PHEOMELANIN PIGMENT IS NOT AN INDICATOR OF FEATHER CORTICOSTERONE CONTENT IN DIURNAL MIGRATORY RAPTORS IN IDAHO

by

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DEDICATION

Dedicated to Dr. Alfred M. Dufty Jr.

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ABSTRACT

In some cases, pigmentation can be used as an indicator of measures of condition. In this study, I tested the relationship between reddish pheomelanin pigmentation and the amount of corticosterone, a hormone associated with stress, sequestered in the feathers. I predicted that with higher corticosterone in feathers individuals would exhibit increased brightness and reduced saturation of pheomelanin pigments in their feathers. I collected spectral data from feathers from American Kestrels (*Falco sparverius*), Cooper's Hawks (*Accipiter cooperii*), and Sharp-shinned Hawks (*A. striatus*) during migration in southwestern Idaho over two years (2010-2011). After extracting corticosterone from these same feathers, I measured concentration using radioimmunoassay and compared this measure of condition to the melanin pigment data using analysis of covariance. I determined that there was no statistical relationship between feather corticosterone and pheomelanin pigmentation. The physiological impact of circulating corticosterone on pheomelanin production during feather development was not apparent in this natural setting.

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LIST OF ABBREIATIONS

ACTH	Adrenocorticotropic Hormone
AHY	After Hatch Year
ANCOVA	Analysis of Covariance
C	Celsius
df	Degrees of Freedom
Fig.	Figure
g	Gram
HPLC	High Performance Liquid Chromatography
IBO	Intermountain Bird Observatory
LOWESS	Locally Weighted Scatterplot Smoother
mL	Milliliter
mm	Millimeter
N	Sample Size
NC	North Carolina
pg	Picogram
SAS	Statistical Analysis System
SD	Standard Deviation
U	Unknown
USGS	United States Geological Survey
UV	Ultra Violet

α	Statistical significance level
α-MSH	α-Melanocyte Stimulating Hormone

INTRODUCTION

Relationships between the expression of pigments and body condition or fitness can be seen in a number of avian species. For instance, female Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*) with brighter carotenoid coloration also have higher hematocrit (Newbrey and Reed 2011). Additionally, pheomelanic red morph Tawny Owls (*Strix aluco*) have lower lifetime fitness (Brommer et al. 2005). Thus, in many cases, coloration in birds can be considered an honest signal of body condition or health.

Coloration in birds is produced by pigments in feathers (or skin), structural components, or a combination thereof. At times, there is also interplay among a number of pigments to result in the final color of birds. For instance, birds may be red in coloration because of the presence of carotenoids, turacins, psittacofulvins, porphyrins, pheomelanin, iron oxides, and/or hemoglobin (Toral et al. 2008). The focus of my research is on the reddish brown pheomelanin-based pigmentation present in a number of diurnal birds of prey.

Bird feathers contain elements and compounds other than just pigments for coloration, however. For instance, hormones are also incorporated into feathers as a reflection of plasma levels (Bortolotti et al. 2008). Interestingly, because these hormones are incorporated into developing feathers at the same time as the pigment, relationships between hormone levels and coloration may develop. For example, Barn Owl (*Tyto alba*) nestlings implanted with corticosterone show less red saturation in the pheomelanic

contour feathers of their breasts (Roulin et al. 2008). This is thought to be caused by tyrosine inhibition or negative feedback of corticosterone with ACTH.

Pheomelanic features are honest signals for communicating condition in a number of avian species. In Tawny Owls, for instance, individuals that are darker red in coloration exhibit greater production and greater maintenance of antibodies (Gasparini et al. 2009). Melanin pigments are indicators of maternal immunocompetence and are hereditary (Roulin et al. 2000; Gasparini et al. 2009). Pheomelanin pigment affects pairing, breeding rate, and reproductive success in certain bird species such as Barn Owls and Tawny Owls (Roulin 1999; Brommer et al. 2005; Roulin and Altwegg 2007). In Eurasian Kestrel (*Falco tinnunculus*) females, browner pheomelanic heads correspond to earlier laying dates (Parejo et al. 2011). In Blue-tailed Bee-eaters (*Merops philippinus*), males with a darker reddish throat patch have a better body condition (Seifferman et al. 2007). These relationships between pheomelanin and body condition are summarized in Table 1.

The role of glucocorticoids in altering expression of pheomelanin in bird feathers is somewhat poorly understood. Barn Owls exhibit lower red saturation after having corticosterone implants (Roulin et al. 2008), but not after nestling handling (Almasi et al. 2010). In contrast, when American Kestrels (*Falco sparverius*) are implanted with corticosterone there is no discernable change in pigmentation (Butler et al. 2010). Barn Swallows (*Hirundo rustica*) also fail to show a relationship between pheomelanism and baseline or induced levels of corticosterone (Jenkins et al. 2013).

The objective of my research was to further understand potential relationships between feather corticosterone and feather coloration in diurnal birds of prey by

conducting an observational experiment on migrating raptors in southwestern Idaho. To do so, I captured individuals of three species of migrating raptors each of which expressed pheomelanin pigmentation, quantified the representation of pheomelanin pigmentation in their feathers, and examined coloration in relation to feather corticosterone levels. If feather pigmentation were indeed related to corticosterone levels, I expected the birds with the lowest feather corticosterone to be darkest, and birds with the highest feather corticosterone to be least pigmented.

Table 1 Pheomelanized features of birds that are and are not correlated with condition with possible mechanisms when known.

Species	Pheomelanized feature	Relationship	Mechanism	Source
American Kestrel	Tail rectrix and back contour	Corticosterone implant yields no effect		Butler et al. 2010
Barn Owl	Belly, breast, underwings, flanks	No assortative mating based on reddish-brown appearance		Roulin 1999
Barn Owl	Body	Less pheomelanic males recruited more often	Perhaps sexual selection	Roulin and Altwegg 2007
Barn Owl	Breast contours	Less saturation with corticosterone implantation	Tyrosine inhibition or negative feedback corticosterone & ACTH	Roulin et al. 2008
Barn Owl	Breast contours	Nestling handling has no effect on melanin expression.		Almasi et al. 2010
Barn Swallow	Breast contours	Baseline, induced, and corticosterone responsiveness are not predicted by melanin.	Competing mechanisms	Jenkins et al. 2013
Blue-tailed Bee-eaters	Throat contours	Darker in males with better body condition	Social environment, dietary amino acids, or minerals	Seifferman et al. 2007
Eurasian Kestrel	Head	Earlier laying date		Parejo et al. 2011
Tawny Owl	Facial disc, back, wings, tail, head, breast,	Pheomelanic morph has lower lifetime fitness	Brown individuals may be more visible to predators	Brommer et al. 2005
Tawny Owls	Body	Dark red birds maintain antibodies longer	Pale birds may have more efficient immunity or dark birds have more exposure to antigens.	Gasparini et al. 2009
Yellow Warbler	Breast contours	Area pheomelanized corresponds to feather condition		Grunst et al. 2014
Yellow Warbler	Breast contours	No relationship with feather corticosterone		Grunst et al. 2015

METHODS

Study Species

I chose three species of diurnal North American raptor for examination of the relationships between pheomelanin and feather corticosterone: American Kestrels, Cooper's Hawks (*Accipiter cooperii*), and Sharp-shinned Hawks (*Accipiter striatus*). I selected these species because each displays pheomelanin pigmentation and was abundant in my study area.

American Kestrel

American Kestrels are the smallest falcons in North America (Clark and Wheeler 2001; Sibley 2003). Males average 24 cm in length, 55 cm in wingspan, and 109 g in mass (Clark and Wheeler 2001). Females average 25 cm in length, 57 cm in wingspan, and 123 g in mass (Clark and Wheeler 2001). American Kestrels are cavity, crevice, and box nesters that feed primarily on insects and small mammals (Clark and Wheeler 2001; Sibley 2003). Generally they lay 4-5 eggs per clutch (Smallwood and Bird 2002). Adults and young migrate from breeding grounds in northern latitudes between mid-August and the end of November (Smallwood and Bird 2002).

American Kestrels are sexually dimorphic. Males have blue wings with black spots and a rufous tail with a black subterminal band. Females have rufous wings with spots and a barred rufous tail (Clark and Wheeler 2001; Smallwood and Bird 2002). The ventral side of both males and females is white to rufous with variable streaking and spotting. Relevant to my study of feather pigmentation, Clark and Wheeler (2001)

describe them as the “only North American falcon with reddish on tail and back.” This red pheomelanin pigment in the tail was the focus of portions of my study on American Kestrels.

