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Source: Ardea, 97(4) : 603-608

Published By: Netherlands Ornithologists' Union

URL: https://doi.org/10.5253/078.097.0428

Flushing effects and seasonal changes on corticosterone levels in adult Long-Eared Owls *Asio otus*

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Romero L.M., Holt D.W. & Petersen J.L. 2009. Flushing effects and seasonal changes on corticosterone levels in adult Long-Eared Owls *Asio otus*. In: Johnson D.H., Van Nieuwenhuyse D. & Duncan J.R. (eds) Proc. Fourth World Owl Conf. Oct–Nov 2007, Groningen, The Netherlands. Ardea 97(4): 603–608.

Long-eared Owls *Asio otus* were flushed and captured from communal winter roosts and nesting seasons to assess both initial and stress-induced corticosterone concentrations. We examined blood samples from 16 males and 8 females in the winter, and 16 males and 11 females in the breeding season. Corticosterone concentrations after flushing owls in either season were not correlated with the elapsed time from initial flush to capture, suggesting that these birds did not interpret flushing as stressful. In contrast, 30 min of handling and restraint during both seasons elicited robust increases in plasma corticosterone concentrations that did not differ by sex. Although stress-induced corticosterone levels did not differ seasonally, baseline levels were 50% lower during the winter compared to breeding, suggesting the breeding season is a more stressful time. These results indicate that capture techniques used in this study with Long-eared Owls were only stressful when successful, and that initial corticosterone concentrations vary seasonally.

Key words: Long-eared Owl, *Asio otus*, corticosterone, glucocorticoids, stress, breeding, non-breeding

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INTRODUCTION

There is considerable interest in using an individual animal's level of stress as a marker for the animal's well-being in its natural habitat. The presumption has been that if an animal is not doing well in its environment, be it from natural or manmade factors, the animal will show physiological signs of being under stress. Although many physiological pathways are activated during stress, such as catecholamine release and gluconeogenesis (Sapolsky *et al.* 2000), studies of wild free-living animals have focused primarily on levels of the glucocorticoid hormones (Wingfield & Romero 2001) since glucocorticoids increase during adverse environmental conditions. For example, natural occurrences such as inclement weather (Wingfield *et al.* 1983, Smith *et al.* 1994, Romero *et al.* 2000) and human-induced influences such as habitat destruction (Wasser *et al.* 1997) and pollution (Hopkins *et al.* 1997, Norris *et al.* 1997) have been shown to increase glucocorticoid levels.

In order to determine whether glucocorticoids are elevated in response to these kinds of stimuli, however, it is important to know the baseline glucocorticoid profile of the species under study. Previous studies of corticosterone (the primary glucocorticoid in birds (Holmes & Phillips 1976) have used a variety of capture techniques with a variety of different species (Wingfield 1994, Wingfield & Romero 2001). Different capture techniques, however, can alter the corticosterone titers that are measured (Romero & Romero 2002). Some nocturnal predators, such as Long-eared Owls *Asio otus*, are often captured by flushing birds from their daytime roosts or nests into pre-erected mist nets. The primary goal of this study was to determine whether flushing was itself stressful to these birds and whether this capture technique could allow measurement of baseline corticosterone concentrations.

In addition, many avian species modulate corticosterone concentrations seasonally (Romero 2002). Thus our second goal was to measure corticosterone

concentrations in different seasons. We captured Long-eared Owls during both non-breeding (winter) and breeding (spring) seasons in order to determine whether seasonal differences exist in either non-stressed or stress-induced corticosterone concentrations.

METHODS

Study area

Our study was conducted in the Missoula and Mission Valleys of western Montana, USA. The valleys are characterized by rolling hills of sagebrush, grasslands, kettle-hole ponds, man-made reservoirs, creeks, and rivers. Basically these are farm, ranch, and conservation lands. Vegetation where the owls' nest consists of mixed native and introduced species. Native trees and shrubs include; Ponderosa Pine *Pinus ponderosa*, Douglas Fir *Psudotsuga menzeseii* Rocky Mountain Juniper *Juniperus scopulorum*, Quaking Aspen *Populus tremuloides*, Black Cottonwood *P. trichocarpa*, Chokecherry *Prunus virginiana*, hawthorn *Crateagus* spp., willow *Salix* spp., Wood Rose *Rosa woodsii*, and Snowberry *Symphoricarpus occidentalis*. Introduced species include Russian Olive *Eleagnus angustifolia*, Siberian Elm *Ulmus pumilla*, Caragana *Caragana aborascens*, apple and pear *Pyrus* spp., and honeysuckle *Lonicera* spp. Farming, ranching, and wildlife management are the predominant occupations affecting the land in the Mission Valley, while urban development affects the land in the Missoula Valley.

