


Review

The potential utility of carotenoid-based coloration as a biomonitor of environmental change

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In the past 30 years, carotenoid-based animal signals have been an intense focus of research because they can potentially broadcast an honest reflection of individual reproductive potential. Our understanding of the underpinning physiological functions of carotenoid compounds is still emerging, however. Here, we argue that wildlife researchers and managers interested in assessing the impact of environmental quality on animal populations should be taking advantage of the signalling function of carotenoid-based morphological traits. Using birds as a model taxonomic group, we build our argument by first reviewing the strong evidence that the expression of avian carotenoid displays provides an integrated measure of a multitude of diet- and health-related parameters. We then present evidence that human-induced rapid environmental change (HIREC) impacts the expression of carotenoid signals across different critical periods of a bird's lifetime. Finally, we argue that variation in signal expression across individuals, populations and species could be quantified relatively easily at a global scale by incorporating such measurements into widespread bird ringing activities. Monitoring the expression of carotenoid-based coloration could help to identify how the environmental factors linked to HIREC can affect avian populations and allow for potentially detrimental effects on biodiversity to be detected prior to demographic change.

Keywords: environmental quality, ornaments, sexual signals, urbanization, wildlife management.

For over three decades, research interest in carotenoid pigments has been expanding continuously. This is not only because of the advent of new technologies, which have made physical and biochemical investigative tools more accessible to researchers, but also because of the key roles carotenoids play in physiological and signalling functions.

The study of carotenoids is particularly prominent in birds. This is because many avian species incorporate carotenoids into their morphology, where they form shades of reds, oranges and yellows in plumage and bare parts (e.g. bill, eye ring,

tarsi) (Goodwin 1984). This coloration is often more pronounced in males and is used by females to assess the quality of a potential mate (Andersson & Simmons 2006, Blount & McGraw 2008). *In vitro* research also suggests that carotenoids could be powerful antioxidants and immune enhancers, and thus could play an important role in maintaining individual health (Britton 2008, Svensson & Wong 2011). Carotenoids are biologically active pigments mostly synthesized *de novo* by plants, and vertebrates such as birds can only acquire them through their diet (Goodwin 1984, Latscha 1990). Consequently, it has been hypothesized that carotenoid-based ornaments should reliably inform conspecifics of the owner's ability to access food, nutritional condition and health status (Endler 1980). The roles played by carotenoids in

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avian physiology and signalling have undergone intensive study (McGraw *et al.* 2006, Blount & McGraw 2008, Britton 2008, Costantini & Møller 2008, Lafountain *et al.* 2015). Although some controversies as to the mechanisms by which carotenoids are used and processed still persist (Koch & Hill 2018, Weaver *et al.* 2018b), it is well established that carotenoid-based ornaments can be honest signals of individual quality (Svensson & Wong 2011, Weaver *et al.* 2018a; see Box S1 for more information about carotenoid pigments and their functions).

Surprisingly, the signalling function of carotenoid-based morphological features has only just begun to be harnessed as a means of monitoring changing environmental quality associated with human-induced rapid environmental change (HIREC) (Lifshitz & St Clair 2016). The idea that ornamental traits could be related to environmental quality was first described in the 1990s (Hill 1995a). If individual foraging efficiency does not vary across habitats, individuals living in habitats of different quality will display between-habitat differences in carotenoid-based coloration indicative of differential levels of fitness. For this reason, mean carotenoid coloration (i.e. average coloration of the population) may provide a measure of environmental quality in terms of its suitability to the survival and reproduction of the animal populations of interest. Alternatively, if the variance of individual foraging efficiency differs across habitats, then the variation in carotenoid coloration could be compounded by prevailing environmental conditions. For example, inefficient foragers in carotenoid-rich environments could perform even worse in carotenoid-poor environments. In this scenario, one would expect to find between-habitat differences in variation in carotenoid-based coloration. With these two non-mutually exclusive hypotheses in mind (differences in mean vs. differences in variation), we argue for the potential of carotenoid signals to be used as early bio-indicators of habitat quality in the present review.

Urbanization is one of the most extreme anthropogenic alterations of natural ecosystems and is well known to have negative impacts on local biodiversity. Urbanization is characterized by the large-scale substitution of natural vegetation by anthropogenic structures, causing reductions in the abundance and richness of primary producers (plants) and secondary consumers (i.e. insects) of carotenoids (Rickman & Connor 2003,

Helden *et al.* 2012). Declines in native vegetation cover and hence lower fruit, seed and insect availability in urban habitats can be expected to reduce carotenoid intake, thereby leading to changes in the expression of carotenoid-based ornamentations (Isaksson 2009). Changes in invertebrate communities are also thought to lead to deficiencies in other nutrients essential for the morphological display of carotenoids, such as protein and lipids (Prum & Torres 2003, Shawkey & Hill 2005, Machovsky-Capuska *et al.* 2016). Furthermore, urban habitats are characterized by high levels of pollution, exaggerated population densities, and a close proximity between humans, pets, domestic stock and wildlife, causing increases in competitive interactions over limited resources and higher risks of pathogen transmission (Marzluff 2001, Faeth *et al.* 2005, Bradley *et al.* 2008, Shanahan *et al.* 2013). All these environmental parameters (i.e. dietary availability in nutrients, pollution and filtered species assemblages including parasites/pathogens) are likely to affect the health of individuals living in urban habitats, and hence the expression of carotenoid signals (Grimm *et al.* 2008). Carotenoid displays are highly plastic phenotypic traits, which allow the signals to fluctuate during the individual's lifetime. Thus, quantification of phenotypic variation at the population level could allow early detection of potential detrimental impacts of urbanization on individuals' condition, importantly, prior to the observation of the drastic and sometimes irreversible effects (e.g. decline, extinction) (Hill 1995a).

Birds are a very useful taxonomic group to investigate the ecological impacts of land-use change (Jetz *et al.* 2007, Lepczyk *et al.* 2017). Taxonomically diverse and individually abundant, birds are easily observable and express a diverse range of responses to changes in urban ecosystems (Marzluff *et al.* 2001, Aronson *et al.* 2014, Gil & Brumm 2014). As a consequence, they can serve as proxies for other species (Weins 1989). Birds are not only an essential component of urban landscapes, they also contribute, through their ostentatious visible and acoustic presence, to building environmental awareness and fostering support for global environmental issues. Birds display a vast array of carotenoid signals and we have a very good general understanding of their physiological and adaptive functions. Because of these attributes, birds make an outstanding

taxonomic group to use carotenoid signals as early indicators of both accidental (for the worse) and planned (for the better) alterations in environmental quality.

