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The effects of sun exposure on carotenoid accumulation and oxidative stress in the retina of the House Finch (Haemorhous mexicanus)

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The effects of sun exposure on carotenoid accumulation and oxidative stress in the retina of the House Finch (*Haemorhous mexicanus*)

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Abstract

Background: Diet-derived carotenoid pigments are concentrated in the retinas of birds and serve a variety of functions, including photoprotection. In domesticated bird species (e.g., chickens and quail), retinal carotenoid pigmentation has been shown to respond to large manipulations in light exposure and provide protection against photodamage. However, it is not known if or how wild birds respond to ecologically relevant variation in sun exposure.

Methods: We manipulated the duration of natural sunlight exposure and dietary carotenoid levels in wild-caught captive House Finches (*Haemorhous mexicanus*), then measured carotenoid accumulation and oxidative stress in the retina.

Results: We found no significant effects of sun exposure on retinal levels of carotenoids or lipid peroxidation, in replicate experiments, in winter (Jan–Mar) and spring/summer (May–June). Dietary carotenoid supplementation in the spring/summer experiment led to significantly higher retinal carotenoid levels, but did not affect lipid peroxidation. Carotenoid levels differed significantly between the winter and spring/summer experiments, with higher retinal and lower plasma carotenoid levels in birds from the later experiment.

Conclusion: Our results suggest that variation in the duration of exposure to direct sunlight have limited influence on intraspecific variation in retinal carotenoid accumulation, but that accumulation may track other seasonal–environmental cues and physiological processes.

Keywords: Carotenoids, *Haemorhous mexicanus*, House Finch, Photostress, Vision

Background

Diet-derived carotenoid pigments accumulate in the retinas of a wide diversity of animals, from lungfish to humans, and play an essential role in the health and function of the visual system (Douglas and Marshall 1999). Carotenoids protect the retina directly by absorbing short-wavelength, high-energy light and indirectly as antioxidants that counter oxidative stress (Krinsky et al. 2003). The effectiveness of these protective mechanisms depends upon the types and concentration of carotenoids and these pigments are ultimately depleted by these processes. Therefore, efficient replenishment (i.e., from diet and internal stores) and accumulation of carotenoids may be essential for long-term visual health and function.

In the avian retina, each cone photoreceptor subtype has a distinctly colored oil droplet pigmented with specific types and concentrations of carotenoids (Goldsmith et al. 1984). These oil droplets are located between the inner and outer segment of the receptor and, in this position; they alter the composition and intensity of light reaching the visual pigment. This filtering provides both spectral-tuning and photoprotective benefits (Hart 2001; Vorobyev 2003). Birds cannot synthesize carotenoids de novo, and

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accumulation in the retina depends upon dietary intake of carotenoids (Toyoda et al. 2002; Knott et al. 2010; Toomey and McGraw 2010). Increased carotenoid accumulation in the avian retina, through dietary supplementation, has been shown to reduce light-induced photoreceptor death and the formation of *N*-retinyl-*N*-retetinylidene ethanolamin (A2E), a marker of light-induced oxidative damage (Thomson et al. 2002a, b; Bhosale et al. 2009).

The photoprotective benefit of carotenoids may come at a cost to visual sensitivity. Cone oil droplet filtering can be quite extensive, absorbing more than 50 % of the light reaching the photoreceptor and potentially limiting color vision under low light conditions (Bowmaker 1977; Vorobyev 2003; Hart and Vorobyev 2005; Toomey et al. 2015). Thus, there may be a trade-off between photoprotection from bright light and color vision under dim conditions. Hart et al. (2006) observed that domestic chickens (*Gallus gallus*) reared under bright lighting conditions developed more intensely pigmented cone oil droplets than birds raised in a dim environment. This result suggests that birds may up- or down-regulate carotenoid accumulation in the retina to match their light environment and meet the competing demands of photoprotection and/or visual sensitivity. However, this study involved a relatively extreme manipulation, exposing birds to consistently dim or bright conditions that differed in intensity by more than four orders of magnitude (Hart et al. 2006). Free-ranging birds are unlikely to experience such extremes, and it is not clear if or how light exposure influences retinal carotenoid accumulation among wild animals.

Long-term light exposure also has the potential to limit carotenoid accumulation through photodegradation. Exposure to ultraviolet light (UV) has been shown to deplete carotenoids from human plasma (White et al. 1988; Biesalski et al. 1996), and light exposure is associated with the fading of carotenoid-based beak coloration in Zebra Finches (*Taeniopygia guttata*) (Blount and Pike 2012). Without compensatory mechanisms of carotenoid accumulation, systemic depletion along with the localized degradation in the retina could cause declines in retinal carotenoid levels. Consistent with this prediction, we have observed that the retinal carotenoid levels of a wild species of bird—the House Finch (*Haemorhous mexicanus*)—are lowest in the late spring and summer, when the animals are exposed to the longest days with the most intense light levels (Toomey and McGraw 2009). However, we cannot rule out other ecophysiological processes that might drive these seasonal patterns. For example, sun exposure has the potential to drive changes in oxidative state through increased body temperature and changes in activity level. Circulating plasma carotenoid levels have been linked to antioxidant capacity in birds (Pérez-Rodríguez 2009; Simons et al. 2012), and it is possible that birds allocate carotenoids away from the eye to other functions when oxidative stress is increased. Thus, the evidence reviewed here offers contrasting predictions about the influence of light on carotenoid accumulation in the avian retina. If carotenoid accumulation is tuned to environmental light levels, we would predict a positive relationship between light exposure and pigment levels, but if photodegradation or shifting allocation patterns are stronger determinants of carotenoid accumulation, we predict the opposite pattern.