Cooper’s Hawk

Cooper’s Hawks are medium-sized *Accipiters* that nest in trees (Sibley 2003). They prey upon small birds and mammals (Clark and Wheeler 2001; Sibley 2003; Curtis et al. 2006). Males average 39 cm in length and 73 cm in wingspan, with a mass of 341 g (Clark and Wheeler 2001). Females measure 45 cm in length on average, 73 cm in wingspan and have an average mass of 528 g (Clark and Wheeler 2001). Clutch sizes average 3 to 4 eggs per clutch (Curtis et al. 2006). Fall migration occurs from the end of August to the beginning of November (Curtis et al. 2006). Juveniles have brown streaking on the ventral side of their bodies, while adults have rufous barring (Curtis et al. 2006). This barring and streaking of the breast contours comprised of pheomelanin pigmentation were the color elements of interest in my study.

Sharp-Shinned Hawk

Sharp-shinned Hawks are the smallest members of the *Accipiter* genus in North America (Bildstein and Meyer 2000; Clark and Wheeler 2001; Sibley 2003). Males have an average length of 26 cm, wingspan of 54 cm, and mass of 101 g (Clark and Wheeler 2001). Females average 31 cm in length, 62 cm in wingspan, and 177 g in mass (Clark and Wheeler 2001). They build nests near the trunks of trees, usually conifers, in which they typically lay 4-5 (up to eight) eggs (Bildstein and Meyer 2000). Sharp-shinned Hawks prey primarily on small birds (Bildstein and Meyer 2000; Clark and Wheeler 2001; Sibley 2003). Fall migration occurs from early August through late November

(Bildstein and Meyer 2000). Adults have reddish barring on the ventral side of their bodies, whereas juveniles have brown to reddish ventral streaking (Bildstein and Meyer 2000; Clark and Wheeler 2001). These reddish pigments on the ventral contours of adult and juvenile Sharp-Shinned Hawks are what I analyzed in this study.

Study Site

I studied relationships between feather corticosterone and feather pigmentation in American Kestrels, Cooper's Hawks, and Sharp-shinned Hawks captured at two migration banding sites located near Boise, Idaho, which were operated by the Intermountain Bird Observatory (IBO). In addition to feather pigments and corticosterone, I recorded the sex, age, and size for each captured bird. All but four of the birds that I studied were captured at Lucky Peak (43°36′N, 116°05′W; elevation 1845 m) in Ada County, Idaho. The remaining four birds (2 Cooper's Hawks and 2 Sharp-shinned Hawks) were from Boise Peak (43°42′N, 116°05′W; elevation 1989 m) in Boise County, Idaho. The trapping stations at both Lucky Peak and Boise Peak are in open areas dominated by shrubs and grassland at the edge of Douglas-fir (*Pseudotsuga menziesii*) forests. Carlisle et al. (2004) provide a detailed description of the topography and plant cover in each area. As the two banding stations are only 11 km apart, there is no reason to believe that different populations of birds migrated through each. Thus, for the purpose of my study, I combined birds from both capture locations. I also pooled data from two years (2010-2011) for analysis.

Capture, Banding, and Feather Collection

Using dho-ghazza, bow-net, and mist net traps baited with House Sparrows (*Passer domesticus*), Eurasian Collared Doves (*Streptopelia decaocto*), and/or Rock

Pigeons (*Columba livia*) as lures (Bloom et al. 2007), IBO staff captured the birds I studied. Many of the captured birds were likely migrants, but there were local breeding populations of each species as well.

Upon capture, birds were banded with USGS aluminum leg bands for identification, and wing chord (to 1 mm), tail length (to 1 mm), and mass (to 1 mm) were recorded for each. Based on Gustafson et al. (1997), IBO staff assigned age and sex for each captured raptor. Briefly, they assigned sex in both Cooper's and Sharped-shinned Hawks based on morphometrics and determined age by plumage and eye color. Individuals with reddish breasts, slate gray backs, and orange to red eyes were classified as After Hatch-Year (AHY). They deciphered sex in American Kestrels using plumage dimorphism. Age of the American Kestrels could sometimes be determined by the appearance of wing molt, but many of the kestrels in my study were aged as Unknown (U).

From each Cooper's Hawk and Sharp-shinned Hawk, I collected three contour feathers from within the breast region that expressed reddish pigment (Figure 1). I plucked the feathers individually from random locations that spanned the lower breast. For American Kestrels, I collected the rectrix immediately adjacent to the outermost from either the left or right side, as these feathers were consistently pigmented with pheomelanin. I chose to pluck entire feathers rather than cut portions because I expected plucking to induce new feather growth quickly (White et al. 1991), whereas cut feathers would not be replaced until the next molt. I initially stored the feathers in plastic zip-lock bags placed in a dark shoe box to prevent fading. I later transferred them to opaque coin envelopes and stored them at room temperature until analysis.

Spectroscopy

After collecting feathers from American Kestrels, Cooper's Hawks, and Sharp-shinned Hawks, I subjected the feathers to spectroscopy using an Ocean Optics USB4000 spectrometer with an Ocean Optics PX-2 pulsed xenon light source to generate colorimetric data. The spectrometer was calibrated to a white standard (ws-1-sl) and light exclusion. Following Seifferman et al. (2007), I used cellophane tape to affix the three contour feathers from each Cooper's Hawk and Sharp-shinned Hawk to black, non-glossed, non-textured cardstock (Figure 1). I did so with the brown to red distal portion of each feather overlapping. I taped the American Kestrel tail feathers individually to black cardstock. Based on my visual determination of the reddest portion of each feather sample, I measured reflectance curves for six randomly selected points within those portions. I made the measurements at a 90° angle of incidence with the probe fixed at 5 mm from the feather (Surmacki et al. 2011). I allowed the instrument 30 s to equilibrate to each point before recording measurements. Ultimately, I transformed the mean reflectance of these six measurements into color variables of Brightness and Saturation using the CLR and RCLR programs in R (Montgomerie 2008a, b). Brightness is best described as total area under the reflectance curve or total light reflectance within the spectral range measured. Essentially, brightness is the sum of reflectance. The equation for Total Brightness where λ max = 700 nm, λ min = 300 nm, and R = percent reflectance is:

$$B_1 = B_T = \int_{\lambda\, min}^{\lambda\, max} R_i = \sum_{\lambda\, min}^{\lambda\, max} R_i$$

Saturation is the proportion of reflectance within the red spectrum between 605 nm and 700 nm and expressed as S1R, spectral purity, or the reflectance ratio:

$$S_{1R} = \sum_{\lambda\,605}^{\lambda\,max} R_i \Big/ \sum_{\lambda\,min}^{\lambda\,max} R_i = \sum_{\lambda\,605}^{\lambda\,max} R_i \Big/ B_1$$

To ensure consistency, I measured coloration of all of the feathers of a given species within the same session and made comparisons only within rather than across species. I ran all calculations within species rather than compare between species. I applied a locally weighted scatterplot smoothing (LOWESS) function to the reflectance curves. As a result of differences in local peaks among species resulting from different measurement sessions, I used different smoothing coefficients (f) for each species (Figure 2). The spectral curves match those of pheomelanin as represented in Toral et al. (2008; Figure 3). Hereafter, for brevity, I refer to total brightness as brightness and red saturation as saturation.

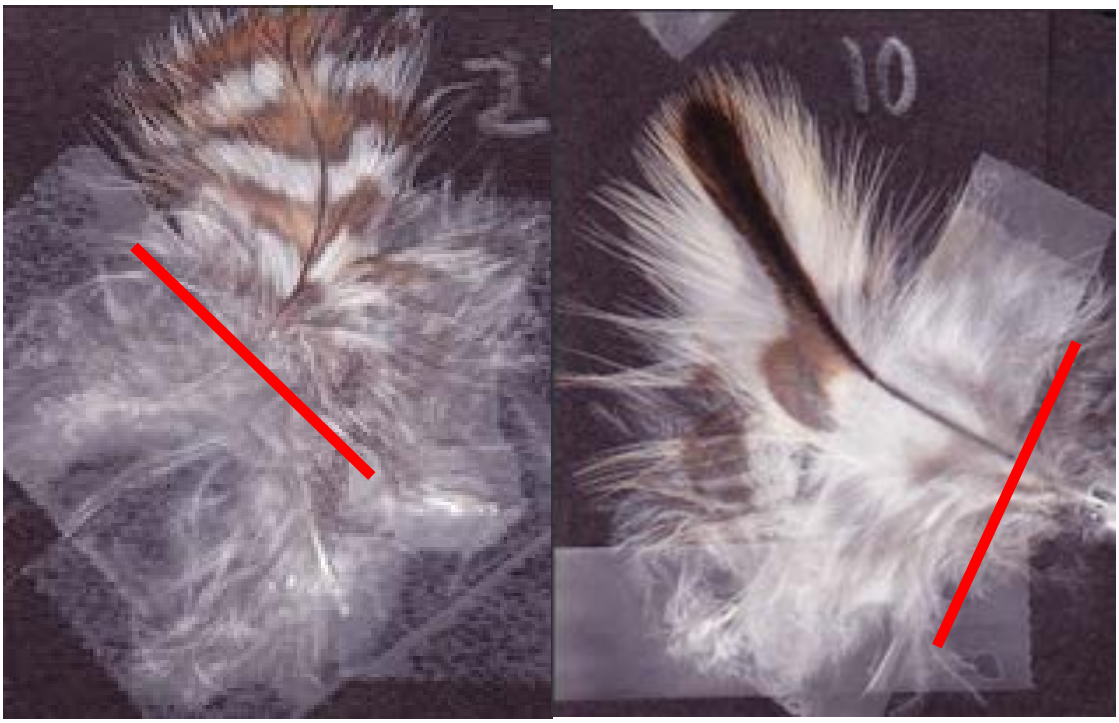

Figure 1 Feathers from a Sharp-shinned Hawk (left) and Cooper's Hawk (right) shown cellophane-taped to black cardstock. Red lines indicate approximate location of removal of plumaceous portion of feathers.

