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

Published By: Netherlands Ornithologists' Union

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

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Corticosterone as a measure of stress in nest-bound and nest-departed Long-eared Owl *Asio otus* chicks

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Holt D.W., Paulson A. & Romero L.M. 2009. Corticosterone as a measure of stress in nest-bound and nest-departed Long-eared Owl *Asio otus* chicks. Ardea 97(4): 593–596.

Long-eared Owl *Asio otus* nestlings usually depart from their nests at approximately 22 days of age, and cannot fly until approximately 35 days of age. Corticosterone has been implicated as a mechanism influencing nest departure in many avian species. We sampled corticosterone concentrations in wild nestling and nest-departed Long-eared Owl chicks to determine if this stress hormone influenced nest departure. Baseline corticosterone titres were found to be similar in nest-bound and nest-departed young (10.69 ± 1.37 vs. 9.29 ± 1.58 ng/ml respectively), suggesting that stress was not the trigger for nest departure. Nest-bound chicks however did show lower stress-induced titres levels than nest-departed chicks (14.62 ± 1.98 vs. 21.58 ± 2.22 ng/ml, respectively). This suggests that nest-bound chicks may have a blunted response, perhaps due to age-related developmental constraints influencing corticosterone secretion.

Key words: corticosterone, Long-eared Owl, *Asio otus*, nestling, nest-bound and nest-departed chicks, stress

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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 (*Megascops asio* 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-

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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 equilibriated 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

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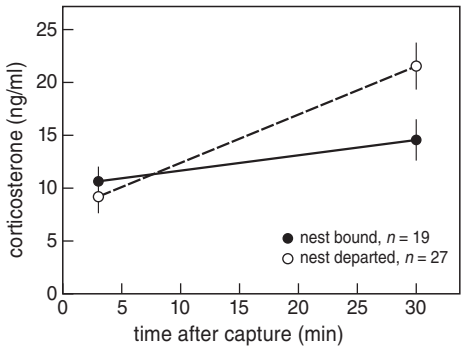


Figure 1. Corticosterone response to capture and restraint in Long-eared Owl chicks both remaining at the nest (nest-bound) and roosting on branches away from the nest (nest-departed). Each point represents the mean ± SE for the samples indicated.

Short-eared Owls, Holt *et al.* (1992) suggested that selective pressures for early nest departure (14–16 d) was probably mammalian predation – conceivably a stressful stimulus. Short-eared Owls do not fledge until approximately 30 days old (Holt *et al.* 1992). Romero *et al.* (2006) reached the same conclusion for Snowy Owls, whose chicks depart their ground nests at about 18–20 days of age, and are flightless for approximately 21– 28 more days on the treeless tundra.

Contrary to the above however, wild American Kestrel *Falco spaverius* corticosterone levels did increase just prior to leaving the nest (Heath 1997). Similar to many small owls, Kestrels are cavity nesters. To our knowledge corticosterone titres have not been evaluated in relation to nest departure on any cavity nesting owl species. The cavity-nesting juvenile Eastern and Western Screech Owls studied by Ritchison *et al.* (1992), Dufty & Belthoff (1997), Belthoff & Dufty (1998) were not part of a nest departure study. In Belthoff & Dufty (1998), increased corticosterone titres in captive birds correlated with juvenile dispersal in free living birds, however juvenile or natal dispersal is different than pre-fledging nest departure.

Although nest-bound Long-eared Owls cannot be sexed by plumage, given our sample sizes, we must have sampled both male and female chicks. We suggest that the sex of the owl probably had no influence on nest-departure. Dufty & Belthoff (1997) reported no difference in corticosterone titres between sexes of young Western Screech Owls at the time of dispersal from their natal area. Similarly, neither brood size or food provisioning likely affected our results because, anywhere from 3–6 chicks departed from each nest and corticosterone titres remained similar for all chicks. Similar results were reported for Snowy Owl chicks (Romero *et al.* 2006). However, in a brood size manipulation study on nesting Barn Owls *Tyto alba*, Roulin *et al.* (1999) detected an effect of brood size on nestlings but not on parents. With two additional young placed in the nest, nestling mortality was higher, and body mass of the surviving male and female nestlings was lower in enlarged than reduced broods.

In another study, there were no differences between corticosterone titres in adult breeding and non-breeding male and female Long-eared Owls (Romero *et al.* 2009). In contrast, Wasser *et al.* (1997) found differences in corticosterone samples between adult 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. Corticosterongehaltes 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 corticosterongehaltes 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 stressprikkels 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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