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Indoor housing during development affects moult, carotenoid circulation and beak colouration of mallard ducks (*Anas platyrhynchos*)

Michael W. Butler* and Kevin J. McGraw

School of Life Sciences, Arizona State University, Tempe, Arizona 85287, USA
**E-mail: Mike.Butler@asu.edu*

ABSTRACT

Developmental conditions are known to influence adult phenotypes, including naturally selected traits such as structural size, as well as sexually selected traits such as song and colouration. Biotic environmental factors, *e.g.*, neonatal nutrition and health, are especially known to shape expression of adult traits, but there are fewer investigations on the role of abiotic conditions (*e.g.*, lighting, climate) during the post-natal period. Moreover, the majority of such studies in birds is limited to galliforms and fail to examine sexual signals. We reared mallard (*Anas platyrhynchos*) drakes under several different husbandry regimes – outdoors with a natural, early-spring photoperiod, or indoors with an artificial, late-spring photoperiod provided by either high-quality or low-quality indoor lights. We tracked growth, moult progress, antioxidant (carotenoid and vitamin A) circulation, and beak colouration (a sexually selected trait) during development and into adulthood. It was found that birds housed outdoors completed moult significantly faster and exhibited more saturated beak colouration at adulthood compared to birds reared indoors under either high or low-quality lighting. Body mass and antioxidant circulation generally did not differ between outdoor-reared birds and those reared indoors on high-quality lighting; however, those reared under high-quality indoor light were heavier than those reared under low-quality light. These results indicate that environmental conditions during ontogeny can impact the development of a sexually attractive phenotype (*e.g.*, faster moult, more colourful bare-parts) in mallards, although the nature of our experimental design does not identify specific causal factors. This finding has implications for the proper husbandry and plumage maintenance of a model avian species.

Keywords: *housing conditions, ontogeny, organisational effects, carotenoid pigmentation*

1. INTRODUCTION

Phenotypes of adult animals often reflect the very recent and current conditions they experience (*e.g.*, body mass is affected by diet), but they may also be impacted by environmental and physiological factors early in life, either *in ovo*, *in utero* or post-natal (Monaghan, 2008). Biotic environmental variables such as nutrition, parasites, and parental care can have strong, permanent effects on characteristics like body size (Ohlsson and Smith, 2001). In addition to naturally selected traits like size, exaggerated sexually selected features might also be influenced by developmental conditions. For example, in birds, neonatal nutrition affects wattle size and colouration in adult pheasants (*Phasianus colchicus*; Ohlsson *et al.*, 2002) and song complexity in swamp sparrows (*Melospiza georgiana*; Soma *et al.*, 2006).

While much work on early-life environmental control of adult phenotype in birds has centred on biotic factors, there is good reason to suspect that abiotic conditions (*e.g.*, temperature, light) experienced during ontogeny also strongly shape trait expression later in life. Temperature can have clear effects on nutritional and thermoregulatory demands of neonates (Østnes and Bech, 1997) and thus adult phenotype. In growing chickens, photoperiod and light intensity can affect numerous developmental variables, including body weight (Keshavarz, 1998; Leeson *et al.*, 2005) and rate of sexual maturation (Lewis *et al.*, 2001; Renema and Robinson, 2001; Lewis and Gous, 2006). Light cues also play a role in the moulting process (Chilgren, 1978; Dawson *et al.*, 2000, 2001). However, to our knowledge, there are no studies that have examined the long-lasting phenotypic effects of the developmental rearing environment in an avian species other than galliforms.

In a previous study (Butler and McGraw, unpublished data), we noted that moult was delayed or incomplete in several male mallards (*Anas platyrhynchos*) that were reared indoors. Similar disrupted