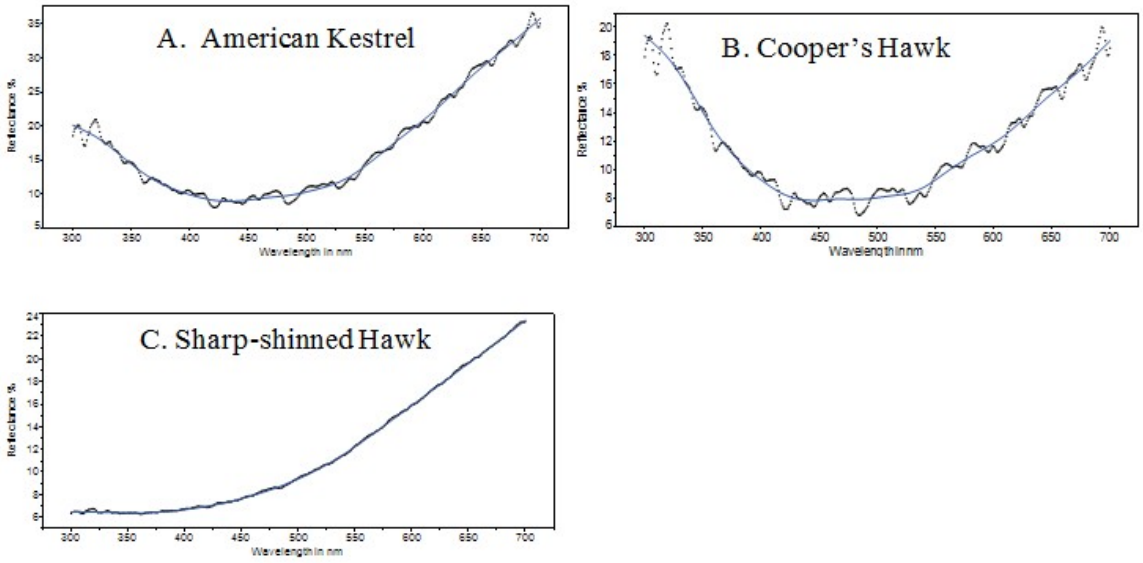

Figure 2 Reflectance curves from the different species. Black dots indicate original data. The blue line is the smoothed curve with LOWESS applied. Smoothing functions: American Kestrel (A) f=0.15, Cooper's Hawk (B) f=0.20, Sharp-shinned Hawk (C) f=0.05.

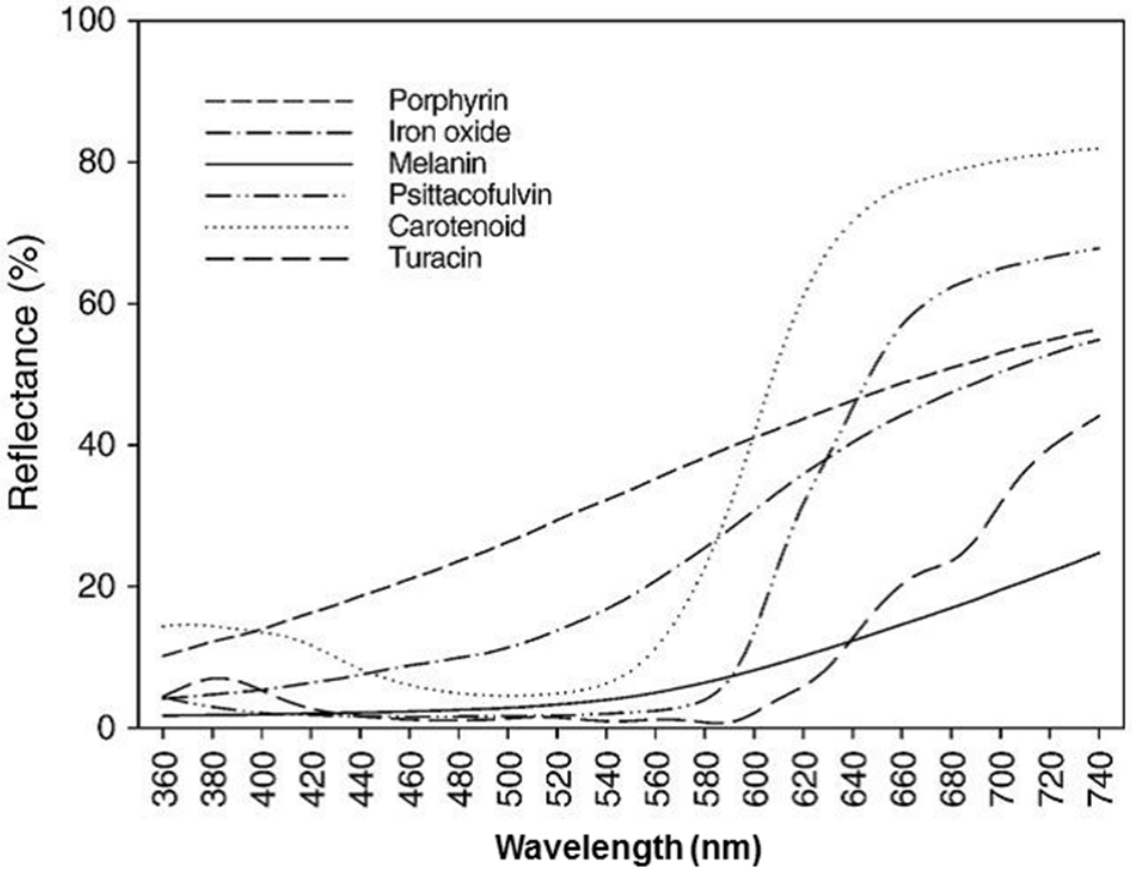

Figure 3 Graph of spectral reflectance for six types of red pigment (from Toral et al. 2008).

Feather Corticosterone Analysis

As I was interested in the potential relationship between feather color and corticosterone, I measured feather corticosterone content using radioimmunoassay following Bortolotti et al. (2008). I prepared the contour feathers for analysis by cutting above the plumaceous portion, which also removed the afterfeather from analysis (see Figure 1). The feathers were then cut into <5 mm^2 pieces within 20-ml glass scintillation vials (to prevent feather material from drifting away during cutting), capped and sonicated in HPLC grade methanol for 30 min. before overnight incubation at 50° C. The following day, under a fume hood, I filtered the feathers and methanol using a faucet aspirator and a vacuum filtration flask with a filter plug in the funnel stem made from

aquarium filter polyester wool. The solvent/hormone extracts were captured in another scintillation vial in the filtration flask elevated to the stem of the funnel by a bed of fish tank gravel. I rinsed the funnel and filter with methanol after processing each sample. The vials containing the extract were removed from the filtration flask with forceps and left uncapped in a 50° C water bath overnight to evaporate the methanol. Using radiolabeled corticosterone through the same filtration process, I found that the extraction efficiency averaged 97.6%. Corticosterone in the extracts were then analyzed by Tracy Marchant of the University of Saskatchewan using radioimmunoassay following procedures outlined in Wayland et al. (2002).

Statistics

I used JMP Pro v 10.0 (SAS Institute, Inc., Cary NC) to conduct analysis of covariance (ANCOVA) to examine potential effects of age, sex, and feather corticosterone on feather coloration (brightness, saturation). In the case of American Kestrels, I used only sex as a covariate because age was unknown for most. For Cooper's Hawks and Sharp-shinned Hawks, I created a categorical variable with four levels that described both age and sex (e.g., adult male, adult female, juvenile male, and juvenile female). For each species, I then tested for equal slopes among age and/or sex categories while examining the relationship between feather corticosterone and color. If there were homogeneous slopes as judged by an interaction term that was not significant, I removed interaction terms and tested for equal intercepts among the levels of the categorical variable. If sex or age/sex categorical factors were significant, I conducted post-hoc comparisons among levels using Student's *t*-tests. I present means ± 1 SD throughout and consider results significant using $\alpha = 0.05$.

RESULTS

Between 31 August and 21 October of 2010 and 2 September and 25 October of 2011, I measured wing chord, tail length, and mass and collected feather samples from 136 (72 female, 64 male) birds captured at Lucky Peak and Boise Peak in Southwestern Idaho.

