
Factors Related to Fecal Corticosterone Levels in California Spotted Owls: Implications for Assessing Chronic Stress

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Abstract: *The California Spotted Owl (*Strix occidentalis occidentalis*) is under consideration for federal protection and has stimulated ecosystem-level management efforts in Sierra Nevada national forests. Because some populations are declining, we used a noninvasive fecal sampling method to estimate stress hormone (i.e., corticosterone) levels within a local population from April to August 2001. Fecal corticosterone levels were similar to those recorded in a previous study of Northern Spotted Owls (*S.o. caurina*) ($\bar{x} = 80.1$ ng/g dry feces, $SE = 75.8$). We then used an information-theoretic approach to identify factors that influence fecal corticosterone levels in Spotted Owls. Our best overall model indicated that nonbreeding owls had higher fecal corticosterone levels than breeding owls early in the breeding season and lower levels later in the breeding season. We collected few samples from breeding owls early in the breeding season, however, which may have influenced the results. Management-related factors reflecting habitat condition and proximity to roads were not correlated with fecal corticosterone. However, factors such as field storage method and sample mass were correlated with the amount of measured fecal corticosterone and should be considered in future studies. Sample vials initially stored on ice had higher levels than those stored immediately in liquid N_2 ($\beta_{storage} = 0.269$ ln[ng/g], 95% CI = 0.026, 0.512). Hormone metabolites were extracted from extremely small samples (0.01 g) by slightly modifying the assay protocol, but the amount of corticosterone detected increased as the sample mass decreased ($\beta_{mass} = -6.248$ ln[ng/g], 95% CI = -8.877, -3.620). Corticosterone levels were significantly higher in 10 cecal samples collected simultaneously with fecal samples (paired difference = 74.7 ng/g, $SE = 45.0$, $p = 0.001$ for a paired t test), so care must be taken to avoid contaminating fecal samples with cecal material. Most of the variation was unexplained by our best model ($R^2 = 0.24$), and additional factors influencing fecal corticosterone levels need to be identified. Therefore, we recommend that well-designed experiments be conducted under controlled conditions to better determine the effect of factors such as sample handling, partial sampling, and diet on fecal corticosterone levels in owls and other birds.*

Key Words: California Spotted Owl, fecal corticosterone, noninvasive, Sierra Nevada, *Strix occidentalis occidentalis*

Factores Relacionados a Niveles de Corticosterona Fecal en Búhos *Strix occidentalis occidentalis*: Implicaciones para la Evaluación de Estrés Crónico

Resumen: *El buho *Strix occidentalis occidentalis* esta siendo considerado para su protección a nivel federal y ha estimulado esfuerzos de gestión a nivel de ecosistema en bosques nacionales en la Sierra Nevada. Debido a que algunas poblaciones están declinando, utilizamos un método no invasivo de muestreo fecal para estimar niveles de hormonas de estrés (es decir, corticosterona) en una población de abril a agosto 2001. Los niveles de corticosterona fecal fueron similares a los registrados en un estudio previo en *Strix o. caurina* ($\bar{x} = 80.1$ ng/g*

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beces secas, $ES = 75.8$). Posteriormente usamos un método información-teórico para identificar factores que influyen en los niveles de corticosterona fecal en *Strix occidentalis occidentalis*. Nuestro mejor modelo indicó que los búbos no reproductivos tenían niveles de corticosterona fecal más altos que los búbos reproductivos al inicio de la época reproductiva y menores niveles al final de la época reproductiva. Sin embargo, recolectamos pocas muestras de búbos reproductivos al inicio de la época reproductiva, lo que pudo haber influido en los resultados. Factores relacionados con la gestión que reflejan la condición del hábitat y la cercanía a caminos no se correlacionaron con la corticosterona fecal. Sin embargo, factores como el método de almacenamiento a campo y la masa de la muestra se correlacionaron con la cantidad de corticosterona fecal medida y deben considerarse en estudios futuros. Frascos con muestras almacenados inicialmente en hielo tuvieron mayores niveles que los almacenados inmediatamente en N_2 ($\beta_{\text{almacenamiento}} = 0.269 \ln[\text{ng/g}]$, 95% CI = 0.026, 0.512). Se extrajeron metabolitos de hormona de muestras extremadamente pequeñas (0.01 g) modificando ligeramente el protocolo de evaluación, pero la cantidad de corticosterona detectada aumentó a medida que disminuyó la masa de la muestra ($\beta_{\text{masa}} = -6.248 \ln[\text{ng/g}]$, 95% CI = -8.877, -3.620). Los niveles de corticosterona fueron significativamente mayores en 10 muestras cecales recolectadas simultáneamente con muestras fecales (diferencia de pares = 74.7 ng/g, SE = 45.0, $p = 0.001$ para una prueba de t pareada) por lo que se debe tener cuidado para evitar que las muestras fecales se contaminen con material cecal. La mayor parte de la variación se explicó por nuestro mejor modelo ($R^2 = 0.24$) y se necesita identificar los factores adicionales que influyen en los niveles de corticosterona fecal. Por lo tanto, recomendamos que se realicen experimentos bien diseñados, bajo condiciones controladas, para entender mejor los efectos de factores tales como el manejo de muestras, muestreo parcial y dieta sobre los niveles de corticosterona en búbos y otras aves.