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patterns of moult in birds housed under indoor lighting conditions have also been observed in other species, such as American goldfinches (*Carduelis tristis*, KJM, personal observation) and Anna's hummingbirds (*Calypte anna*; M. Meadows, personal communication). Therefore, we elected to examine phenotypic differences in male mallard ducks (*Anas platyrhynchos*) reared in three different rearing environments. Mallards serve as model organisms in population ecology (Gunnarsson *et al.*, 2006; Pöysä *et al.*, 2004) and environmental toxicology (Levengood and Skowron, 2007; Newsted *et al.*, 2007), are precocial and therefore easy to raise in the absence of parental feeding, and also display colourful patches, some of which serve as sexually selected traits. They have also been reared in a variety of settings, including outdoors (André *et al.*, 2007), sequential indoor and outdoor conditions (Klint, 1980), or indoor conditions (Schrank *et al.*, 1990). Females choose males that moult into their nuptial plumage earlier in the year and those with more colourful yellow beaks (Omland, 1996a). Beak colour in mallards is due to carotenoid pigmentation, which is a sensitive indicator of nutritional and health state in many birds (Hill, 2006) and is positively associated with immune function in mallard drakes (Peters *et al.*, 2004a).

We studied ducklings growing for 16 weeks under three different rearing conditions: (1) low-quality indoor light; (2) high-quality indoor light; and (3) outdoor light. We tracked body mass, moult timing, and antioxidant (carotenoid and vitamin A) circulation throughout development in addition to beak colour expression at adulthood. Though several other factors also differed among the treatments (*e.g.*, outdoor birds experienced not only different levels of light, but also differences in temperature, humidity, *etc.*), which limits our ability to ascribe precise relationships between particular environmental variables and phenotypic expression, we were able to describe which of the three treatments resulted in appearances that were most similar to wild mallards. Based on our previous work, we predicted that birds housed outdoors would moult into their alternate (breeding) plumage at ages similar to wild mallards, while birds in low- and high-quality indoor light would exhibit severe and moderate delays, respectively, in time to moult. For similar reasons, we predicted that outdoor birds would circulate higher levels of carotenoids, particularly during the development of beak colouration, than birds reared in high-quality lighting, which would in turn circulate higher levels than those reared in low-quality light. Finally, we predicted that body mass would be larger in low-intensity light birds, as lower light intensities have resulted in larger body weights in chickens due to some unidentified mechanism (Lien *et al.*, 2007).

2. METHODS

We acquired one-day-old mallard ducklings (ssp *platyrhynchos*) of unknown sex from McMurray Hatchery (Webster City, Iowa) on 7 November 2007 and reared them in two indoor rooms in randomly selected groups of 4–6 ducklings per cage ($60 \times 60 \times 60$ cm) to mimic natural family groups until they were six weeks old (when siblings tend to separate in the wild). Rooms had no access to natural light, and there were eight fluorescent bulbs fixed to the ceiling in each room set to a 14L : 10D light cycle, with bulbs identical to those experienced by the low-quality light group (see below). Additionally, there was one infrared heat lamp per cage placed 20 cm from the cage bedding that was left on 24 h per day for the first 3 weeks to maintain an ambient temperature of approximately 30°C. Food (Mazuri Waterfowl Starter for the first 7 weeks, and Mazuri Waterfowl Maintenance thereafter) and drinking tap water were provided *ad libitum* throughout the study.

From weeks 7–21, which span the remainder of the developmental period and the beginning of the adult stage, all 19 males (identified at this point by the presence of a phallus) were individually housed in these cages under one of the following three lighting conditions: (1) LOW – receiving low-intensity indoor illumination (fluorescent bulbs with a colour rendering index [CRI], or measure of the light source's approximation of a natural light source, of 60 [with 100 perfectly approximating sunlight], with plastic covers over the bulbs; $n = 6$ ducklings); (2) HIGH – receiving high-intensity indoor illumination (92 CRI, no plastic coverings over the bulbs, in a different room from the LOW group; $n = 6$ ducklings); or (3) OUTDOOR – receiving outdoor illumination ($n = 7$ ducklings), by housing them outdoors in enclosures consisting of chain-link fencing on all four sides and a solid aluminium top. Outdoor photoperiod ranged from 10L : 14D in December (6 weeks old) to 11.5L : 12.5D in February (16 weeks old) to 12.5L : 11.5D in April (21 weeks old), while indoor photoperiod was set to 14L : 10D for birds aged 6–13 weeks, and 13L : 11D for birds aged 13–21 weeks, in order to have indoor birds more closely track the photoperiod they would experience if they were maturing at a natural time in the wild (May–July). Indoor temperatures were held relatively constant at approximately 24°C, while outdoor temps averaged lows of 7, 8, and 12°C and highs of 17, 20, and 25°C during the periods of 7–12, 13–16, and 17–21 weeks old, respectively. Therefore, while indoor