Capture techniques

Male and female Long-eared Owls were captured in the Missoula (Deschamp Ranch, Missoula, 46°54'N, 114°07'W) and Mission (Ninepipe National Wildlife Refuge, Charlo, 47°27'N, 114°07'W; and Pablo National Wildlife Refuge, Polson, 47°38'N, 114°11'W) valleys of Montana, USA. The yearly Long-eared Owl research has been separated into three seasons (DWH, this study); non-breeding (October–February/March); breeding (February–July); and migration/post-breeding movement (June–October). Birds were captured during the winter from 21–23 January and 15–21 December 1997, and during breeding from 14–18 May 1997 and 17–22 April 1999. All trapping occurred during the day (between 9:00 and 17:00) while birds were either using daytime roosts or while females were brooding chicks on their nests. Both laboratory (Breuner *et al.* 1999, Joseph & Meier 1973, Rich & Romero 2001, Romero & Remage-Healey 2000) and field studies (e.g. Wingfield *et al.* 1992, Wingfield *et al.* 1994) indicate that baseline corticosterone concentrations are uniformly low throughout the day, although this has never been verified in a nocturnal avian species. However, one laboratory study of the Western Screech Owl (*Megascops kennicottii*) suggested that baseline samples are similar during day and night (Dufty & Belthoff 1997). Communal winter roosts (of generally 4–10 owls) and nests were in isolated stands of predominantly native or in man-made shelterbelts and deciduous thickets. Wintering birds did not always remain in the same area to breed (Holt 1997), so we could not capture the same owls in two different seasons.

During the winter, we erected 10 cm mesh mist nets of various lengths along natural flight corridors within a roosting area. We then flushed the owls from their roosts towards the nets. If the owls missed the nets, they would generally drop into a new roost within the same tree stand. After processing the captured birds (which took 10–20 min), we flushed the remaining birds a second time, but the time from flush to capture was always recorded from the initial flush at each site. Flushing continued every 10–20 min until the owls were either captured or until 60 min had elapsed from the first time the bird was flushed (one bird flew into the net after 60 min while we were removing the net). The owls were not continuously flushed for 60 min. For all birds, we initiated timing from when the bird initially began to move, although birds could potentially have initiated a stress response earlier when we first entered the roost area. Sexes were distinguished by plumage characteristics and confirmed through mark-recapture techniques (DWH unpubl. data, and DWH in Marks *et al.* 1994: 2).

During breeding, females were captured by flushing them from the nest into a mist net. If females missed the net, we would try to lure them back to the nest by remaining at the nest and hoping their nest defence behaviour drew them into a net. Although this could be stressful (Silverin 1998), this trapping technique has not caused any nest abandonment or reduced fledging success in 22 yrs of study (DWH unpubl. data). Breeding males were captured by flushing them from their daytime roosts and by erecting mist nets around the nest and inducing them to initiate nest-defense – similar to females.

Blood samples

Blood samples were taken from all birds as soon as possible from the time they were captured in the net, with the majority sampled within 2–3 min of capture. Birds were then held in the hand for 30 min, representing a

30 min period of restraint, which is known to induce stress and stimulate corticosterone release (Wingfield & Romero 2001). During this period birds were weighed, measured, sexed, and banded. At the end of the restraint period (i.e. 30 min post-capture) birds were bled a second time to measure stress-induced corticosterone levels. All birds were captured only once during the study.

All blood samples were collected by puncturing the brachial vein with a sterile 18 gauge hypodermic needle. Upwelling blood was collected in microhematocrit tubes and stored on ice. Cotton stanched blood flow between bleeds. Blood samples were centrifuged at approximately 400 g to separate plasma from red blood cells within 12 hours of collection. The plasma was then removed, stored frozen, and transferred to Tufts University for analysis.