American Kestrel

I analyzed morphometrics, corticosterone, and characteristics of pheomelanin pigments in feathers from 37 American Kestrels, including individuals of both sexes (Table 2). The female American Kestrels (N = 12) had wing chords and tails that ranged between 192-201 mm, and 115-130 mm in length, respectively. They weighed 115-149 g. Brightness and saturation for these females ranged between 55.40-84.13 and 0.38-0.42, respectively (Table 2). Male American Kestrels (N = 25) had wing chords between 176-199 mm in length, tails that were 110-131 mm in length, and body mass from 101-152 g. These males ranged in brightness from 58.49-77.86 and saturation from 0.39-0.43 (Table 2).

The interaction term in the ANCOVA for both brightness and saturation was not significant; thus, there was no lack of homogeneity of slopes (Table 3, Figs. 4, 5), so I removed the interaction term for each subsequent analysis. With the interaction terms removed, there was no significant effect of corticosterone or sex on either of the color variables (Table 4).

Table 2 Morphometrics, feather corticosterone, and feather color variables ($\bar{x}$ ± SD) for male and female American Kestrels captured at Lucky Peak and Boise Peak, Idaho during 2010 and 2011. Sample sizes for females, males, and total are in parentheses.

Attribute	Female (12)	Male (25)	Total (37)
Wing Chord (mm)	196.8 ± 2.62	189.3 ± 5.49	191.8 ± 5.91
Tail (mm)	124.6 ± 5.05	122.4 ± 5.72	123.1 ± 5.53
Mass (g)	131.8 ± 8.64	117.0 ± 11.43	122.2 ± 12.47
Corticosterone (pg/mm)	8.16 ± 3.93	7.51 ± 3.29	7.72 ± 3.47
Brightness ($R_{300-700}$)	70.08 ± 9.72	67.61 ± 5.53	68.41 ± 7.12
Saturation	0.40 ± 0.01	0.40 ± 0.01	0.40 ± 0.01

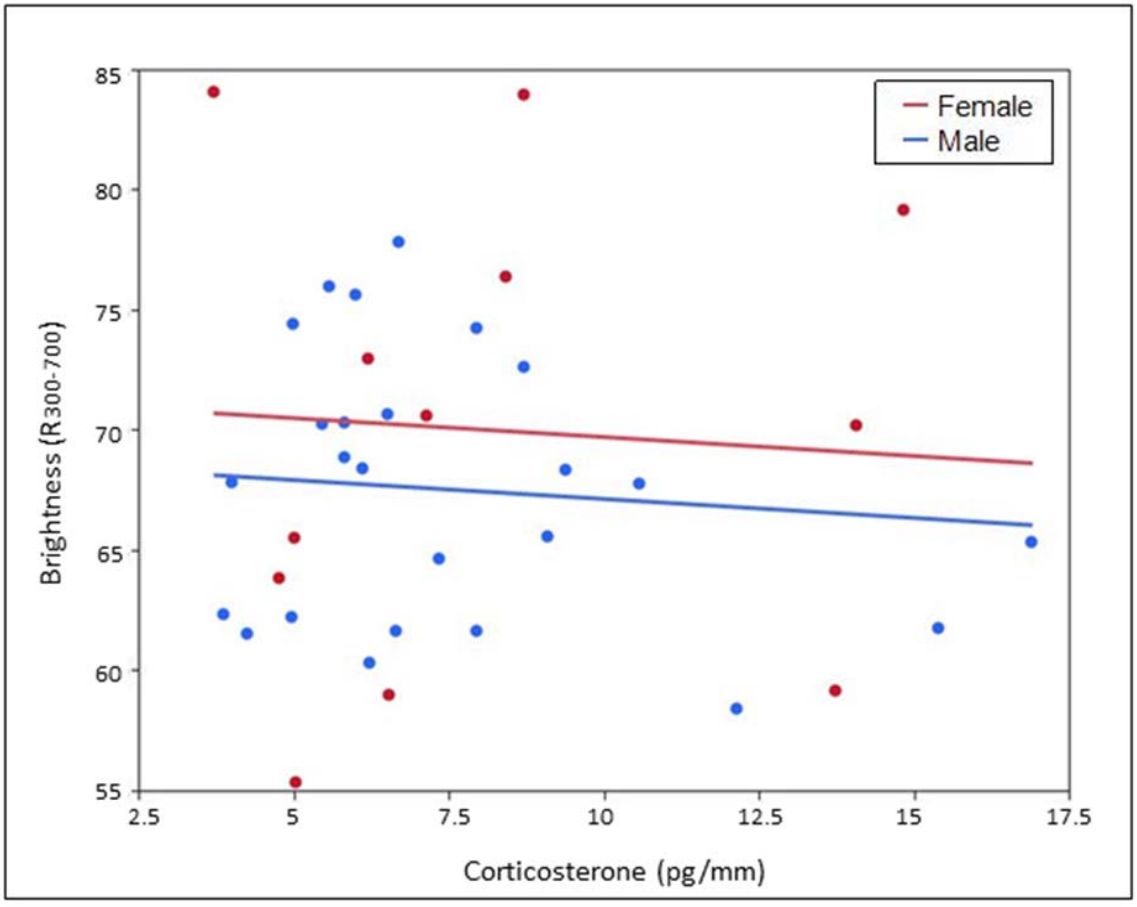

Figure 4 Relationship between sex, feather corticosterone (pg/mm), and brightness ($R_{300-700}$) of pheomelanin-pigmented feathers from American Kestrels captured during 2010-2011 at Lucky Peak and Boise Peak, Idaho.

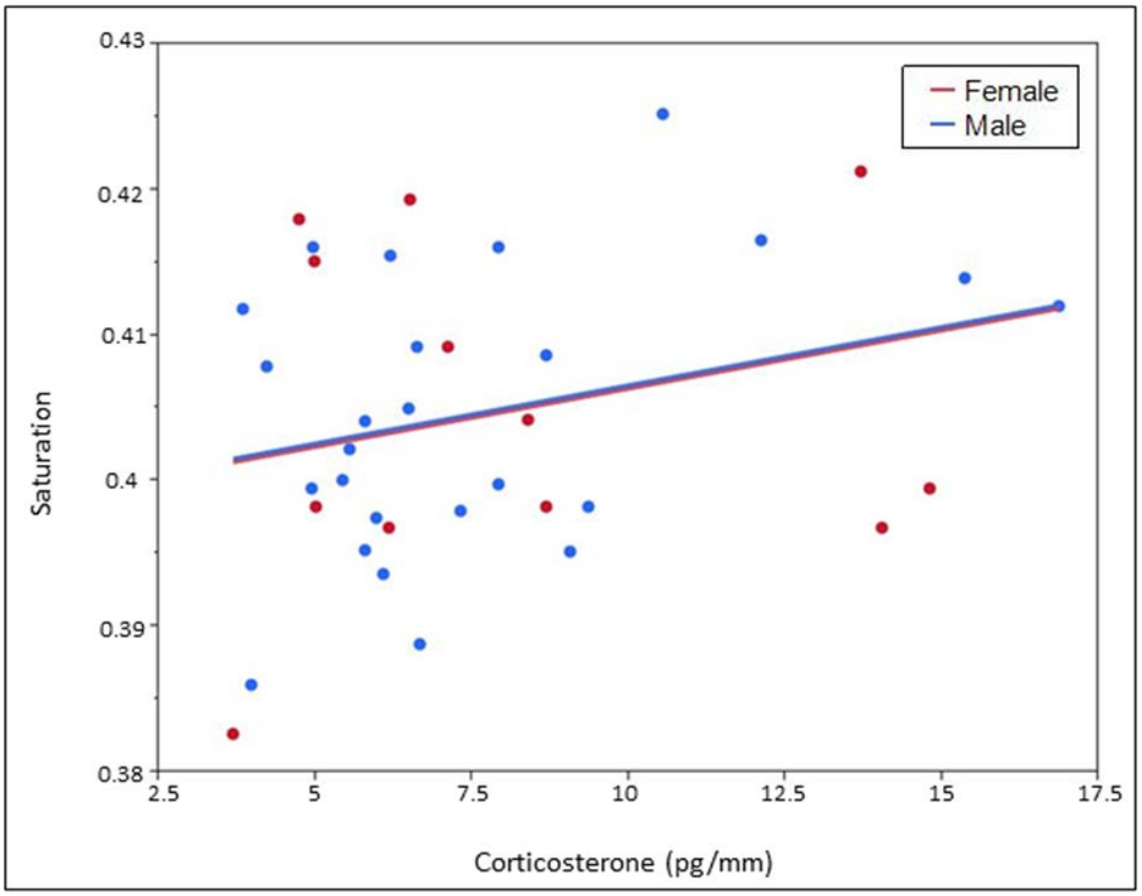

Figure 5 Relationship between sex, feather corticosterone (pg/mm), and saturation of pheomelanin pigmented feathers from American Kestrels captured during 2010-2011 at Lucky Peak and Boise Peak, Idaho.

