with water, shelled corn, alfalfa hay, and commercial deer feed (Purina Mills, Inc., St. Louis, MO) *ad libitum* throughout each trial (Millspaugh et al. 2002, Washburn and Millspaugh 2002). We collected fecal samples every

hour if feces were available. Samples were homogenized, placed in a cooler with ice packs, and transported to our laboratory at the University of Missouri-Columbia within 3 hr of collection. Samples were frozen at -20° C until processed.

Deer—seasonal patterns.—We collected fresh fecal samples on a weekly basis for 1 year (15 Oct 2001 to 9 Oct 2002) from 17 captive white-tailed deer (5 ad M, 6 ad F, and 6 juv of unknown sex) housed in 3 large enclosures (approx. 4 ha in size). Deer herds within each enclosure were composed of similar density, social structure (composed of both mature males and females), and had a similar sex ratio (approx. 3 F for every M). Within each enclosure deer had access to native and planted forages and open water and were fed supplemental grain. About 40% of the 4-ha plot contained mature oak and hickory trees; the rest was old field (20%) and unimproved pasture (40%). Juvenile deer were easily identifiable apart from adults based on body size, but not individual marked. Adult deer were individually identifiable through ear tags or markings, thus, allowing us to link and record fecal samples with individual animals. We attempted to collect fresh fecal samples (<30 min since defecation) from both juveniles (who we were unable to individually identify) and known adult individuals within each enclosure $\geq$2 times/week by visually monitoring deer from a distance with aid of binoculars during daytime hours (late morning to afternoon). During sample collection efforts, to avoid overrepresenting one individual deer, we attempted to balance sampling across all individuals. Samples were homogenized, placed in a cooler with ice packs, and transported to our laboratory at the University of Missouri-Columbia within 3 hr of collection. Samples were frozen at -20° C until processed.

Doves—diel rhythms.—We conducted 2 diel rhythm trials with 1 male and 1 female after-hatching-year mourning dove. We have previously reported diel rhythms in FGMs for doves housed in identical conditions during autumn (Oct), winter (Mar), and summer (Jul) months (Washburn et al. 2003, Jachowski et al. 2014). Thus, for this study, we focused on monitoring diel rhythms during the spring. For a 48-hour period (9–11 May 2002), we collected dove droppings (consisting of feces and urine) every hour, when available, using a stainless steel spatula. Samples were frozen at -20° C within 10 min of collection.

Doves—seasonal patterns.—We collected fresh dove fecal samples (<1 hr old) on a weekly basis from individually housed captive after-hatching-year mourning doves (4 M and 4 F) for 1 year (17 Jul 2001 to 9 Jul 2002). Fecal samples were frozen at -20° C within 10 min of collection.

Fecal Sample Preparation

Each frozen dove dropping and an approximately 10-g subsample of each frozen deer fecal sample were individually placed in a lyophilizer (Freeze-dry Specialties, Inc., Osseo, MN) for 24 hr. Once freeze-dried, samples were ground, sifted through a stainless steel mesh to remove large particles, and thoroughly mixed. Glucocorticoids were extracted using a modification of the method described by Schwarzenberger et al. (1991). We placed dried feces in a test tube with 2.0 mL of 90% methanol and vortexed the sample for 30 min. We centrifuged samples at 500*g* for 20 min, saved the supernatant, and stored at -84° C until assayed.

Fecal Glucocorticoid Metabolite Assays

We used I^{125} corticosterone radioimmunoassay kits (ICN 07-120103; ICN Biomedicals, Costa Mesa, CA) previously validated for use with white-tailed deer (Millspaugh et al. 2002) and mourning doves (Washburn et al. 2003) to quantify fecal glucocorticoid metabolite concentrations. We followed the ICN protocol for the I^{125} corticosterone radioimmunoassay, except that we halved the volume of all reagents. For the white-tailed deer diel-rhythm study, we analyzed fecal samples ($n = 179$) in 3 assays, with each assay including all the samples from an individual deer. Inter-assay variation was 4.4% and average intra-assay variation was 1.3%. For the deer seasonal rhythm study, we analyzed fecal samples ($n = 444$) in 7 assays, with each assay including all the samples from an individual deer. Inter-assay variation was 5.5% and average intra-assay variation was 1.2%. For the mourning dove diel-rhythm study, we analyzed fecal samples ($n = 88$) in 2 assays, with each assay including all the samples from an individual dove. Inter-assay variation was 6.1% and average intra-assay variation was 1.2%. For the dove seasonal study, we analyzed fecal samples ($n = 385$) in 5 assays, with each assay including all the samples from an individual dove. Inter-assay variation was 4.0% and average intra-assay variation was 1.1%.