Here, we review systematically the findings from studies on carotenoid displays in birds, focusing on the parameters most likely to influence the production of these signals in human-disturbed habitats, and using urbanization as the main example to illustrate how carotenoid signals can assist environmental management. We emphasize the mechanisms by which diet- and health-related constraints modulate the expression of carotenoid signals across different critical periods of a bird's lifetime. We also highlight, *inter alia*, the role of non-carotenoid substances on the expression of carotenoid signals. First, by synthesizing the parameters that constrain the expression of carotenoid signals, we aim to highlight how these parameters can affect an individual's diverse array of morphological signals at different critical life stages. We then describe how variation in these carotenoid signals could be measured fairly easily and how these methods could be incorporated into currently widespread bird-ringing schemes. Finally, we aim to build a convincing argument that wildlife managers could have at their fingertips a highly integrated tool for proactively monitoring environment quality.

DATA SOURCES

An extensive literature search covering 38 years of research (1980–2018) was conducted to analyse findings on the associations between carotenoid-based coloration in birds, environmental parameters and urbanization. The data search was initiated in July 2017 and was last updated in February 2018. Specialized web search engines such as Web of Science, Google Scholar, Wiley, ScienceDirect and Springer Link were used to perform the search. Different combinations of a list of keywords were entered for the search: 'carotenoid', 'colouration/coloration', 'bird', 'avian', 'ornament', 'display', 'bare part' and 'signal'. Searches were also performed for specific parameters by including new keywords such as 'diet quality', 'parasite', 'pollution' and 'immune response'. The thoroughness of the search was also ensured by analysing the references of all articles, book chapters and reviews covering the subject. We found 119 papers after screening (Tables S1–S5).

PARAMETERS CONSTRAINING CAROTENOID SIGNAL EXPRESSION

The role of diet

Environmental availability in carotenoids

One important property of carotenoid pigments is that they cannot be synthesized *de novo* by birds and must be obtained through the diet. Consequently, the expression of carotenoid-based coloration in birds should be limited by the availability of dietary pigments in the environment.

It is common to observe a loss in coloration when birds are brought from the wild into captivity and are kept on a carotenoid-free or carotenoid-low diet (Hill 2006). Indeed, McGraw *et al.* (2001, 2004) showed that classic seed diets provided in captivity contain low levels of carotenoids (0.1–7 µg/g of seed) and were associated in American Goldfinches *Carduelis tristis* with a low concentration of plasma carotenoids (~8 µg/mL) compared with wild birds (~60 µg/mL). Subsequent research on a variety of avian species focused on establishing causal relationships between carotenoid availability and the expression of ornamental display. The resulting body of work confirmed that supplemented individuals typically develop more colourful traits compared with unsupplemented conspecifics (Table S1). Although the vast majority of this work has focused on experimentally induced changes in feather coloration, carotenoid supplementation has also been shown to modify the appearance of mouth flanges, bill, ceres and tarsi in songbirds and raptors (Table S1).

Environmental limitations on the expression of ornamental displays were further demonstrated by studies showing direct relationships between the amount of carotenoid available and the level of display coloration (Navara & Hill 2003, Alonso-Álvarez *et al.* 2004, McGraw *et al.* 2004, Shawkey *et al.* 2006, Hill *et al.* 2009). Alonso-Álvarez *et al.* (2004) showed that Zebra Finches *Taeniopygia guttata* supplemented with higher concentrations of carotenoids in their drinking water (12.5, 25, 50, 100 and 200 mg/L) displayed redder bills than birds on lower doses. Hill *et al.* (2002) also showed that wild male House Finches *Haemorhous mexicanus* with higher gut concentrations of carotenoids exhibited proportionally brighter feathers. In free-ranging male Northern Cardinals *Cardinalis*

cardinalis, variation in breast feather brightness is reduced after a harsh winter, controlled by a negative association with abundance in fruits (Linville & Breitwisch 1997). These convergent findings strongly suggest that environmental availability of carotenoids limits the expression of carotenoid-based ornaments in birds.

Total caloric intake

To produce colourful signals, ingested carotenoid pigments must be absorbed, transported and deposited at the integument (Hill 1999), all of which require energy. This is even more relevant for red xanthophylls, which must be metabolically produced from dietarily absorbed yellow xanthophyll substrates in most bird species (Hill 1996, Weaver *et al.* 2018a). As described above, the acquisition of carotenoids from the environment depends upon the availability of these pigments in the habitat. However, if the next part of the production process (described as 'carotenoid utilization', Hill 1999) is costly, then energetic constraints that are dependent on the overall nutritional state (i.e. total caloric intake) of the individual, and are independent of dietary access to carotenoids, could arise.

The concept that carotenoid displays might be constrained by the amount of energy an individual must invest to utilize them is supported by studies that have manipulated nutritional stress (Table S2). In captivity, birds undergoing short periods of food deprivation grow less colourful feathers than birds fed *ad libitum* (Hill 2000, McGraw *et al.* 2005, Shawkey *et al.* 2006). Comparable effects were also observed on bare part coloration, such as bills and eye-rings (Pérez-Rodríguez & Viñuela 2008, Hill *et al.* 2009). Although some of these studies suggest a direct impact of nutritional stress on coloration, independent of carotenoid access (Hill 2000, McGraw *et al.* 2005), it is also possible that energetic state and carotenoid availability interact with one another (Shawkey *et al.* 2006, Hill *et al.* 2009). Shawkey *et al.* (2006) showed that when carotenoids were supplemented at low or high concentration and access to food resources was limited to random intervals through the day, restricted food access only significantly affected coloration when carotenoid pigments were provided at low concentrations. This finding suggests that nutritional stress only affects coloration when access to carotenoids in the environment is limited.

