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DO CONDITIONS ON THE MEXICAN MOULTING GROUNDS INFLUENCE CAROTENOID CONTENT AND COMPOSITION OF BULLOCK'S ORIOLE (*ICTERUS BULLOCKII*) FEATHERS?

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by

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ABSTRACT

The Bullock's oriole (*Icterus bullockii*) has an interesting moult migration strategy, in which it stops mid-way to the wintering grounds, in the Mexican monsoon region to replace feathers (moult) before continuing migration south to overwinter. Moult-migrants, such as Bullock's orioles, undergo such a strategy because food resources are more abundant in the monsoon region during early fall than on the breeding grounds, allowing better conditions for moult. During this time, Bullock's orioles produce bright orange/yellow carotenoid-based plumage. Carotenoid colouration is an honest signal of individual quality and is important in social interactions and female mate choice across taxa. Here, we asked whether diet and habitat quality during moult (inferred by stable isotopes) were associated with feather carotenoid content and composition. Liquid chromatography mass spectrometry (LC-MS) was used to determine carotenoid concentrations in the breast feathers of after second year males of a Bullock's oriole population breeding in British Columbia. Feathers were also analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes to infer habitat conditions during moult. We also used reflectance spectrometry to calculate feather hue, red chroma, and brightness. Perhaps surprisingly, I found no direct relationship between features of colouration (as measured by reflectance spectrometry) and carotenoid concentration or composition. There was, however, a positive relationship between $\delta^{15}N$ and concentration of lutein, as well as a negative relationship between $\delta^{15}N$ and proportion of canthaxanthin. These results suggest that feeding at a higher trophic level during moult –perhaps having a diet high in insects –allows for a greater amount of lutein to be deposited in feathers. Feeding more on insects may also indicate a lack of β-carotene or dietary canthaxanthin, or an inability to effectively metabolize β-carotene to canthaxanthin in that environment, possibly due to stress. This study highlights the importance of moulting in high-quality habitats for species that exhibit carotenoid-based colouration. A moult migration strategy may have evolved to take advantage of superior moult conditions in the Mexican monsoon region, which can directly influence the ability to obtain bright colouration, critical for inter- and intra-sexual interactions.

Thesis Supervisor: Associate Professor Matthew Reudink

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INTRODUCTION

Sexual selection is a strong evolutionary force that can lead to the elaboration of ornamental traits. Individuals with larger, more elaborate, and/or more colourful ornamental traits tend to be favoured by sexual selection, as these traits may be honest indicators of individual condition or quality, which function in intersexual competition and/or mate choice. Ornamental plumage colouration, the bright and vibrant feathers exhibited by many birds, has long fascinated evolutionary biologists (Hill and McGraw 2006). There are various mechanisms that produce colourful plumage, such as deposition of pigments, including melanin, porphyrins, and carotenoids in feathers, and light refraction due to feather structure (Hill and McGraw 2006).

Carotenoids are bright red, yellow, and orange organic pigments that have many functions in animals, including producing colour, boosting immune response, and acting as antioxidants (Navara and Hill 2003; García-de Blas et al. 2014). Because animals cannot synthesize carotenoids hey must be obtained through the diet (Völker 1938). In birds, carotenoids are ingested, and in some cases, subsequently modified into different carotenoids, and used in the skin, bill, and feathers to produce colouration. To produce feather colouration, carotenoids can be deposited directly from the diet as yellow dietary carotenoids, or they can be modified prior to deposition in feathers. Lutein, zeaxanthin, β-carotene, and β-cryptoxanthin, the main dietary carotenoids, can be modified to the yellow carotenoids, 3'-dehydrolutein, canary xanthophyll, and canary xanthophyll B, or modified to the red keto-carotenoids such as α-doradexanthin, astaxanthin, canthaxanthin, and adonirubin (Hill and McGraw 2006; Appendix A). The main modifications to dietary carotenoids include oxidation of hydroxyl groups to ketones, which produces modified yellow carotenoids, or the addition of ketones via oxidation, producing red keto-carotenoids. These metabolic conversions appear to be condition dependent, and indicate that orange/red feathers are most costly to produce (Hill and Johnson 2012).