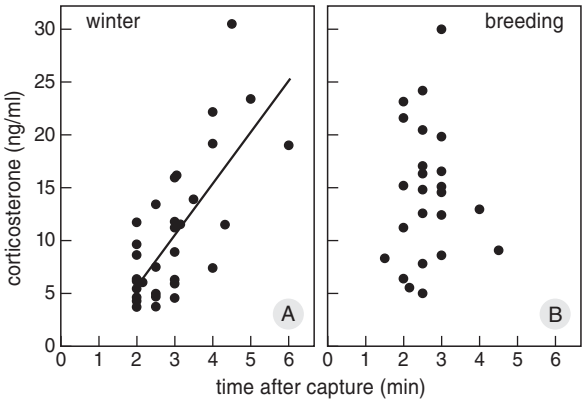

Figure 1. Relationship between the time after capture and corticosterone levels in the initial blood sample taken from Long-eared Owls during the winter (A) and while breeding (B).

Sample analysis

Plasma samples were analyzed for corticosterone using a radioimmunoassay as described by Wingfield *et al.* (1992). Briefly, samples were equilibrated with a small amount of tritiated corticosterone to monitor the recovery and corticosterone was extracted using dichloromethane. A known amount of tritiated corticosterone and corticosterone antibody were added to each sample and corticosterone concentrations were determined from the bound/unbound ratio in comparison to a standard curve. Samples from both years were mixed and run in two assays, with inter- and intra-assay variations of 12% and 8%, respectively.

Statistics

Only birds from whom the initial blood sample was taken within 3 min of capture, and again after 30 min post-restraint, were included in these analyses. Differences in corticosterone levels between males and females, between winter and breeding seasons, and over the course of the 30 min restraint period were analyzed by repeated measures ANOVA after testing for heteroscedasticity. Correlations between corticosterone levels and both time after flushing and time after capture were determined using linear regression. Alpha levels were considered significant at $P < 0.05$.

RESULTS

Capture techniques

There were no sex differences in either initial or restraint-stress-induced concentrations in the winter ($F_{1,22} = 0.15$, $P = 0.70$) for 16 males (initial concentrations of 7.5 ± 0.9 and concentrations after 30 min of restraint of 41.7 ± 4.5) and 8 females (initial concentrations of 7.1 ± 1.0 and concentrations after 30 min of restraint of 39.1 ± 7.8). In addition, there were no sex differences during breeding ($F_{1,25} = 0.89$, $P = 0.36$) for 16 males (initial concentrations of 14.6 ± 1.1 and concentrations after 30 min of restraint of 41.7 ± 2.7) and 11 females (initial concentrations of 20.4 ± 3.5 and concentrations after 30 min of restraint of 42.8 ± 4.9). Furthermore, there was no interaction between sex and the time course of corticosterone release at either season ($F_{1,22} = 0.06$, $P = 0.8$ for winter birds, and $F_{1,25} = 1.0$, $P = 0.32$ for breeding birds). Because male and female corticosterone levels did not differ, nor did sex interact with the increase in corticosterone after restraint, the sexes were combined in all further analyses. Furthermore, corticosterone concentrations were not correlated with body mass (data not presented), so body mass was ignored.

The elapsed time after capture before taking the first blood sample had a significant effect on initial corticosterone levels during the winter (Fig. 1). Corticosterone levels increased the longer it took to complete the first bleed ($F = 34.4$, $P < 0.0001$, $R^2 = 0.53$, $n = 33$). However, when the analysis was restricted to only those birds bled within 3 min of capture, there was no significant increase ($F = 2.52$, $P = 0.13$, $R^2 = 0.11$, $n = 24$). During breeding, all but 2 birds were bled within 3 min of capture and there was no significant increase in corticosterone levels with elapsed time (Fig. 1; $F = 0.35$, $P = 0.56$, $R^2 = 0.012$, $n = 30$).