A few correlational studies in the field have corroborated the idea that some relationship exists between energetic state and the capacity to utilize available carotenoids over and above carotenoid availability. By using the growth rate of tail feathers as a proxy to estimate the nutritional state of birds (tail ptilochronology, Grubb 1995), two studies found that free-ranging birds with wider growth bars on their tail feathers also displayed more colourful plumage compared with individuals with narrower growth bars (Hill & Montgomerie 1994, Senar *et al.* 2003). Thus, it was argued that individuals growing brighter feathers were also in better nutritional condition than duller birds. It is impossible to quantify the impact of carotenoid intake on feather coloration in those studies, and it could be that the individuals with wider growth bars were able to increase their intake in carotenoids by being better foragers. To disentangle the impact of carotenoid intake and nutritional state, Pagani-Núñez and Senar (2014) quantified the proportion of spiders and caterpillars that Great Tit *Parus major* parents fed to their nestlings. Nestlings fed a higher proportion of spiders grew yellower breast feathers than nestlings fed lower proportions. As spiders contain lower levels of carotenoids (compared with caterpillars, see Arnold *et al.* 2010 and Eeva *et al.* 2010) but do improve nestling condition (Pagani-Núñez & Senar 2014), this finding indicates that diet quality, independent of pigment access, can modulate the expression of carotenoid-based coloration.

Non-carotenoid nutrient intake

Diet quality also probably affects the expression of carotenoid displays not only because it limits the amount of energy available for carotenoid conversion and utilization but also because these displays rely upon the dietary availability of non-carotenoid substances to be expressed effectively. Indeed, at the molecular level, carotenoids are found in association with protein complexes (forming carotenoproteins) in the chromoplasts and chloroplasts of plant foods (Erdman *et al.* 1993). When foods are digested, these complexes are broken down, and carotenoids are typically incorporated into lipid micelles before being moved into mucosal cells inside the intestines (Erdman *et al.* 1993, Parker 1996, During & Harrison 2004). Micelle formation, which is dependent upon bile flow stimulated by dietary fat, is crucial to carotenoid absorption. This means that low levels of dietary fat could

reduce carotenoid absorption and points to the interdependency of carotenoid and non-carotenoid availability on signal expression (Erdman *et al.* 1993). Assimilated carotenoid molecules are then bonded with lipoproteins to be transported through the bloodstream (Erdman *et al.* 1993, Parker 1996, During & Harrison 2004).

Protein is of particular interest, first, because it is known to play a structural role in carotenoid pigmentation, and second, because there is increasing evidence that it is limited in urban landscapes (due to changing arthropod communities) (McIntyre *et al.* 2001, Faeth *et al.* 2005, Heiss *et al.* 2009, Machovsky-Capuska *et al.* 2016). Feathers are almost entirely made up of protein deposited as keratin and forming feather barbs (Murphy & Taruscio 1995). Yellow coloration is produced by the absorption of light by carotenoids and by the reflection of light from white structural keratin tissues (Shawkey & Hill 2005). Transmission electron micrographs of carotenoid-coloured skin of several avian species have revealed that the uppermost strata of the dermis is formed of lipid-filled cells (containing carotenoid pigments), below which lies a thick array of large collagen fibres, supposedly arranged to scatter longer-wavelength colours, such as yellow, orange and red (Prum & Torres 2003). Thus, yellow to orange skin is most probably produced by a combination of structural coloration and carotenoid pigmentation, which may make such ornaments dependent jointly on dietary carotenoid, protein and lipid intake.

Protein intake may also affect additional visual attributes of the signal. One study found a relationship between plasma protein concentration and UV chroma in male nestling Blue Tits *Cyanistes caeruleus* during the phase of rapid feather production (Peters *et al.* 2007). Male nestlings with lower protein plasma concentrations developed tail feathers that were more UV/blue, suggesting that individuals withdrawing more protein from the circulation for feather production were able to develop better quality structural colours in their plumage. It remains to be investigated whether the UV component of the signal contained within the structural section of carotenoid-based ornaments could be related to, and modulated by, the concentration in plasma proteins. More generally, the role played by microstructures in the production of carotenoid displays has been largely overlooked and there is a need for more research on the contribution of these anatomical structures to the properties of displays.

As previously mentioned, lipids also have properties that make their levels in the diet essential to carotenoid signalling in birds. McGraw and Parker (2006) investigated the effect of cholesterol levels on ornamental coloration in Zebra Finches. Cholesterol is a lipoprotein that is involved in the transport of carotenoid pigments from the gut to the coloured integument through the blood (Parker 1996). These investigators supplemented the seed diet with powdered cholesterol in order to double blood cholesterol levels and compared bill coloration between experimental and control groups. Cholesterol-supplemented birds developed redder bills than at pre-treatment and compared with those of control birds. They also examined the effect of experimentally reducing cholesterol blood-levels by supplementation with atorvastatin, which inhibits the enzyme responsible for catalysing cholesterol biosynthesis. They showed that bill coloration of atorvastatin-supplemented finches faded significantly, whereas those of control birds remained unchanged. The authors previously showed that food deprivation reduced carotenoid transport (McGraw *et al.* 2005), suggesting that nutritional stress could impact lipoprotein production. They hypothesized that the degree to which birds manufacture lipoproteins from substrates acquired through the diet could be another costly physiological process that contributes to constraining carotenoid displays. Along those lines, recent work focusing on digestive efficiency and lipid absorption in wild male House Finches showed that birds displaying redder feathers are more efficient at absorbing fats from their diet (Madonia *et al.* 2017). Higher levels of lipid absorption could allow better absorption of carotenoids and better transport through blood circulation, which could ultimately improve ornamental coloration. The assumption that a link exists between carotenoid-based coloration and lipid metabolism is also reinforced by recent evidence showing that high-density lipoprotein receptors are essential to mediate the transport of carotenoid molecules to coloured tissues (Toomey *et al.* 2017). However, it is also worth noting that the link between circulating lipids and carotenoid signal expression may not be present in all species (e.g. raptors, Casagrande *et al.* 2011, 2012).

This work provides experimental and correlational evidence that diet quality (in terms of dietary carotenoid intake, total caloric intake, protein and lipid intake) affects the production of

carotenoid signals by influencing the individual's ability to transport, metabolize and process pigments and/or by altering the morphological structure itself at the anatomical level.