The purpose of the studies presented here was to determine if and how variation in sun light exposure influences carotenoid accumulation and oxidative damage in the retina of a wild bird species and to test the contrasting predictions of accumulation and degradation. We carried out two separate experiments, where we exposed wild-caught captive adult House Finches to short or long daily bouts of direct sunlight exposure for 2 months and measured their resulting plasma and retinal carotenoid levels with high-performance liquid chromatography (HPLC) and retinal lipid peroxidation levels with a thiobarbituric acid reactive substances assay (TBARS). Our manipulation resulted in a twofold to fourfold difference in sun exposure (measured in lux), between the treatment groups, which is much smaller than previous studies (e.g., Hart et al. 2006), but was intended to approximate a range of sun light exposure experienced by House Finches in the natural environment. Our first experiment was conducted over the winter months (Jan–Mar), while the second experiment was done in the late spring–summer (May–July, which is a period characterized by long, cloud-free days, with intense irradiance; Arizona Meteorological Network 2011) and included a manipulation of dietary carotenoid levels to test for possible interactive effects of light exposure and dietary carotenoid availability on retinal carotenoid accumulation and oxidative stress.

Methods

Experiment 1

Capture and housing of study animals

In October 2007, we captured 16 adult male and 13 adult female House Finches on the campus of Arizona State University (ASU) in baited basket traps following the methods described in Toomey and McGraw (2009). These birds were housed as male/female pairs or singly ($n = 3$ males) in small cages (0.6 m × 0.4 m × 0.3 m) on top shelves of movable racks. These racks were kept in an outdoor enclosure within an animal run designed for large mammals. This space included areas of direct sun exposure and shaded areas under a metal roof. The birds were provided with ad libitum access to tap water for drinking and a maintenance diet (ZuPreem small bird maintenance diet, Premium Nutritional Products

Inc. Mission, KS, USA) that contained two predominant carotenoid types—lutein (1.15 ± 0.12 μg g^{-1}) and zeaxanthin (0.52 ± 0.06 μg g^{-1}).

Sun exposure manipulation

To manipulate sun light exposure among the birds, we controlled the amount of time during each weekday that birds were exposed to direct sunlight versus shade, with the intention of mimicking variation in light exposure that birds might experience in the natural environment. We arranged the caged birds on two mobile racks (Metro-MaxQ, InterMetro Industries Corp., Wilkes-Barre, PA, USA) and placed one rack in the direct sun for a period of 3 h per day (low-light exposure, $n = 8$ males, 7 females), while the other rack was kept in direct sunlight for 8 h per day (high-light exposure, $n = 8$ males, 6 females). The 8-h experimental sun-exposure period was from 08:30 to 16:30 h, while the 3-h period was randomized among days to occur sometime within that same 8-h time span. At night (16:30–08:30 h) and all day on weekends (Saturday and Sunday), both treatment groups were kept in the shade for the entire day. To track levels of light exposure in each treatment group, we attached data loggers (HOBO UA-002-64, Onset Computer Co. Bourne, MA) to each rack and recorded light intensity (lux) and temperature at 4-min intervals throughout the study. The mean daily light intensity and temperature profiles for each treatment group are shown in Fig. 1a, b. We continued this sun exposure regime for 8 weeks, a duration that we have previously used to examine the effects of diet and health manipulations on retinal carotenoid accumulation in House Finches (Toomey and McGraw 2010; Toomey et al. 2010).

Body mass, food consumption, and carotenoid measurements

To examine the possible influence of sun exposure on body mass and food intake of the birds, which might affect carotenoid intake/use in ways independent from direct sun exposure, we weighed the birds before starting the manipulation (week 0), in the middle of the study (week 4), and at the conclusion of the study (week 8). In week 3 of the manipulation, we measured the mass of food consumed by each pair of birds in a 24-h period. On weeks 0, 4, and 8, we collected plasma samples (~40 μL) from each bird and determined circulating carotenoid levels with HPLC following Toomey and McGraw (2009). At the conclusion of the study, we euthanized all birds, dissected out the retina of the left eye, and measured retinal carotenoid concentrations by HPLC (Toomey and McGraw 2009). As in previous studies, we observed six major types of carotenoids in the House Finch retina and we report concentration per whole retina (Toomey and McGraw 2009, 2010). The galloxanthin measurement includes both galloxanthin (41.1 ± 0.07 %) and an apocarotenoid of undetermined structure (58.9 ± 0.07 %) with a short wavelength shifted spectrum that has recently been described in Toomey et al. (2015).

Oxidative-stress measurement

Oxidative stress in the retina was measured using a miniaturized thiobarbituric acid reactive substances (TBARS) assay modified from a commercially available kit (Oxi-Tek TBARS assay kit, ZeptoMetrix Corp., Buffalo, NY). The TBARS assay provides a measure of oxidative stress by quantifying levels of lipid peroxidation products, specifically malondialdehyde (MDA), a major marker of oxidative stress (Janero 1990; also see Alonso-Álvarez et al. 2010 for its use in avian research). Briefly, whole retinas were dissected out of the right eye, weighed to the nearest 0.0001 g with a digital balance, and then homogenized in 500 μL of phosphate buffered saline. A 30 μL aliquot of this homogenate was mixed with 30 μL of 8.1 % sodium dodecyl sulfate (SDS) and 750 μL of thiobarbituric acid (TBA) buffer reagent. Samples were then incubated at 95 °C for 60 min, placed on ice for 10 min, and centrifuged at 3000 r min^{-1} for 15 min. We measured absorbance of the supernatant at 540 nm and calculated concentration by comparison to a standard curve of known concentrations of MDA (expressed in nmol mg^{-1} of MDA equivalents).

Statistical analyses

We compared mean daily light intensities and temperatures and the food consumption of the high- and low-light exposure treatments using a Student's t test. We compared changes in body mass and plasma carotenoid levels over time between the sexes and treatment groups using repeated-measures analyses of variance (rmANOVA). We compared retinal carotenoid levels between the sexes and treatment groups with a multivariate analysis of variance (MANOVA), with the six major retinal carotenoid types as the dependent variable. We compared lipid peroxidation levels between the sexes and treatment groups using analysis of covariance (ANCOVA), with total retinal carotenoid concentration as the covariate. Lipid peroxidation values were natural-log transformed to meet the assumptions of normality. ANOVA models initially included all possible interaction terms, if the three-way interaction terms were non-significant, we removed them from the models. We retained all two-way interactions and main effects in the final models. All statistical analyses were carried out in R 2.12 (R Foundation for Statistical Computing 2010), values are reported as mean ± SE, and the alpha level was set at 0.05. Detailed tables of the group means, standard error, and sample sizes are presented in the Additional file 1: Tables S1–4.