Statistical Analyses

We used a 2-stage analysis for evaluating factors that could have contributed to observed variation in deer FGMs across seasons. We first evaluated whether FGMs differed between adult and juvenile deer using a standard analysis of variance (ANOVA; SAS Statistical Software Version 9.3, Cary, NC). Second, for adult deer, we tested whether FGMs differed by sex of individual and month of sampling using a mixed-model ANOVA. Within our model, sex and month of sampling were fixed effects, day was a repeated effect, and individual sampled was treated as a random effect. In addition, because previous research showed that adult male deer differ in FGMs over the course of the year as a result of the rut (McCoy and Ditchkoff 2012), we additionally evaluated support for an interaction of sex and month of sampling. For juvenile deer, we were unable to determine individuals and sex, so a repeated-measures ANOVA with repeated effect of day was used to test for effect of month sampled.

We evaluated the effect of sex and month of sampling on dove FGMs using a mixed-model ANOVA. Similar to analysis of data from deer, within our model, day was the repeated effect and individual sampled was treated as a random effect. Prior to analysis, we evaluated the dependent variable (FGM) and log-transformed it to approximate a normally distribution. To appropriately balance our ability to detect relationships of management significance versus our need to minimize Type I error, we considered differences significant at $P \leq 0.100$.

RESULTS

Deer Diel Rhythms

During each 48-hour sampling session, we collected an average of 22.38 samples from each of our 2 study individuals (SD = 4.75, range = 16–29). Despite their exposure to similar controlled environments, and collection of a similar number of samples for each individual (an average of 21.5/month, SD = 4.4, range = 16–26 from individual 1, and an average of 23.3/month, SD = 5.6, range = 18–29 from individual 2) each season, we observed contrasting patterns in hourly FGMs between individuals across each season of investigation (Fig. 1). During the autumn (Fig. 1A) and winter (Fig. 1B) sampling periods, individual 1 exhibited a distinct peak of approximately 120 ng/g toward the middle of the 48-hour sampling period, compared with individual 2 that exhibited a consistent physiological state. Opposite patterns were observed during spring (Fig. 1C) and summer (Fig. 1D), when individual 2 exhibited greater variability and overall higher FGMs compared with individual 1, which had consistently lower FGMs. Thus, the FGMs of either individual were not consistently higher or lower, nor did they consistently exhibit diel fluctuations within a given 48-hour period, compared with the other individual.

Deer Seasonal Rhythms

We collected and assayed 444 samples from adult and juvenile deer between October 2001 and November 2002. For adult deer, 2 samples resulted in extremely high FGM values (981 ng/g and 322 ng/g) that were larger than previous experimental challenge results of 250 ng/g (Millspaugh et al. 2002), and were censored from the analysis. On average, juvenile deer ($\bar{x} = 53.32$, SD = 33.88, range = 246.45–9.52) maintained slightly higher FGMs over the course of our study compared with adults ($\bar{x} = 46.64$ ng/g, SD = 29.10, range = 197.81–10.13; $F_{1,\ 439} = 5.19$, $P = 0.023$).

We analyzed 324 samples from 11 adult deer (5 M and 6 F; $\bar{x} = 29.27$/individual, SD = 21.64, range = 1–55) collected between October 2001 and November 2002 ($\bar{x} = 26.8$/month, SD = 4.7, range = 20–36). We found no significant difference in FGMs based on sex of the individual deer ($F_{1,\ 289} = 1.89$, $P = 0.170$). In contrast, there was strong evidence of monthly differences ($F_{11,\ 289} = 3.87$, $P \leq 0.001$), with a distinct peak during spring months of April and May when FGMs were nearly 2 times higher than late winter (i.e., Jan and Feb) and late summer values (i.e., Jul and Aug; Fig. 2A). An additional peak in FGMs was observed in December (Fig. 2). We found no support for an interactive effect of sex and month of sampling ($F_{11,\ 289} = 0.95$, $P = 0.497$).