Palabras Clave: Buho de California, corticosterona fecal, no invasivo, Sierra Nevada, *Strix occidentalis occidentalis*

Introduction

The Spotted Owl (*Strix occidentalis*) is a well-studied species because its habitat contains commercially valuable timber (Simberloff 1987; Gutiérrez et al. 1995). Habitat loss due to timber harvest led to the listing of the Northern (*S. o. caurina*) and Mexican (*S. o. lucida*) Spotted Owls as federally threatened under the U.S. Endangered Species Act (U.S. Department of the Interior 1990, 1993). Similarly, timber harvest and fire suppression have altered the habitat of the California Spotted Owl (*S. o. occidentalis*; Verner et al. 1992).

Recent studies indicate that local California Spotted Owl populations may be declining and that population changes are mainly influenced by annual variation in fecundity (Blakesley et al. 2001; Seamans et al. 2001). On our study area, owls frequently failed to reproduce, and annual population fecundity rates ranged from 0.0 to 0.8 female young fledged per adult female (Seamans et al. 2001).

Chronically high glucocorticoid hormone levels may be a physiological mechanism influencing reproductive output in wildlife (Wingfield & Ramenofsky 1999). Under normal conditions, glucocorticoids adaptively mediate an individual's long-term stress response (Sapolsky et al. 2000). However, exposure to stressors for more than a few days can lead to chronically elevated glucocorticoid levels, and high glucocorticoid levels can suppress growth, disease resistance, and reproductive function (Wingfield & Ramenofsky 1999; Sapolsky et al. 2000).

Corticosterone is the primary avian glucocorticoid, and experimental studies have confirmed that elevated corticosterone levels can suppress reproductive development and behavior in captive and wild birds (Wilson & Follet 1976; Cain & Lien 1985; Silverin 1986).

Wingfield et al. (1998) hypothesized that elevated corticosterone levels may cause an individual to postpone or abandon its current life-history stage (breeding, migration, molting). For example, increased corticosterone secretion during the breeding season caused by chronic stress from inclement weather, food shortage, or human disturbance could lead birds to abandon or forego nesting. In fact, elevated blood corticosterone levels in wild birds have been correlated with exposure to potential chronic environmental stressors, such as low-quality habitat (Marra & Holberton 1998), ecotourist visitation (Fowler 1999), and food deprivation (Vleck et al. 2000).

Using a fecal sampling method, Wasser et al. (1997) found that elevated fecal corticosterone levels in male Northern Spotted Owls were correlated with proximity to roads and past logging activity. Due to its noninvasive nature, fecal sampling may be a particularly useful technique to assess chronic stress in wild birds. Fecal samples may also provide a more integrated measure of recent glucocorticoid secretion (Harper & Austad 2000). Over the course of an induced stress response, fecal corticosterone levels in captive birds have paralleled blood corticosterone levels after a time lag of several hours (Wasser et al. 1997; Ludders et al. 2001).

We hypothesized that nonbreeding California Spotted Owls have higher fecal corticosterone levels than breeding owls and that certain environmental factors are correlated with elevated fecal corticosterone levels. We were particularly interested in habitat conditions and proximity to roads because of their management implications. Therefore, we collected fecal samples from a well-studied population of owls to estimate corticosterone levels within the population, to identify factors that influenced these levels, and to estimate the overall variation explained by these factors.

Methods

Study Area

Our study area (925 km²) was located on the Eldorado and Tahoe National Forests, 16 km northeast of Georgetown, California, and on the California portion of the Lake Tahoe Basin Management Unit (Lake Tahoe). This has been the site of a long-term study of California Spotted Owl demography (Seamans et al. 2001). The topography was mountainous, with owl locations ranging in elevation from 1018 to 2201 m. Vegetation was typical of Sierran middle-elevation, mixed-conifer forest with some red fir (*Abies magnifica* A. Murr) forest at higher elevations. Dominant tree species were sugar pine (*Pinus lambertiana* Dougl.), ponderosa pine (*P. ponderosa* Doubl. Ex Laws.), Douglas-fir (*Pseudotsuga menziesii* [Mirb.]), incense cedar (*Calocedrus decurrens* [Torr.] Florin), black oak (*Quercus kelloggii* Newb.), white fir (*A. concolor* [Gord. and Gland] Lindl. Ex Hildebr.), and red fir. Land ownership was approximately 60% U.S. Forest Service and 40% private land, often distributed in a checkerboard pattern.

Field Study

The study was conducted from April to August 2001, with early April marking the onset of Spotted Owl nesting on the study area. We located owls by imitating their calls to elicit a response and then following responsive birds to their roost or nest (Forsman 1983). When possible, we placed a clean polyethylene sheet beneath a roosting owl to facilitate fecal sample collection. The sampling technician remained at least 10 m from the owl and waited a minimum of 2 hours for defecation. Upon defecation the fecal portion of excreta was removed from the urine portion (Wasser et al. 1997) and placed in a cryogenic vial. The vial was either immediately placed in liquid N₂ or on ice until it could be placed in liquid N₂, and initial storage method was recorded for each sample.

We also opportunistically collected cecal discharges that occurred within a few minutes of a fecal sample collection to assess the similarity of cecal and fecal corticos-

terone levels. Owls have a well-developed cecum branching from the intestine near the cloaca (DeGolier et al. 1999) and periodically discharge cecal material with a different texture, color, and smell than feces (P. E. Redig, personal communication).

Most individual owls were color-marked and had known reproductive histories (Seamans et al. 2001). In the case of unmarked birds, the sex of the bird was determined by the pitch of the owl's call (Forsman et al. 1984). We determined nest and roost site locations and reproductive status using the methods described by Franklin et al. (1996).