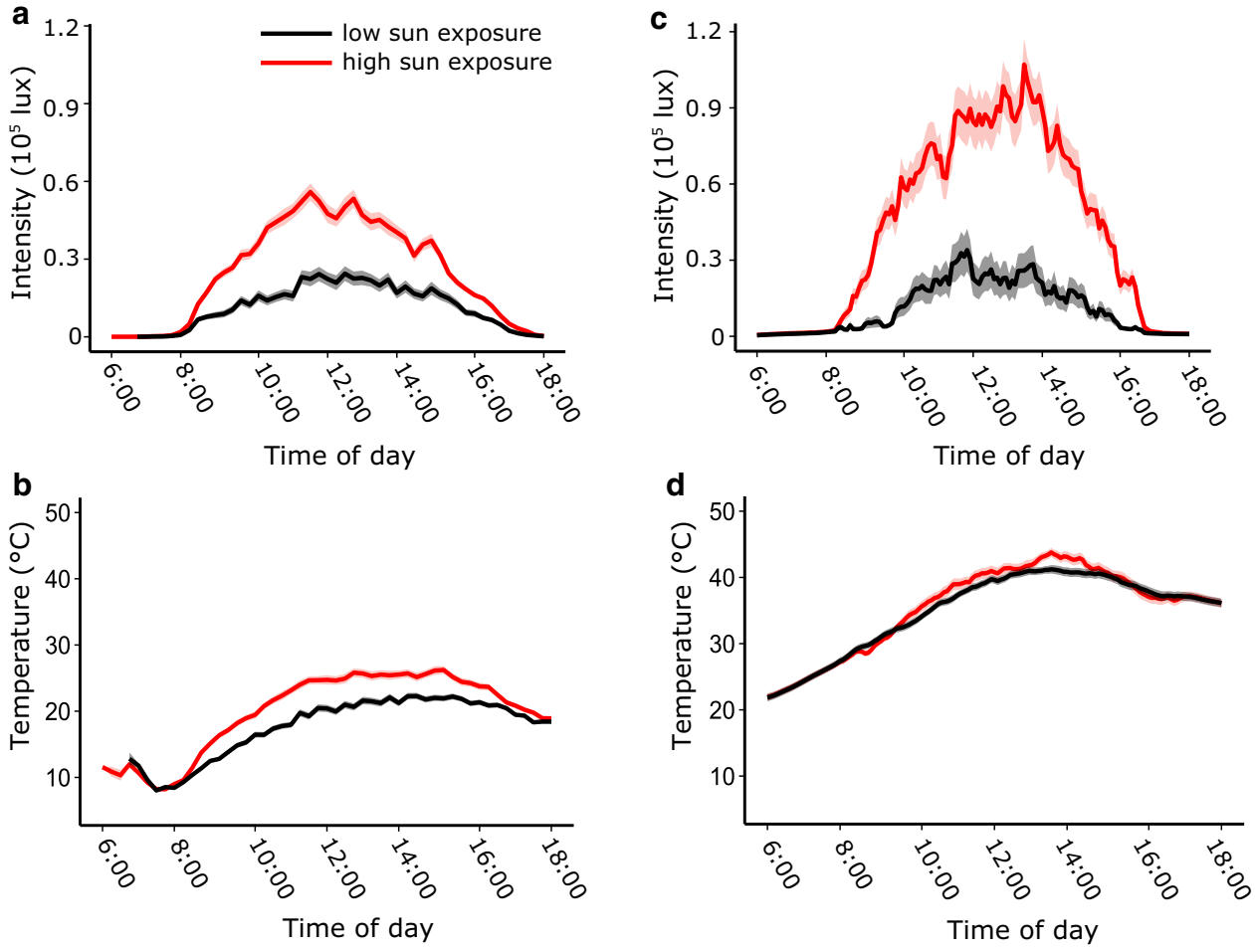

Fig. 1 Mean ± SE daily light-intensity and temperature profiles for the high sun exposure (*red*) and low sun exposure (*black*) treatment. **a** The light intensity and **b** temperature profiles from experiment 1 and **c** light intensity and **d** temperature profiles from experiment 2

Experiment 2

Capture and housing of study animals

In April 2010, we captured 32 adult male House Finches at a private residence ~1.2 km from the ASU campus as described above. We limited this study to male birds because we found no significant difference between the sexes in experiment 1 (see more below) and because we wanted to avoid taking females that were actively laying eggs at this time of year (Hill 1993). The birds were housed as pairs in the same cage types with the same base diets as described above. For this experiment, the cage racks were kept in a large outdoor aviary at the same facility as experiment 1 that offered similar areas of sun and shade.

Light-exposure manipulation

The sun exposure manipulation mimicked experiment 1, with 16 males receiving 3 h per day of direct sun (low sun exposure) and 16 males receiving 8 h per day (high sun exposure). However, this experiment was conducted in the summer when outdoor temperatures in the direct sun can reach 46 °C. To counter these extreme temperatures, we used a combination of fans and a misting system to cool the birds in the direct sunlight. Unfortunately, during week 6, this cooling system failed and resulted in the death of five birds in the high-light treatment. After this incident, the birds were monitored continuously for signs of heat stress (e.g., gaping, lethargy) and removed from the direct sun for 30-min intervals if necessary. These cooling bouts were infrequent, occurring at a maximum of three times per day and are included in the calculation of mean light and temperature levels presented in Table 1.