Table 3 Initial results of ANCOVA examining potential effects of sex and feather corticosterone on Brightness and Saturation of pheomelanin pigments in American Kestrel feathers.

	Brightness			Saturation		
Factors	df	*F* Ratio	*P*	df	*F* Ratio	*P*
Corticosterone	1,33	0.062	0.804	1,33	1.916	0.176
Sex	1,33	0.931	0.342	1,33	0.000	0.987
Corticosterone*Sex	1,33	0.838	0.367	1,33	0.934	0.341

Table 4 Final ANCOVA results for examining potential effects of sex and feather corticosterone on Brightness and Saturation of pheomelanin in American Kestrel feathers.

	Brightness			Saturation		
Factors	df	*F* Ratio	*P*	df	*F* Ratio	*P*
Corticosterone	1,34	0.206	0.653	1,34	2.644	0.113
Sex	1,34	1.030	0.317	1,34	0.004	0.948

Cooper's Hawk

I analyzed morphometrics, feather corticosterone, and feather brightness and saturation for 31 Cooper's Hawks, including individuals of both sexes and of the adult and juvenile age classes (Table 5). Juvenile females (N=8) had wing chords from 245-264 mm in length, and tails between 201-224 mm in length. They weighed between 375-505 g. Brightness and saturation ranged between 45.26-91.03 and 0.31-0.38, respectively. Adult female Cooper's Hawks (N=14) had wing chords that were 245-264 mm in length and tails that were 199-219 mm in length. They weighed 411-540 g. Brightness and saturation were 65.52-102.23 and 0.35-0.38, respectively. Juvenile male Cooper's Hawks (N=7) had wing chords between 215-230 mm in length and had tails ranging 180-204 mm in length. They weighed 209-311 g. Brightness and saturation ranged from 48.16-93.13 and 0.30-0.34, respectively. Adult male Cooper's Hawks (N=2) had wing chords that were 224-233 mm in length and tails that were both 187 mm in length. They weighed 283-286 g. Brightness and saturation were 47.73-81.66 and 0.37-0.42, respectively.

The interaction term in the ANCOVA for the effect of corticosterone and age/sex class on breast feather brightness and saturation was not significant (Table 6, Figs. 6, 7); therefore, it was removed from subsequent analysis. With the interaction term removed,

corticosterone had no effect on brightness and saturation (Table 7). However, age/sex class had significant effects on the color variables (Table 7).

Because age/sex class had a significant effect on color, I compared levels to determine which contributed to this effect. Adult male and female Cooper's Hawks did not differ in brightness (Fig. 8). The brightness of juvenile Cooper's Hawks did not differ by sex, but both sexes of juveniles had significantly lower brightness than adult females (Fig. 8). Saturation values were highest in adult male Cooper's Hawks and significantly lower in adult females. Saturation in juveniles of both sexes were significantly lower than in adults but did not differ from each other (Fig. 9).

Table 5 Morphometrics, feather corticosterone, and feather color variables ($\bar{x}$ ± SD) for male and female Cooper's Hawks captured at Lucky Peak and Boise Peak, Idaho during 2010 and 2011. Sample sizes are in parentheses.

Attribute	Adult Female (14)	Juv. Female (8)	Adult Male (2)	Juv. Male (7)	Total (31)
Wing Chord (mm)	254.1 ± 4.68	254.4 ± 6.86	228.5 ± 6.36	220.3 ± 4.72	244.9 ± 15.77
Tail (mm)	207.2 ± 6.04	214.1 ± 7.68	187.0 ± 0.00	192.1 ± 8.19	204.3 ± 11.28
Mass (g)	476.7 ± 38.40	431.4 ± 39.95	284.5 ± 2.12	269.6 ± 41.31	405.8 ± 95.84
Corticosterone (pg/mm)	4.85 ± 3.75	3.6 ± 0.99	3.15 ± 1.27	3.00 ± 0.36	4.00 ± 2.66
Brightness ($R_{300-700}$)	81.03 ± 12.75	59.62 ± 14.88	64.7 ± 24.00	58.05 ± 15.88	69.26 ± 17.66
Saturation	0.37 ± 0.01	0.33 ± 0.02	0.40 ± 0.03	0.32 ± 0.01	0.35 ± 0.03

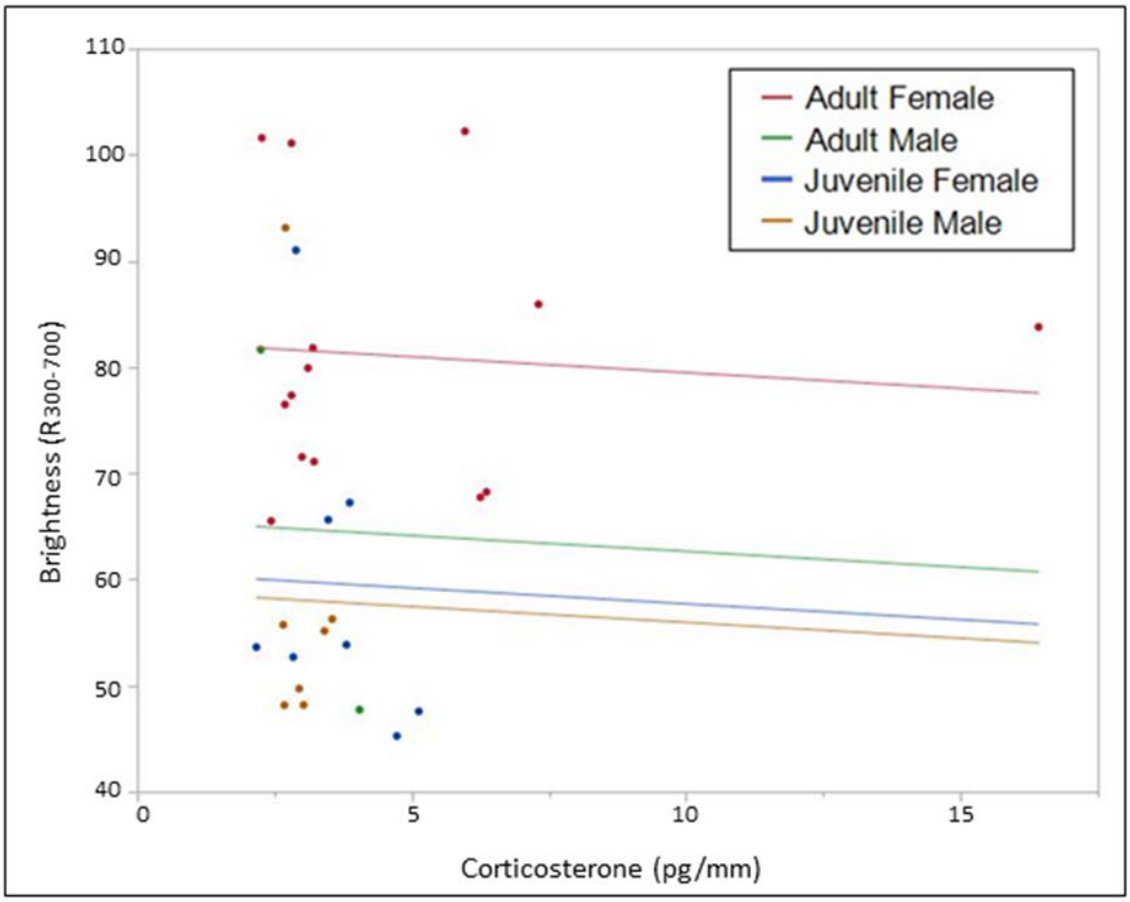

Figure 6 Relationship between age, sex, feather corticosterone (pg/mm), and brightness ($R_{300-700}$) of pheomelanin-pigmented feathers from Cooper's Hawks captured during 2010-2011 at Lucky Peak and Boise Peak, Idaho.