We initially hoped to collect at least three samples from each individual on our study area, but in many cases we were unable to do so because of unsuccessful collection attempts. For example, birds failed to defecate after several hours of waiting, feces were caught by intervening foliage, or we were unable to locate birds during a survey.

Laboratory Analyses

Frozen fecal samples were placed in a lyophilizer (Freeze-dry Specialties, Osseo, Minnesota) for 24 hours. Once freeze-dried, samples were ground, sifted through a stainless steel sieve to remove large particles, and thoroughly mixed. Glucocorticoids were extracted from feces with a modification of the technique described by Schwarzenberger et al. (1991). Dried feces (approximately 0.1 g) were placed in a test tube with 2.0 mL of 90% methanol and vortexed at high speed in a multitube vortexer for 30 minutes. Extremely small fecal samples (<0.010 g; $n = 25$) were placed in a test tube with 0.5 mL of 90% methanol and vortexed at high speed in a multitube vortexer for 30 minutes (B. E. Washburn, personal communication). Samples were then centrifuged at 2500 rpm for 20 minutes, and the supernatant was saved and stored at -84° C until assayed 1–2 days later.

An I¹²⁵ corticosterone radioimmunoassay (RIA) kit (ICN #07-120103, ICN Biomedicals, Costa Mesa, California), previously validated for use in Spotted Owls (Wasser et al. 1997, 2000), was used to quantify fecal glucocorticoid metabolites. The ICN protocol for the I¹²⁵ corticosterone RIA was followed, except the volume of all reagents was halved (Wasser et al. 2000). Standard curves were produced from six standards (0.125 to 5 ng/mL). Three fecal samples were diluted to 1:4, 1:8, 1:16, and 1:32, and all regression slopes were parallel to the standard curve ($p > 0.05$; Neter et al. 1990). A dilution of 1:8 displaced approximately 50% of the I¹²⁵-labeled hormone from the antibody. This dilution maximized the precision of the assay, and all fecal samples were diluted in assay diluent to this level prior to assay, except for the extremely small fecal samples, which were diluted to 1:4. Assay sensitivity was 1.25 ng/g dry feces. All samples were run in duplicate, and the intraassay coefficient of variation

(COV) was 1.6%. Four assay runs were required to analyze all of the samples. For three control samples analyzed in each assay run, the interassay COV was 9.6%.

A Priori Model Development

We constructed a priori candidate models representing multiple hypotheses of covariate effects (e.g., number of habitat patches) on owl fecal corticosterone levels (Burnham & Anderson 2002). We used linear mixed models in which owl territory was treated as a random blocking effect because samples from a territorial pair may not have been independent and all other covariates were treated as fixed effects (Littell et al. 1996). The individual owl was the sampling unit in a repeated-measures design. To account for possible heterogeneous sampling variances among individuals, we first used a restricted-maximum-likelihood estimation to select the appropriate covariance structure for the global model (using PROC MIXED in SAS 8.2, SAS Institute, Cary, North Carolina; Littell et al. 1996). This covariance structure was then incorporated into a maximum-likelihood-based comparison of our candidate models. The general form of the models was $CORT = \mathbf{X}\beta + \mathbf{Z}\mathbf{u} + \mathbf{e}$, where \mathbf{X} is the fixed parameter design matrix, β a vector of fixed parameters, \mathbf{Z} a design matrix for the random-effect (i.e., territory) parameters, \mathbf{u} a vector of random effect parameters, and \mathbf{e} a vector of random errors (Littell et al. 1996).

Based on the literature on Spotted Owl biology, general avian corticosterone levels, and our own field observations, we developed a list of covariates that may affect fecal corticosterone levels. These covariates were classed into two groups, nonmanagement and management, predicated upon the ability of land managers to manipulate them (Table 1). We were most interested in the comparison of management covariates but expected the nonmanagement covariates to have a greater influence on corticosterone levels. Therefore, to control for the nonmanagement effects, we used a two-stage approach. In the first stage, we compared models containing only nonmanagement covariates. In the second stage, we added management covariates to the best first-stage model to create a set of competing overall models. A similar strategy has been used in demographic studies of Spotted Owls in which recapture probabilities were modeled first, and the best recapture model was incorporated into the selection of the best survival models (Franklin et al. 2000; Seamans et al. 2001).

We used an information-theoretic approach to objectively compare our models. An adjusted Akaike's information criterion (AIC_c) was used to correct for a relatively small sample size compared to the overall number of model parameters (Burnham & Anderson 2002:66). Akaike weights (w_i) were used to estimate the relative likelihood of each model given the observed data (Burn-

Table 1. Covariates used in a priori models to estimate fecal corticosterone levels in California Spotted Owls in the central Sierra Nevada, California, April–August 2001.

Variable	Description
Nonmanagement	
biological	
sex	sex (female, 0; male, 1)
breed	breeding status (breeding, 0; not breeding, 1)
date	day of year (Julian date)
fitness	past reproductive success or "fitness" (average no. young fledged per year in past years when reproductive output was determined)
elev	elevation of owl's territory center (m)
sampling effects	
collect	no. of minutes elapsed between a technician's initial hooting and the collection of a sample
storage	storage method (sample placed in liquid N ₂ within 6 hours of collection, 0; or more than 6 hours after collection, 1)
mass	dry mass of sample (g)
Management	
habitat	
area	amount of spotted owl habitat within territory (km ²)
core	amount of interior spotted owl habitat (>100 m from an edge) within territory (km ²)
edge	amount of edge between spotted owl habitat and all other vegetation types within territory (km)
patch	no. of discrete patches of spotted owl habitat within territory
roads	
road1	distance to nearest paved road (m)
road2	distance to nearest improved surface road, including paved (m)
road3	distance to nearest road of any type (m)
roads	length of all roads within territory (km)

ham & Anderson 2002). This approach allowed us to simultaneously compare multiple hypotheses.