Carotenoid supplementation

To examine if and how dietary carotenoid levels might interact with sun exposure and influence carotenoid accumulation and oxidative stress in the retina, we

Table 1 Light and temperature conditions among treatment groups in experiments 1 and 2

Experiment	Dates	Mean day length (min)	Sun exposure treatment	Daily hours of direct sun exposure	Mean light intensity (lux)[a]	Min–max light intensity (lux)	Mean temperature (°C)[a]	Min–max temperature (°C)
1	7 Jan–3 Mar 2008	642.88 ± 3.69	High	8	12,746.93 ± 197.76	0–176,356	15.69 ± 0.059	1.7–49.4
			Low	3	5714.55 ± 176.73	0–209,424	14.37 ± 0.047	1.5–48.4
2	10 May–5 July 2010	852.10 ± 1.44	High	8	21,561.29 ± 369.69	0–220,225	31.42 ± 0.066	11.5–56.8
			Low	3	5584.77 ± 183.33	0–231,468	31.07 ± 0.061	11.9–54.9

[a] Differed significantly between treatment groups and experiments ($t > 4.02$, $p < 0.0001$)

supplemented the diets of eight of the birds in each light treatment with zeaxanthin. Zeaxanthin was chosen because it is a common carotenoid in finch retinas (Toomey and McGraw 2009) as well as the putative precursor for many of the other retinal carotenoid types (Schiedt et al. 1991; Bhosale et al. 2007). Zeaxanthin (17.5 μg mL^{-1}, OptiSharp™, DSM Inc. Heerlen, Netherlands) was given in the drinking water along with a vitamin supplement (Vita-Sol, United Pet Group EIO, Tampa, FL) for carotenoid-treated birds; control animals received only the vitamin supplement in their water. We used a carotenoid dose that was intermediate to the high levels of our previous investigations of diet (Toomey and McGraw 2010), because the high temperatures in the current study were likely to result in increased water consumption (Bartholomew and Cade 1956) and we wanted to avoid subjecting the birds to an unnaturally high daily dose. To further reduce the confounding effects of temperature, the drinking-water treatments were administered each weekday evening, after the light exposure manipulation was finished and when birds from both light treatments were in the shade. We replaced the supplemented water with plain tap water each morning, prior to the light-exposure manipulation, to ensure that differences in carotenoid accumulation were not driven by the rate of water consumption while the birds were differentially exposed to direct sun.

Body mass, food consumption, carotenoid, and oxidative stress measurements

Body mass, plasma and retinal carotenoid levels, and retinal oxidative stress were measured as described for experiment 1. Food consumption was measured as in experiment 1, but during weeks 2 and 6 in this experiment.

Statistical analyses

Statistical analyses were carried out as described for experiment 1, with the inclusion of carotenoid supplementation level and the interaction of sun exposure and supplementation levels as factors in each test. Also, as all of the birds in experiment 2 were males, sex was not included as a factor in ANOVA models. We natural-log transformed the plasma carotenoid measures to meet the assumptions of parametric statistics. Detailed tables of the group means, standard error, and sample sizes are presented in the Additional file 1: Tables S5–8.

Comparisons between experiments 1 and 2

Among wild house finches, retinal carotenoid levels vary with the seasons, with a minimum in the early spring (March) and a peak in the late fall (November; Toomey and McGraw 2009). However, it is not clear from this observation what factors (e.g. diet, health, and reproductive state) drive these seasonal differences. A comparison of individuals from experiments 1 and 2 offers the opportunity to examine the influence of season, while controlling for dietary carotenoid availability. For this comparison, we limited our analyses to male finches receiving the non-supplemented diet, leaving us with 16 males from experiment 1 and 15 from experiment 2. The only differences between the experimental groups were the year, date, time in captivity prior to the sun manipulation (66 and 35 days respectively), and the sex of their cage mate (see above). We compared retinal carotenoid accumulation between the experiments and sun exposure treatments in a MANOVA and used univariate ANOVA to compare total plasma carotenoid levels and retinal lipid peroxidation levels.

Results

Experiment 1

Light intensity and temperature

In the high sun exposure treatment, birds experienced 2.2× greater mean light intensities than the low sun exposure group (Table 1; Fig. 1a). Similarly, birds in the high sun exposure treatment experienced 3.4 °C higher temperatures, on average, than low sun exposure birds (Table 1; Fig. 1b).

Body mass and food consumption

There was no significant effect of sun exposure on body mass of the finches (rmANOVA: treatment × date – $F_{2,51} = 0.34$, $p = 0.71$) or food consumption in a 24-h period ($t = -0.99$, $p = 0.34$). Body

mass changed significantly over the course of the study (rmANOVA: date – $F_{2,51} = 16.78$, $p < 0.0001$, Fig. 2a), but there was no significant interaction with sun exposure (rmANOVA: sun exposure × date – $F_{2,49} = 0.28$, $p = 0.76$). Body mass did not differ significantly between the sexes ($F_{1,25} = 1.01$, $p = 0.32$). The loss and recovery of body mass (Fig. 2a) commonly occurs when wild House Finches are brought into captivity (e.g., Toomey and McGraw 2010) and is unlikely to be related to the specific conditions in this study.

Retinal and plasma carotenoid accumulation

Retinal carotenoid concentration did not differ significantly between the sexes (Table 2) or between the high- and low-sun-exposed birds (Table 2; Fig. 3a). However, plasma carotenoid levels did change significantly over the course of the study and there was a significant interaction

Table 2 Results of MANOVA analyses testing the effect of sun exposure, sex, and their interaction on retinal carotenoid accumulation in experiments 1 and 2

Factor	Wilks' λ	df	*p*
Experiment 1			
Sun exposure	0.89	6, 18	0.90
Sex	0.78	6, 18	0.58
Sun exposure × sex	0.82	6, 18	0.69
Experiment 2			
Sun exposure	0.71	6, 23	0.21
Diet	0.57	6, 23	*0.033*
Sun exposure × diet	0.81	6, 23	0.50

