
Noninvasive Physiological Measures of Disturbance in the Northern Spotted Owl

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Introduction

The Northern Spotted Owl (*Strix occidentalis caurina*) continues to decline across its entire range at a rate exceeding 4% per year, with a 1% annual acceleration (Burnham et al. 1994). One possible factor impeding its recovery is the lack of adequate measures that reflect the impact of habitat disturbance on this species (Hanson et al. 1993). Measures of reproductive success between individual owl sites and areas are critical to evaluating long-term effects of management in species such as the Northern Spotted Owl. The considerable time-span from nesting to fledging, however, makes it difficult to pinpoint individual factors associated with nest failure among the myriad sources of disturbance over the course of the breeding season. At the least, large numbers of breeding pairs and considerable time are required to use reproductive success to index such disturbance impacts. More immediate measures of disturbance could complement measures of reproductive success, providing a proactive tool to guide adaptive management in a manner that does not compromise owl survivorship and reproduction. We demonstrate that noninvasive hormone measurements of physiological stress in feces could provide an objective, readily accessible and cost effective means of quantifying impacts of disturbance in Northern Spotted Owls that can directly address pivotal conservation questions surrounding this and other listed species.

It is well-documented that physiological stress occurs in response to environmental pressure and affects resistance to disease, survival, and reproduction negatively in a variety of species (Moberg 1985; Munck et al. 1984;

Wingfield & Farner 1993). Until recently, however, the application of physiological stress measures to free-ranging threatened or endangered species has been severely limited by the invasiveness of sample collection procedures such as repeated capture and blood withdrawal. Fecal steroid measures provide an ideal alternative to serum measures because they are relatively easy to collect longitudinally, without disturbing study subjects. Results illustrate that fecal stress hormone disturbance measures can be used as a proactive management tool, enabling the industry and wildlife managers to optimize the trade-offs between resource utilization and proliferation of species at risk.

Methods

Captive Studies

Two studies were conducted in captivity to establish the reliability of our fecal corticosterone measures as an index of physiological stress in Northern Spotted Owls. The first study injected a captive female Northern Spotted Owl, housed at the Woodland Park Zoological Gardens, with adrenal corticotropic hormone (ACTH). This hormone is typically secreted into blood by the pituitary gland in response to an external stressor; it then travels to the adrenal gland, causing it to secrete corticosterone—the primary glucocorticoid stress hormone in birds. We injected 100 IU ACTH (Sigma, St. Louis, MO) in the owl's jugular vein at 0700 hours. All fecal samples were then collected hourly from time 0 until 48 hours post-infusion.

The second study examined the correspondence between blood and fecal corticosterone measures in the same captive owl. The female was moved from her usual

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enclosure to the Department of Animal Health for the duration of study. The move was undoubtedly stressful. However, every effort was made thereafter to minimize the owl's stress level. Blood sampling occurred 3 times per week in the morning immediately after a fresh fecal sample was obtained. The bird was captured by hand and held for blood withdrawal from the jugular vein within 3 minutes after capture.

The fecal sample, which tends to be encapsulated in a membrane, was removed from the urine/urate portion of the excreta and stored frozen. Approximately 0.1 g of the sample was then extracted using the techniques described by Wasser et al. (1994). Blood samples were centrifuged within 30 minutes after collection; the serum was then withdrawn and stored frozen until assayed. All fecal and serum samples were assayed using the ICN ^{125}I corticosterone assay (Costa Mesa, California). This anti-serum was shown to exhibit both parallelism and accuracy for owl serum and feces. Inter-assay coefficient of variation (CV) was 5.54 for a high sample (27% bound) and 3.0 for a low sample (62% bound; $n = 5$). Intra-assay CV was 5.18 ($n = 30$ pairs).

Wild Studies

Fecal samples were serially collected between October 1994 and September 1995 from 16 radiomarked Northern Spotted Owl pairs and 2 territorial singles on the Yakama Indian Reservation (YIR). Serial sampling also occurred during 1994 in the east side of the Wenatchee National Forest, checkerboarded by private lands (WNF+). Spot sampling occurred throughout Washington and Oregon during owl censusing by crews from the Washington Department of Fish and Wildlife, U.S. Fish and Wildlife Service, U.S. Forest Service, Washington Department of Natural Resources, and private industry scientists. Field crews noted their time of arrival in vicinity of the owl, time of owl defecation and sample collection, owl behavior, and possible sources of disturbance, including the crew's own activities. The fecal pellet was removed as described for captive studies and placed in a 7-mL vial containing 2.5 mL 90% ethanol as a preservative (Wasser et al. 1988). Preserved samples were generally transported at ambient temperature in 2-5 hours and then stored frozen until shipped to our laboratory, where they were lyophilized and ~ 0.05 g of the fecal powder was extracted as in captive studies.