Nonmanagement Models

BIOLOGICAL MODELS

We hypothesized that high corticosterone levels may suppress reproduction in Spotted Owls, and thus we expected levels to be higher in nonbreeding birds (model N1; Table 2). Additionally, owls that have experienced high reproductive success during past years on our study area may have intrinsically lower corticosterone levels (models N2–3). We hypothesized that levels would be higher in males than females because this pattern was observed in Northern Spotted Owls (model N4; Wasser et al. 1997). Age was not considered in our models because the precise age of most owls was unknown.

Table 2. A priori candidate models representing hypothesized nonmanagement covariate effects on fecal corticosterone levels in California Spotted Owls in the central Sierra Nevada, California, April–August 2001.

Hypothesized model	Predicted effects
Biological	
N1. breed	$\beta_{\text{breed}} > 0$
N2. fitness	$\beta_{\text{fitness}} < 0$
N3. fitness + breed	$\beta_{\text{fitness}} < 0, \beta_{\text{breed}} > 0$
N4. sex	$\beta_{\text{sex}} > 0$
N5. date	$\beta_{\text{date}} > 0$
N6. date + date ²	$\beta_{\text{date}} > 0, \beta_{\text{date}^2} < 0$
N7. sex + breed	$\beta_{\text{sex}} > 0, \beta_{\text{breed}} > 0$
N8. sex + date	$\beta_{\text{sex}} > 0, \beta_{\text{date}} > 0$
N9. breed + date	$\beta_{\text{breed}} > 0, \beta_{\text{date}} > 0$
N10. sex*breed	$\beta_{\text{sex}} > 0, \beta_{\text{breed}} > 0, \beta_{\text{sex*breed}} > 0$
N11. sex*date	$\beta_{\text{sex}} > 0, \beta_{\text{date}} > 0, \beta_{\text{sex*date}} < 0$
N12. breed*date	$\beta_{\text{breed}} > 0, \beta_{\text{date}} > 0, \beta_{\text{breed*date}} < 0$
N13. sex*breed*date	$\beta_{\text{sex}} > 0, \beta_{\text{breed}} > 0, \beta_{\text{date}} > 0,$ $\beta_{\text{sex*breed*date}} < 0$
N14. elev	$\beta_{\text{elev}} < 0$
N15. elev + elev ²	$\beta_{\text{elev}} < 0, \beta_{\text{elev}^2} > 0$
Sampling effects	
N16. collect	$\beta_{\text{collect}} > 0$
N17. collect*sex	$\beta_{\text{collect}} > 0, \beta_{\text{sex}} > 0, \beta_{\text{collect*sex}} > 0$
N18. collect*breed	$\beta_{\text{collect}} > 0, \beta_{\text{breed}} > 0, \beta_{\text{collect*breed}} < 0$
N19. collect*date	$\beta_{\text{collect}} > 0, \beta_{\text{date}} > 0, \beta_{\text{collect*date}} < 0$
N20. storage	$\beta_{\text{storage}} > 0$
N21. mass	$\beta_{\text{mass}} < 0$
N22. storage + mass	$\beta_{\text{storage}} > 0, \beta_{\text{mass}} < 0$

We expected corticosterone levels to change over time (models N5–6) because fecal levels in female Northern Spotted Owls varied seasonally in a quadratic manner (Wasser et al. 1997). However, male fecal corticosterone levels did not vary over the course of the breeding season (Wasser et al. 1997). Therefore, we included additive and interactive models involving sex, breeding status, and date (models N7–13). Avian blood corticosterone levels are also known to fluctuate on a daily cycle (Dufty & Belthoff 1997; Breuner et al. 1999). However, on many occasions we collected multiple samples from the same individual within the same day. To avoid pseudoreplication in our analyses, we calculated a single mass-weighted daily average for these cases and did not include time of day in our set of candidate models. We tested for time-of-day effects by performing regressions of fecal corticosterone levels versus time of day.

Elevation may affect physiological stress in owls in two ways. First, owls at lower elevations may be more heat-stressed than those at higher elevations during the hot, dry summers in our study area (model N14; Barrows 1981). Second, owls at higher elevations may experience greater food stress due to reduced prey diversity (model N15). Owls at high elevations in our study area feed primarily on northern flying squirrels (*Glaucomys sabrinus*), whereas those at lower elevations feed on

both dusky-footed woodrats (*Neotoma fuscipes*) and flying squirrels (M. Seamans, unpublished data).

SAMPLING EFFECTS

Hooting to locate owls and our physical presence near a roosting owl during sample collection may have been a source of stress. Because secreted corticosterone appeared in the feces of Spotted Owls at some time within 2 hours of secretion (Wasser et al. 2000), the amount of time required to collect the sample might affect the detection of such potential corticosterone secretion (model N16). In addition, we hypothesized that male owls or breeding pairs would be more sensitive to the sample collection process, so we also included interactive models (N17–19). We developed a model (N20) to account for initial storage method, which might affect the amount of measurable metabolites in the sample. Finally, extremely small samples required an adjustment in the lab protocol that may have influenced the assay results (models N21–22) (B. E. Washburn, personal communication).

