

RESEARCH ARTICLE

Red coloration varies with dietary carotenoid access and nutritional condition in kittiwakes

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ABSTRACT

Carotenoid-based ornaments are common signaling features in animals. Although the mechanisms that link color-based signals to individual condition is key to understanding the evolution and function of these ornaments, they are most often poorly known. Several hypotheses have been posited. They include: (i) the role of foraging abilities on carotenoid acquisition and thereby carotenoid-based ornaments, and (ii) the role of internal processes linked to individual quality on the allocation and conversion of carotenoids in integuments. Here, we tested the influence of dietary carotenoid access versus internal process on gape coloration in black-legged kittiwakes (*Rissa tridactyla*). This seabird displays a vibrant red gape, whose coloration varies with individual quality in males and is due to the deposition of red ketocarotenoids, such as astaxanthin. We decreased hydroxycarotenoid and ketocarotenoid levels in plasma, but increased efficiency in internal processes linked to nutritional condition, by supplementing breeding males with capelin, a natural energy-rich fish prey. We found that, despite having lower carotenoid levels in plasma, supplemented birds developed redder coloration than control birds, but only in the year when dietary levels of astaxanthin in the natural diet were low. In contrast, in the astaxanthin-rich year, supplemented males had a less-red gape than unsupplemented birds. These results suggest that inter-individual differences in internal processes may be sufficient to maintain the honesty of gape coloration under conditions of low dietary astaxanthin levels. Nonetheless, when inter-individual variations in dietary astaxanthin levels are elevated (such as in the crustacean-rich year), carotenoid access seems a more limiting factor to the expression of gape coloration than internal processes. Therefore, our study revealed a complex mechanism of gape color production in kittiwakes, and suggests that the main factor maintaining the condition dependency of this ornaments may vary with environmental conditions and diet composition.

KEY WORDS: Ketocarotenoid, Astaxanthin, Zeaxanthin, Bare part, Gull

INTRODUCTION

The yellow, orange or red coloration of numerous bird species results from the deposition of carotenoid pigments into integuments (McGraw, 2006). These colorations are textbook examples of sexual

selection and have repeatedly been shown to depend on condition and health, and thus to play pivotal roles as honest signals of individual quality (Hill, 2006b; McGraw, 2006; Moller et al., 2000; Pérez-Rodríguez et al., 2013; Svensson and Wong, 2011). However, although special interest has been paid to elucidate the proximal sources of their variability (García-de Blas et al., 2016; Hill, 2006a; Simons et al., 2012), the mechanisms maintaining their honesty are not fully understood (Koch and Hill, 2018; Svensson and Wong, 2011; Weaver et al., 2017).

The earliest hypothesis explaining how carotenoid-based coloration relates to individual quality posits that the expression of full color is limited by dietary carotenoid access ('the foraging hypothesis'; Endler, 1980; Hill, 1992). Birds cannot synthesize carotenoids *de novo*, and thus must acquire carotenoids in their diet. According to this hypothesis, only individuals with good foraging ability would be able to obtain sufficient carotenoids to develop full coloration. Accordingly, in siskins (*Carduelis spinus*), blue tits (*Cyanistes caeruleus*) and brown boobies (*Sula leucogaster brewsteri*), carotenoid-based coloration seems related to foraging skills (García-Navas et al., 2012; Michael et al., 2018; Senar and Escobar, 2002). In addition, experimental provisioning of carotenoids consistently produces changes in coloration (Hill, 2006a; Koch et al., 2016; Simons et al., 2012), indicating that dietary carotenoid availability can limit color expression. Nonetheless, previous studies have also shown that inter-individual variations in carotenoid-based coloration persist under uniform diet (Karu et al., 2007; McGraw and Hill, 2001), suggesting that dietary factors may not be the only determinants maintaining the honesty of carotenoid-based coloration. Therefore, although the importance of dietary carotenoid access in maintaining the honesty of carotenoid-based coloration in wild birds has been debated for decades, it remains largely unresolved (Koch and Hill, 2018).