Because carotenoids are also necessary for many other functions, including immune processes, vision, growth, lipogenesis, glycolysis, and energy homeostasis, becoming colourful involves trade-offs among these shared pathways (Hill and Johnson

2012). Individuals using large quantities of carotenoids for colouration must be in good health to do so. For example, Baeta et al. (2008) examined trade-offs between load of an intestinal parasite (*Isospora*), and bill colouration in the blackbird (*Turdus merula*) Carotenoid supplemented male blackbirds had bills that were more brightly coloured bills, as well as lower parasite loads. Infected birds without supplementation had reduced bill colouration, while supplemented infected birds maintained their bill colouration. Restriction of carotenoids in the diet of male house finches (*Haemorhous mexicanus*) resulted in decreased hue, saturation, and brightness of carotenoid-based plumage (Hill 2000). Interestingly, excessively high levels of carotenoids in American goldfinches (*Carduelis tristis*) resulted in tissue damage (Huggins et al., 2010).

While individual condition is essential for the expression of optimal colouration, individuals may also be constrained by habitat quality during moult. Arriero et al. (2006) examined how carotenoid-based plumage colouration varied across habitats in Eurasian blue tit (*Parus caeruleus*) nestlings and found that tits situated in young and structurally simple forests showed reduced plumage colouration. Nestlings with more intense yellow plumage were also of a larger body size and had a stronger immune response upon injection of phytohemagglutinin (PHA, binds to T-cells and stimulates metabolic activity). Eeva et al. (2009) analyzed plumage colour in great tit nestlings in polluted and unpolluted areas. In unpolluted areas, higher levels of plumage carotenoid chroma values and higher plasma carotenoid levels were observed compared to the polluted area. Nestlings supplemented with carotenoid rich diets grew better (in terms of body mass, wing length, plasma lutein, and colouration) in the polluted area only. Similarly, great tits (*Parus major)* raised in a deciduous forest were more yellow than those raised in a coniferous forest due to the increased availability of larvae in the former habitat (Slagsvold and Lifjeld 1985). Ferns and Hinsley (2008) examined blue tits and great tits (*Cyanistes caeruleus*) in low and high quality habitats (small and large woods) and analyzed yellow ventral plumage variation with respect to moult and/or diet. Blue tits consumed 47% more and great tits 81% more caterpillar flesh in high quality habitats compared to lower quality habitats. Surprisingly, unlike the results found by Arriero (2006), this did not result in a significant difference in the chroma of feathers produced by birds living in the two habitats. In house finches, those occupying environments with

high carotenoid availability have larger red patches than those living in dry, hot environments (Hill et al., 2002). Habitat plays an important role in colouration both directly, as moulting in a poor-quality habitat can result in an insufficient amount of carotenoids being consumed and deposited, and indirectly, through malnourishment, exposure to parasites, high stress load, or other factors, ultimately resulting in a need to utilize carotenoids elsewhere.

Migratory birds vary vastly in the timing and location of their moult. Which feathers are replaced and when can vary greatly among species and even within species across populations. In the arid west, several species hereafter referred to as moult-migrants interrupt their migration to moult in the Mexican monsoon region, located in southwestern USA and northwestern Mexico (Leu and Thompson, 2002; Rohwer et al., 2005; Pyle et al., 2009; Pillar et al., 2015). However, this is a vast region that encompasses a range of habitats; individuals may be able to optimize their colouration by selecting a high quality moult location. The challenge, however, is tracking habitat use across seasons in animals moving hundreds or thousands of kilometres between breeding, moulting, and wintering areas.

Because habitat can influence food and carotenoid availability, moving to areas with high food availability during moult could provide a strong selective advantage. Although small migratory birds are difficult to track due to their small sizes and the vast distances they travel, stable isotope analysis has become quite useful for linking different phases of the annual cycle and determining diet and habitat use. This technique provides information on prior location and habitat use (Wassenaar 2008), as stable isotopes are incorporated into a growing animal's tissue from the food and water it ingests. Because stable isotopes in feathers are inert once the feathers are grown, analyzing stable isotopes of carbon and nitrogen in feathers can provide information on diet and habitat use at the time of moult.