Territory-Scale Management Models

On a study area similar to our own, Franklin et al. (2000) found that the amount of core owl habitat (>100 m from an edge), the amount of edge between owl habitat and all other vegetation types, and the number of owl habitat patches within a territory were important predictors of Northern Spotted Owl reproductive output. We hypothesized that these measures of habitat condition, as well as the total amount of owl habitat within a territory, would also affect corticosterone levels (models M1–5; Table 3). We included interactive models for each habitat covariate with both sex and breeding status (models M6–7, for example). We defined owl habitat as conifer forest with a dominant tree size of 61.0 cm diameter at breast height (dbh) and canopy cover of $\geq 70\%$, which constitutes prime Spotted Owl nesting habitat (Franklin et al. 2000).

Wasser et al. (1997) found that male Northern Spotted Owls near a major road had significantly higher fecal corticosterone levels than other males; no such effect was observed for females. Therefore, we included models reflecting road conditions (models M8–12) and models containing an interaction between road conditions and both sex and breeding status (models 13–14, for example).

For each habitat and road model, we included three forms—linear, quadratic, and pseudothreshold—because the covariates may be related to a response in several ways (see Franklin et al. 2000). Despite the potential for identifying spurious relationships in the data, we opted to include such a large number of management models because of the exploratory nature of our study (Burnham & Anderson 2002:41). To our knowledge, our study was

Table 3. A priori candidate models representing hypothesized management covariate effects on fecal corticosterone levels in California Spotted Owls in the central Sierra Nevada, California, April–August 2001.

Hypothesized model ^a	Predicted effects		
	linear	quadratic	pseudobreshold
Habitat			
M1. area	$\beta_{\text{area}} < 0$	$\beta_{\text{area}} < 0, \beta_{(\text{area})^2} > 0$	$\beta_{\ln(\text{area})} < 0$
M2. core	$\beta_{\text{core}} < 0$	$\beta_{\text{core}} < 0, \beta_{(\text{core})^2} > 0$	$\beta_{\ln(\text{core})} < 0$
M3. edge	$\beta_{\text{edge}} > 0$	$\beta_{\text{edge}} > 0, \beta_{(\text{edge})^2} < 0$	$\beta_{\ln(\text{edge})} > 0$
M4. patch	$\beta_{\text{patch}} > 0$	$\beta_{\text{patch}} > 0, \beta_{(\text{patch})^2} < 0$	$\beta_{\ln(\text{patch})} > 0$
M5. area + core + edge + patch			
M6. sex*area ^b	$\beta_{\text{sex}} > 0, \beta_{\text{area}} < 0$ $\beta_{\text{sex*area}} < 0$	$\beta_{\text{sex}} > 0, \beta_{\text{area}} < 0, \beta_{(\text{area})^2} > 0$ $\beta_{\text{sex*area}} < 0, \beta_{\text{sex*(area)}^2} > 0$	$\beta_{\text{sex}} > 0, \beta_{\ln(\text{area})} < 0$ $\beta_{\text{sex*ln}(\text{area})} < 0$
M7. breed*area ^c	$\beta_{\text{breed}} > 0, \beta_{\text{area}} < 0$ $\beta_{\text{breed*area}} > 0$	$\beta_{\text{breed}} > 0, \beta_{\text{area}} < 0, \beta_{(\text{area})^2} > 0$ $\beta_{\text{breed*area}} > 0, \beta_{\text{breed*(area)}^2} < 0$	$\beta_{\text{breed}} > 0, \beta_{\ln(\text{area})} < 0$ $\beta_{\text{breed*ln}(\text{area})} > 0$
Roads			
M8. road1	$\beta_{\text{road1}} < 0$	$\beta_{\text{road1}} < 0, \beta_{(\text{road1})^2} > 0$	$\beta_{\ln(\text{road1})} < 0$
M9. road2	$\beta_{\text{road2}} < 0$	$\beta_{\text{road2}} < 0, \beta_{(\text{road2})^2} > 0$	$\beta_{\ln(\text{road2})} < 0$
M10. road3	$\beta_{\text{road3}} < 0$	$\beta_{\text{road3}} < 0, \beta_{(\text{road3})^2} > 0$	$\beta_{\ln(\text{road3})} < 0$
M11. roads	$\beta_{\text{roads}} > 0$	$\beta_{\text{roads}} > 0, \beta_{(\text{roads})^2} < 0$	$\beta_{\ln(\text{roads})} > 0$
M12. road1 + road2 + road3 + roads			
M13. sex*road1 ^d	$\beta_{\text{sex}} > 0, \beta_{\text{road1}} < 0$ $\beta_{\text{sex*road1}} < 0$	$\beta_{\text{sex}} > 0, \beta_{\text{road1}} < 0, \beta_{(\text{road1})^2} > 0$ $\beta_{\text{sex*road1}} < 0, \beta_{\text{sex*(road1)}^2} > 0$	$\beta_{\text{sex}} > 0, \beta_{\ln(\text{road1})} < 0$ $\beta_{\text{sex*ln}(\text{road1})} < 0$
M14. breed*road1 ^e	$\beta_{\text{breed}} > 0, \beta_{\text{road1}} < 0$ $\beta_{\text{breed*road1}} > 0$	$\beta_{\text{breed}} > 0, \beta_{\text{road1}} < 0, \beta_{(\text{road1})^2} > 0$ $\beta_{\text{breed*road1}} > 0, \beta_{\text{breed*(road1)}^2} < 0$	$\beta_{\text{breed}} > 0, \beta_{\ln(\text{road1})} < 0$ $\beta_{\text{breed*ln}(\text{road1})} > 0$

^aAll hypothesized models also include the terms from the best nonmanagement model.