The role of health

Parasitic infections

The presence and abundance of parasites in the environment are expected to be affected by HIRECs (e.g. through increases in population density and interspecies interactions) and are known to modify the expression of carotenoid signals (Hill 2006). In 1982, Hamilton and Zuk first proposed that individuals infected by parasites might display duller ornaments than disease-free individuals, thus signalling weaker heritable parasitic resistance compared with brighter individuals (Hamilton & Zuk 1982). Since then, much work has assessed the relationship between parasitic infection and carotenoid-based coloration, most of which has focused on endoparasites. The most clear-cut evidence confirming Hamilton and Zuk's original concept has emerged from studies of coccidian infections. Compared with other parasites, the mechanisms by which coccidian infections impact carotenoid signal expression are relatively well known. Coccidiosis causes a disruption in the absorption of carotenoids across the gut lining and slows their transport to colourful integuments by inhibiting the production of high-density lipoproteins (Allen 1987a, 1987b). It is therefore not surprising to find that most experimental studies focusing on infection by coccidia have revealed negative relationships with carotenoid-based coloration. Indeed, in Red Junglefowl *Gallus gallus*, Red Grouse *Lagopus lagopus scoticus* and passerines, feather and bill colorations are reliably reduced by coccidian infections (Table S3). Similar results have been seen in Red Junglefowl and Red Grouse infected with nematodes (Zuk *et al.* 1990, Martinez-Padilla *et al.* 2007, Mougeot *et al.* 2007, 2010).

Although the relationship between intestinal endoparasites and ornamental coloration has stood up to empirical testing, the same is not true for blood parasites (hematozoans) and ectoparasites. To date, much of the work on the relationship between blood parasites and bird coloration has involved correlational approaches in free-ranging birds, and a mix of negative and positive correlations have been found (Table S3). In terms of

ectoparasitism, whereas several species of songbird have been found to display duller plumages when experimentally infected or left untreated against mites (i.e. *Proctophyllodes* sp.), other experimental studies show no differences in coloration (Table S3). As the presence of ectoparasites most often correlates with plumage coloration, there is a possibility that the presence of ectoparasites simply correlates with whatever is causing the plumage to become drabber (Thompson *et al.* 1997, Harper 1999). More generally, it could be that our knowledge of the impact of hematozoans (Hill 2006) and ectoparasites (Harper 1999, Blanco *et al.* 2001, Figuerola *et al.* 2003) on avian health is still too limited to envision by which biochemical routes blood parasites and ectoparasites might interact with carotenoid pathways. In addition, it is possible that the effects of hematozoans and ectoparasites on carotenoid-based morphological features are context-specific as well as species-specific.

Allocation strategies and temporal integration of carotenoid pigmentation

Work on the relationship between parasites and carotenoid-based coloration has historically led to a more general hypothesis regarding the impact of an individual's health status on carotenoid signalling. The assumption is that when the immune system, in which carotenoids are expected to play a role through their antioxidant and immunostimulant properties (Britton 2008), is activated by the presence of parasites and pathogens in an organism, a physiological trade-off occurs between allocating carotenoid molecules to coloration or health-related functions (Folstad & Karter 1992). Based on this knowledge, Lozano (1994) proposed that the immune defences of species with carotenoid-based ornaments should be limited by carotenoid availability and that activation of the immune system should reduce the amount of carotenoids available for ornamentation.

Several studies have tested the hypothesis in birds via activation of the immune system (specifically injection of sheep red blood cells (Saino *et al.* 2000), Complete Freund's Adjuvant (George *et al.* 2017) or lipopolysaccharide of *Escherichia coli* (Alonso-Álvarez *et al.* 2004)) or by comparing the immune defences of individuals with bright and dull ornaments (Dunn *et al.* 2010) (Table S4). In general, activation of the immune system led to reduced colour intensity of ornaments, and

colourful individuals typically had higher plasma concentrations of antibodies (IgG) or mounted greater inflammatory responses compared with duller individuals (Table S4). However, there are many studies that did not find any correlations between immunity and carotenoid pigmentation (Table S4). Indeed, in many species of small passerines, feather coloration often did not relate to any measures of innate immune defences (i.e. inflammation) or humoral immunity (Table S4), adding to the controversy regarding whether the resource trade-off hypothesis is the best mechanism for maintaining honesty from carotenoid signals (Weaver *et al.* 2017, Koch & Hill 2018).

However, it is interesting to note that, in a majority of studies focusing on immune responsiveness, carotenoid-based skin coloration tends to relate more often to some measures of immunity, unlike feather ornaments (Table S4). One explanation could be that production of coloured feathers reflects a one-time, fixed resource allocation that occurs during moulting (Iverson & Karubian 2017). In contrast, the production of pigmented skin might require a continuous allocation to maintain or renew the signal (Iverson & Karubian 2017). Consequently, ornaments based on live tissues are more likely to change quickly according to the current state of the bearers and thus convey up-to-date information about their health and condition (Svobodová *et al.* 2013, Iverson & Karubian 2017). Consistent with this idea, the yellow saturation of the bills of wild American Goldfinches started decreasing 7 h after capture (Rosenthal *et al.* 2012), and the colour of the bill of Eurasian Blackbirds *Turdus merula* decreased significantly over the course of a 7-day-long experimental immune challenge (Faivre *et al.* 2003).

These results could help to clarify the currently ambiguous relationship between parasitism and coloration. If feather ornamentation can only give information about the condition of individuals at the time of production, it is then likely that recent infection by pathogens affecting the health of individuals might not be immediately reflected by a change in feather coloration. Thus, it is possible that different ornaments might relate to different aspects of the individual's health and quality at different time points; for example, some ornaments might inform about the overall condition of an individual and others might be dependent on the individual's current health status.

If it turns out to be true that different morphological displays (i.e. feather vs. bare parts) incorporate physiological information at different time points of an individual's lifetime trajectory (e.g. nestling vs. yearling vs. adulthood) and integrate physiological information over different timescales (i.e. a few days vs. a few months), then carotenoid signals could inform us not only of present but also temporally more distant environmental conditions, and specifically of when in an animal's development, these conditions are most unfavourable.