Other studies have suggested that carotenoid-based colors are limited by internal processes, rather than by dietary carotenoid access (Hudon, 1994; Lozano, 1994). First, the trade-off hypothesis suggests that because carotenoids may act as immunostimulants and antioxidants (Blount et al., 2003; Chew, 1993; McGraw and Ardia, 2003; Young and Lowe, 2001), only individuals with a strong immune system and effective antioxidant functions can allocate sufficient carotenoids away from these critical physiological functions to achieve full coloration (Lozano, 1994; Moller et al., 2000). However, the role of carotenoids in physiological functions is still highly contentious (Koch and Hill, 2018), and meta-analyses have revealed poor associations between carotenoid-based coloration and immune function or oxidative stress in birds (Costantini and Møller, 2008; Simons et al., 2012; Weaver et al., 2018b). Second, a more recent hypothesis suggests that the honesty of carotenoid-based coloration is maintained through its reliance on vital cellular processes (the 'shared pathway hypothesis') (Hill, 2011). This hypothesis notably applies to animals that use carotenoids that they biochemically convert before deposition into

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integuments (Weaver et al., 2018b). For instance, most species that display carotenoid-based red coloration have to bioconvert yellow carotenoids (e.g. zeaxanthin, lutein, β -cryptoxanthin) present in the diet into red carotenoids (e.g. astaxanthin, canthaxanthin) (Hill, 1996; Weaver et al., 2018a). This metabolic conversion of carotenoids requires efficient cellular respiration (Hill, 2014; Johnson and Hill, 2013; Mundy et al., 2016), a core process with major impacts on the organism's performance (Hill, 2014; Salin et al., 2015, 2012). According to this hypothesis, carotenoid-based ornamentations are honest cues of individual quality because of their fundamental dependence on the physiological state of the individual.

The debate on the proximate mechanisms maintaining the honesty of carotenoid-based signals continues, and there is still no consensus. In natural conditions, the foraging hypothesis is difficult to tease apart from hypotheses based on internal processes (the trade-off and shared pathway hypotheses) (Linville and Breitwisch, 1997), because for most species, dietary carotenoid availability is usually confounded with food availability and thereby physiological state (Arnold et al., 2010; Ilyina et al., 2013). In this study, we therefore supplemented black-legged kittiwakes (*Rissa tridactyla*) with an energy-rich but carotenoid-poor diet to determine whether supplemented birds develop more intense coloration than unsupplemented birds. This could be consistent under either hypothesis invoking internal processes, but not under the foraging hypothesis. The black-legged kittiwake is a seabird that displays vibrant bare parts. Male gape coloration has repeatedly been shown to be positively associated with several traits related to individual condition (Blévin et al., 2014; Doutrelant et al., 2013; Leclaire et al., 2011a,b, 2013), suggesting that gape coloration might be an honest signal of individual quality used in inter- or intra-sexual selection, such as mate choice, reproductive investment or competition for nesting sites. Black-legged kittiwakes feed primarily on small schooling fish [e.g. Pacific sandlance (*Ammodytes hexapterus*), capelin (*Mallotus villosus*) and Pacific herring (*Clupea pallasii*)] and secondarily on ketocarotenoid-rich crustaceans such as krill and copepods (Hatch, 2013). As their integument coloration is due to red ketocarotenoids (Leclaire et al., 2015), kittiwakes seem to develop redder integuments when feeding mostly on crustaceans (S.L., personal observation). This observation suggests that, unlike most other red-colored birds that have to convert yellow carotenoids into red carotenoids (Hill, 1996), kittiwakes can directly deposit dietary red ketocarotenoids into integuments.

To determine whether internal processes associated with nutritional condition are the main factors maintaining the honesty of gape coloration in males, we used data from an ongoing and long-term capelin supplementation experiment. Preliminary analyses showed that, compared with a natural diet, capelin supplementation leads to lower hydroxycarotenoid and ketocarotenoid plasma levels in kittiwakes (see Results). However, capelin (*Mallotus villosus*) are lipid-rich fish prey, and their availability in the ocean is strongly correlated with kittiwake reproductive success (Hatch, 2013). Consequently, dietary supplementation with capelin has positive effects on body mass and energy expenditure rate (Jodice et al., 2002; Schultner et al., 2013; Welcker et al., 2015), and on several traits related to reproductive performance, including fledging success and chick growth (Gill and Hatch, 2002; Gill et al., 2002; Merklings et al., 2012; White et al., 2010). In addition, although the effects of capelin supplementation on adult physiological traits have not yet been determined, increased immunity and antioxidant levels have been observed in supplemented chicks (Gasparini et al., 2006; Young et al., 2017). We can therefore speculate that capelin-supplemented adults are in better condition overall and less