^bAlso modeled sex*core, sex*edge, and sex*patch.

^cAlso modeled breed*core, breed*edge, and breed*patch.

^dAlso modeled sex*road2, sex*road3, and sex*roads.

^eAlso modeled breed*road2, breed*road3, and breed*roads.

only the second to investigate fecal corticosterone levels in any species of wild bird.

Estimating Management Covariates

We defined an owl territory as a circle with a radius of one-half the mean nearest-neighbor distance between territory centers (Hunter et al. 1995; Peery et al. 1999; Franklin et al. 2000). A territory center was the location of a nest site or, for non-nesting owls, the average Universal Transverse Mercator location of roost sites separated by at least one week in time. Most roost sites within a territory were near each other within the same forest stand. Mean nearest-neighbor distance was estimated from the year of highest owl density on our study area (1996).

We quantified habitat variables with a U.S. Forest Service Geographic Information System (GIS)-based vegetation map derived from Landsat satellite data (U.S. Forest Service Remote Sensing Lab, Sacramento, California) and performed all habitat analyses with ArcView 3.2 (Environmental Systems Research Institute, Redlands, California). Forest stands with different tree species, average dominant tree size, and/or canopy closure were represented by map polygons. Any stand with $\geq 10\%$ conifer cover was classified as conifer forest. Stands with a dominant tree size of ≥ 61.0 cm dbh were classified as mature for-

est, and stands with a dominant tree size between 30.5 and 60.9 cm dbh were classified as medium-sized forest.

To assess map accuracy, we surveyed randomly chosen points >80 m from a polygon edge. Random points were located with a Garmin 12XL Global Positioning System unit (Garmin International, Olathe, Kansas). At 81 locations of medium-sized and mature conifer forest, we recorded the species and dbh of all trees >15.2 cm dbh within 20×100 m plots centered at the random point and oriented in a random direction. We measured canopy cover with a vertical densitometer (Stumpf 1993) at 1-m intervals along a transect spanning the length of the plot. At 67 locations for all other vegetation types, we verified a priori classification by visual inspection.

Owl habitat (i.e., mature, high-cover conifer forest) map polygons were correct 85% of the time. However, there was a high rate of map omission for mature, high-cover conifer forest (i.e., 13 of 30 ground plots identified as this forest type were not classified as such on the map). Thus, the map indicated that several owl territories contained little or no mature, high-cover forest, which contradicted our direct field observations. Because California Spotted Owls forage and roost in most mature conifer forest and in medium-sized, high-cover ($\geq 70\%$) forest (Gutiérrez et al. 1992), we redefined owl habitat as all mature forest or medium-sized, high-cover forest. With

our redefinition, 27 of the 30 mature, high-cover forest plots were now classified as owl habitat on the map, and the map accuracy rate for owl habitat polygons increased to 90%. We then used the program Patch Analyst (Elkie et al. 1999) to calculate area, core, edge, and patch for each territory (Table 1).

To quantify the road covariates, we obtained GIS road coverages from the Eldorado and Tahoe National Forests and Lake Tahoe. We visually inspected a GIS map containing road and owl locations to determine that nearby roads were accurately located for each owl. We then used program XTools (Oregon Department of Forestry, Salem, Oregon) to calculate road1, road2, road3, and roads for each territory (Table 1).

Results

We collected 174 fecal samples from 64 owls on 45 territories. Thirty-two owls were males, and 32 were females. In addition, 50 were nonbreeding owls and 14 were breeding owls. On 19 occasions, multiple samples ($n = 55$) were collected from the same individual on the same day. After averaging all same-day corticosterone values, we had 138 samples, with the number of samples per individual ranging from 1 to 6. Regression analyses showed that fecal corticosterone was not strongly related to time of day in either a linear ($R^2 < 0.01$) or quadratic ($R^2 = 0.04$) manner.

The average fecal corticosterone level was 80.1 ng/g dry feces (SE = 75.8). Unlike a previous study of Northern Spotted Owls (Wasser et al. 1997), male and female corticosterone levels were similar (male $\bar{x} = 81.1 \pm 83.9$ ng/g; female $\bar{x} = 78.9 \pm 65.4$ ng/g). On 10 occasions, we also collected cecal samples simultaneously with fecal samples. Fecal corticosterone was significantly higher in cecal samples (cecal $\bar{x} = 186.7 \pm 110.5$ ng/g; fecal $\bar{x} = 112.0 \pm 99.9$ ng/g; $p = 0.001$ for a paired t test). Therefore, we excluded cecal samples from subsequent analyses.

The highest fecal corticosterone level observed in our study (575.70 ng/g dry feces) was collected in the evening from a male at a nest site within 200 m of active logging operations. This was the only sample collected during our study under such circumstances.