We analyzed 118 samples collected between October 2001 and November 2002 from 6 juvenile deer ($\bar{x} = 9.9$/month, SD = 2.9, range = 6–16). In contrast to adult deer, we found no significant difference in FGMs based on month of sampling ($F_{11,\ 106} = 0.85$, $P = 0.595$). In particular, there was no distinct increase in early spring (the month of May) as observed in adults, and overall there was more variability in FGMs over the course of the year (Fig. 2B).

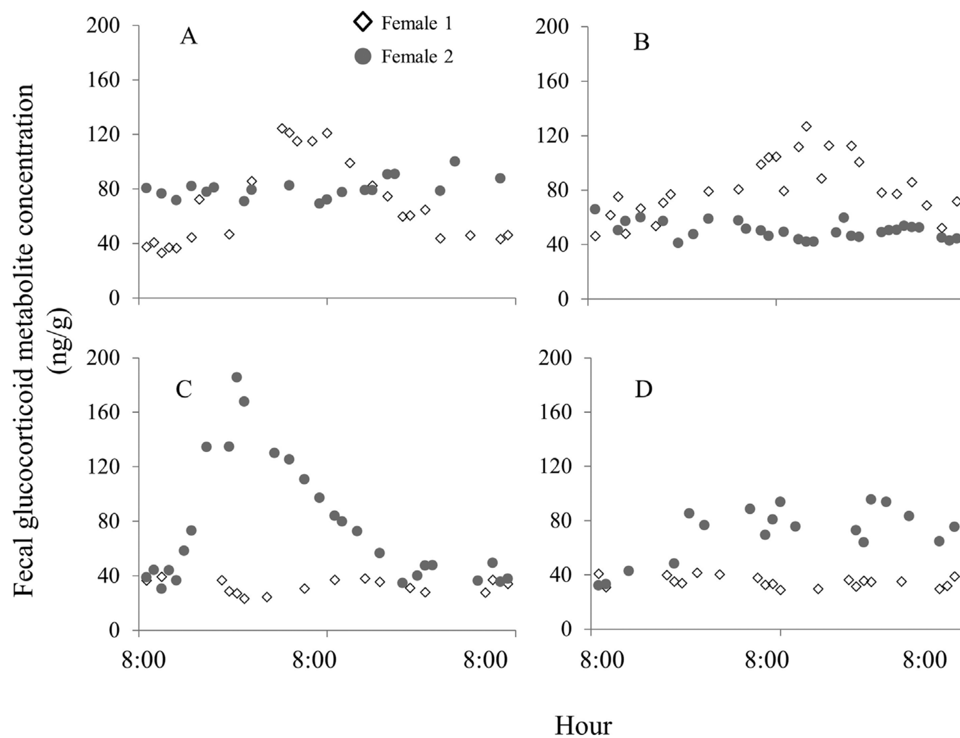

Figure 1. Diel rhythms in fecal glucocorticoid metabolite concentrations for 2 female deer housed individually in a wooden holding pen (8 × 22 m) for 72 hr at the Charles W. Green Conservation Area, located near Ashland, Missouri, USA. Samples were collected at hourly intervals over a 48-hour period during 4 seasons: autumn (A; 4–5 Nov 2001 and 11–13 Nov 2001), winter (B; 25–27 Feb 2002 and 3–5 Mar 2002), spring (C; 28–30 Apr 2002 and 5–7 May 2002), and summer (D; 14–16 Jul 2002 and 21–23 Jul 2002).