Significant terms are denoted in italics

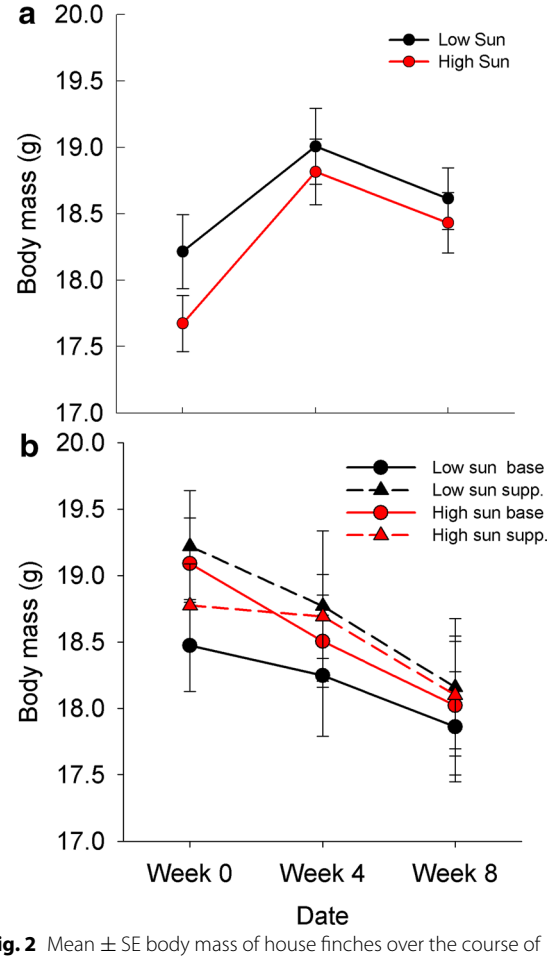

Fig. 2 Mean ± SE body mass of house finches over the course of experiments 1 (**a**) and 2 (**b**). Week 0 measurements were performed just prior to the beginning of sun exposure and dietary (experiment 2 only) manipulations. In experiment 2 (**b**) "supp." birds received a zeaxanthin supplement in their drinking water

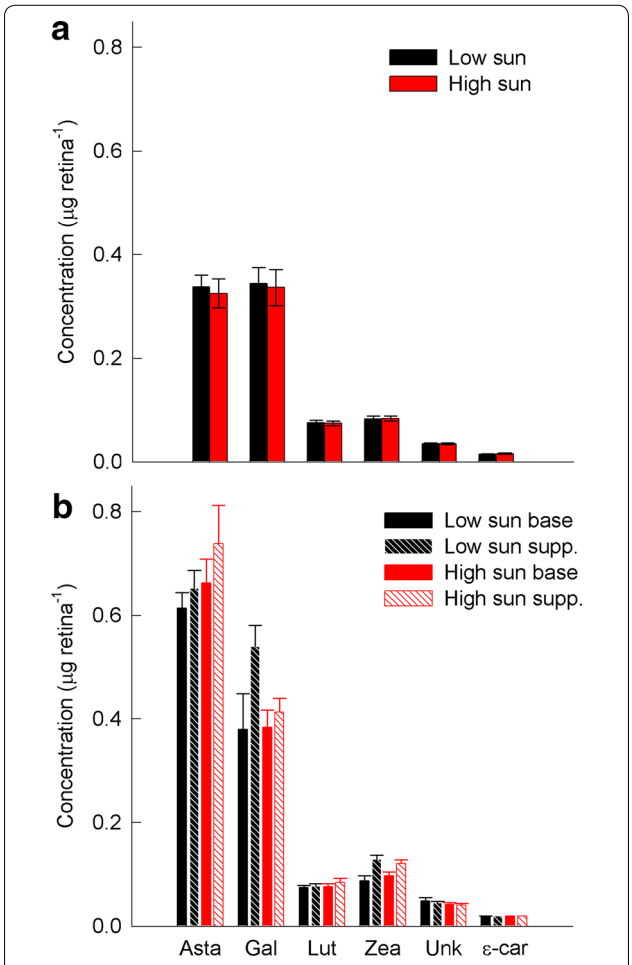

Fig. 3 Mean ± SE retinal carotenoid levels of house finches in experiments 1 (**a**) and 2 (**b**). In experiment 2 (**b**) "supp." birds received a zeaxanthin supplement in their drinking water. The major retinal carotenoid types measured in house finches were astaxanthin (Asta), galloxanthins (Gal), lutien (Lut), zeaxanthin (Zea), an unidentified carotenoid (Unk), and ε-carotene (ε-car)

between sun exposure, sex, and date (rmANOVA: sun exposure × sex × date − $F_{2,48} = 4.04$, $p = 0.024$, Fig. 4a). In general, plasma carotenoid levels tended to decline from week 0 to week 4 and then increase from week 4 to week 8. However, females in the low-sun exposure condition diverged from the other treatment × sex groups and showed little change in circulating carotenoid levels between weeks 4 and 8 (Fig. 4a).

Retinal oxidative stress

Lipid peroxidation levels in the retina did not differ significantly between sun exposure treatments or the sexes and was not significantly correlated with total retinal carotenoid concentrations (Table 3; Fig. 5a).

Experiment 2

Light intensity and temperature

Birds in the high sun exposure treatment experienced 3.8× greater mean light intensities and significantly higher temperatures than did those in the low sun exposure group (Table 1; Fig. 1c). However, the difference in mean temperatures among treatments was <1 °C (Table 1; Fig. 1d).

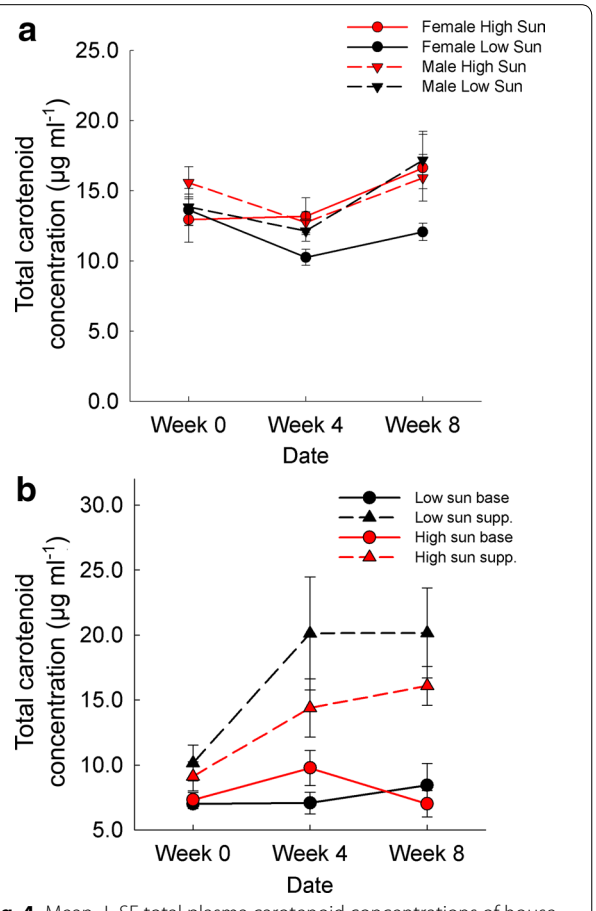

Fig. 4 Mean ± SE total plasma carotenoid concentrations of house finches over the course of experiments 1 (**a**) and 2 (**b**). Week 0 measurements were performed just prior to the beginning of sun exposure and dietary (experiment 2 only) manipulations. In experiment 2 (**b**) "supp." birds received a zeaxanthin supplement in their drinking water