challenged by physiological trade-offs than unfed birds. In addition, capelin-supplemented birds might be able to convert carotenoids more efficiently than unfed birds, because the metabolic conversion of carotenoids requires specific enzymes and a series of oxidation steps that is likely to demand energy (Hill, 1996). Accordingly, a food-restricted diet seems to reduce the capacity of male house finches (*Carpodacus mexicanus*) to metabolically convert carotenoids (Hill, 2000). A diet rich in fish oil has also been shown to improve mitochondrial functions (Stanley et al., 2012; Yu et al., 2014), which is a key determinant in the ability to bioconvert carotenoids (Koch et al., 2017). If the condition dependency of gape coloration in kittiwake males is mainly due to internal processes rather than to dietary carotenoid access, we thus expect capelin-supplemented males to be able to display redder gape than unsupplemented males, despite lower carotenoid levels in plasma. To test this prediction, we compared body condition, carotenoid levels and gape coloration between fed and unfed males. In addition, as a prerequisite, we ensured that, under natural conditions, the honesty of gape coloration in males was maintained regardless of the dietary conditions. We studied three different pre-laying seasons that differed markedly in the proportion of fish versus crustacean in the diet, and expect, within a year, gape coloration to covary with body condition, despite being on average redder in years of high-crustacean abundance.

MATERIALS AND METHODS

Study site

The study was conducted in the 2010, 2017 and 2018 breeding seasons on a population of black-legged kittiwakes [*Rissa tridactyla* (Linnaeus 1758)] nesting on an abandoned US Air Force radar tower on Middleton Island in the Gulf of Alaska (59°26'N, 146°20'W). Artificial nest sites created on the upper walls of the tower were observed from inside the building through sliding one-way windows (Gill and Hatch, 2002). This enabled us to easily capture and monitor breeders and chicks. All nest sites were checked twice daily to record events such as laying. Study birds were sexed by molecular methods (Merklings et al., 2012) or sex-specific behaviors (copulation and courtship feeding) during the pre-laying period (Jodice et al., 2000). Experiments were carried out in accordance with US laws and under permits from the US Fish and Wildlife Service and State of Alaska.

Food supplementation

The amount of food available to breeders was manipulated as part of a large-scale food supplementation program in kittiwakes (Gill and Hatch, 2002). Breeding pairs were divided into two groups: fed pairs were provided with supplemental food whereas unfed pairs received none. The supplemental food consisted of adult Atlantic capelin (*Mallotus villosus*), an energy-rich natural prey of kittiwakes, bought frozen and thawed to ambient temperature before feeding. Supplemental feeding started on 3 May 2010, 6 May 2017 and 6 May 2018, approximately 4 weeks before laying (mean laying dates of A-eggs: 29 May 2010, 3 June 2017, 30 May 2018). Feeding stopped on 15 August. Feeding occurred three times daily (at 09:00, 14:00 and 17:00 h). During each feeding session, fish were provided singly through a plastic tube passing through the wall at each nest site (see pictures in Gill and Hatch, 2002). Feeding continued until satiation of the bird(s) present at the nest.

Unfed males (199 in total; $n=72$ in 2010, 62 in 2017, 65 in 2018) and fed males (55 in total; $n=15$ in 2010, 24 in 2017, 16 in 2018) were caught between 11 and 30 May. Time between the onset of feeding and capture was (mean \pm s.e.m.) 15 \pm 1 days (range: 10–21 days) in 2010, 14 \pm 1 days (range: 6–18 days) in 2017 and 9 \pm 0 days (range: 7–11 days) in 2018. All males were captured prior to their mate laying

the pair's first egg. At capture, birds were weighed to the nearest 5 g with a Pesola® scale, tarsus length was measured to the nearest millimeter with a caliper and integument color was measured as described below. In addition, in 2010 and 2017, blood was collected from the alar vein with a 1 ml syringe and a 25 gauge needle (maximum amount of blood collected: 1 ml).