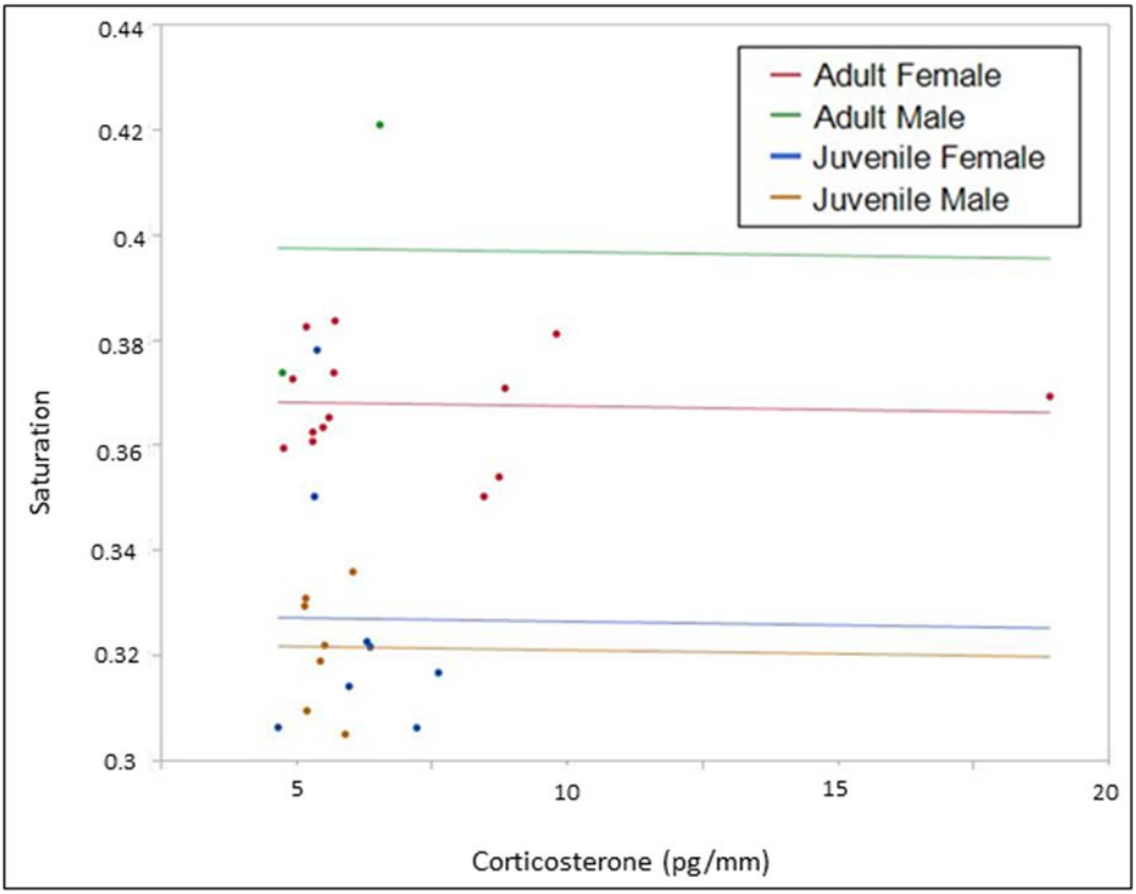

Figure 7 Relationship between sex, age, feather corticosterone (pg/mm), and saturation of pheomelanin-pigmented feathers from Cooper's Hawks captured during 2010-2011 at Lucky Peak and Boise Peak, Idaho.

Table 6 Initial results of ANCOVA examining potential effects of age, sex, and feather corticosterone on Brightness and Saturation of pheomelanin pigments in Cooper's Hawk feathers.

	Brightness			Saturation		
Factors	df	*F* Ratio	*P*	df	*F* Ratio	*P*
Corticosterone	1,23	3.029	0.095	1,23	0.478	0.496
Age/Sex	3,23	5.636	0.005*	3,23	18.504	<0.001*
Corticosterone*Age/Sex	3,23	1.477	0.247	3,23	2.225	0.112

Table 7 Final ANCOVA results for examining potential effects of age, sex and feather corticosterone on Brightness and Saturation of pheomelanin in Cooper's Hawk feathers.

	Brightness			Saturation		
Factors	df	*F* Ratio	*P*	df	*F* Ratio	*P*
Corticosterone	1,26	0.077	0.783	1,26	0.013	0.911
Age/Sex	3,26	5.195	0.006*	3,26	20.599	<0.001*

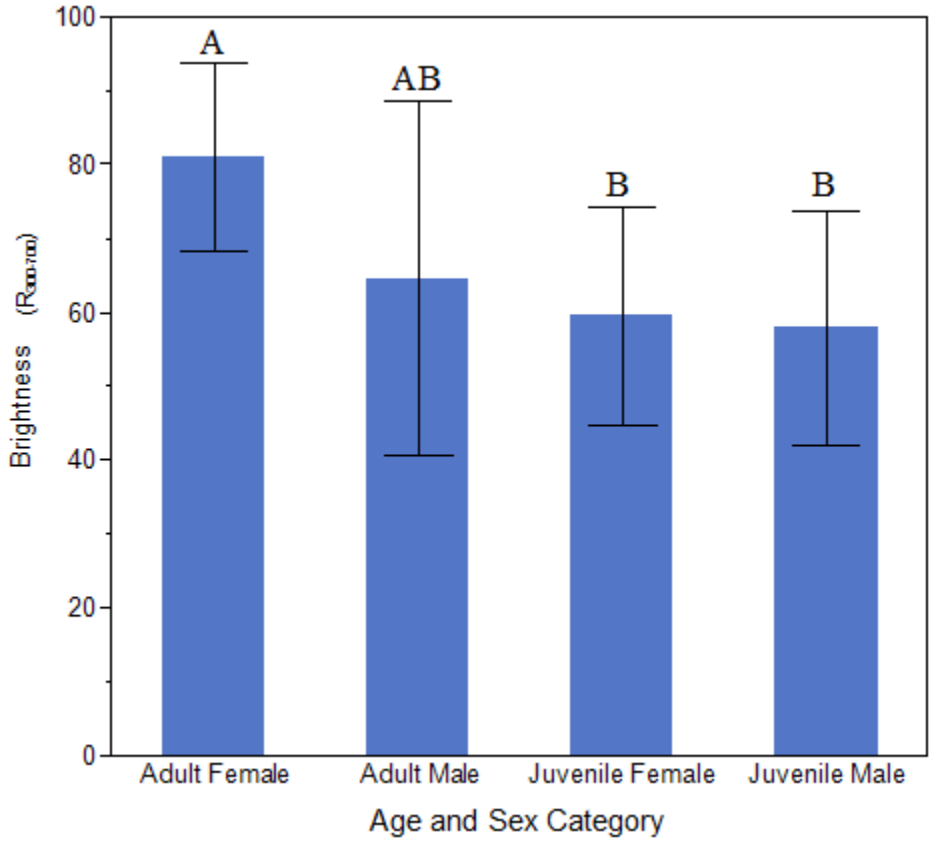

Figure 8 Feather brightness (mean ± SD) in adult female (N=14), adult male (N=2), juvenile female (N=8), and juvenile male (N=7) Cooper's Hawks (N = 31) captured during 2010-2011 at Lucky Peak and Boise Peak, Idaho.

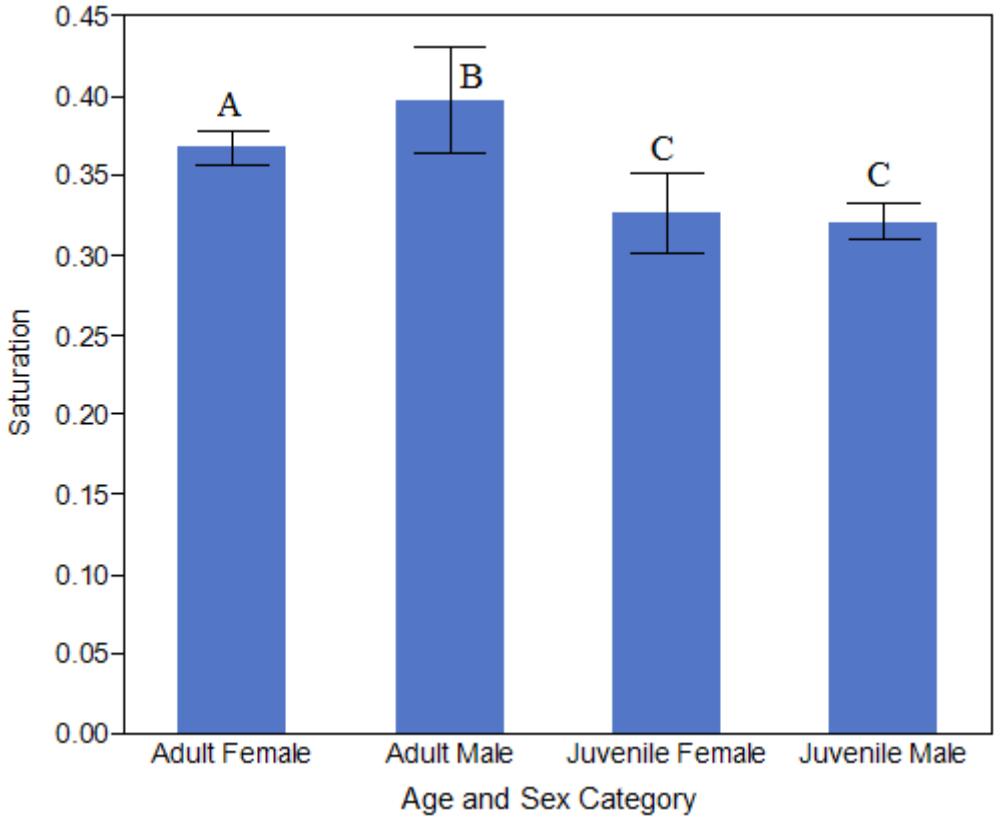

Figure 9 Saturation in (mean ± SD) in adult female (N=14), adult male (N=2), juvenile female (N=8), and juvenile male (N=7) Cooper's Hawks (N = 31) captured during 2010-2011 at Lucky Peak and Boise Peak, Idaho.