Stable isotope analysis makes use of the fact that a handful of elements have forms that vary in mass –referred to as isotopes –that can be distinguished from each other on analysis. Analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes in an animal's tissues can provide information about the individual's diet at the time during that tissue was grown. Carbon ($\delta^{13}C$) stable isotope values can indicate habitat types due

to differences in plant water stress and photosynthetic systems (Lajtha and Marshall 1994). Specifically, low $\delta^{13}C$ values indicate a diet containing high amounts of C_3 plants (or prey that feeds on C_3 plants) and plants experiencing little water stress, while high $\delta^{13}C$ values indicate diets with more C_4 plants (Lajtha and Marshall 1994). Differing $\delta^{13}C$ values are also indicative of marine versus terrestrial dietary input (Lajtha and Marshall 1994). Nitrogen ($\delta^{15}N$) stable isotope analysis is associated with the trophic level of an organism. Organisms that are more carnivorous have a greater $\delta^{15}N$ values (Post 2002, Poupin et al., 2011). Hydrogen ($\delta^{2}H$) stable isotope analysis can provide rough latitudinal approximation (Hobson 2008). Moving from southeast to northwest North America, $\delta^{2}H$ values become more negative (Hobson 2008). Stable isotope analysis of bird tissues has been used to establish migratory connectivity between breeding and wintering populations (Hobson et al., 2004), determine quality of winter habitat (Marra et al. 1998), and in turn, discern carry-over effects (Marra et al. 1998; Reudink et al., 2009a): events that occur during one season that influence the performance of individuals the following season. Recently, hydrogen isotope analysis was used to confirm that a northern population of Bullock's orioles (*Icterus bullockii)* moults between their breeding and wintering grounds, likely in the Mexican monsoon region (Pillar et al., 2015).

While Pillar et al. (2015) demonstrated that Bullock's orioles moult-migrate, it remains unclear how specific habitat and moult conditions affect an individual's ability to obtain carotenoids for plumage colouration. Here, we use carbon and nitrogen stable isotope analysis to ask whether conditions during moult influence carotenoid content and composition in Bullock's orioles. Bullock's orioles are large songbirds that breed as far north as the southern interior of British Columbia, before overwintering as far south as Costa Rica. However, these birds stop and fully moult in the Mexican monsoon region prior to reaching their wintering sites (Rohwer et al., 2005; Pillar et al., 2015). After second year (ASY) males exhibit bright orange plumage on their breast, belly, rump, and face, while females of all ages have a pale yellow head and breast. Second year (SY) males look similar to females, but also exhibit patches of ASY-like plumage. In this species, older males, who are more orange than younger males, secure higher reproductive success (Richardson and Burke 1999; Whittingham and Dunn 2000).

I predict that Bullock's orioles that moulted in high-quality, wet habitats in the Mexican monsoon region (indicated by low $\delta^{13}C$ values) will have more carotenoids deposited in their feathers than those that moulted in low-quality, xeric habitats (high $\delta^{13}C$ values). In addition, I predict that high concentrations of carotenoids, especially keto-carotenoids such as canthaxanthin, should produce feathers with orange-shifted hue, high red chroma, and low brightness. However, the relationship between carotenoid concentration and $\delta^{15}N$ may be either positive or negative, depending on the richness of carotenoids in insects and plants during moult.

MATERIALS AND METHODS

Fieldwork

Fieldwork was conducted in Kamloops, British Columbia, Canada (50.68 N 120.34 W). Adult male Bullock's orioles were captured in mistnets with the use of conspecific playback and decoys or by positioning nets near oriole feeders. Breast feathers (n = 10 feathers per bird) were collected from each individual during the summer (May through July) of 2012 (n = 18) and 2013 (n = 10). Identification of the correct sex and age class was made using following descriptions provided by Rohwer and Manning (1990) and Pyle (1997). Individuals were banded with a single Canadian Wildlife Service aluminum band and three unique colour bands for individual identification.