birds with both high and low-intensity illumination experienced similar temperatures, diel temperature fluctuations, and photoperiods to each other, outdoor birds differed from both groups by having generally cooler temperatures, a greater variation in daily temperature, shorter photoperiods, and within-day differences in light intensity. This study was not designed to test for the relative importance of these (and possibly other) variables in determining phenotype. Rather, we were assessing how these different common methods of mallard husbandry affect development. Therefore, we controlled indoor variables to most closely approximate optimal levels of temperature and photoperiod, while outdoor birds were reared in the late winter/early spring to accommodate the logistical constraints of rearing mallards in Arizona, where summer temperatures routinely reach 43°C. At 21 weeks of age, all birds were moved indoors and housed under the low-light conditions (above) for four weeks, as they were transitioning to take part in another study.

To track moult progress of alternate plumage development, as typified by the iridescent green head feathers, we took digital photographs (Nikon Coolpix P3, Nikon Inc., Melville, NY) of the head and neck (left side in lateral profile) of each duck at 12, 14, and 16 weeks of age, as this is when mallards generally acquire their alternate plumage. Pictures were taken with no flash against a uniformly gray background under standardised illumination. Using Adobe Photoshop v. 8 software (Adobe Systems Inc., San Jose, CA), we quantified percent alternate plumage moult by selecting the area on the head that contained newly moulted iridescent green feathers, and divided that number of pixels by the total number of pixels in the entire head (*e.g.*, mottled brown feathers above the white neck ring or the black-tipped brown breast and back contour feathers). Photos were also taken of beaks (top-down view) in weeks 16 (the end of development) and 25 (9 weeks after the completion of development), in order to quantify beak saturation (a colour metric that captures variation in carotenoid concentration; Andersson and Prager, 2006). To do this, we selected the lateral sides of the beak with the lasso marquee in Photoshop, acquired Red/Green/Blue values using the Histogram function, and used the Colour Picker tool to transform these to Hue/Saturation/Brightness scores. While this method of colour quantification does not capture UV light reflectance, it does record data relevant to carotenoid concentration and does so from the entire colourful portion of the beak, as opposed to just from a very small area, as would be the case with many spectrometers. At 12, 14, 16, and 25 weeks of age, we measured body mass to the nearest gram with a digital scale (PL202-S, Mettler Toledo, Switzerland) and took a blood sample for plasma antioxidant analyses. To collect blood, we punctured the alar vein and collected approximately 250 µL in heparanised capillary tubes. We spun down the blood for 3 minutes at 10,000 rpm and stored the plasma at −80°C until analysis.

To analyse carotenoid and vitamin A content of the plasma, we followed the hexane:methyl tert-butyl ether extraction method and high performance liquid chromatography procedures of McGraw *et al.* (2008). Detectable amounts of lutein, zeaxanthin, a lutein derivative, and β-cryptoxanthin existed in duck plasma at all time points measured. All carotenoid types were correlated within a time period (all $r > 0.6$, all $P < 0.006$), so we used total carotenoid titre as our measurement of circulating carotenoid levels. Similarly, we were able to quantify circulating levels of vitamin A, which was not correlated with total carotenoid titre ($P > 0.8$), so we considered it separately in statistical analyses.

We used repeated-measures general linear models to examine the effect of rearing environment on four different dependent variables: total carotenoid titre, percent head-plumage moult, vitamin A titre, and body mass. For each model, rearing environment was the between-groups factor and age was the within-subjects factor. We used one-way analysis of variance to look for treatment differences in beak colour saturation at 25 weeks old, and we also used analysis of covariance to examine the effect of average circulating carotenoid levels, rearing environment, and their interaction on beak saturation at 16 weeks old. To examine the relationships between variables with significant interactions, we used Tukey's post-hoc tests. Finally, we performed a regression of body mass on percent moult at 16 weeks of age (roughly when moult is complete in wild mallards; Drilling *et al.*, 2002) to see if extent of moult was related to mass. All variables met assumptions of parametric statistics, and means are presented with standard errors.