We recorded the time from flushing to capture for 38 birds (all bled within 3 min of capture) over both seasons (Fig. 2). There was no relationship between

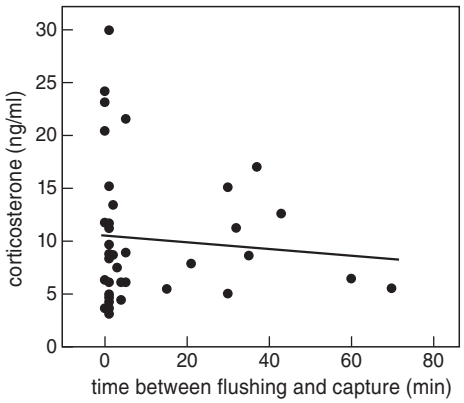

Figure 2. Relationship between the time since flushing and corticosterone levels in initial blood samples taken within 3 min of capture in Long-eared Owls.

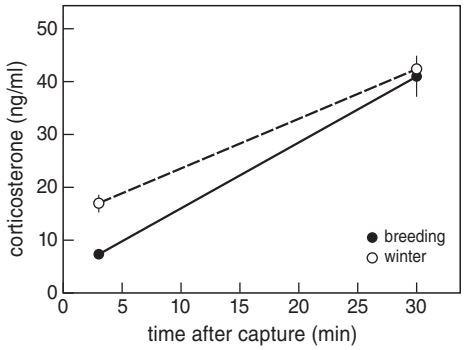

Figure 3. The corticosterone response to capture and handling during winter and breeding in Long-eared Owls. $n = 24$ and 27 for winter and breeding seasons, respectively. Each point represents the mean ± SE.

time after flushing and corticosterone levels during the initial bleed ($F = 0.27$, $P = 0.60$, $R^2 = 0.008$, $n = 38$). Furthermore, all baseline samples collected 20–70 min after flushing were lower than corticosterone concentrations after 30 min of restraint (see Fig. 3).

Corticosterone in different seasons

Since samples collected under 3 min did not differ significantly (see above), these baseline samples were grouped together for statistical purposes. Long-eared Owls dramatically elevate corticosterone concentrations (Fig. 3) over the course of a 30 min period of handling and restraint during both seasons (for overall effect of bleeding time, $F_{1,49} = 164$, $P < 0.0001$, $n = 24$ and 27 for winter and breeding, respectively). Indeed, corticosterone levels increased almost 3-fold for the breeding season and almost 6-fold for the winter season (Fig. 3). All samples from birds whose initial bleed was taken more than 3 min post capture were removed from the analysis in order to calculate a repeated measures ANOVA.

Long-eared Owls also showed a significant seasonal difference in their initial corticosterone levels (Fig. 3). Samples taken within 3 min of capture were over twice as high during breeding than they were during the winter (for overall effect of season, $F_{1,49} = 4.57$, $P < 0.04$). However, there was no difference between stress-induced corticosterone levels at 30 min (for interaction between season and bleeding time, $F_{1,49} = 3.32$, $P = 0.75$).

DISCUSSION

Long-eared Owls showed no sex differences in either initial (samples taken within 3 min of capture) or restraint-stress-induced corticosterone concentrations. This parallels data from many passerine species (Wingfield *et al.* 1994, Astheimer *et al.* 1995, Romero *et al.* 1997), but other species have shown distinct sex differences, especially during breeding (e.g. Wingfield *et al.* 1992, Schoech *et al.* 1999, O'Reilly & Wingfield 2001). Few studies have measured corticosterone concentrations in free-living raptors, and to our knowledge, this is the first report of plasma corticosterone concentrations in an adult owl. Furthermore, Dufty & Belthoff (1997), did not see a sex difference in captive juvenile Western Screech Owls. Although it is unknown why such a large variation existed in our initial corticosterone concentrations, all owls were roosting and undisturbed prior to flushing, making it unlikely that birds were stressed prior to our arrival.

It does not appear that simply flushing Long-eared Owls from daytime roosts results in elevated corticosterone (Fig. 2). Owls captured approximately 30 min after the initial flush did not have elevated corticosterone levels when bled within 3 min. Animals other than humans may also disturb the owls' daytime roosting, and owls may simply move without becoming stressed. Our data support this hypothesis. Other studies have shown that activities that humans might perceive as stressful are not necessarily perceived that way by the animal (Silverin 1998).