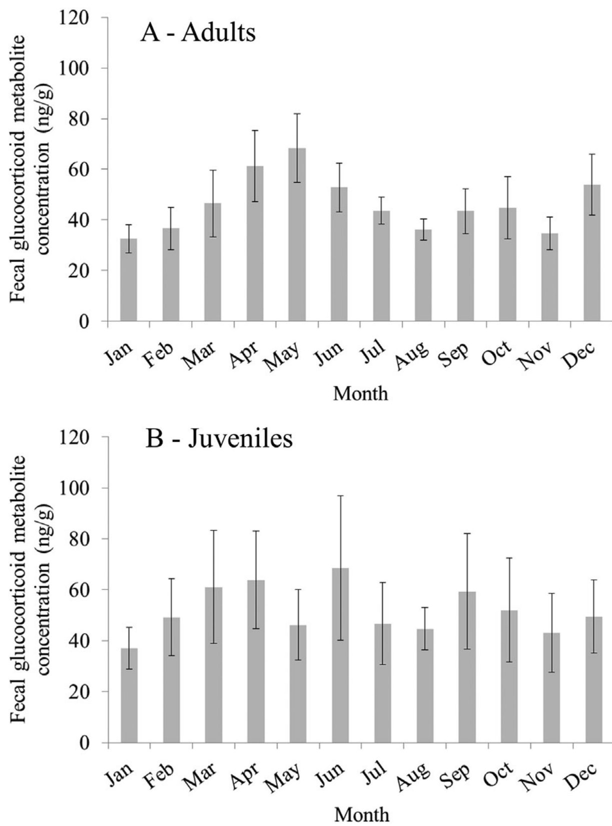

Figure 2. Average (with 95% CI) monthly fecal glucocorticoid metabolite in (A) 11 captive white-tailed deer (5 ad M, 6 ad F), and (B) 6 juveniles (of unknown sex) housed in 3 large enclosures near Ashland, Missouri, USA. Fresh (<30 min old) fecal samples were collected on a weekly basis for 1 year (15 Oct 2001 to 9 Oct 2002).

Dove Diel Rhythms

We analyzed 88 samples collected over a 48-hour period from 1 male (43 samples) and 1 female (45 samples) mourning dove in May 2002. Both doves monitored exhibited high variability in FGMs between hours sampled, with no distinct diel pattern in FGMs (Fig. 3). However, both individuals did exhibit an overall increasing trend in FGMs over the 48-hour period (Fig. 3).

Dove Seasonal Rhythms

We analyzed 385 samples collected from mourning doves between July 2001 and July 2002 ($\bar{x} = 32.1$/month, SD = 4.8, range = 24–39). We observed no effect of sex of the individual ($F_{1, 355} = 0.82$, $P = 0.370$), but even under captive conditions, we found support for a difference in FGMs based on month of sampling ($F_{11, 355} = 8.51$, $P \leq 0.001$). Dove FGMs were nearly 50% higher during the late spring months of May and June compared with winter and early spring months (Jan–Apr; Fig. 4), and twice as high as during late summer, autumn, and early winter months of August through December (Fig. 4).

DISCUSSION

Our findings further emphasize that prior to conducting a study involving FGMs, it is important to ask: What are "normal" FGM values within the context of the animal's environment and prevailing stressors? Across both species, we observed distinct seasonal, but not daily patterns in FGMs. Although seasonal bio-rhythms were rarely accounted for in previous studies involving FGMs because of logistical constraints (Touma and Palme 2005), the predictable (although species-specific) patterns we observed suggest that with adequate foresight they can be accounted for in future studies. Problematically, despite previous thinking that fine-scale fluctuations in FGMs are often "smoothed" out in feces compared with blood plasma sampling methods (Palme 2005), variability in FGMs within a 48-hour period was high and did not follow a distinct pattern in either species. Such hourly differences together with the high amount of individual variability we observed should be of concern for future studies involving the use of FGMs.

The seasonal variability observed in our study was extreme, and suggests that failure to account for such rhythms could bias interpretation of FGM assay results. For example, peaks in FGMs by captive, habituated white-tailed deer fluctuated to the extent that they were consistently in the region of what has previously been referred to as an elevated stress response in this deer herd (>50–90 ng/g; Millspaugh et al. 2002)

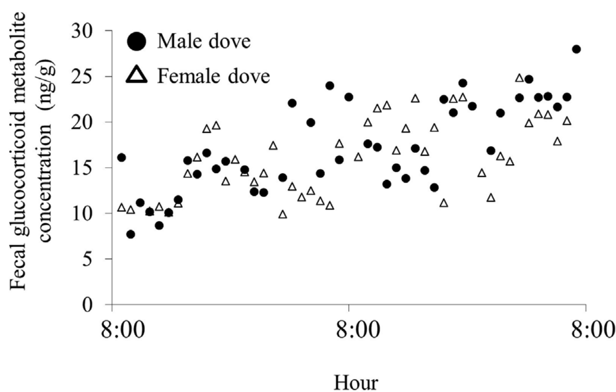

Figure 3. Fecal glucocorticoid metabolite concentrations for 1 male and 1 female mourning dove housed individually in outdoor pens near Ashland, Missouri, USA. Samples were collected at hourly periods for 48 hr between 9 and 11 May 2002.