After performing the initial model-selection analyses with fecal corticosterone as the response variable, a statistical reviewer suggested that the data be checked for normal distribution and constant variance. The residuals from the best overall model were not normally distributed (Shapiro-Wilk test; $W = 0.685$, $p < 0.0001$), and the variances of the residuals increased with the mean (score test [Cook & Weisberg 1999]; $\gamma = 20.98$, $p < 0.0005$). After we performed a natural-log transformation of fecal corticosterone and repeated the analyses, the residuals from

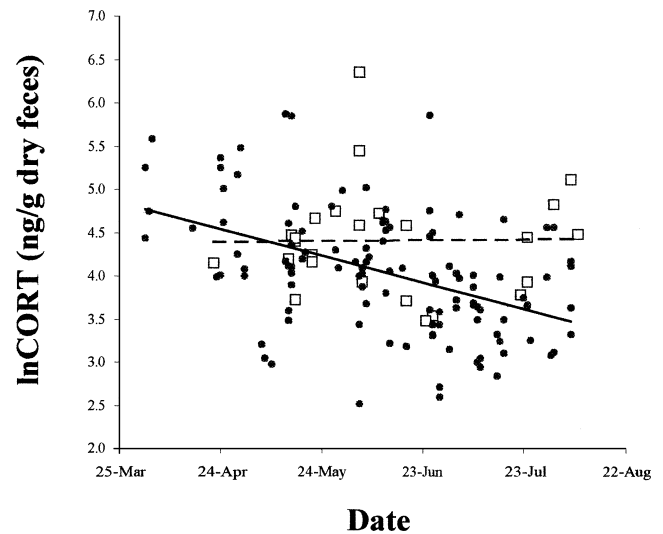


Figure 1. Natural log of fecal corticosterone levels (CORT) in California Spotted Owls, April–August 2001. Both nonbreeding (●) and breeding (□) owls are shown. Best-fit regression lines are shown for nonbreeding (solid line) and breeding owls (dashed line).

the best model were normally distributed ($W = 0.986$, $p = 0.18$), and the variances of the residuals were constant ($\gamma = 0.05$, $p = 0.83$).

The best covariance structure for the global model contained a single variance parameter for the fixed effects, σ^2 , indicating that there was little correlation among repeated samples from an individual. The best first-stage model (breed + date + breed*date) was 3.5 times as likely as the nearest competitor (storage + mass; see Table 4). Nonbreeding owls had higher corticosterone levels early in the breeding season and lower levels later in the breeding season (breed + date + breed*date model; $\beta_{\text{breed*date}} = -0.011 \ln[\text{ng/g}/\text{day}]$, 95% CI = $-0.020, -0.002$; see Fig. 1). However, only one sample was collected from a breeding owl during the first month of our study. Samples initially stored on ice had higher levels than those stored immediately on liquid N_2 (storage + mass model; $\beta_{\text{storage}} = 0.269 \ln[\text{ng/g}]$, 95% CI = $0.026, 0.512$), and small samples had higher levels than large samples ($\beta_{\text{mass}} = -6.248 \ln[\text{ng/g}]$, 95% CI = $-8.877, -3.620$; see Fig. 2).

In addition to the base terms, the best overall model contained $\ln(\text{edge})$. Fecal corticosterone was positively correlated with $\ln(\text{edge})$ (overall model; $\beta_{\ln(\text{edge})} = 0.200 \ln[\text{ng/g}]$, 95% CI = $-0.013, 0.414$). However, the management covariates contributed little explanatory power to our base model. The base model was only 1.28 AIC_c units behind the best overall model (Table 4), indicating substantial support for the simple base model (Burnham & Anderson 2002). In addition, a direct comparison of the base model with a model containing only $\ln(\text{edge})$ showed that $\ln(\text{edge})$ was comparatively

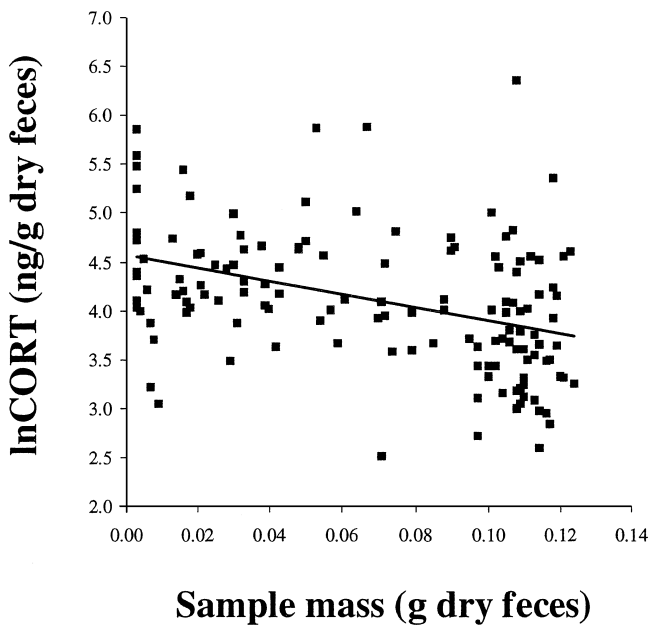


Figure 2. Natural log of fecal corticosterone levels (CORT) in California Spotted Owls versus mass of sample. A best-fit regression line is shown.

unimportant (base model, $R^2 = 0.22$; ln[edge] model, $R^2 = 0.02$; overall model, $R^2 = 0.24$).

Discussion

Habitat and road covariates were not highly correlated with fecal corticosterone levels despite the inclusion of many habitat and road models in our analyses. We found no evidence of an adverse road effect on Spotted Owl fecal corticosterone levels, in contrast to previous research (Wasser et al. 1997). This may have been due to differences in road traffic volume or forest structure between the two study areas. This also may have been evidence that owls on our study area are habituated to road traffic. Alternatively, the previous study may not have controlled for other influential factors; almost all explanatory power in our models was provided by biological and sampling factors.

