Corticosterone as a Measure of Stress in Nest-Bound and Nest-Departed Long-Eared Owl Asio otus Chicks

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Source: Ardea, 97(4) : 593-596

Published By: Netherlands Ornithologists' Union

URL: https://doi.org/10.5253/078.097.0426
INTRODUCTION

Glucocorticoids have been shown to be released by animals during times of stress, such as food shortage, habitat alterations, pollution, predation, research, and adverse weather (Wingfield et al. 1983, Smith et al. 1994, Hopkins et al. 1997, Norris et al. 1997, Wasser et al. 1997, Romero et al. 2000). Indeed, corticosterone, the primary avian glucocorticoid (Holmes & Phillips 1976) is rapidly secreted into the bloodstream following a stressful stimulus (Sapolsky et al. 2000). Non-stressed corticosterone levels appear to function primarily for physiological maintenance, whereas stress-induced corticosterone levels are believed to help regulate emergency functions (Dallman et al. 1993, Sapolsky et al. 2000). Perhaps the most important response to a stressful stimulus is behaviour, such as escaping a predator, fleeing inclement weather, or temporarily or permanently abandoning an area. The relationship between corticosterone release and these behaviours was supported by several studies (e.g. Astheimer et al. 1992, Wingfield & Ramenofsky 1997, Breuner et al. 1998, Romero & Wingfield 2001).

In addition to emergency behaviours in response to stressors, corticosterone may also play a role in behaviours linked to normal life history events. For instance, in owls, corticosterone has been implicated in natal and juvenile dispersal for Eastern and Western Screech Owls (Megascopsasio and M. kennicottii) (Ritchison et al. 1992, Dufty & Belthoff 1997, Belthoff & Dufty 1998) and pre-fledging nest departure in Snowy Owls Bubo scandiacus (Romero et al. 2006).

Long-eared Owl Asio otus is a nocturnal, open-country foraging species (Holt 1997). Only female Long-eared Owls incubate eggs for approximately 25–30 days, and the young hatch asynchronously (Marks et al. 1994, Holt et al. 1999). Females feed and brood nestlings for the first three weeks of life, while males provide food. After young Long-eared Owls branch or depart from their nests at about 22 days old (Seidensticker et al. 2006), they hide in surrounding vegetation for about two more weeks, and begin their first flights at about 35 days old (Seidensticker et al. 2006).

In many bird species, it has been suggested that nest-bound chicks may leave their nests early because of some stressful stimulus. For example, food competi-
tion, food shortage, reduced food delivery by parents, predation, sibling rivalry, size differences among nestlings, among others reasons (Holt et al. 1992, Heath 1997, Romero et al. 2006). Because Long-eared Owls exhibit early nest departure behaviour, we were able to test the influence of corticosterone titres in nest-bound and nest-departed chicks before fledging. Our hypothesis was that an increase in corticosterone titres was the trigger that initiated nest departure.

**METHODS**

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 ponds, man-made reservoirs, creeks, and rivers. Basically these are farm, ranch, and conservation lands. We located four owl nests in the Missoula Valley (46°54' N, 114°07'W) in 1997, and nine nests in the Mission Valley (47°27'N, 114°07'W and 47°38'N, 114°11'W) in 1998. Within these nests, we acquired blood samples from 46 chicks; 21 in 1997 and 25 in 1998. Of these, 19 chicks were sampled within the nest and 27 chicks that had departed the nest. All nests were approached by foot and climbed with a ladder or freehand. Chicks were passed down to colleagues to be sampled. Blood samples were taken from the brachial vein with a sterile 18 gauge hypodermic needle. We collected 60 to 120 μl of blood in heparinized microhematocrit tubes. We then staunched the blood flow with cotton. Time is a critical factor when collecting blood for comparing corticosterone levels to a stress response (Romero & Romero 2002). All chicks were sampled within three min of the initial start of the climb or handling and then again 30 min later. The period between the initial bleeding and 30 min was the restraint period and is known to induce a stress response and consequently the release of corticosterone (Wingfield & Romero 2001). Blood was centrifuged within 12 h at 400 g to separate plasma from red blood cells. Plasma was stored frozen and transferred to Tufts University for analysis. Plasma was analyzed for corticosterone titres using a previously published radioimmunoassay (see Wingfield et al. 1992, Romero et al. 2006). Briefly, plasma samples were equilibrated with small amounts of tritiated corticosterone and then extracted with redistilled dichloromethane. Samples were dried with nitrogen, and then re-suspended in a sodium phosphate buffer. Bound and unbound fractions were separated with dextran-coated charcoal. To determine plasma corticosterone concentrations, bound fractions were counted, compared to a standard curve and adjusted by the percent recovery (see Romero et al. 2006). All samples were included in a single assay and the intra-assay variability was 4.6%.