CAROTENOID DISPLAYS AS BIO-INDICATORS OF ENVIRONMENT QUALITY

With the ever-increasing downward pressure of HIREC on animal populations and biodiversity, developing tools to identify proactively the effects of environmental perturbations on population health, *sensu* demographics, is now a priority. Whereas some species appear to be benefitting from rampant environmental change and turning into perceived public and/or real impactful pests, many others are decreasing to the point of becoming a conservation concern and may require the implementation of management and conservation programmes (Shochat *et al.* 2010, Wong & Candolin 2015). Although the dramatic end-result consequences of HIREC are relatively easy to observe (decline and extinction of animal populations), we currently lack the tools to detect early changes in population health. Indeed, current management plans tend to be reactive, rather than proactive, and have often been implemented too late (Liu & Heino 2013, Adams 2016). We also lack the means of evaluating the effectiveness of experimentally modifying the environment, in the early stages of, or perhaps even prior to, changes in population abundance. The necessity to identify these tools is also highlighted by works such as that carried out by the Group on Earth Observations Biodiversity Observation Network (GEO BON). They introduced the framework of Essential Biodiversity Variables and the concept of 'species traits', the dynamics of which could provide an indicator of early stress in response to changing environments if consistently monitored (Kissling *et al.* 2018). It is now crucial to develop tools that enable proactive monitoring and identification of unfavourable environmental conditions before

observing deleterious effects on population demographics (Grant *et al.* 2017).

We argue that measuring the coloration of carotenoid displays systematically and considering any phenotypic variation in the light of what is known about the environmental impacts of HIREC offers just such an opportunity. We know that carotenoid-based ornamental coloration in birds advertises individual survival and reproductive success (Hill 1991, Nolan *et al.* 1998, Hórak *et al.* 2001, Figuerola & Carlos Senar 2007, Simons *et al.* 2012), which in turn necessarily affect population demography. As such, these signals have the potential to provide early warnings of future demographic changes in bird populations. We have also argued here that carotenoid signals can provide an integrated measure of a vast number of environmental and physiological challenges (Fig. 1), sparing us the difficulties of identifying and quantifying each one's independent contribution to individual fitness. We now aim to build our case further by synthesizing the growing body of evidence that carotenoid signal expression varies across habitats of differing quality.

Chemical pollution and urbanization

Pollution is known to impair habitat quality and is a common threat to wildlife biodiversity. Its impact on the expression of carotenoid displays in many species of bird and fish has been thoroughly reviewed, and the potential for carotenoid-based coloration to be used for visual condition assessments evoked (Lifshitz & St Clair 2016, Marasco & Costantini 2016, Pacyna *et al.* 2018).

One of the main mechanisms by which pollution interferes with signalling is oxidative stress (Marasco & Costantini 2016; Fig. 1). Oxidative stress occurs in the cell when free-radical damage accumulates past the antioxidant defences of the organism. Both non-organic and organic pollutants have the ability to increase oxidative stress in contaminated individuals by inducing the generation of new free radicals or via depletion of antioxidants (e.g. via oxidation of non-protein and protein thiols) (Marasco & Costantini 2016). For example, experimental exposure to PCBs in American Kestrels *Falco sparverius* affected coloration in exposed parents and their offspring (Bortolotti *et al.* 2003). Similarly, feathers of Great Tits with higher concentrations of mercury were found to be less colourful (Giraudeau *et al.* 2015b). The

suggested mechanism behind these changes in coloration is exposure to pollutants, which raise levels of oxidative stress and lead to the re-allocation of carotenoid molecules from coloration to the immune and/or antioxidant systems (Bortolotti *et al.* 2003, Giraudeau *et al.* 2015b).

Research on Great Tit nestlings also showed that nestlings from highly polluted areas are typically less yellow than nestlings in unpolluted areas (Table S5). Although no direct relationship had been found between heavy metal content in either blood, feather or faeces and feather coloration (Eeva *et al.* 2008, Koivula *et al.* 2011, Bauerová *et al.* 2017), it was shown that abundance in carotenoid-rich caterpillars was impacted by environmental levels of heavy metal pollution (Eeva *et al.* 1998, 2005, 2008). Therefore, it is possible that the reduced coloration of nestlings is due to secondary pollution effects that lead to a reduction in dietary intake of carotenoid-rich invertebrates (Eeva *et al.* 2014). Carotenoid signals have also been shown to respond quickly to major environmental catastrophes such as the *Prestige* oil-spill in Spain and to reflect oil-induced sublethal effects in breeding colonies of Yellow-legged Gulls *Larus michahellis* (Pérez *et al.* 2010a, 2010b). These examples reinforce the idea that using carotenoid signals as a measure of habitat quality would allow wildlife managers to observe the short- and long-term effects of the sum of many factors (both indirect and direct) through the quantification of a single integrated bio-indicator.

Urbanization is another concerning human-induced environmental modification that generates habitats of differing quality. Urban habitats have increased rapidly over the last 100 years and it is expected that they could expand by a further 20–50% by 2050 worldwide (Grimm *et al.* 2008, Burgin 2016). Urban expansion is associated with population declines and extinction of the most vulnerable species (Marzluff 2001, McKinney 2008), making it vital to design cities that support biodiversity. As carotenoid displays reflect the health and condition of their bearers in a given environment, we hypothesize that it will be possible to estimate the early detrimental or beneficial effects of different urban designs on wildlife by monitoring the expression of ornamental traits in urban bird populations.

Support for this proposal can be found in a handful of studies quantifying variation in carotenoid-based coloration along gradients of