Table 3 Results of ANOVA analyses testing the effects of sun exposure, sex, and their interaction on retinal lipid peroxidation in experiments 1 and 2

Factor	*F*	df	*p*
Experiment 1			
Sun exposure	0.14	1, 20	0.23
Sex	0.002	1, 20	0.88
Total retinal carotenoids	0.006	1, 20	0.81
Sun exposure × sex	0.004	1, 20	0.83
Sun exposure × total retinal carotenoids	0.0002	1, 20	0.96
Sex × total retinal carotenoids	0.22	1, 20	0.15
Experiment 2			
Sun exposure	0.027	1, 21	0.55
Diet	0.0093	1, 21	0.72
Sun exposure × diet	0.0058	1, 21	0.94

Body mass and food consumption

Body mass again declined over the course of the experiment, and there was a significant three-way interaction between date, carotenoid supplementation, and sun exposure treatment (diet × treatment × date: $F_{2,50} = 5.07$, $p = 0.0099$; Fig. 2b). This interaction likely reflects variation in body mass among the treatment groups at the beginning of the experiment; however within sampling periods, there were no significant differences in body mass among dietary and sun exposure treatment groups (Tukey post hoc, $p > 0.98$, Fig. 2b). Consistent with the decline in mass, food consumption declined significantly between the May and June sampling periods from 9.74 ± 0.40 to 6.91 ± 0.18 g day^{-1} cage^{-1} ($F_{1,15} = 57.80$, $p < 0.0001$), but did not differ significantly between diet treatments ($F_{1,13} = 0.045$, $p = 0.83$) or sun exposure treatments ($F_{1,13} = 5.29$, $p = 0.55$).

Retinal and plasma carotenoid accumulation

Retinal carotenoid accumulation was significantly higher in zeaxanthin-supplemented birds than in control, unsupplemented birds (Table 2; Fig. 3b). Specifically, zeaxanthin-supplemented birds had significantly higher levels of galloxanthin ($F_{1,29} = 4.23$, $p = 0.049$) and zeaxanthin ($F_{1,29} = 14.68$, $p < 0.001$) in the retina. There was no significant effect of sun exposure on retinal carotenoid accumulation, or a significant interaction of sun exposure and carotenoid supplementation (Table 2). Zeaxanthin supplementation significantly increased circulating plasma carotenoid levels (rmANOVA:

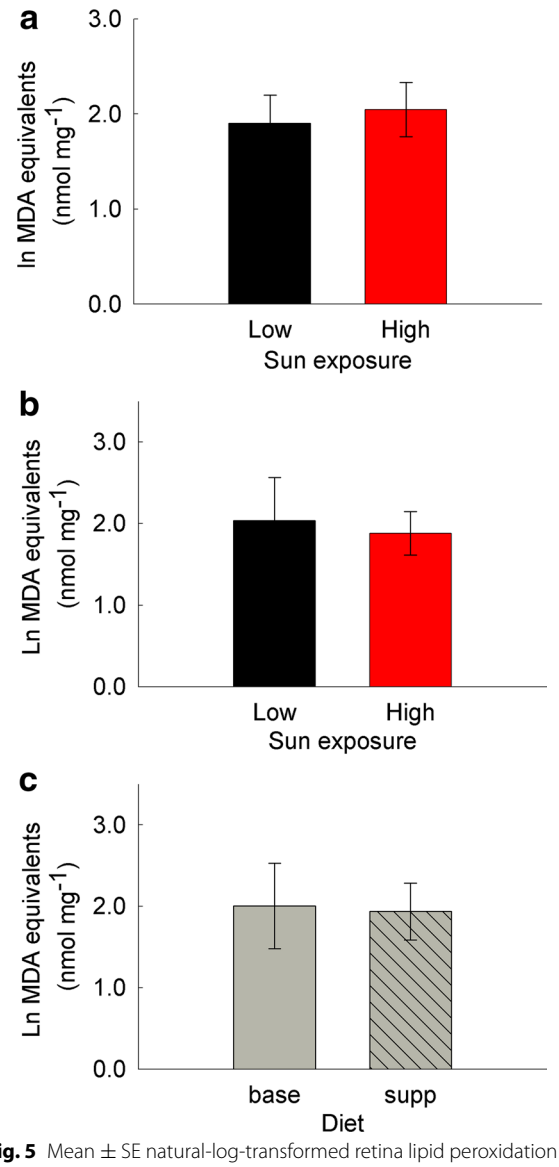

Fig. 5 Mean ± SE natural-log-transformed retina lipid peroxidation (MDA equivalents) levels in retinas of low- and high-sun exposed house finches in **a** experiment 1 and **b** experiment 2. **c** Lipid peroxidation levels of house finches receiving the low carotenoid base diet (base) or a zeaxanthin supplement (supp)

diet × date – $F_{2,52} = 7.09$, $p = 0.0019$, Fig. 4b), but there was no significant effect of sun exposure ($F_{1,28} = 0.78$, $p = 0.38$) or interaction between sun exposure and diet on total plasma carotenoid levels ($F_{1,28} = 1.56$, $p = 0.22$).