Stable Isotope Analyses

For all feathers, analysis of stable-carbon and stable-nitrogen isotope ratios was performed at the Smithsonian Institution Isotope Mass Spectrometry Lab in Suitland, Maryland, USA. Feathers and claws were washed in a 2:1 chloroform-methanol solution and allowed to dry. Approximately 0.30-0.40 mg of the distal tip of the feather was sampled, excluding the rachis. Using a Thermo high temperature conversion elemental analyzer (TC/EA; Thermo Scientific) at 1350 °C, samples were first pyrolyzed and sequentially analyzed by a Thermo Delta V Advantage isotope ratio mass spectrometer. All isotope ratios are reported in δ notation in units per mil (%) relative to international standards. For carbon stable isotope analysis, following Reudink et al. (2009c), each claw sample was weighed and converted to CO_2 (in an oxidation/reduction furnace) before separation and quantification of $\delta^{13}C$ by gas chromatography equipped with an isotope-ratio mass spectrometer (Lajtha and Marshall 1994; Norris et al. 2004). For each stable carbon and nitrogen analysis, between every 10 samples, two house standards were run (acetanalide and urea). Repeatability of carbon and nitrogen samples was found to be ± 0.2% (based on repeatability of measurements of standards; Reudink et al., 2015). Nitrogen ($\delta^{15}N$) was used because ^{15}N is preferentially incorporated with increasing trophic level, resulting in greater $\delta^{15}N$ values at higher trophic levels (Post 2002, Poupin

et al., 2011). Furthermore, $\delta^{15}N$ values are negatively correlated with rainfall and positively associated with temperature, so that $\delta^{15}N$ level may be indicative of the aridity of a biome (Sealy et al., 1987; Craine et al., 2009; Reudink et al., 2015).

Colour Analysis

Following Reudink et al. (2009b), reflectance spectrometry was performed on feathers using an Ocean Optics JAZ spectrometer attached to a PX-2 xenon pulsed light source to record reflectance spectra across the bird visual spectrum (300-700 nm) from which variables of hue, red chroma, and brightness were calculated. Feathers were mounted on a minimally reflective black background (<5% reflectance) and the probe was kept at a 90° angle. Ten measurements were taken in the centre of the orange region on the stack of 10 breast feathers for each bird. In between measurements for each bird, readings from dark (sealed black velvet lined box) and white (spectralon) standards were used for standardization. Hue, which describes the colour (i.e., yellow, orange, red), was calculated as arctan $([(R_{415\text{-}510} - R_{320\text{-}415})/R_{320\text{-}700}]/[(R_{575\text{-}700} - R_{415\text{-}575})/R_{320\text{-}700}])$. Red chroma, which describes the degree of colour saturation, was calculated as the amount of red light reflected relative to the overall reflectance: $(R_{575\text{-}700})/(R_{300\text{-}700})$. Brightness was calculated as the mean amount of light reflected across all wavelengths: (mean $R_{300\text{-}700}$).

Standards

Carotenoid standards and vials were purchased from Sigma-Aldrich, Oakville, ON, Canada, and VWR, Mississauga, ON, Canada, respectively. Standards were made by weighing out canthaxanthin, lutein, or xanthophyll into a volumetric flask and filling with a chloroform ($CHCl_3$) and methanol (MeOH) solution (1:1, *v/v*). Standards were serial diluted, ranging from 3 to 100 ppm.

Extractions

Feathers were blotched with hexane and allowed to dry. Once dry, feathers were cut and weighed into glass vials. Anti-bumping granules were added along with acidic

pyridine (3 drops of HCl in 50 mL pyridine). The mixture was refluxed for 3 hours. Once cooled, the carotenoids were transferred to hexane by adding hexane and mixing, then washing three times with water. The hexane layer was transferred to a new vial and the drying agent sodium sulfate was added. The extraction was evaporated to dryness with nitrogen gas, and then a known amount of $CHCl_3$ and MeOH solution (1:1, *v/v*) was added. The antioxidant butylated hydroxytoulene (BHT) was also added to prevent oxidation of the carotenoids (2 μL of 10 mg/mL BHT per sample). The extractions were kept in glass vials and stored in a dark freezer until use.