3. RESULTS

There were significant effects of housing treatment ($F_{2,16} = 72.18$, $P < 0.0001$), age ($F_{2,32} = 89.17$, $P < 0.0001$) and the treatment-by-age interaction ($F_{4,32} = 41.83$, $P < 0.0001$) on adult breeding plumage development. The significant interaction term meant that we compared differences by age within each rearing environment and differences among rearing environments within each age grouping. During weeks 14 and 16, OUTDOOR birds had a greater percent head plumage moult

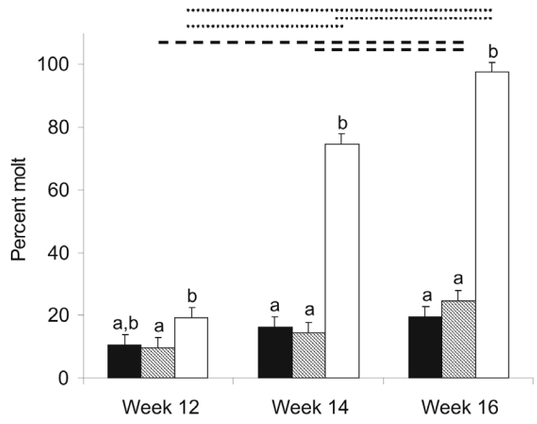

Figure 1 Percent moult as a function of housing treatment and age. Filled bars represent LOW birds, striped bars represent HIGH birds, and open bars represent OUTDOOR birds. Different letters above the bars represent statistically different ($P < 0.05$, Tukey's post-hoc tests) groupings within an age class. Dashed lines and dotted lines represent statistically different ($P < 0.05$, Tukey's post-hoc tests) age classes within HIGH birds and OUTDOOR birds, respectively. All bars are means $\pm$ SE.

than either LOW or HIGH ducks (Figure 1), with the difference most striking at week 16 when moult for OUTDOOR birds was 98% complete, but LOW and HIGH birds were on average only 20% and 25% complete (Figure 2). Also, during week 12, OUTDOOR ducks were at a more advanced stage of moult than HIGH birds. HIGH birds showed a more advanced head plumage moult at week 16 than either week 12 or 14. OUTDOOR birds showed a more advanced moult between weeks 12 and 14 and weeks 14 and 16. There was no association between body mass at 16 weeks of age and percent moult ($F_{1,17} = 0.58$, $P = 0.46$).

Rearing environment significantly affected colour saturation of the bill at week 16 ($F_{2,15} = 4.305$, $P = 0.0393$; Figure 3a), with OUTDOOR birds having more saturated beaks than HIGH birds, and LOW birds having intermediate beak saturation. At week 25, previous rearing environment also affected beak saturation ($F_{2,15} = 5.64$, $P = 0.0149$; Figure 3b),