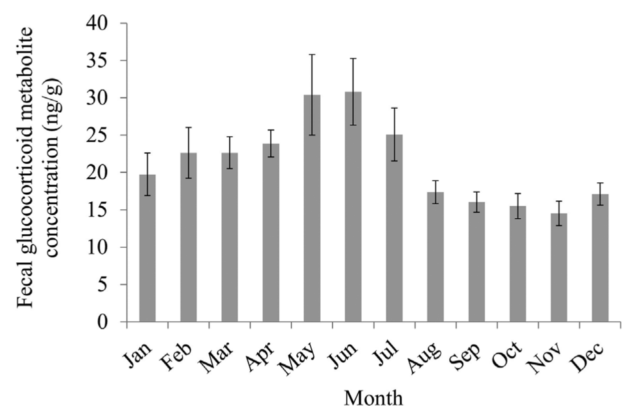

Figure 4. Mean (with 95% CIs) for monthly fecal glucocorticoid metabolite concentrations observed in 4 male and 4 female mourning doves housed individually in outdoor pens near Ashland, Missouri, USA. Samples were collected at daily intervals for 1 year (17 Jul 2001 to 9 Jul 2002).

during certain times of day and seasons. Although we did not observe support for seasonal, sex-specific differences in FGMs similar to previous studies (e.g., McCoy and Ditchkoff 2012), a peak in adult deer FGMs in December potentially corresponded with the onset of colder winter temperatures and the latter stages of the rut—a time known to result in peak FGMs in males (McCoy and Ditchkoff 2012). Conversely, it is likely that the observed peak in FGMs of adult deer in May likely corresponded with female parturition and birth of fawns. We did not observe a similar pattern in juveniles. Their May values were consistently lower than those of adult deer, but in June, the juveniles had a dramatic increase in FGM values and variability. A June spike in FGMs of juveniles might correspond with independence from their mothers and associated dietary switching, as well as potential stress of increased heat and difficulty thermoregulating compared with adults.

Differing rhythms, and increased variability in juvenile FGMs overall, suggest that care should be taken when designing sampling schemes and attempting to make interpretations from FGMs based on samples taken from multiple age classes. For example, the relatively common practice of collecting fecal samples from unknown, random individuals typically necessitates pooling data across a population (e.g., Creel et al. 2002, Jachowski et al. 2013). However, our results illustrate that to make interpretations and inference, some knowledge of age or sex of the individual is likely required, particularly when samples include both mature and immature individuals.

The high variability among individuals in diel rhythms we observed for both species suggests that even when individuals were of similar age classes and kept in identical conditions, high levels of variability existed that could influence interpretation of studies that involve limited sampling. When investigated, individual variation in stress hormone production is relatively common, and has been attributed to a variety of individual-based factors, such as past experience (Poessel et al. 2011), social standing (Creel 2001), or shyness or boldness (Clary et al. 2014). Although additional investigations with larger sample size of individuals is warranted, the relatively high magnitude of inter-individual variation we observed suggests that current efforts that pool samples from multiple animals in a population could mask potentially important individual responses to stressors. Further, if sampling is biased toward a segment of the population that does not respond or responds more severely to a stressor, inference can be biased. Ideally, future studies would focus sampling on individual focal animals where it is possible to gain a longitudinal perspective of how an individual responds to stressors—thereby gaining insight into intrinsic factors that currently limit our ability to make population-level inference based on FGMs. Studies that are unable to focus on individual animals might still provide useful data, but we encourage interpretation that considers the limitations and assumptions inherent in such pooled data.