Comparisons between nest-bound and nest-departed chicks were made using a mixed model repeated measure ANOVA with nest identity as a random variable to control for potential nest effects. We followed the main ANOVA with Tukey’s post hoc tests. Alpha levels were set at P < 0.05.

**RESULTS**

Both nest-bound and nest-departed chicks increased their corticosterone titres over 30 min of handling and restraint ($F_{1,44} = 24.2, P < 0.0001$), although the increase was much more robust in the nest-departed chicks (Fig. 1). Although there was no overall difference in nest-bound and nest-departed chicks ($F_{1,44} = 1.94, P = 0.17$), there was a significant interaction between nest status and the corticosterone response to restraint ($F_{1,44} = 5.26, P < 0.03$). Post hoc analysis indicated that baseline titres were not different, but nest-bound chicks had significantly lower corticosterone titres at 30 min than did nest-departed chicks.

**DISCUSSION**

There is an abundance of evidence that a stressful stimulus elicits a corticosterone response in a wide variety of animals (Harvey et al. 1984, Sapolsky et al. 2000, Romero et al. 2006), including habitat alterations in Northern Spotted Owls *Strix occidentalis caurina* Wasser et al. (1997).

In the tree-nesting Long-eared Owl, chicks depart their nests at approximately 22 days (Seidensticker et al. 2006) and do not fledge until about 35 days old. It is unknown why Long-eared Owl chicks depart their nests approximately two weeks before they can fly. Given that there was no difference in corticosterone values between nest-bound and nest-departed Long-eared Owl chicks, stress does not appear to be the mechanism driving pre-fledging nest-departure. Seidensticker et al. (2006) felt that pre-fledging nest-departure was probably an artifact of predation pressure and not necessarily a stressful nest situation.

Previous studies on pre-fledging nest departure in ground-nesting Short-eared Owl *Asio flammeus* and Snowy Owl chicks suggest predation pressure was the selective force driving this behaviour. In ground-nesting
Similar results were reported for Snowy Owl chicks; corticosterone titres remained similar for all chicks. Anywhere from 3–6 chicks departed from each nest and food provisioning likely affected our results because, from their natal area. Similarly, neither brood size or sex of the young Western Screech Owls at the time of dispersal appear to influence pre-fledging nest-departure in Northern Spotted Owls and attributed their results to differences in male and female parental duties.

Our results fit a growing pattern of juvenile birds having a dampened corticosterone response to restraint that slowly changes to match the adult response as the chicks age. Studies of Northern Mockingbirds Mimus polyglottos (Sims & Holberton 2000), Common Redpoll Carduelis flammea (Romero et al. 1998), and White-crowned Sparrows Zonotrichia leucophrys (Wada et al. 2007) all show that younger chicks have lower corticosterone responses than older chicks. Consequently, the lower corticosterone titres after restraint in our nest-bound chicks may also represent a developmental process.

In summary, our data show that stress does not appear to influence pre-fledging nest-departure in Long-eared Owl chicks. In observations of 204 Long-eared Owl nests over 23 years, DWH has not witnessed brood reduction. Thus, we do not think that aspects of stress, brood size or sex of the young induce pre-fledging nest departure. Rather, other factors such as predation may be responsible.

**ACKNOWLEDGEMENTS**

We thank the many volunteers who have helped trap and band Long-eared Owls over the past 23 years. We also thank the private land owners, Flathead Indian Reservation Department of Wildlife, Montana Department of Fish Wildlife and Parks, and the United States Fish and Wildlife Service, for permission to work on their lands. Partial funding was provided by US National Science Foundation grants (IBN-9975502 and IOB-0542099) to LMR.

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**SAMENVATTING**

Jonge Ransuilen Asio otus verlaten het nest wanneer ze ongeveer 22 dagen oud zijn. Het duurt dan nog bijna twee weken voordat ze kunnen vliegen. Men veronderstelt dat het stresshormoon corticosteron een rol speelt bij het vertrek van jonge vogels uit het nest. Om dit te onderzoeken werden hormoonspiegels gemeten bij jonge Ransuilen die nog op het nest zaten en bij jongen die het nest al hadden verlaten. Corticosterongehalten verschilden niet tussen beide groepen (10,69 ± 1,37 en 9,29 ± 1,58 ng/ml) wat erop wijst dat het verlaten van het nest niet door stress gestuurd wordt. Dertig minuten nadat de jonge uilen waren gepakt, en daarmee bloot gesteld waren aan stress, waren de corticosterongehalten minder toegenomen bij de vogels op het nest dan bij de jongen die het nest al hadden verlaten (14,62 ± 1,98 en 21,58 ± 2,22 ng/ml). Kennelijk reageren jongen op het nest zwakker op stressprikkel dan jongen die niet meer aan het nest gebonden zijn. Mogelijk komt dit omdat de vorming van corticosteron gebonden is aan de leeftijd van de vogels.
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Printed by Van Denderen, Groningen, The Netherlands, December 2009