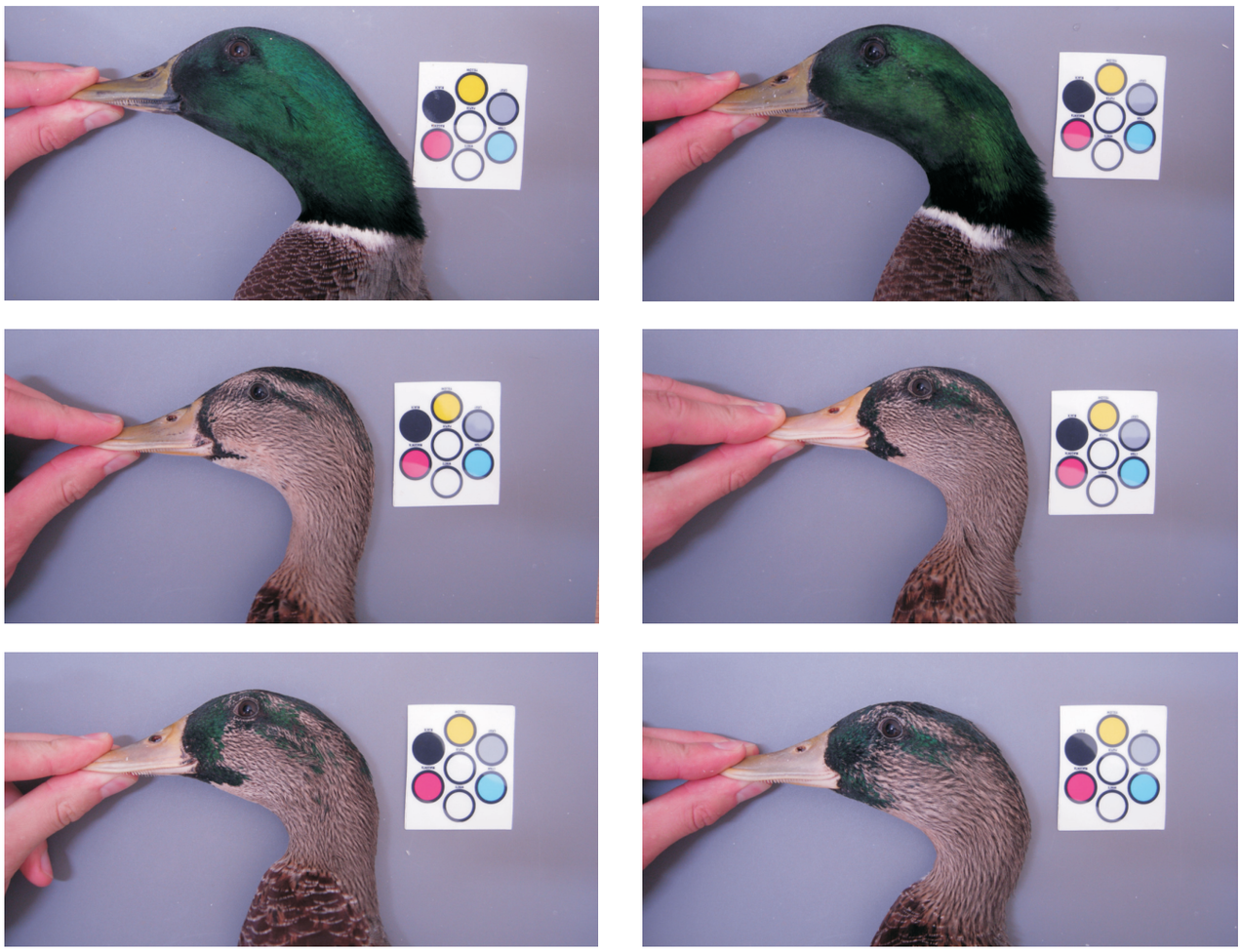

Figure 2 Representative pictures of drakes for quantifying moult progress from the three treatment groups (OUTDOOR, LOW and HIGH) when all birds were 16 weeks of age. OUTDOOR birds had an average of 98% of their head moult complete at this age, while LOW and HIGH birds had 20% and 25% of their head moult complete, respectively.

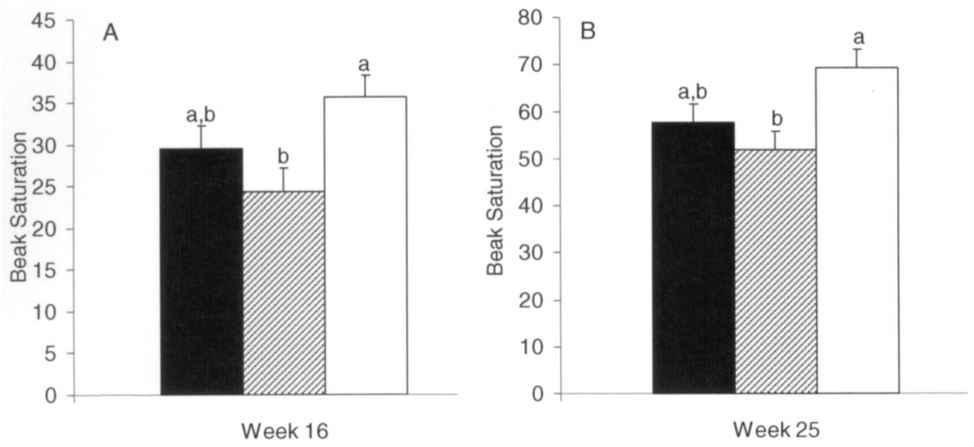

Figure 3 (**A**) Bill saturation as a function of housing treatment and age. Filled bars represent LOW birds, striped bars represent HIGH birds, and open bars represent OUTDOOR birds, all at 16 weeks old. (**B**) Similar to (A), except values represent birds that are 25 weeks old. Different letters above the bars represent statistically different ($P < 0.05$, Tukey's post-hoc tests) groupings. All bars are means $\pm$ SE.

with OUTDOOR birds having more saturated beaks than HIGH and with LOW birds having intermediate beak saturations not significantly different from HIGH or OUTDOOR. Beak saturation was not associated with average circulating carotenoid levels at 16 weeks of age ($F_{1,15} = 0.01$, $P = 0.93$).