The low amount of seasonal fluctuation in dove FGMs that we observed further emphasizes the need to evaluate how species- or population-specific rhythms are potentially influenced by site-specific conditions. Although we observed relatively slight annual peaks in mourning dove FGMs during spring months of May and June, other avian species have exhibited more distinct seasonal rhythms in the release of stress hormones (Romero and Wingfield 1998, Liebl et al. 2013). This suggests that the release of stress hormones and its potential adaptive benefit varies between species (Boonstra 2013), where mourning doves are less likely to exhibit pronounced seasonal rhythms. However, by housing the mourning doves individually in our study, we removed the potential stressor of breeding and rearing young that can be correlated with seasonal fluctuations in stress hormones (Ouyang et al. 2011). Further, because our experiment was in a captive setting, we could not account for potential extrinsic factors that could drive seasonal fluctuations in FGMs, such as diet (Dantzer et al. 2011) or exposure to parasites (Raouf et al. 2006). Additional research on dove FGM rhythms is needed, and we suggest researchers develop a more nuanced understanding of seasonal FGM biorhythms (or lack thereof) and the potential intrinsic and extrinsic drivers of those patterns for a given species under particular conditions.

Baselines and mechanisms behind observed FGM fluctuations likely need to be determined on a site-specific basis, even when methodologies are apparently standardized. Using methodologies nearly identical to ours, McCoy and Ditchkoff (2012) previously showed that in a large (258.2-ha) fenced reserve that was hunted but retained a high density of white-tailed deer, male deer FGMs were elevated (43.06 ng/g) during rut (Jan–Feb) compared with before (Nov–Dec) and following (Feb–Mar) the rut. By contrast, in our study of captive deer, January and February were months when we observed lowest FGMs, and we observed no significant difference between males and females. These contrasting findings further emphasize the importance of identifying site-specific stressors and baselines prior to assuming that an increase in FGMs is indicative of chronic stress. For example, although social stressors of high density and competition between males (or hunting pressure) could explain elevated values during rut reported by McCoy and Ditchkoff (2012), their reported values are within what was considered a basal range of FGMs by Millspaugh et al. (2002), and well within the normal bounds of variation based on our findings. Thus, interpreting a normal FGM baseline and biorhythm present in one population might not occur in other populations, or be less apparent because of other overriding stressors (Goymann 2012). Therefore, we encourage researchers to avoid making generalizations and comparisons across studies or populations, and instead undertake robust pre- versus post-treatment sampling that accounts for site-specific intrinsic and extrinsic conditions that likely vary over time.

ACKNOWLEDGMENTS

Financial and logistical support for this project was provided by the University of Missouri (MU) Department of Fisheries and Wildlife Sciences, a MU Life Science Mission Enhancement Postdoctoral Fellowship, a MU Research

Board Grant, the Missouri Department of Conservation (Federal Aid in Wildlife Restoration Project W-13-R), and the 2001 Webless Migratory Game Bird Research Program (U.S. Fish and Wildlife Service and the U.S. Geological Survey—Biological Resources Division). Fecal glucocorticoid assays were conducted in the Wildlife Stress Physiology Laboratory in the Department of Fisheries and Wildlife Sciences at the University of Missouri-Columbia. We thank B. Crampton, S. Kistner, B. Hoenes, T. Bonnot, T. Mong, R. Woods, T. Meyer, L. Miller, J. Schulz, R. Woeck, and C. Rittenhouse for their assistance in pen construction and/or feces collection. We thank the Associate Editor E. Rominger and 2 anonymous reviewers for helpful comments that improved this manuscript.

LITERATURE CITED

Beringer, J., J. J. Millspaugh, J. Sartwell, and R. Woeck. 2004. Real-time video recording of food selection by captive white-tailed deer. Wildlife Society Bulletin 32:648–654.

Boonstra, R. 2013. Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. Functional Ecology 27:11–23.

Boonstra, R., A. H. Hubbs, E. A. Lacey, and C. J. McColl. 2001. Seasonal changes in glucocorticoid and testosterone concentrations in free-living arctic ground squirrels from the boreal forest of the Yukon. Canadian Journal of Zoology 79:49–58.

Busch, D. S., and L. S. Hayward. 2009. Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biological Conservation 142:2844–2853.

Carere, C., T. G. G. Groothuis, E. Möstl, S. Daan, and J. M. Koolhaas. 2003. Fecal corticosteroids in a territorial bird selected for different personalities: daily rhythm and the response to social stress. Hormones and Behavior 43:540–548.

Chelini, M. O. M., E. Otta, C. Yamakita, and R. Palme. 2010. Sex differences in the excretion of fecal glucocorticoid metabolites in the Syrian hamster. Journal of Comparative Physiology B 180:919–925.