Corticosterone hormone levels were compared between males and females centered within or beyond 0.41 km of a major logging road on the YIR during the 1994-1995 field seasons. The 0.41-km distance was based on U.S. Fish and Wildlife Service guidelines as the distance at which disturbance is likely to affect an owl. Site centers were nest trees for 12 of the 16 pairs (including 5 that eventually failed in their nesting attempts). The remaining four sites were activity centers

of radiomarked owls. The 1995 field season also discriminated between owls centered within versus beyond 0.41 km from recent (within 10 years) timber harvest on the YIR. Mean corticosterone levels were additionally compared between pairs being affected by two very different harvesting regimens in the WNF+ versus the YIR. Clear cutting is common in patches throughout the WNF+. In contrast, the Yakama Nation utilizes selective harvesting methods that typically retain >80% canopy closure despite harvests of about 5 billion board feet from its 157,831-ha managed forest area over the past 50 years (King 1993). Finally, the ad libitum spot samples collected throughout Washington and Oregon were used to compare corticosterone levels across time for each sex.

Results

Captive Studies

The adrenal response to the "ACTH challenge," measured by fecal corticosterone levels, paralleled that expected in blood. A rapid and significant increase in corticosterone excretion occurred in the fecal sample (from 20 to 140 ng/g) within 2 hours post-injection, sharply peaking at 720 ng/g 12 hours post-injection of ACTH. Corticosterone levels then returned to baseline (20 ng/g) by 26 hours post-injection. The ACTH challenge results demonstrate that the corticosterone metabolites we are measuring in feces accurately reflect the adrenal stress response. The 2-hour lag time is also consistent with the 2-hour lag time found between intravenous injection and fecal excretion of ^3H -corticosterone in surrogate Barred Owl (*Strix varia*) and Great Horned Owl (*Bubo virginianus*) species (Wasser & Monfort, unpublished data).

Serum and fecal corticosterone levels showed comparable responses following the transfer of the captive owl from her exhibit to the Department of Animal Health. A large, acute rise in serum and fecal corticosterone levels occurred in the first sample following the transfer; both serum and fecal corticosterone levels then returned to and hovered around baseline for the duration of the study ($r = 0.89$; $p < 0.0001$; $n = 37$). These combined results suggest that our fecal corticosterone measure provides a reliable index of physiological stress in the Northern Spotted Owl.

Wild Studies

Consistent with the literature for other avian species (Wingfield & Farner 1993), fecal corticosterone levels were significantly higher in adult male versus female owls in the wild ($p < 0.03$; $F = 4.89$, ANOVA). There were effects of proximity of forest management activity to the center of the owl's home range, which were largely confined to males; significant differences were

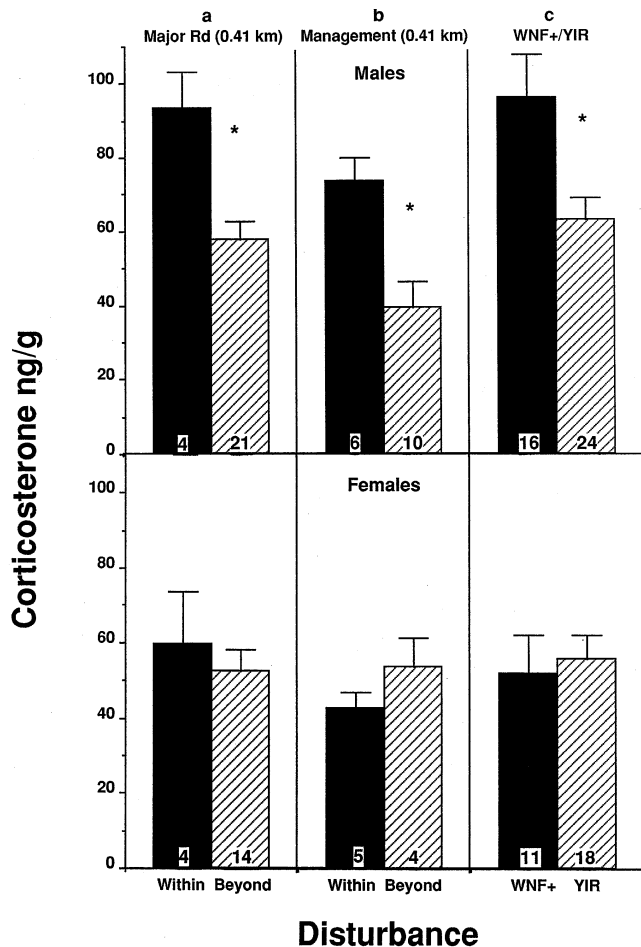


Figure 1. Mean fecal corticosterone levels in male and female Northern Spotted Owls under three separate conditions: center of home range on the Yakama Indian Reservation (YIR) was within versus beyond a 0.41 km radius from a major logging road (a) or timber harvest activity (b) and YIR versus the more intensively harvested Wenatchee National Forest/private industry lands checkerboard (WNF+), all on the eastern slope of the Cascade Mountains (c). Asterisk is $p < 0.01$. Sample sizes shown in bars refer to number of individuals, with an average of three samples per individual.