LC-MS

Analysis was performed on an Agilent 1200 series HPLC system (Agilent Technologies, Mississauga, ON, Canada) coupled to an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) spectrometer, equipped with electrospray ionization (ESI) source (gas temperature, 300 °C; drying gas, 8 L/min; nebulizer 35 psig; sheath gas temperature, 350 °C; sheath gas flow, 11 L/min; Vcap, 3500 V). Carotenoids were analyzed in positive ion mode, and mass spectra were collected between 100 and 600 m/z. A sample of 5 μL of blanks, extractions, and standards (3 to 100 ppm) were injected into the LC, with the flow rate set at 1.0 mL/min. Separation was achieved with a *Luna C8 (2)* column (100 mm × 4.6 mm; 3 μm particle size; Agilent, Canada) kept at a constant temperature of 60 ± 0.2 °C. The auto-sampler and column were set for 60 °C. Mobile phase (A) consisted of pure methanol and mobile phase (B) was composed of 70:30 *v/v* methanol with 0.1% ammonium acetate. Gradient elution was programed as follows: 95% B at time zero, 80% B at 10 min, 65% B at 15 min, 40% B at 20 min, 10% B at 24 min, and 95% B again at 25 min, with the effluent flowing into the Q-TOF MS.

Data was obtained by taking the area divided by the retention time of the peak of interest. Using the data produced from the standards, a calibration curve was produced for each canthaxanthin, lutein, and xanthophyll. The calibration curves were used to determine the given carotenoid content in the extraction sample, which in turn was used to determine the amount of carotenoid in μg per mg of feather extracted. We examined the repeatability of our analysis by using linear regression on repeated samples. For

repeatability varied among carotenoids for both concentration ($r^2 = 0.30 – 0.53$, n = 7) and proportion of each carotenoid ($r^2 = 0.19 – 0.31$, n = 6).

Statistical Analysis

Canthaxanthin, lutein, and xanthophyll concentrations, as well as total carotenoid content, were natural log transformed due to strong deviations from normality. I used linear regression to examine whether hue, red chroma, and brightness were predicted by carotenoid concentration or by the proportion of each specific carotenoid relative to the total concentration of all three carotenoids. To examine whether carbon or nitrogen stable isotope values predicted carotenoid content or concentration, I constructed linear mixed models with carotenoid content or the proportion of carotenoid present as the response variable, $\delta^{13}C$ and $\delta^{15}N$ as fixed effects, and individual as a random effect (due to repeated measurements of three individuals in different years). Year was also included as an effect; however, it was not significant in any test and was subsequently removed. All statistical analyses were performed using JMP v12.0 (SAS 2012).

RESULTS

Carotenoid concentration and composition in adult male breast feathers

Concentrations of canthaxanthin, lutein, and xanthophyll ranged from 0.19 to 3.32 μg/mg, 0.42 to 11.4 μg/mg, and 0.20 to 9.88 μg/mg, with means and standard deviations of 0.59 ± 0.62 μg/mg, 3.75 ± 3.08 μg/mg, and 2.23 ± 1.99 μg/mg, respectively (Fig. 1a).

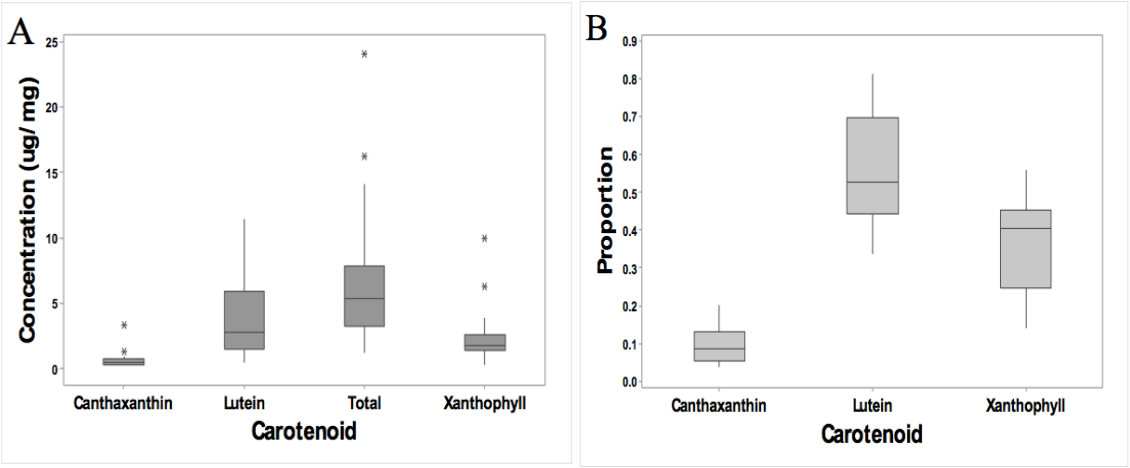

Figure 1. Distribution of carotenoids in adult male Bullock's oriole breast feathers. A) The concentration of carotenoids in μg per mg of feather extracted. B) Proportion (carotenoid concentration divided by total carotenoid concentration) of each carotenoid. Boxes show the interquartile range, representing the middle 50% of the data, while whiskers represent the upper and lower 25% of the distribution; outliers are represented by stars.