Rearing environment did not have an effect on circulating carotenoid levels ($F_{2,16} = 0.71$, $P = 0.51$), although there was a significant effect of age ($F_{2,32} = 3.31$, $P = 0.049$) and an interaction between rearing environment and age ($F_{4,32} = 5.08$, $P = 0.0028$). Within treatments, we found that LOW birds had lower carotenoid levels at 16 weeks of age than at either 12 or 14 weeks old, that OUTDOOR birds had higher levels at 14 weeks old than 12 weeks old, and within age classes, we found that at 12 weeks old, LOW birds circulated more carotenoids than either the HIGH or OUTDOOR group (Figure 4). There was no effect of rearing environment ($F_{2,16} = 2.53$, $P = 0.1110$), age ($F_{2,32} = 2.52$, $P = 0.0965$), or the age-by-treatment interaction ($F_{4,32} = 1.25$, $P = 0.31$) on circulating vitamin A

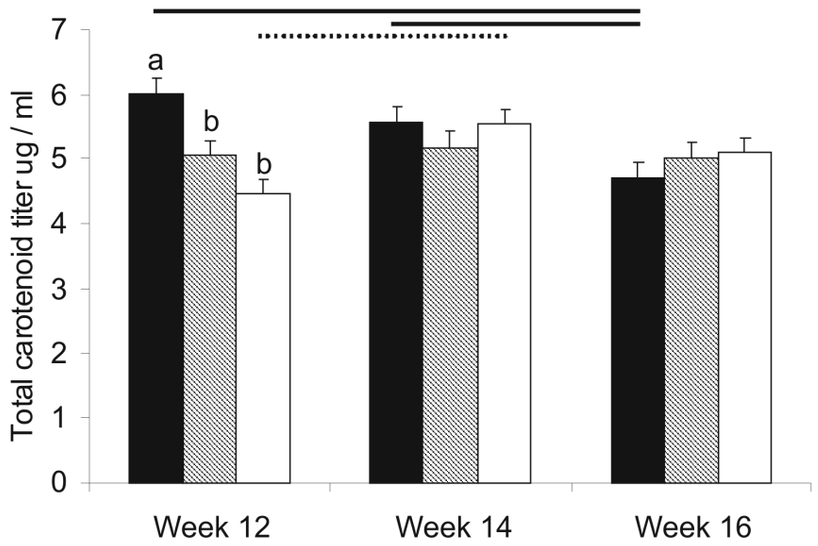

Figure 4 Total circulating carotenoid levels as a function of housing treatment and age in mallard ducklings. Filled bars represent LOW birds, striped bars represent HIGH birds, and open bars represent OUTDOOR birds. Different letters above the bars represent statistically different ($P < 0.05$, Tukey's post-hoc tests) groupings within an age class. Solid lines and dotted lines represent statistically different ($P < 0.05$, Tukey's post-hoc tests) age classes within LOW birds and OUTDOOR birds, respectively. All bars are means $\pm$ SE.