Sharp-Shinned Hawk

Female juvenile Sharp-shinned Hawks (N=22) had wing chords 191-211 mm in length, tails that ranged from 155-170 mm in length, and weighed 142-204 g (Table 8). Brightness and saturation ranged from 26.99-73.88 and 0.33-0.40, respectively. Adult female Sharp-shinned Hawks (N=15) had wing chords that ranged 195-213 mm in length and tails that were 153-170 mm in length. They weighed 159-211 g. Brightness and saturation were 42.27-93.04 and 0.32-0.39, respectively. Juvenile male Sharp-shinned Hawks (N=16) had wing chords that were 165-179 mm in length, and tails 125-148 mm in length (Table 8). They weighed 90-115 g. Brightness and saturation were 22.77-61.74 and 0.32-0.41, respectively. Adult male Sharp-shinned Hawks (N=13) had wing chords

166-179 mm in length and tails 129-145 in length (Table 8). They weighed 94-112 g. Brightness and saturation were 47.59-88.89 and 0.34-0.40, respectively.

There was no significant interaction between corticosterone and age/sex class on breast feather brightness and saturation in the ANCOVA indicating homogeneity of slopes (Table 9, Figs. 10, 11). With the interaction term removed, corticosterone had no effect on brightness or saturation (Table 10). Age/sex class significantly affected brightness but not saturation (Table 10).

Because brightness differed as a function of age/sex class in Sharp-shinned Hawks, I investigated levels using post-hoc *t*-test. Adult males and females had significantly greater brightness than juveniles but did not differ from each other (Fig. 12). Juvenile female Sharp-shinned Hawks had significantly brighter feathers than juvenile males (Fig. 12).

Table 8 Morphometrics, feather corticosterone, and feather color variables ($\bar{x}$ ± SD) for male and female Sharp-shinned Hawks captured at Lucky Peak and Boise Peak, Idaho during 2010 and 2011. Sample sizes are in parentheses.

Attribute	Adult Female (15)	Juvenile Female (22)	Adult Male (13)	Juvenile Male (16)	Total (66)
Wing Chord (mm)	205.6 ± 4.5	203.2 ± 4.7	173.6 ± 4.07	169.9 ± 4.17	189.8 ± 16.89
Tail (mm)	160.9 ± 4.3	162.2 ± 4.2	136.9 ± 4.42	138.0 ± 4.99	151.1 ± 12.86
Mass (g)	186.8 ± 12.9	173.2 ± 16.7	103.8 ± 5.39	100.9 ± 7.5	145.1 ± 40.43
Corticosterone (pg/mm)	3.76 ± 1.85	5.30 ± 3.62	5.20 ± 3.26	5.80 ± 4.51	4.49 ± 3.30
Brightness ($R_{300-700}$)	67.69 ± 12.27	47.00 ± 10.56	65.27 ± 12.26	50.35 ± 17.52	53.18 ± 16.30
Saturation	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.36 ± 0.02	0.36 ± 0.02

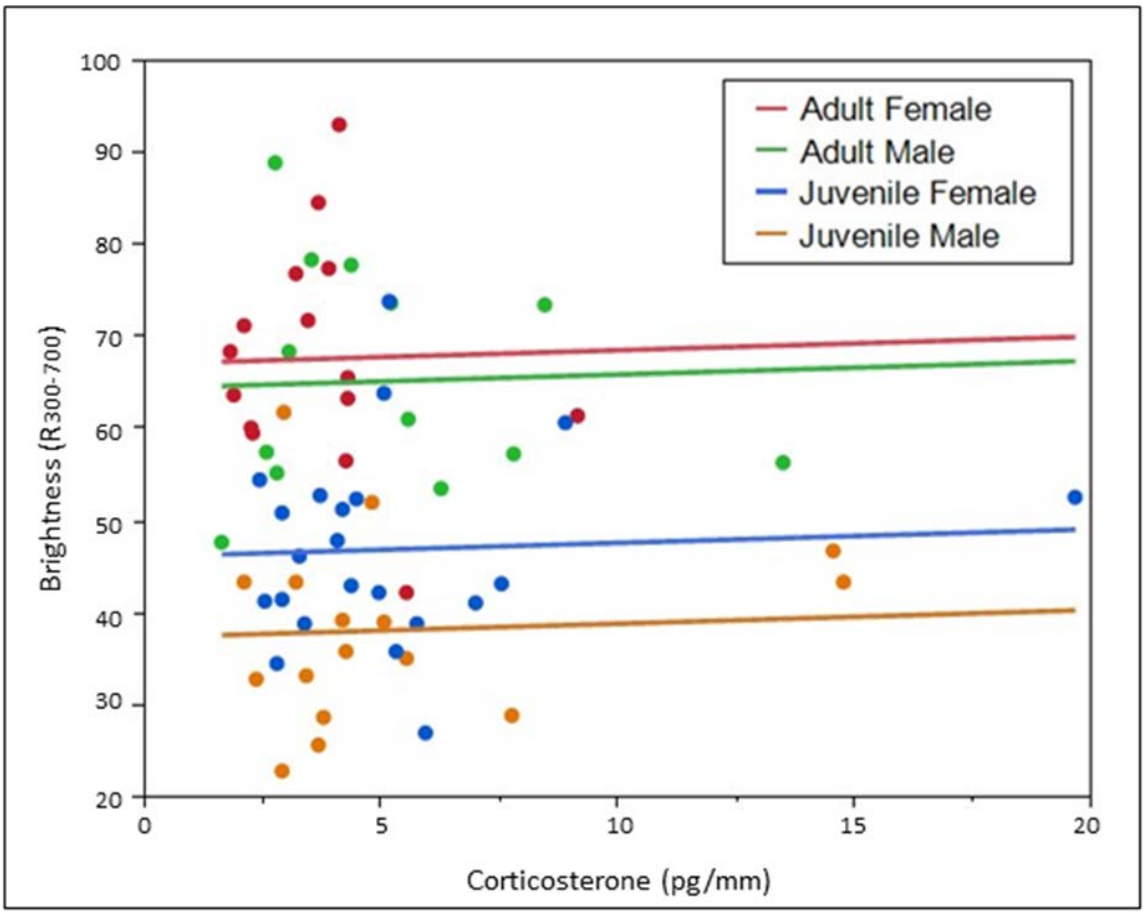

Figure 10 Relationship between age, sex, feather corticosterone (pg/mm), and brightness ($R_{300-700}$) of pheomelanin-pigmented feathers from Sharp-shinned Hawks captured during 2010-2011 at Lucky Peak and Boise Peak, Idaho.

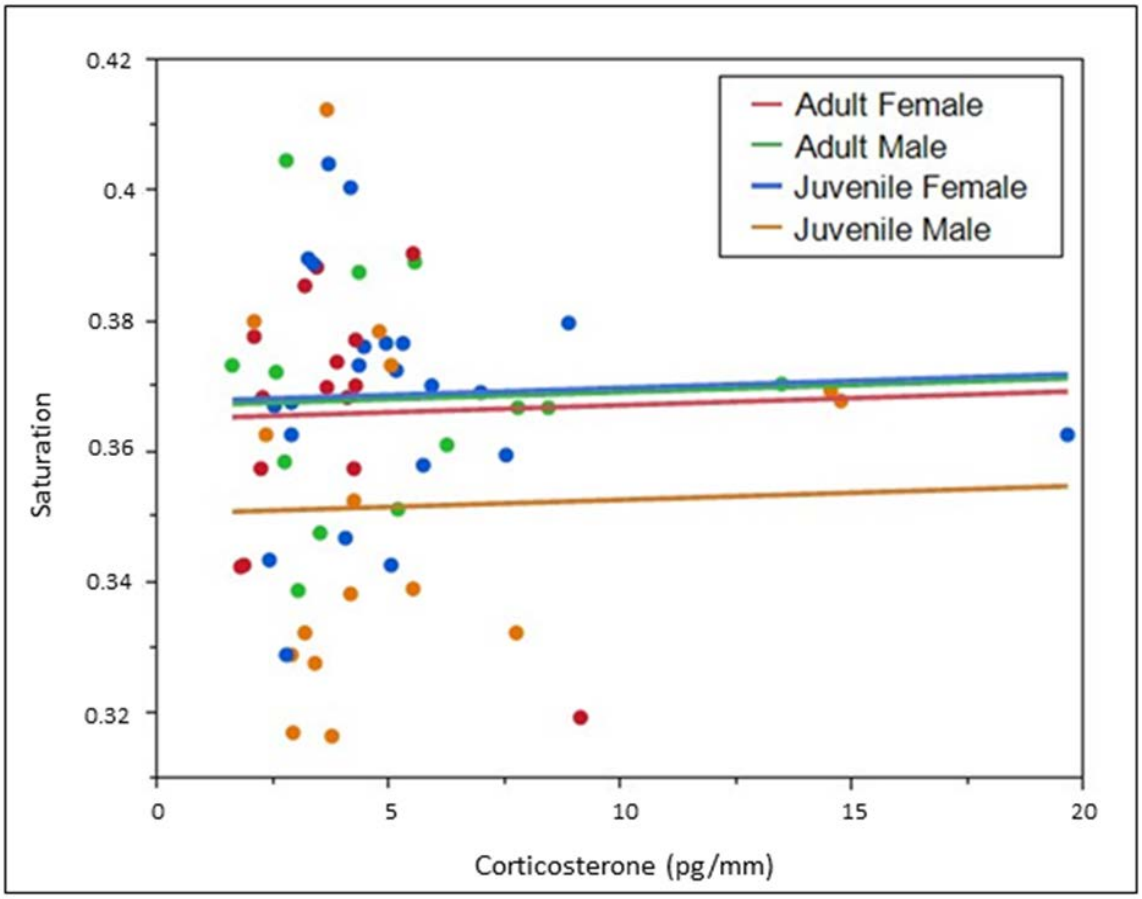

Figure 11 Relationship between sex, feather corticosterone (pg/mm), and saturation of pheomelanin-pigmented feathers from Sharp-shinned Hawks captured during 2010-2011 at Lucky Peak and Boise Peak, Idaho.