We observed higher corticosterone levels in nonbreeding owls early in the breeding season, which did not contradict the hypothesis that elevated corticosterone levels may prevent birds from breeding (Wingfield et al. 1998). Unfortunately, logistic constraints and behavioral responses of birds limited our collection of samples from breeding birds early in the season. Heavy snowfall in early April prevented access to much of the study area early in the breeding season, breeding females were difficult to sample during the incubation period because they seldom left the nest, and proportionally fewer birds nested in 2001 than in other years. In fact, the inclement weather in

Table 4. Information-theoretic ranking of nonmanagement and overall models estimating California Spotted Owl fecal corticosterone levels in California Spotted Owls in the central Sierra Nevada, California, April–August 2001.

Model	K ^a	AIC _c ^b	ΔAIC _c	w _i ^c
Nonmanagement				
breed*date	6	276.14	0.00	0.577
storage + mass	5	278.65	2.51	0.164
breed + date	5	278.85	2.71	0.149
mass	4	281.30	5.16	0.044
sex*breed*date	9	282.51	6.37	0.024
Overall ^d				
ln(edge)	7	274.86	0.00	0.071
ln(area)	7	275.46	0.60	0.053
breed*ln(edge)	8	275.82	0.96	0.044
road1	7	275.96	1.10	0.041
edge	7	276.06	1.20	0.039
base model	6	276.14	1.28	0.037
edge + edge ²	8	276.22	1.36	0.036
ln(road1)	7	276.36	1.50	0.034
breed*ln(area)	8	276.52	1.66	0.031
ln(road2)	7	276.56	1.70	0.030
ln(core)	7	276.66	1.80	0.029
patch	7	276.66	1.80	0.029
area	7	277.06	2.20	0.024

^aNumber of parameters in model, including two variance parameters (one each for random and fixed effects).

^bAkaike's information criterion (AIC) adjusted for small sample size.

^cAkaike's information criterion weights.

^dAll overall models included the terms from the best nonmanagement model (i.e., breed, date, breed*date).

early April may have contributed to the elevated corticosterone levels in nonbreeding birds. Furthermore, the direction of a potential cause-and-effect relationship could be reversed, such that entering into breeding condition suppresses corticosterone secretion in breeding owls. In future studies, comparison of the two mechanisms will require estimation of basal corticosterone levels prior to the onset of the breeding season and sampling both breeding and nonbreeding owls early in the breeding season.

Our results suggest that some factors related to sampling must be considered in future avian fecal corticosterone studies. Because cecal samples contained greater quantities of corticosterone metabolites than fecal samples, they must not be used in the same analyses with fecal samples or allowed to contaminate them. The method of field storage may have influenced corticosterone level. Samples should be placed into cold storage within a few hours, or portable liquid N₂ containers should be used for field storage. Corticosterone metabolites in extremely small (0.01 g) samples were extracted and quantified by slightly adjusting the lab protocol, but the amount of corticosterone detected may have been overestimated. Therefore, future studies should either correct for sample-mass effects or small samples should not be used.

There was a large amount of variation present in our data, much of it unexplained by our predictive models.

In addition, samples taken from the same bird within the same day sometimes differed greatly (e.g., by nearly 200 ng/g dry feces in one instance), although time of day did not explain this variation. Some variation was undoubtedly due to factors that were unknown, such as individual differences in metabolism, susceptibility to stress, past experience, and recent exposure to stressors. Partial sampling of a fecal mass may also have been important. Frequently, only a portion of a fecal mass would be available for sampling because some of the feces would be caught by intervening foliage. If corticosterone metabolites were not uniformly distributed within the feces, this would have affected our measured levels. Recent food intake may have been important because fecal metabolite concentrations depend on the total mass of the feces. Rates of prey delivery to Mexican Spotted Owl nests, which may be indicative of prey capture rate, were not uniform over a 24-hour period or during nocturnal hours only (Delaney et al. 1999).

Conclusion

Noninvasive fecal monitoring of stress hormone levels in wildlife is less intrusive than repeated capture, handling, and blood withdrawal, and its use in wildlife research continues to be informative (Millsbaugh et al. 2001; Creel et al. 2002). In our study, anecdotal evidence (i.e., an extremely high level detected on one occasion during logging activity) suggested that the method may be useful for monitoring gross effects due to intense or prolonged disturbance on individual Spotted Owls. In addition, fecal samples appeared to be an integrated measure of recent corticosterone secretion because fecal corticosterone was not affected by time of day.

There was a large amount of variation in fecal corticosterone levels, however, much of it unexplained by our hypothesized a priori models. Only a few of our model terms (breeding status, interaction between breeding status and date, and sample mass) had a parameter estimate whose 95% confidence interval did not overlap 0. In contrast to a previous study, territory-scale measures of habitat condition and road proximity (factors that may be responsible for chronic stress) were not correlated with fecal corticosterone. Either this population of owls was not responding to these environmental factors, or high levels of inherent and/or sampling variation were masking our ability to detect relationships. Detecting subtle relationships between these factors and wild Spotted Owl fecal corticosterone levels will require substantial data-collection effort to obtain the necessary sample sizes, given that our collection efforts were often time-intensive and unsuccessful. In our opinion, future studies should initially focus on carefully designed experiments under controlled laboratory conditions that will yield more precise information on fac-

tors that may affect fecal corticosterone levels—such as sample handling, partial sampling, and food intake—and the sensitivity of this species to chronic stress at different times of the year. If necessary, a more common but closely related surrogate species, such as the Barred Owl (*S. varia*), could be used in such studies.

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