Retinal oxidative stress

Retinal lipid peroxidation levels did not differ significantly between sun exposure or diet treatments (Fig. 5b, c), and there was no significant interaction between sun exposure and zeaxanthin supplementation (Table 3).

Comparisons between experiments 1 and 2

Retinal carotenoid accumulation differed significantly between males in experiment 1 and 2 (Wilks' $\lambda = 0.15$, df = 6, 22, $p < 0.0001$); specifically, unsupplemented males in experiment 2 had significantly higher levels of astaxanthin, an unknown carotenoid, and ε-carotene than males in experiment 1 (Table 4; Fig. 6a). In contrast, birds in experiment 1 circulated significantly higher levels of carotenoids in their plasma than birds in experiment 2 ($F_{1,25} = 25.26$, $p < 0.0001$, Fig. 6b). There was no significant difference in the levels of retina lipid peroxidation ($F_{1,25} = 0.023$, $p = 0.88$) or body mass at week 8 ($F_{1,25} = 2.99$, $p = 0.096$) between experiments 1 and 2.

Discussion

Our goal in this pair of experiments was to examine if and how exposure to intense natural sun light and dietary carotenoid availability influence the accumulation of carotenoids in retinas of a wild bird, the House Finch. We found that: (1) manipulating direct sunlight exposure did not significantly affect carotenoid levels or lipid peroxidation in the retina, (2) dietary carotenoid supplementation increased retinal carotenoid accumulation but did not influence retinal lipid peroxidation levels, and (3) carotenoid levels in the retina differed seasonally, despite similar diet and housing conditions in the two experiments.

Contrary to our predictions, we found no significant differences in retinal carotenoid or lipid peroxidation levels between birds exposed to long versus short daily bouts of direct sunlight exposure. This suggests that the exposure to direct sunlight may not be a major factor driving the variation in retinal carotenoid accumulation that we have previously observed among free-living house finches (Toomey and McGraw 2009). However, interpreting these negative results requires careful consideration of the design of our study and the biology of the House Finch. Our manipulation (twofold to threefold change in average light exposure) was intended to mimic the variation a wild bird might experience and was much smaller than previous studies that have demonstrated physiological changes in the avian eye. Hart et al. (2006) observed significant increases in the carotenoid pigmentation (i.e., light-absorbance properties) of the cone oil droplets of chickens reared under relatively constant exposure to bright light averaging 70,250 lux compared to birds reared in dim light averaging 14 lux (Hart et al. 2006). However, such a large and persistent difference (>5000-fold) in light intensity is a condition that wild birds, especially desert dwelling House Finches, are unlikely to encounter in the natural environment.

In addition to the intensity and duration of the light manipulation, there are several other aspects of our study design that contrast with previous studies. We

Table 4 Results of ANOVA analyses testing the effects of light exposure, experiment, and their interaction on the accumulation of specific types of retinal carotenoids

Factor	*F*	df	*p*
Astaxanthin			
Light treatment	1.68	1, 27	0.21
Experiment	64.76	1, 27	*<0.0001*
Light treatment × experiment	0.084	1, 27	0.77
Galloxanthin			
Light treatment	0.44	1, 27	0.51
Experiment	1.49	1, 27	0.23
Light treatment × experiment	0.55	1, 27	0.46
Lutein			
Light treatment	1.10	1, 27	0.30
Experiment	0.064	1, 27	0.80
Light treatment × experiment	1.28	1, 27	0.27
Zeaxanthin			
Light treatment	0.0045	1, 27	0.95
Experiment	1.38	1, 27	0.25
Light treatment × experiment	1.21	1, 27	0.28
Unknown			
Light treatment	1.03	1, 27	0.32
Experiment	8.37	1, 27	*0.0074*
Light treatment × experiment	0.12	1, 27	0.73
ε-Carotene			
Light treatment	1.69	1, 27	0.21
Experiment	31.22	1, 27	*<0.0001*
Light treatment × experiment	1.23	1, 27	0.28

Significant terms are denoted in italics

manipulated light exposure over the course of 8 weeks in adult birds, which is a significantly shorter period than the 30-week manipulation of young chickens employed by Hart et al. (2006). Carotenoids in the avian retina are quite stable (Toomey and McGraw 2010), and it is possible that much longer-term changes in sun exposure are required to alter accumulation. Studies that have demonstrated light-mediated effects on the avian eye (e.g., Harrison et al. 1968; Hart et al. 2006; Blatchford et al. 2009) have all used young domesticated chickens. It is possible that the influence of light is limited to the developmental period and would explain why we did not detect changes among the adult birds in our study. Consistent with this hypothesis, there is growing evidence linking developmental conditions (i.e. dietary carotenoid levels) to adult carotenoid assimilation and accumulation efficiency in birds (Blount et al. 2003; Butler and McGraw 2011). Additionally, it is possible that the effects of light exposure were localized to specific regions of the retina that we were unable to detect with our whole retina measurement. Dietary carotenoid supplementation tends to enhance carotenoid pigmentation specifically in the dorsal retina (Knott et al. 2010), and the effects of light exposure on retinal oil droplet absorbance are most pronounced in the ventral retina (Hart et al. 2006).

Contrary to our predictions, the duration of direct sunlight exposure did not significantly affect lipid peroxidation levels in the retina, and we found no significant relationship between retinal carotenoid accumulation and lipid peroxidation, however, the transience of lipid peroxidation, time course of our manipulations, and our terminal sampling regime may have limited our ability to detect this effect. In the rat (*Rattus norvegicus*) retina, lipid peroxide levels peak 3 h after the application of a stressor (ischemia–reperfusion) and return to normal 48 h after the stressor has been removed (Shibuki et al. 2000). Thus, the extended duration of our manipulations may have allowed for adaptation to and recovery from light-induced lipid peroxidation (i.e. each evening following the light treatments). It is also important to consider that the house finch is native to the Sonoran desert (Hill 1993) and may be well-adapted to the stresses of intense sunlight that is characteristic of this open desert habitat.