Table 9 Initial results of ANCOVA examining potential effects of age, sex, and feather corticosterone on Brightness and Saturation of pheomelanin pigments in Sharp-shinned Hawk feathers.

Factors	Brightness			Saturation		
	df	*F* Ratio	*P*	df	*F* Ratio	*P*
Corticosterone	1,57	0.170	0.681	1,57	0.158	0.692
Age/Sex	3,57	20.642	<0.0001*	3,57	2.438	0.074
Corticosterone*Age/Sex	3,57	0.543	0.655	3,57	0.601	0.617

Table 10 Final results of ANCOVA for examining potential effects of age, sex, and feather corticosterone on Brightness and Saturation of pheomelanin in Sharp-shinned Hawk feathers.

Factors	Brightness			Saturation		
	df	*F* Ratio	*P*	df	*F* Ratio	*P*
Corticosterone	1,60	0.1118	0.681	1,60	0.158	0.692
Age/Sex	3,60	20.642	<0.001*	3,60	2.438	0.073

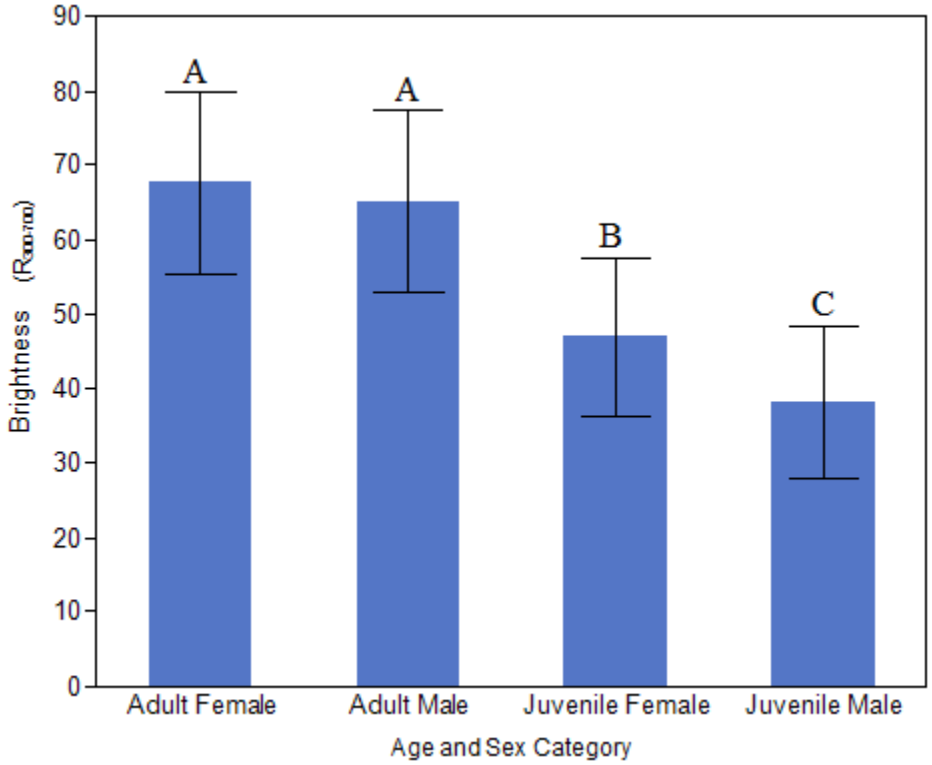

Figure 12 Bar graph of means and standard deviation of brightness ($R_{300-700}$) in adult female (N = 15), adult male (N = 13), juvenile female (N = 22), and juvenile male (N = 16) Sharp-shinned Hawks (N = 66) captured during 2010-2011 at Lucky Peak and Boise Peak, Idaho.

DISCUSSION

The main objective of my study was to examine potential relationships between feather corticosterone and the saturation and brightness of pheomelanin in feathers from male and female American Kestrels, Cooper's Hawks, and Sharp-shinned Hawks captured during migration in Idaho. Using feather corticosterone, I was able to obtain a measure of corticosterone that reflected the birds' overall hormone level throughout the period that the feather was grown and during which time the melanin was deposited. While I found wide variation in feather corticosterone values, the hormone levels present in feathers was not a significant predictor of brightness or saturation in any of the species I examined. However, in both Cooper's Hawks and Sharp-shinned Hawks, age and sex class were related to color. This was to be expected because of the well-known transition from juvenile to adult plumage in these species.

Previous studies confirm that in some cases there is a relationship between stress and pigmentation in birds. These include the effect of food stress and maternal weight on eumelanin pigmentation in Eurasian Kestrel nestlings described by Fargallo et al. (2007). Dietary deficiency in the amino acid precursors to melanin may reduce melanization (McGraw 2006). Because food stress also causes corticosterone elevation (Jenni-Eiermann et al. 2008), which in turn inhibits tyrosinase, one expects a possible relationship between corticosterone and melanization. Nonetheless, my results concurred with Jenkins et al. (2013) who found no relationship between baseline or stress-induced plasma corticosterone and pheomelanic feathers in breast feathers of Barn Swallows. My

results were also consistent with Butler et al. (2010) where plasma corticosterone levels were unrelated to melanin color and UV chroma in nestling American Kestrels.

Possible reasons that some studies showed a relationship between pheomelanin and corticosterone while others did not may include study design and other physiological reasons. Many of the studies that have shown variation in hormones affecting changes in melanin were manipulative experiments (McGraw 2006; Roulin et al. 2008; San-Jose and Fitze 2013). Perhaps the experimental manipulation caused the corticosterone to vary enough to affect melanization. A physiological reason that the expected relationship between feather corticosterone and feather pigmentation is not apparent might be the down regulation of corticosterone during molt (Romero et al. 2005), during which the hormone is reduced to an extent that it does not create a significant effect on pigmentation during feather development. Furthermore, melanin pigments are very strongly hereditary (Roulin and Dijkstra 2003).

Future studies should focus on the complex (often contradictory) interactions of the hormones involved in melanogenesis. Field studies would do well to focus on the hormones and feather development in nestlings as well as in molting adults. More work may be necessary to determine what visual cues most highly influence sexual selection in diurnal raptors for targeted research on pigmentation. If feather corticosterone is to be used in future experiments regarding feather color, one might consider using only the section of feather that was analyzed spectrally.

Another factor that further research should address is the potential fading of feathers that occurs during the months between feather growth and feather collection. For instance, in my study, there was up to several months between the time when feather

growth was completed and when I captured birds migrating through the banding station. Accounting for this requires capturing the birds while the feathers are growing to get a baseline for pigmentation (Hasegawa et al. 2008).

Future research might consider using a different method of obtaining color metrics, as the probing method I employed measured only a small area of each feather. Perhaps an analysis of a digital image would yield results representing a greater proportion of the feather. High Performance Liquid Chromatography (HPLC) is also a procedure that would measure the total products of eu- and pheomelanins in the feathers (McGraw 2006). However, spectroscopy and digital image analysis probably have an advantage over HPLC quantification by representing how the color is perceived.

There are also hormones other than corticosterone that could potentially affect melanin production in bird feathers. For instance, androgens may play an important role in melanization (McGraw 2006), but few studies have quantified this possible relationship. Perhaps future studies should explore the possibility of extracting androgens like testosterone from feathers (Koren et al. 2012) and examining their relationship with coloration.

CONCLUSION

While I expected to see an effect of corticosterone on pheomelanin features in feathers of these three species of diurnal raptors, there was no evidence of a relationship. Pheomelanin did vary among age and sex classes in some of the species, however. The relationship between melanin and the endocrine system remains unclear. More studies must be done on the mechanisms of melanogenesis and the degree to which each of the potential mechanisms plays a role.

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