On the other hand, corticosterone in Long-eared Owls is clearly sensitive to capture, handling, and restraint (Fig. 3). Initial corticosterone concentrations, however, are much lower during the winter, consistent with data from a variety of other avian species (reviewed by Romero 2002), including captive

American Kestrels *Falco sparverius* (Rehder *et al.* 1986). Although we lack a theoretical understanding of seasonal changes in corticosterone concentrations (Romero 2002), Long-eared Owls apparently seasonally modulate initial corticosterone levels as do many passerines. However, Long-eared Owls do not seasonally modulate stress-induced corticosterone concentrations. Regardless of season, 30 min of handling and restraint stimulated corticosterone concentrations to similar levels. This contrasts with data from other species, where the stress-induced corticosterone concentrations also varied seasonally (e.g. Wingfield *et al.* 1992, Romero *et al.* 1997).

In conclusion, these data indicate that corticosterone concentrations can be monitored in Long-eared Owls. Encouragingly, whereas capture and handling clearly elicit a corticosterone response, the data suggest that flushing birds does not, which should be important for management considerations. Finally, it is clear that in order to use corticosterone concentrations as a long-term background measure for assessing the health of Long-eared Owls, as well as other species, account must be taken of the natural seasonal variation of corticosterone levels in free ranging wild animals.

ACKNOWLEDGEMENTS

We thank Mike Maples and Anne Paulson for help trapping owls. We thank private land owners, Flathead Indian Reservation Department of Wildlife, Montana Fish Wildlife and Parks, and United States Fish and Wildlife Service for access to lands. This study was funded in part by grants from The Owl Research Institute, Tufts University, and the U.S. National Science Foundation (IOB-0542099) to LMR. This study was also approved by the Tufts University Animal Care and Use Committee.

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SAMENVATTING

Het doel van dit onderzoek was om door bepaling van het gehalte aan stresshormonen (de corticosteronspiegel) vast te stellen in hoeverre vangacties stress bij uilen veroorzaken en in hoeverre de corticosteronspiegel tussen de seizoenen varieert. Hiertoe werden Ransuilen *Asio otus* opgejaagd van hun winterslaapplaats (16 mannetjes en 8 vrouwtjes) of van het nest (16 mannetjes en 11 vrouwtjes). In geen van de seizoenen was de corticosteronspiegel gecorreleerd met de tijd die verstreken was tussen het verjagen en vangen, wat erop wijst dat het opjagen niet als stressvol werd ervaren door de vogels. Wel waren in de winter de gehaltes 50% lager dan in de zomer, wat mogelijk een gevolg is van de stress in het broedseizoen. Tijdens het vasthouden en behandelen van de uilen schoot de corticosteronspiegel omhoog, een patroon dat zowel in de winter en tijdens de nestfase zichtbaar was en zowel bij mannetjes als vrouwtjes optrad.

ARDEA

TIJDSCHRIFT DER NEDERLANDSE ORNITHOLOGISCHE UNIE (NOU)

ARDEA is the scientific journal of the Netherlands Ornithologists' Union (NOU), published bi-annually in spring and autumn. Next to the regular issues, special issues are produced frequently. The NOU was founded in 1901 as a non-profit ornithological society, composed of persons interested in field ornithology, ecology and biology of birds. All members of the NOU receive ARDEA and LIMOSA and are invited to attend scientific meetings held two or three times per year.

World Owl Conference Special

Editors – David H. Johnson, Dries Van Nieuwenhuyse and James R. Duncan, in cooperation with Jouke Prop and Rob G. Bijlsma

Technical editor – Jouke Prop

Dutch summaries – Arie L. Spaans, Dries Van Nieuwenhuyse, Jouke Prop, Rob G. Bijlsma, or authors

Graphs and layout – Dick Visser

Drawings – Jos Zwarts

Cover photos - Serge Sorbi
front – Snowy Owl
back – Snowy Owl, Great Grey Owl and young Tengmalm's Owl

Production – Hein Bloem, Johan de Jong and Arnold van den Burg

Printed by Van Denderen, Groningen, The Netherlands, December 2009