Consistent with our previous study of House Finch retinas (Toomey and McGraw 2010, 2011, 2012), dietary carotenoid supplementation led to significantly higher retinal carotenoid levels. Specifically, supplementation with dietary zeaxanthin increased galloxanthin and zeaxathin levels in the retina. We have speculated that the specificity of these diet-driven increases may be attributable to differing rates of carotenoid degradation and replacement in the retina (Toomey and McGraw 2010). However, there was no significant interaction between sun exposure and dietary carotenoid levels; thus the photodegradation of specific carotenoids in the retina is unlikely to be driving this pattern of specific diet-enhanced carotenoid accumulation.

Under similar dietary and housing conditions, male finches in experiment 2 (May–July) had significantly higher retinal carotenoid levels, but lower plasma carotenoid concentrations, than the males in experiment 1 (Jan–Mar). This pattern of seasonal retinal accumulation is consistent with observations of wild birds (Toomey and McGraw 2009), where retinal carotenoid levels tend to be lowest in the late winter and increase through the summer and fall seasons. These seasonal differences could be driven by of the increased day length and more intense sunlight in the summer months and may have swamped the effects of our finer-scale light manipulations. Thus, the high retinal carotenoid levels found in males from the summer experiment are consistent with a seasonal up-regulation of accumulation to optimize photoprotection and visual performance (Hart et al. 2006). The relatively lower plasma carotenoid levels among these same

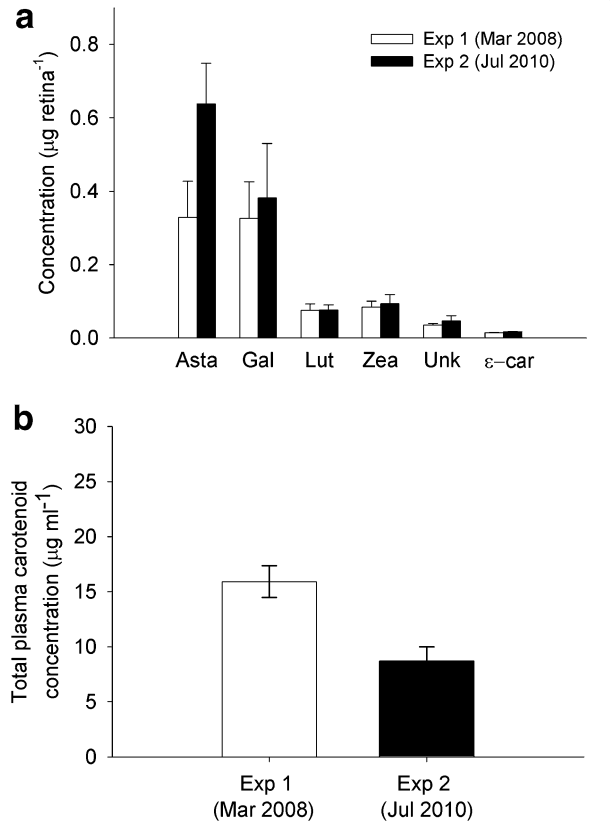

Fig. 6 **a** Mean ± SE retinal carotenoid concentration of unsupplemented male house finches in experiments 1 and 2. Carotenoid types abbreviated as in Fig. 3. **b** Mean ± SE plasma carotenoid concentrations of unsupplemented male house finches in experiments one and two at the conclusion (week 8) of each study

males might reflect light-related depletion of plasma carotenoids. However, Blount and Pike (2012) reported increased circulating carotenoid levels in zebra finches in response to UV light exposure. Yet, any effects of light intensity between seasons in our study are confounded with changes in temperature and day length that are also important cues in the timing of reproduction in the House Finch (Hamner 1966) and may indirectly influence carotenoid physiology.

The timing of two experiments corresponds to distinct phases in the reproductive cycle of the house finch, which suggests that a proximate hormonal mechanism could mediate seasonal differences in carotenoid accumulation. Experiment 1 occurred at the beginning of the breeding season, when birds are pairing and testosterone levels are highest in males (Hamner 1966; Duckworth et al. 2004), whereas experiment 2 took place during the nestling and post-nesting phase, when house finches become photorefractory and testosterone levels typically drop (Hamner 1968; Duckworth et al. 2004). There is a growing body of evidence that testosterone alters carotenoid bioavailability (Blas et al. 2006; McGraw et al. 2006; Casagrande et al. 2011), and experimentally elevated testosterone levels are known inhibit the accumulation of carotenoids in house finch plumage (Stoehr and Hill 2001). Thus, reproductive state and possibly testosterone may influence the accumulation of carotenoids in the retina.

Additionally, the social environment in the experiments may have impacted carotenoid allocation to the retina. Males in experiment 1 were housed with females, while males in experiment 2 were housed only with other males. Zebra Finches in mixed versus single sex conditions have been shown to significantly shift their allocation of carotenoids, with males in mixed groups increasing carotenoid-based bill coloration (Gautier et al. 2008). In wild populations of Red Grouse (*Lagopus lagopus scoticus*), levels of circulating carotenoids are correlated with levels of intersexual competition, but not directly linked to circulating testosterone levels (Martínez-Padilla et al. 2014). Thus social environment may influence the allocation and availability of carotenoids independent of hormonal mechanisms.

Conclusion

Taken together, these studies indicate that the duration of intense sun light exposure does not influence the accumulation of carotenoids in retinas of adult House Finches. The House Finch retina also appears to be buffered against the oxidative stresses of intense sun exposure, which may reflect an adaptation to its bright desert environment. However, the comparison of experiments one and two suggests a role for seasonal cues in determining retinal carotenoid accumulation. There are several potential mechanisms that could drive these seasonal differences, including the direct influence of day length on the retina and/or hormone-mediated shifts in carotenoid allocation.

Additional file

Additional file 1: Tables S1–8. Detailed data tables from each of the experimental comparisons.

Authors' contributions
MBT and KJM conceived the study, designed the experiments, and wrote the manuscript. MBT conducted the experiments and analyzed the data. Both authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Ethics approval
Birds we captured and housed under approval of United States Fish and Wildlife Service permit #MB088806-1 and Arizona State Game and Fish scientific collecting permit SP727468. The experimental protocols were approved by the Arizona State University Institutional Animal Care and Use Committee (protocol #09-1054R).

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