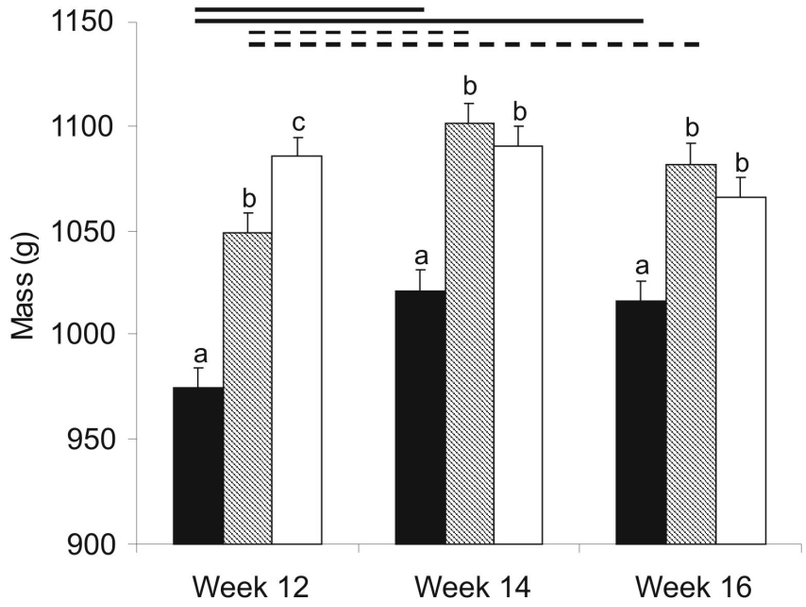

Figure 5 Mass as a function of housing treatment and age. Filled bars represent LOW birds, striped bars represent HIGH birds, and open bars represent OUTDOOR birds. Different letters above the bars represent statistically different ($P < 0.05$, Tukey's post-hoc tests) groupings within an age class. Solid lines and dashed lines represent statistically different ($P < 0.05$, Tukey's post-hoc tests) age classes within LOW birds and HIGH birds, respectively. All bars are means $\pm$ SE.

levels (Week 12: $0.70 \pm 0.015\,\mu g/ml$; Week 14: $0.75 \pm 0.015\,\mu g/ml$; Week 16: $0.73 \pm 0.015\,\mu g/mL$).

Rearing environment did not significantly affect body mass ($F_{2,16} = 2.81$, $P = 0.09$), but there were significant effects of age ($F_{2,32} = 9.61$, $P = 0.0005$) and the age-by-treatment interaction ($F_{4,32} = 3.39$, $P = 0.020$). Within treatments, we found that HIGH birds and LOW birds were heavier at weeks 14 and 16 than at week 12. In week 12, LOW birds weighed less than HIGH birds, which in turn weighed less than OUTDOOR birds. At 14 weeks of age and at 16 weeks of age, LOW birds weighed less than both HIGH and OUTDOOR birds (Figure 5).

4. DISCUSSION

Differences in housing conditions during the middle and late stages of development affected several aspects of phenotype in mallard drakes. The most striking difference among treatment groups was the difference in moult, where birds reared outdoors moulted into their adult breeding plumage much faster and more completely than birds reared indoors. Additionally, birds reared outdoors also had the most saturated beaks, a condition that persisted even after all birds were housed similarly under indoor light for several weeks just after the onset of adulthood. Birds reared under low-quality lighting conditions were the smallest at all time points, although they did circulate higher levels of carotenoids at 12 weeks old.

Given our very coarse manipulation of rearing environment in this experiment, there could be several environmental factors that contributed to the observed moult patterns–photoperiod, temperature, and light intensity, to name a few. Though moult in adult temperate birds is largely associated with photoperiod (Dawson *et al.*, 2001), our findings suggest that longer photoperiods do not accelerate the juvenile moult in mallards, contrary to work in blackcaps (*Sylvia atricapilla*; Pulido and Coppack, 2003). Instead, we presume that the less intense light offered by the bulbs in indoor-housed birds was the primary factor responsible for retarding moult, compared to sun-exposed outdoor birds. More intense light advances onset of moult in turkeys (*Meleagris gallopavo*; USDA, 1954) and sets biorhythms (including moult) in equatorial stonechats (*Saxicola torquata axillaris*; Gwinner and Scheuerlein, 1998). However, we cannot rule out possible effects of the absence of daily transitions in light intensity or of the less variable temperatures experienced by indoor birds on reduced speed and completion of the alternate moult.