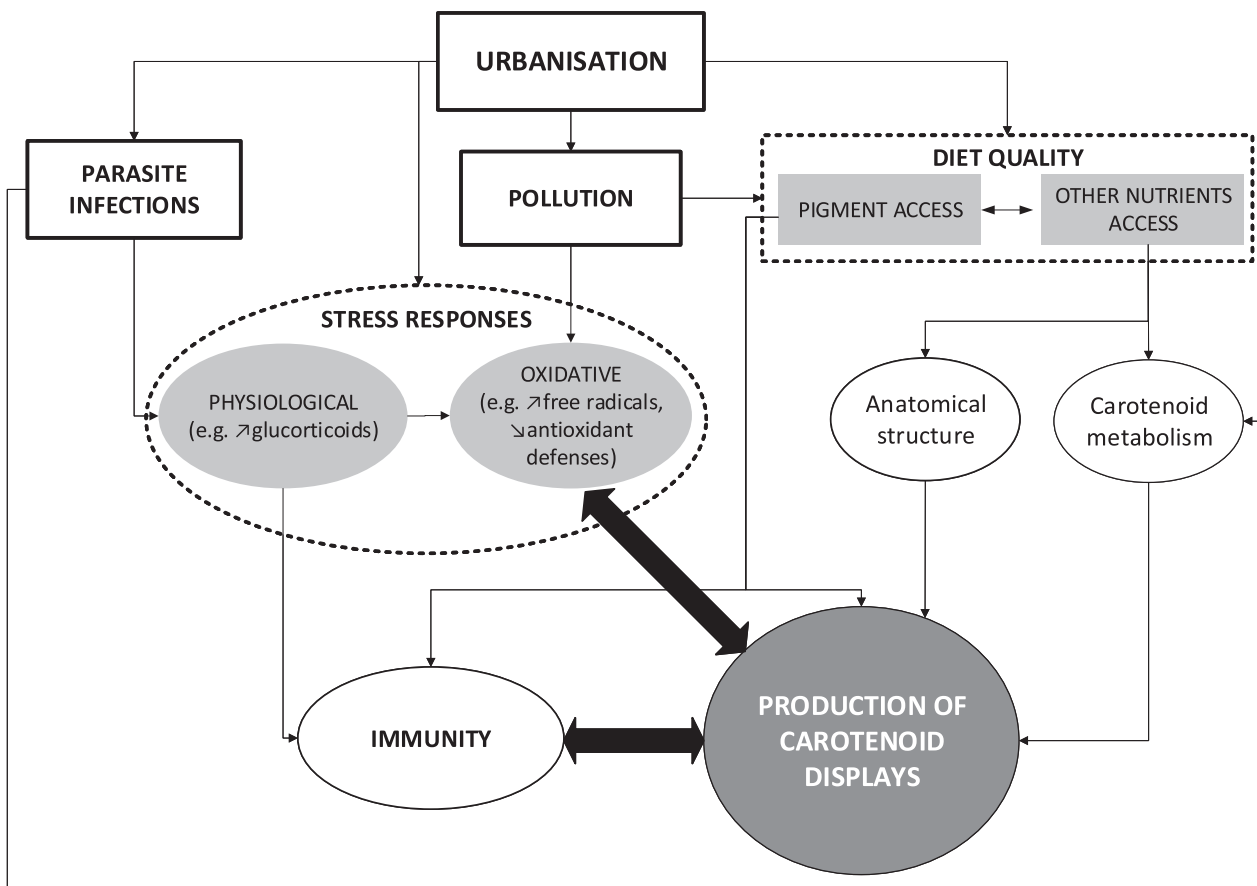


Figure 1. Schematic of the potential mechanisms and interactions between environmental constraints and production of carotenoid-based coloration. Thick black arrow: hypothesized trade-off.

urbanization. Recent work focusing on environmental stress (e.g. urbanization) and its link to oxidative balance (a factor potentially affecting carotenoid signals, Fig. 1) and sexual signals has highlighted that urban dwellers tended to experience oxidative stress and display impaired signal coloration (Isaksson 2015, Hutton & McGraw 2016). More specifically, in passerines, all studies found that populations sampled in urban environments were less colourful than populations from more rural habitats (Table S5). For example, Isaksson *et al.* (2005) showed that urban Great Tits displayed pale yellow feathers, suburban birds displayed an intermediate pigmentation and rural birds had the most yellow chromatic feather coloration. Urbanization levels were also positively correlated with levels of air pollution. Yet another set of studies indicate that reduced caterpillar abundance (an important source of carotenoids in Great Tits) in highly polluted and urbanized

environments cause reduced coloration in urban populations relative to rural ones via decreased dietary carotenoid availability (Eeva *et al.* 1998, 2005, 2008).

An alternative possibility is that food resources in urban areas could be reduced in quality rather than in quantity. Pursuing this line of thought, Hōrak *et al.* (2000) manipulated the size of broods of urban and rural Great Tits. In rural environments, nestlings from experimentally reduced broods had yellower breast feathers than those from increased broods or control broods. This result was independent of body mass, suggesting that differences in coloration were attributable to differential availability of carotenoid-rich food sources and not to differential food abundance. A cross-fostering experiment partially supported this conclusion, with nestlings of rural origin becoming less yellow when raised in an urban environment (Hōrak *et al.* 2000), but with the reverse not being

true: urban nestlings raised in rural nests did not become more colourful. The authors proposed that urban mothers had fewer carotenoids to deposit in the yolk due to the reduced availability in their diet. Thus, urban nestlings would have to invest a greater proportion of their dietary carotenoids on maintenance rather than on coloration, even when raised in better quality environments. Convergent evidence for the lesser quality of urban food resources has emerged from a comparative analysis of the carotenoid content of tree leaves and caterpillars available in the environment of urban and rural Great Tits (Isaksson & Andersson 2007, Isaksson 2009). First, the total carotenoid content of the leaves of urban trees was significantly lower than in the leaves of rural trees (Isaksson 2009). Second, although caterpillars were more abundant and heavier in urban environments, they were simultaneously poorer in carotenoids than those found in rural habitats. Thus, differences in feather coloration and caterpillar carotenoid content could be caused by the lower carotenoid concentration found in the leaves of urban trees (Isaksson 2009).

This work suggests that the carotenoid content of food items present in urban environment is lower than the content of similar items available in rural habitats. As described above (section Non-carotenoid nutrient intake), however, dietary macronutrients could be equally important for the expression of carotenoid signals. It is therefore likely that macronutrient deficiencies, associated more generally with urban settings, could alter the expression of carotenoid ornamental displays in urban birds.

First, through its impact on the abundance and species richness of arthropod communities (Rickman & Connor 2003, Helden *et al.* 2012), substitution of natural vegetation by man-made structures modifies accessibility to protein-rich nutrients (McIntyre *et al.* 2001, Shochat *et al.* 2004). Second, urban birds rely heavily on human discards (Marzluff 2001, Faeth *et al.* 2005, Shochat *et al.* 2006), which are often high in lipids and carbohydrates (Pierotti & Annett 1987). In combination, these two effects mean that birds living in urban habitats might be confronted with a dietary reduction in protein relative to lipid and carbohydrate intake. Free-ranging urban Common (Indian) Mynas *Acridotheres tristis* select almost exclusively a high-protein food, and compete to gain access to it, when given the choice between

high-protein, high-carbohydrate and high-lipid artificial pellets (Machovsky-Capuska *et al.* 2016). Moreover, protein-deprived mynas selectively consumed novel foods containing higher proportions of protein (Peneaux *et al.* 2017). Urban habitats may therefore be deficient in protein-rich foods, making this macronutrient a potentially limiting resource for ornamental production.