Plumage colour and feather carotenoid concentration and composition

Carotenoid content and composition (canthaxanthin, lutein, and xanthophyll concentration, total concentration, and proportion canthaxanthin, lutein, and xanthophyll, Fig. 1b) of feathers did not predict any feather colour variable. (brightness, red chroma, or hue; all $p > 0.12$; Table 1).

Table 1. Relationships between plumage colour and carotenoid concentration and composition.

	Brightness	**Red chroma**	**Hue**
Total	$r^2 = 0.10$	$r^2 = 0.02$	$r^2 = 0.003$
	$p = 0.12$	$p = 0.55$	$p = 0.80$
Canthaxanthin	$r^2 = 0.07$	$r^2 = 0.07$	$r^2 = 0.03$
	$p = 0.20$	$p = 0.20$	$p = 0.45$
Lutein	$r^2 = 0.05$	$r^2 = 0.009$	$r^2 = 0.004$
	$p = 0.28$	$p = 0.66$	$p = 0.78$
Xanthophyll	$r^2 = 0.13$	$r^2 = 0.004$	$r^2 = 0.005$
	$p = 0.07$	$p = 0.76$	$p = 0.73$
% Canthaxanthin	$r^2 = 0.004$	$r^2 = 0.04$	$r^2 = 0.06$
	$p = 0.77$	$p = 0.32$	$p = 0.25$
% Lutein	$r^2 = 0.02$	$r^2 = 0.0003$	$r^2 = 0.002$
	$p = 0.55$	$p = 0.94$	$p = 0.83$
% Xanthophyll	$r^2 = 0.03$	$r^2 = 0.003$	$r^2 = 0.001$
	$p = 0.43$	$p = 0.79$	$p = 0.87$

Feather isotopes ($\delta^{13}C$, $\delta^{15}N$) and carotenoid concentration and composition

$\delta^{13}C$ was not associated with carotenoid concentration or percentage composition of carotenoids deposited in feathers (all $p > 0.07$; Table 2). However, $\delta^{15}N$ was correlated positively with lutein concentration ($p = 0.04$) and negatively with the proportion of canthaxanthin in feathers ($p = 0.02$; Table 1, Fig. 2).

Table 2. Relationships between feather isotopes ($\delta^{13}C$, $\delta^{15}N$) and carotenoid concentration and composition.

	$\delta^{13}C$	$\delta^{15}N$
Total	$F_{1, 7.325} = 0.68$ $p = 0.43$	$F_{1, 9.589} = 3.73$ $p = 0.43$
Canthaxanthin	$F_{1, 16.65} = 2.33$ $p = 0.15$	$F_{1, 18.59} = 0.02$ $p = 0.89$
Lutein	$F_{1, 16.36} = 0.33$ $p = 0.58$	$F_{1, 18.84} = 4.79$ $p = 0.04$
Xanthophyll	$F_{1, 4.935} = 1.19$ $p = 0.32$	$F_{1, 6.428} = 1.02$ $p = 0.35$
% Canthaxanthin	$F_{1, 20.99} = 3.65$ $p = 0.07$	$F_{1, 20.83} = 6.70$ $p = 0.02$
% Lutein	$F_{1, 18.42} = 0.06$ $p = 0.81$	$F_{1, 20.12} = 2.31$ $p = 0.14$
% Xanthophyll	$F_{1, 20.56} = 0.28$ $p = 0.60$	$F_{1, 20.99} = 0.34$ $p = 0.57$