A series of endogenous factors (*e.g.*, body condition, hormones, nutrient status, stress levels) may have also responded to our housing treatments and governed the observed treatment differences in phenotype. Body condition has previously been

linked to moult transitions and rates in birds (Czapulak, 2002), but we found little evidence that condition *per se* was linked to moult speed or completeness in this study, as OUTDOOR birds had more complete moults, but did not weigh significantly more than HIGH birds during weeks 14 and 16. Hormones such as oestrogen can affect the rate and pattern of plumage development in birds, including mallards (reviewed by Kimball and Ligon, 1999). While rearing environment may have affected hormone production or clearance, we did not measure any hormones and lack the data to address this mechanism. Regardless, speed of moult is important in mate choice (Mulder and Magrath, 1994; Omland, 1996a; Robertson *et al.*, 1998) and thus a more natural rearing environment may be important in future studies regarding mate choice or sexually selected traits.

The purity (or saturation) of drake beak colouration, a trait important in mate choice (Omland, 1996a, 1996b), also differed significantly among our treatments, with outdoor-reared birds displaying the most yellow bills. This was true both after the developmental experiment itself as well as nine weeks after it was completed, which suggests that our housing conditions had long-lasting effects (as opposed to transient effects that could have washed out during the four weeks in which birds were all housed under standardised conditions from weeks 21–25). Several parameters are known to affect current carotenoid colour expression in the bare parts of adult birds (*e.g.*, diet, parasites; Hill, 2006), and some studies have even examined organisational effects of factors like diet on adult pigmentation (Blount *et al.*, 2003; Koutsos *et al.*, 2003), but ours is the first to show the effect of an abiotic environmental condition *during development* on carotenoid colour expression. The closest comparison is the fact that an environmental variable like cold temperature inhibits carotenoid colouration in the beaks of adult zebra finches (*Taeniopygia guttata*; Eraud *et al.*, 2007). Ultimately, indoor housing produced a phenotype that may be less preferred by females to those reared in a natural outdoor setting.

Mechanistically, based on prior work on endogenous controls of avian carotenoid colouration (McGraw *et al.*, 2001, 2004; McGraw and Parker, 2006), one might expect that carotenoid circulation through the body would also have been impaired by indoor housing, thus generating the beak-colour differences. However, we failed to find consistent effects of rearing conditions on plasma carotenoid concentration. In week 16, carotenoid levels did not differ among groups but beak colour did, and pigment levels in circulation for individual birds did not predict their colour intensity at that time. If anything, contrary to predictions, LOW birds circulated more carotenoids than other groups, at least during week 12. The uncoupling of carotenoid colouration from circulating pigment levels was also uncovered in previous studies of neonatal nutrition and adult colouration in zebra finches (Blount *et al.*, 2003) and chickens (Koutsos *et al.*, 2003). It is possible in our study that, as in Blount *et al.* (2003) and Koutsos *et al.* (2003), conditions very early in life shaped the phenotypic effects we observed, and that by failing to draw blood during the first few weeks of the study we missed a key window into the mechanism of colour change. Because sex hormones can affect both moult (see above) and carotenoid circulation/colouration in birds (Blas *et al.*, 2006; McGraw *et al.*, 2006), it will be exciting to test for links among these variables in future developmental studies of mallards.

Most differences among groups in our study were clear when comparing outdoor- versus indoor-reared birds, but we did find a specific effect of indoor lighting quality on body mass. Low-light birds were always lower in mass than high-light birds. While greater light intensity has been associated with a reduction in mass in chickens (Renema and Robinson, 2001), and red or white light exposure results in poults of lower mass than green or blue lighting (Rozenboim *et al.*, 1999), phenotypic differences arising from differences in the ambient light's spectral similarity to the sun have not been explicitly tested. We found that indoor illumination with a greater CRI resulted in male mallards having higher body masses, similar to those birds reared outside. Food was available *ad libitum* and there seemed to be no difference in the rate of disappearance of food between treatment groups (MWB, personal observation), so we do not believe this effect was due to food intake.

In summary, we found that outdoor housing was favourable for raising mallard ducklings to develop an optimal adult phenotype, even when the photoperiod was unnatural and temperatures were lower. Outdoor birds were heavier, moulted sooner and more completely, and had more saturated bill colouration compared to birds reared with either low or high-quality indoor lighting. While we were not able to experimentally isolate which aspects of the outdoor rearing environment (*e.g.*, transitional periods of dim light at dawn and dusk, light intensity, spectral composition, humidity, within-day temperature variation) played an important role in regulating moult or other aspects of development, we did demonstrate the importance of housing conditions on the development of the adult phenotype in mallard drakes.

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