Clary, D., L. J. Skyner, C. P. Ryan, L. E. Gardiner, W. G. Anderson, and J. F. Hare. 2014. Shyness-boldness, but not exploration, predicts glucocorticoid stress response in Richardson's ground squirrels (*Urocitellus richardsonii*). Ethology 120:1101–1109.

Creel, S. 2001. Social dominance and stress hormones. Trends in Ecology and Evolution 16:491–497.

Creel, S., J. E. Fox, A. Hardy, J. Sands, B. Garrott, and R. O. Peterson. 2002. Snowmobile activity and glucocorticoid stress responses in wolves and elk. Conservation Biology 16:809–814.

Dantzer, B., A. G. McAdam, R. Palme, S. Boutin, and R. Boonstra. 2011. How does diet affect fecal steroid hormone metabolite concentrations? An experimental examination in red squirrels. General and Comparative Endocrinology 174:124–131.

Goymann, W. 2012. On the use of non-invasive hormone research in uncontrolled, natural environments: the problem with sex, diet, metabolic rate and the individual. Methods in Ecology and Evolution 3:757–765.

Harper, J. M., and S. N. Austad. 2001. Effect of capture and season on fecal glucocorticoid levels in deer mice (*Peromyscus maniculatus*) and red-backed voles (*Clethrionomys gapperi*). General and Comparative Endocrinology 123:337–344.

Jachowski, D. S., T. W. Mong, B. E. Washburn, J. H. Schulz, and J. J. Millspaugh. 2014. Evaluating the effect of short-term capture and handling on fecal glucocorticoid metabolite levels in mourning doves. Wildlife Biology in Practice 10:39–50.

Jachowski, D. S., R. Slotow and J. J. Millspaugh. 2012. Physiological stress and refuge behavior by African elephants. PLoS ONE 7:e31818.

Jachowski, D. S., R. Slotow and J. J. Millspaugh. 2013. Delayed physiological acclimatization by African elephants following reintroduction. Animal Conservation 16:575–583.

Khonmee, J., J. L. Brown, S. Rojanasthien, D. Thumasanukul, A. Kongphoemphun, B. Siriaroonrat, W. Tipkantha, V. Punyapornwithaya, and C. Thitaram. 2014. Seasonality of fecal androgen and glucocorticoid metabolite excretion in male goral (*Naemorhedus griseus*) in Thailand. Animal Reproduction Science 146:70–78.

Liebl, A. L., T. Shimizu, and L. B. Martin. 2013. Covariation among glucocorticoid regulatory elements varies seasonally in house sparrows. General and Comparative Endocrinology 183:32–37.

McCoy, J. C., and S. S. Ditchkoff. 2012. Patterns of fecal hormones in a fenced population of white-tailed deer. Wildlife Society Bulletin 36:641–646.

McEwen, B. S., and J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. Hormones and Behavior 4:2–15

Millspaugh, J. J., and B. E. Washburn. 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. General and Comparative Endocrinology 138:189–199.

Millspaugh, J. J., B. E. Washburn, M. A. Milanick, J. Beringer, L. P. Hansen, and T. M. Meyer. 2002. Non-invasive techniques for stress assessments in white-tailed deer. Wildlife Society Bulletin 30:899–907.

Millspaugh, J. J., R. J. Woods, K. E. Hunt, K. J. Raedeke, G. C. Brundige, B. E. Washburn, and S. K. Wasser. 2001. Fecal glucocorticoid assays and the physiological stress response in elk. Wildlife Society Bulletin 29:899–907

Mirarchi, R. E. 1993. Sexing, aging, and miscellaneous research techniques. Pages 399–408 *in* T. S. Baskett, M. W. Sayre, R. E. Tomlinson, and R. E. Mirarchi, editors. Ecology and management of the mourning dove. Wildlife Management Institute, Washington, D.C., USA.

Mong, T. W., J. H. Schulz, and J. J. Millspaugh. 2002. Design and construction of mourning dove research pens. Transactions of the Missouri Academy of Sciences 36:21–24.

Mooring, M. S., M. L. Patton, V. A. Lance, B. M. Hall, E. W. Schaad, G. A. Fetter, S. S. Fortin, and K. M. McPeak. 2006. Glucocorticoids of bison bulls in relation to social status. Hormones and Behavior 49:369–375.

Nixon, C. M., L. P. Hansen, P. A. Brewer, and J. E. Chelsvig. 1991. Ecology of white-tailed deer in an intensively farmed region of Illinois. Wildlife Monographs 118.