Human-induced alterations such as chemical pollution and urbanization are known to impair individuals' condition (Marzluff 2001, Bradley *et al.* 2008, Shanahan *et al.* 2013). It is also clear that HIREC affects abundance and quality of carotenoid-rich food items in the environment. Furthermore, the processes associated with urban conversion generate perturbations in the natural food chains and modify the abundance of non-carotenoid substances that could play a role in ornamental production, such as lipid and protein. As an integrated signal of environmental perturbations, subsequent changes in carotenoid-based coloration could thus inform wildlife managers of these disturbances, in advance of them impacting population sizes.

Carotenoid signals as bio-indicators: advantages and practicalities

Highly integrative

Carotenoid signals represent the outcome of a multiplicity of internal and external factors, which can help to circumvent the difficulties of quantifying these factors directly and separately. For example, carotenoid displays reflect dietary intake and their expression is dependent on the availability of both carotenoid and non-carotenoid nutrients (Fig. 1). However, it is notably difficult and/or extremely labour-intensive to obtain reliable measures of food availability, particularly insect abundance (Watson 2003, Razeng & Watson 2012). Moreover, the relationship between food availability and food intake is not straightforward and a multitude of internal and external factors can determine whether an animal consumes the food available in its environment.

Carotenoid signals also give an insight into the health condition of the bearer. The relationship between parasitism/immunocompetency and ornamental coloration is complex, however. In many cases, the impact of parasites (e.g. ectoparasites or blood parasites) on carotenoid-based coloration seem to be species-specific, making the

identification of the parasite, or group of parasites, responsible for a change in coloration difficult. Moreover, each individual's innate ability to fight infections (immunocompetency) makes the response to parasitic onslaughts specific to an individual.

Measuring carotenoid signals would circumvent problems associated with measuring diet quality, abundance/presence of parasites/pathogens and immune responsiveness, providing access to an integrated output from all factors (without measuring each one independently), yielding a single integrated indicator of reproductive potential. Although it might be argued that measuring an integrated proxy might make it difficult to identify (and therefore remedy) specific factors detrimental to population health, we argue here that with sufficient measures of carotenoid signal variation and its associations (such as pollution levels, population density levels, vegetation cover) insight into specific causes should gradually become clear.

Time-sensitivity

The high phenotypic plasticity of carotenoid displays, especially in skin compared with feathers, makes their expression sensitive to a bearer's condition and reproductive potential across an individual's lifetime (Hill 1995a, 1995b, Buchanan 2000, Pérez-Rodríguez 2008), raising the possibility of using them to identify at which development stage an individual is deleteriously affected by its environment. Displays requiring metabolized carotenoids or continuous allocation of dietary pigments to stay coloured should be more sensitive to changes in the bearer's condition and thus should reflect its current health condition (Svobodová *et al.* 2013, Iverson & Karubian 2017, Weaver *et al.* 2018a). On the other hand, ornaments produced at a specific time point, such as feathers grown following moult, convey information on the individual's past condition (Svobodová *et al.* 2013). By measuring multiple ornaments, and both skin and feathers, one could assess more accurately the life history of the conditions in a given environment.

Non-invasive

Characterization of carotenoid displays via digital photography or spectrophotometry can become a non-invasive, easy-to-monitor, proactive tool. To avoid trapping birds solely to measure carotenoid signals, we argue that reflectance of coloured

ornaments should become a standard component when trapping birds for research, ringing routines (e.g. alongside measuring and ringing) or other monitoring and conservation programmes. Many bird-ringing schemes exist all over the world and millions of ringing records are received each year in North America (The North American Bird Banding Program), Europe (EURING), South Africa (The South African Bird Ringing Unit) and Australia (Australian Bird and Bat Banding Scheme), for example. Using ringing activities as an opportunity to quantify carotenoid display coloration and centralizing this information in a publicly available database would allow researchers worldwide to examine the relationships between signal variation and environmental variables, such as population densities and climate, at a global scale.

While spectrophotometers can be expensive and complex to use other than for research purposes, digital photography is a common and readily available measurement tool. As carotenoid-based coloration is primarily reflected in the visible spectrum, data collected from digital photographs are often sufficient to capture most of the variation in coloration and have been found to correlate with results obtained from spectrophotometers (Simons *et al.* 2012, Fairhurst *et al.* 2014, Bergeron & Fuller 2018). Any high-definition digital camera can be used to make quantitative measurements of coloration and Troscianko and Stevens (2015) have provided thorough explanations on how to calibrate camera and images to make such measurements using a wide range of consumer cameras. Digital photography under field conditions will also require setting-up standardized parameters such as distance to lens and light conditions, and colour and luminance standards in the photographic frames.

Standardized conditions can easily be obtained from household goods once the mechanics of producing repeatable photos of 3D objects are understood. Repeatability is essential to ensure comparability across images but can be difficult to produce with non-flat objects and living, moving animals. It is crucial to have both standardized lighting conditions and maintain similar distance and angle to the camera. For example, simply holding the animal below the camera lens will lead to unpredictable and/or unrepeatable orientation and distance between the body part recorded and the camera.

An example of a device used to create standardized conditions for digital photography is illustrated in Fig. 2 (also see Eeva *et al.* 2008). First, to create standardized lighting conditions, we painted a white-lidded bucket (30 × 30 × 35 cm (length × width × height)) in several layers of black to obscure external light and we fixed LED lights to the inside of the lid to provide the only source of light inside the apparatus (Fig. 2a). Two openings were made: one on the top large enough to fit a camera lens and one in the side large enough to pass a hand holding a bird through (Fig. 2a,c). A piece of black opaque fabric was added to cover the side opening (Fig. 2b). The fabric was cut lengthways to allow the hand holding the bird, but not the external light, to enter the bucket. Then, to ensure repeatability across images, we standardized the position of the recorded display inside the apparatus. A transparent shelf was attached to the inside wall and a small opening was made to place the recorded body part in a standard position (while removing physical obstacles and potential light interferences (e.g. glare) between the measured display and the camera lens) (Figs 2a,d and 3). The shelf allowed us to maintain both the same angle and the same distance to the camera by simply introducing the animal through the opening under the shelf and placing it gently against the lower side of the transparent barrier. On the upper side of the transparent shelf, a colour standard was placed (Figs 2d and 3). Here the X-Rite ColorChecker® Passport Photo was chosen for its compact size and non-reflective surfaces that reduce the risk of glare.