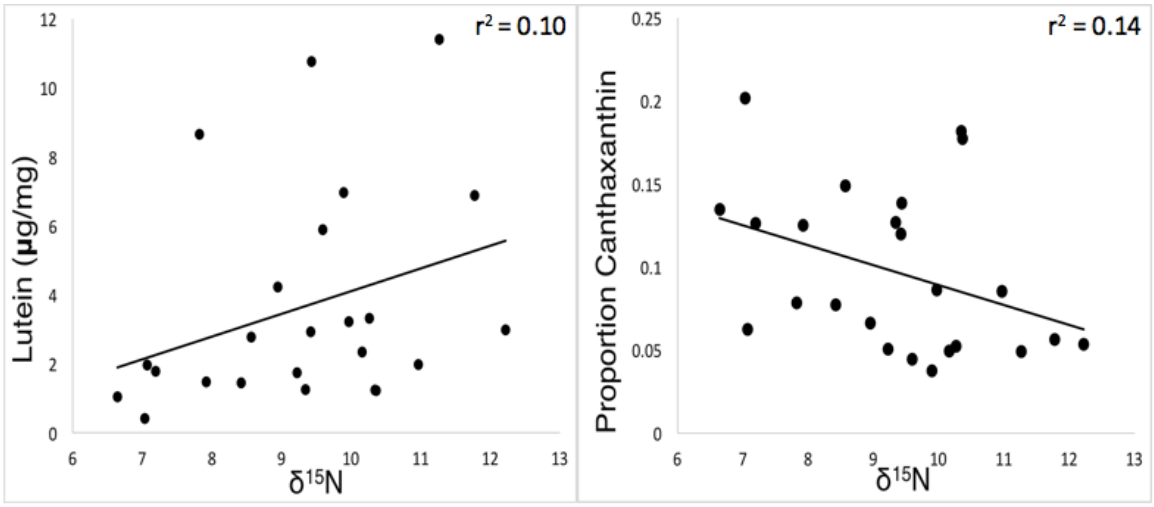

Figure 2. Positive relationship between $\delta^{15}N$ and lutein concentration, and negative relationship between $\delta^{15}N$ and the proportion of canthaxanthin in Bullock's oriole breast feathers.

DISCUSSION

To our knowledge, this paper is the first to demonstrate that conditions experienced during moult in the Mexican Monsoon region, as inferred by feather stable isotopes, are directly associated with carotenoid content and composition. Carotenoids are important for immune function as well as colouration, providing an honest signal to both competitors and potential mates (Navara and Hill 2003; Hill and Johnson 2012; García-de Blas et al. 2014), and are important for signaling across a diversity of animal taxa (Alfonso et al., 2013; Sefc et al., 2014). Highly ornamented individuals are generally socially dominant, secure more mates (both social and extra-pair), and attract older females with greater reproductive capacity (Whittingham and Dunn 2000), and are more likely to secure paternity at their own nest (e.g., house finch, McGraw and Hill 2000; American redstart, Reudink et al., 2009b). Because of the importance of obtaining high-quality sexual ornaments, individuals should seek out the highest-quality, most nutrient abundant environment in which to moult in order to achieve vibrant plumage colouration. Thus, while a moult-migration strategy may have evolved to compensate for low post-breeding food availability in the arid West (Rohwer et al., 2005), habitat selection on the individual level may be critical for obtaining high-quality plumage, which can then carry-over to influence individual success in subsequent seasons.

Carry-over effects (events occurring during one season that affect the performance in a following season) can drive differences in fitness through effects on survival and reproduction at both the population and individual level (Harrison et al., 2011). For example, in the American redstart (*Setophaga ruticilla*), acquisition of a high-quality winter habitats results in the earlier arrival to breeding grounds, and consequently, positively influences reproductive success (Marra et al., 1998; Reudink et al., 2009b; Reudink et al., 2009c). Similarly, non-breeding habitat can influence migratory timing in black-throated blue warblers (*Setophaga caerulescens*) (Bearhop et al., 2004) and reproductive success in black-tailed godwits (*Limosa limosa islandica*; Gunnarsson et al., 2005). Critically, yellow warblers (*Setophaga petechia)* undergo a pre-alternate moult of body feathers on the wintering grounds and Jones et al. (2014) demonstrated that birds overwintering in higher-quality habitat (inferred via stable isotope analysis) produced more colourful feathers (higher chroma), which are important for mate choice during

breeding. Thus, in moult-migrant birds, this strategy may have evolved to ensure that birds can acquire carotenoids and nutrients necessary for producing colourful plumage, thus carrying-over to influence reproductive success.