Ouyang, J. Q., P. J. Sharp, A. Dawson, M. Quetting, and M. Hau. 2011. Hormone levels predict individual differences in reproductive success in a passerine bird. Proceedings of the Royal Society B: Biological Sciences. doi: 10.1098/rspb.2010.2490

Owen, M. A., N. M. Czekala, R. R. Swaisgood, K. Steinman, and D. G. Lindburg. 2005. Seasonal and diurnal dynamics of glucocorticoids and behavior in giant pandas. Ursus 16:208–221.

Palme, R. 2005. Measuring fecal steroids: guidelines for practical application. Annals of the New York Academy of Sciences 1046: 75–80.

Poessel, S. A., D. E. Biggins, R. M. Santymire, T. M., Livieri, K. R. Crooks, and L. Angeloni. 2011. Environmental enrichment affects adrenocortical stress responses in the endangered black-footed ferret. General and Comparative Endocrinology 172:526–533.

Pravosudov, V. V., A. S. Kitaysky, C. J. Saldanha, J. C. Wingfield, and N. S. Clayton. 2002. The effect of photoperiod on adrenocortical stress response in mountain chickadees (*Poecile gambeli*). General and Comparative Endocrinology 126:242–248.

Raminelli, J. L., M. B. C. de Sousa, M. S. Cunha, and M. F. V. Barbosa. 2001. Morning and afternoon patterns of fecal cortisol excretion among reproductive and non-reproductive male and female common marmosets, *Callitrix jacchus*. Biological Rhythm Research 32:159–167.

Raouf, S. A., L. C. Smith, M. B. Brown, J. C. Wingfield, and C. R. Brown. 2006. Glucocorticoid hormone levels increase with group size and parasite load in cliff swallows. Animal Behaviour 71:39–48.

Reeves, H. M., A. D. Geis, and F. C. Kniffin. 1968. Mourning dove capture and banding. U.S. Fish and Wildlife Service, Special Scientific Report-Wildlife 117, Washington, D.C., USA.

Romero, L. M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. General and Comparative Endocrinology 128:1–24.

Romero, L. M., and J. C. Wingfield. 1998. Seasonal changes in adrenal sensitivity alter corticosterone levels in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 119:31–36.

Schatz, S., and R. Palme. 2001. Measurement of faecal cortisol metabolites in cats and dogs: a noninvasive method for evaluating adrenocortical function. Veterinary Research Communications 25: 271–287.
Schulz, J. H., S. L. Sheriff, Z. He, C. E. Braun, R. D. Drobney, R. E. Tomlinson, D. D. Dolton, and R. A. Montgomery. 1995. Accuracy of techniques used to assign mourning dove age and gender. Journal of Wildlife Management 59:759–765.
Schwarzenberger, F., E. Möstl, E. Bamberg, J. Pammer, and O. Schmehlik. 1991. Concentrations of progestagens and oestrogens in the faeces of pregnant Lipizzan, Trotter and Thoroughbred mares. Journal of Reproduction and Fertility Supplement 44:489–499.
Sheriff, M. J., B. Dantzer, B. Delehanty, R. Palme, and R. Boonstra. 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166:869–887.
Touma, C., and R. Palme. 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Annals of the New York Academy of Sciences 1046:54–74.
Touma, C., N. Sachser, E. Mostl, and R. Palme. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. General and Comparative Enocrinology 130:267–278.
von der Ohe, C. G., and C. Servheen. 2002. Measuring stress in mammals using fecal glucocorticoids: opportunities and challenges. Wildlife Society Bulletin 30:1215–1225.
Washburn, B. E., and J. J. Millspaugh. 2002. Effects of simulated environmental conditions on glucocorticoid metabolite measurements in white-tailed deer feces. General and Comparative Endocrinology 127:217–222.
Washburn, B. E., J. J. Millspaugh, J. H. Schulz, S. B. Jones, and T. Mong. 2003. Using fecal glucocorticoids for stress assessment in mourning doves. Condor 105:696–706.
Westmoreland, D., L. B. Best, and D. E. Blockstein. 1986. Multiple brooding as a reproductive strategy: time-conserving adaptations in mourning doves. The Auk 103:196–203.

Associate Editor: Rominger.