found between males centered within versus beyond 0.41 km from a major logging road ($p < 0.008$; $F = 8.54$, ANOVA) or recent (10 years to present) timber activity ($p < 0.004$; $F = 12.05$, ANOVA; Fig. 1a and b, respectively). Fecal corticosterone concentrations were also significantly greater ($p < 0.01$; $F = 7.7$, ANOVA) among males in the more intensively harvested WNF+ versus the YIR (Fig. 1c); but again, no differences were found among females. (No effect of observer presence on fecal corticosterone levels were found. However, one of the highest corticosterone samples (approaching levels 4–5

times higher than the means reported in Fig. 1) was obtained from a male approximately 1 hour after being captured, to change his radiotag, and released.

Finally, spot samples collected ad libitum by censusers across Washington and Oregon revealed no effect of season on males ($n = 91$). A significant effect of season was found for females, however, regardless of their nesting behavior ($p < 0.05$; $F = 3.2$, quadratic regression; $n = 74$). Mean (\pm SE) fecal corticosterone levels more than doubled from 39 ± 7 ng/g (maximum 91 ng/g) to 90 ± 17 ng/g (maximum 274 ng/g) during the 1.5-month time interval (June through mid-July) when young began to fledge, gradually falling back to 37 ± 8 ng/g by August.

Discussion

A late start and mid-season interruptions because of fires in July and August 1994 limited our sample size and prohibited us from making many collections during the critical post-fledging periods on the YIR. The resultant small sample size means our results should be interpreted with caution until replicated. Nevertheless, both captive and field data suggest that fecal stress hormone measures can provide a powerful, unbiased tool for evaluating impacts of disturbance, including forest practices, on the Northern Spotted Owl. The impacts on fecal corticosterone levels shown for males as a function of distance from timber management activity (Fig. 1a & b) suggest that fecal corticosterone measures may be particularly useful to address the controversial question regarding the distance surrounding the owl's nest that should be protected from timber management activity (Hanson et al. 1993). Higher corticosterone levels in males whose home ranges were in proximity to clear-cut versus selectively logged areas (Fig. 1c) suggest that impacts of different timber harvesting methods on owl survivorship may additionally become discernible with these fecal stress hormone measures. The temporal rise in fecal corticosterone levels during the 1.5-month interval when young began to fledge suggests that fecal corticosterone measures may also prove useful in addressing the equally controversial question regarding the window of time when timber harvest practices should be restricted over the breeding season of these birds.

The disturbance impacts tied to home range were largely confined to adult males, perhaps reflecting the predominantly male role in territorial defense. In contrast, physiological stress associated with offspring survival was largely confined to adult females, reflecting the predominantly female role in parental care. These sex differences are consistent with reports from a variety of avian species (Wingfield & Farner 1993).

Fecal hormone analyses appear to provide quantitative evaluation of habitat disturbance effects on Northern Spotted Owls that previously were inaccessible. These

measures may become particularly important with the adoption of habitat conservation plans (HCPs). By providing a reliable, accessible, and cost effective means of measuring physiological effects of disturbance, fecal stress measures may provide a tool that can guide adaptive management, reducing impacts of front loaded timber cuts in mature forest stands proposed by a number of HCPs. Better means of monitoring such impacts could help alleviate concerns over such policies expressed by the conservation community (Bean et al. 1991).

Reproductive hormone measures in feces have already proven to have broad applicability across a wide array of avian and mammalian species (e.g., Brown et al. 1994; Monfort et al. 1993; Schwarzenberger et al. 1995; Wasser 1995, 1996; Wasser et al. 1995, 1996). Because of the strong association of physiological stress with impaired health and reproductive performance among vertebrates (Moberg 1985; Munck et al. 1984; Wingfield & Farner 1993), accurate noninvasive measurement of physiological stress hormones in feces provides an important advancement of this fecal hormone technology. Fecal stress hormone measures, thus, may provide means to correct management problems before they become too severe—outcomes that should facilitate the objectives of private industry and wildlife conservation alike.

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