Recent work by Friedman et al. (2014) on the New World orioles (*Icterus*) found that species characterized as yellow had no keto-carotenoids present, while species exhibiting orange had keto-carotenoids present. When looking across both yellow and orange species, keto-carotenoid concentration predicted colouration ($p < 0.05$), but variation in hue among "orange" taxa was not explained by variation in keto-carotenoid concentration. Thus, the presence of keto-carotenoids differentiated yellow and orange colouration, but the concentration of keto-carotenoids within orange taxa did not explain variation in hue, similar to the lack of relationship we observed between carotenoid concentration and hue. However, in other species, pigment concentration can accurately reflect variation in feather hue, as has been demonstrated in the American goldfinch (*Spinus tristis*), the house finch (*Haemorhous mexicanus*), and the Baltimore oriole (*Icterus galbula;* McGraw and Gregory 2004; McGraw et al., 2006; Hudon et al., 2013). Saks et al. (2003) demonstrated that 32-51% of variation in chroma and hue in yellow areas of greenfinch (*Chloris chloris*) feathers was explained by carotenoid content.

Bullock's orioles typically feed on insects, nectar, and fruit during both the winter and breeding seasons (Rising & Williams 1999). Although diet during moult has not been studied, the time of moult corresponds to the monsoon season in northwestern Mexico and southwestern USA, when food is abundant due to the large increase in precipitation (Rohwer and Manning 1990; Rohwer et al., 2005). Increasing $\delta^{15}N$ values, indicating a higher trophic level during moult, were positively associated with lutein concentration deposited in Bullock's oriole feathers. Lutein is one of the main dietary carotenoids and does not need to be metabolically converted after digestion prior to being deposited into feathers. Thus, one possibility is that individuals eating a greater quantity of lutein-containing insects may have been able to deposit a greater amount of lutein into their feathers. Alternatively, individuals eating greater numbers of insects may have been in better physiological condition and better able to utilize carotenoids for colouration.

Additionally, individuals with lower $\delta^{15}N$ values, indicating a lower trophic level during moult, deposited a greater proportion of their total carotenoid as canthaxanthin.

This may be due to feeding on canthaxanthin or β-carotene rich fruit during moult (β-carotene can be metabolically converted into canthaxanthin), which would increase the amount of canthaxanthin deposited into feathers, but would result in lower $\delta^{15}N$ values. Another possible explanation is that feeding on more insects or canthaxanthin- or β-carotene-poor fruit during moult (perhaps due to poor environmental conditions) may result in a lack of dietary canthaxanthin or its precursors, resulting in a decreased proportion of canthaxanthin deposited into feathers. Finally, having a greater proportion of insects in the diet may be a potential indicator of stress, as this would restrain the individual to effectively metabolize β-carotene to canthaxanthin for deposition into feathers (Hill and Johnson 2012), or allocating those carotenoids for use elsewhere (e.g., immune function).

Carotenoid concentration repeatability on the LC-MS was relatively low (r^2 = 0.30 – 0.53), which may have affected the results; however, our sample size for repeatability analysis was only seven samples. More samples should be run to examine the true repeatability of this technique and its utility for carotenoid analysis.

Carotenoid plumage colouration is an important condition-dependent trait that functions in inter- and intra-sexual communication and can be strongly influenced by conditions at the time of moult. Here, we demonstrate that plumage colouration in Bullock's orioles is directly associated with conditions experienced during moult stopover in the Mexican monsoon region. We suggest that habitat selection and moult conditions may carry-over to influence sexual selection and mating dynamics in the subsequent season. Further, our results are consistent with the idea that a moult migration strategy may have evolved because both food and carotenoids are more readily available during the post-breeding period in the Mexican monsoon region than in the arid West.

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APPENDIX

Appendix A Modifications of dietary carotenoids (LaFountain et al. 2013). A) β-carotene to canthaxanthin, via the intermediate, echinenone; B) zeaxanthin to astaxanthin, via the intermediate, adonixanthin; C) β-cryptoxanthin to adonirubin, via the intermediate, 3-hydroxy-echinenone.