Once standardized, images could be made available in a database to researchers to analyse and compare on a large scale. Image processing software (e.g. Adobe Photoshop® (Adobe Inc., San Jose, CA, USA) or ImageJ (Schneider *et al.* 2012)) can be used to analyse any standardized images and collect RGB values that are typically used to calculate hue (H), saturation (S) and brightness (B) (Giraudeau *et al.* 2015a; García-Heras *et al.* 2017). The programs MATLAB (The MathWorks, Natick, MA, USA) and R (R Core Team 2019) can also be created to facilitate the extraction of HSB values from standardized images (such as shown in Fig. 3). Similarly, Troscianko and Stevens (2015) created a free image processing toolbox which allows the calibration of standardized photographs and contains image analysis tools that can extract HSB values.

Caveats and future directions

Some factors limit the use of carotenoid signals as an indicator of population health and habitat quality. First and most clearly, the method is not applicable to species that do not display carotenoid pigmentation. Second, it is only applicable to the sex that displays carotenoid traits, most often males (Andersson & Simmons 2006, Blount & McGraw 2008). Nevertheless, male breeding success can have a significant effect on demographics, so variation in male coloration might be informative and worthy of follow up. Third, carotenoid signals integrate the effects of multiple internal and external factors. This is, on the one hand, an advantage (see above) but, on the other hand, means that variation in carotenoid coloration does not have a one-to-one correspondence to any particular external or internal cause. Changes need to be followed up therefore with other investigations, and what we know about the influencing factors (see above) provides a very good place to start looking (diet, pollution, etc.). It is also important to bear in mind that relationships between environmental factors and carotenoid coloration can be complex. For example, high levels of pollution are typically associated with a loss in coloration in birds via disturbances to the oxidative balance or by affecting food quality (see examples in section Chemical pollution and urbanization and Fig. 1). However, low-dose exposure to a contaminant can stimulate a bird's defence mechanisms (Marasco & Costantini 2016) and enhance signal coloration (Vallverdú-Coll *et al.* 2016). The sensitivity of displays can also vary among species and among displays within a species (see examples of differential time-sensitivity in section Allocation strategies and temporal integration of carotenoid pigmentation). Hence, biomonitoring of carotenoid signals provides a useful early warning system for demographic health, but the information obtained must be considered in the light of species-specific characteristics and be followed up with environmental monitoring to yield the full benefits of the approach.

We recommend that a first step could involve creating a database containing information on the properties and characteristics of carotenoid-based ornaments in species that are typically monitored during ringing. This will allow researchers and managers to gain and expand knowledge on the type of ornaments exhibited, the pigment



Figure 2. Illustrations of a device used to create standardized conditions for digital photography.



Figure 3. Example of a standardized image captured with the described device in carotenoid signals as bio-indicators: advantages and practicalities, section Non-invasive, for the measurement of the eye-patch coloration of a Common Myna (*Acridotheres tristis*).

composition, the sensitivity and environmental and physiological mechanisms known to control ornamental expression. This could be paired with information on how to measure coloration and create standardized conditions for repeatability. This database would provide a stepping stone toward the collection of baseline coloration measurements in populations of interest.

CONCLUSIONS

In response to the dramatic impact of HIREC on biodiversity and a generally reactive-based approach to wildlife management, and in line with the GEO BON initiative (Kissling *et al.* 2018), we urgently need to develop new proactive monitoring tools. These tools should be made available to wildlife managers to assess environmental impacts on animal populations before population densities begin to change. We have argued here that measuring carotenoid displays could allow early assessment of population health.

Carotenoid signals are condition-dependent. The expression of these dynamic traits relies on carotenoid availability in the environment (i.e. diet), the concentrations in which carotenoids are absorbed, the availability of essential macronutrients (e.g. lipid, protein), as well as the presence of pathogens in the environment. There is also evidence that urbanization, pollution and nutritionally unbalanced diets affect the expression of carotenoid signals. We have highlighted the functional role of carotenoid displays, i.e. to constitute an honest signal of individual fitness in terms of potential to contribute to population growth. Thus, we should capitalize on this adaptive function and begin using variation in carotenoid signals as early predictors of population health.

We propose that incorporating coloration measurements into ringing schemes would support the creation of a large database of signal variation in avian populations, which would provide an invaluable opportunity to explore on a global scale the relationship between predictors of individual fitness, demography and environmental variation.

We thank *Ibis* Associate Editor Jesús Martínez-Padilla and two other anonymous reviewers for providing

valuable comments that helped us improve the content of our review.

AUTHOR CONTRIBUTIONS

Chloe Peneaux: Conceptualization (lead); Data curation (lead); Investigation (lead); Visualization (equal); Writing-original draft (equal); Writing-review & editing (equal). **Philip Hansbro:** Supervision (supporting); Writing-review & editing (supporting). **Andrea Griffin:** Conceptualization (supporting); Supervision (lead); Visualization (equal); Writing-original draft (equal); Writing-review & editing (equal).

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Received 24 October 2019;
revision accepted 23 June 2020.
Associate Editor Petra Sumasgutner.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Summary of the effects of dietary carotenoid availability on carotenoid-based ornaments in birds.

Table S2. Summary of the effects of dietary parameters on carotenoid-based ornaments in birds.

Table S3. Summary of the effects of parasitism on carotenoid-based ornaments in birds.

Table S4. Illustrative examples of studies investigating the relationship between immune immunocompetency and responsiveness and carotenoid-based ornaments in birds.

Table S5. Summary of the effects of environment quality on carotenoid-based ornaments in birds.

Box S1. Biochemical and physiological properties of carotenoids.