Integument color measurements

Gape color was measured with a reflectance spectrometer (Ocean Optics USB2000), a deuterium–halogen light source (DH2000, Top Sensor System) and a 200 µm fiber optic reflectance probe held at 45 deg to the integument surface. Reflectance was measured using SpectraSuite software (Ocean Optics) and in relation to dark and white (Spectralon®, Labsphere) standards. The spectrometer was recalibrated between each bird. We measured gape color at the intersection between the upper and lower mandibles. In 2010, the color of each individual was measured once, while in 2017 and 2018, color measurements were taken three times for each individual, and the three measurements were averaged.

Reflectance spectra of the gape have peaks in both UV and visible wavelengths (Leclaire et al., 2011b). From the smoothed reflectance spectra (span=0.15), we used the R PAVO package (Maia et al., 2013) to calculate red chroma as the relative contribution of the red spectral range to the total brightness ($R_{\lambda 605-700}/R_{\lambda 300-700}$, where R indicates reflectance), and mean brightness as the mean relative reflectance between 300 and 700 nm (Montgomerie, 2006). Red chroma and brightness were correlated (Spearman's correlation test: $\rho = -0.52$, $P < 0.0001$). For consistency with previous studies in kittiwakes, we also calculated carotenoid chroma as $(R_{\lambda 700} - R_{\lambda 450})/R_{\lambda 700}$ (Andersson and Prager, 2006; Butler et al., 2011; Maia et al., 2013), which is associated with fitness-related traits and circulating carotenoid levels in kittiwakes (Leclaire et al., 2015, 2011b). Gape carotenoid chroma was positively correlated with gape red chroma (Spearman's correlation test: $\rho = 0.83$, $P < 0.0001$). Results using carotenoid chroma were similar to those using red chroma (Figs S1–S3).

Plasma antioxidant levels

We analyzed carotenoid levels in the 2010 and 2017 plasma samples following the protocol described in McGraw et al. (2008). Briefly, we thawed and added 15 µl of plasma to 100 µl of ethanol in a microcentrifuge tube and vortexed for 5 s. Afterward, we added 100 µl of methyl *tert*-butyl ether and vortexed again for 5 s. We then centrifuged tubes for 3 min at 16,000 *g*. We transferred the supernatant to a fresh screw cap tube and evaporated to dryness with a nitrogen evaporator in a hood. Next, we resuspended the supernatant in 200 µl mobile phase, vortexed for 5 s, and injected 50 µl into a high-performance liquid chromatograph (HPLC; Waters Alliance® Instrument, Waters Corporation, Milford, MA, USA). We used a 5 µm Waters Carotenoid C-30 column (4.6×250 mm ID) to determine the types and amounts of carotenoids present. Pigment concentrations were calculated based on external curves constructed from known amounts of purified reference carotenoids. We detected eight different carotenoids in plasma: lutein, iso-lutein, zeaxanthin, β-cryptoxanthin, astaxanthin, iso-astaxanthin, anhydrolutein and β-carotene. Within individuals, most carotenoid levels (except β-carotene levels) were correlated to each other, except in fed males in 2017, where carotenoid levels were poorly correlated to each other (Fig. S4).

Diet analysis

Diet samples of unfed birds were obtained in May 2010, 2017 and 2018 as regurgitations from birds captured for measurements and banding at the colony ($n = 51, 67$ and 40 samples, respectively).

Regurgitated food samples consisted of slightly to moderately digested masses of recently ingested prey. Samples were frozen for later identification in the laboratory. Ideally, diet composition should be expressed in terms of percent of biomass at the time of ingestion. That is not feasible for regurgitated food samples because it is difficult to separate fleshy material precisely and because of variable residence times and differential digestion in the gut (Barrett et al., 2007; Duffy and Jackson, 1986). For each prey type, we therefore used presence–absence data in each sample and expressed it as the prey type relative occurrence [as $100 \times (\text{number of samples containing prey type}) / (\text{total of prey-type identifications in all samples})$; see supplement 2 in Hatch, 2013 for a discussion of this measure].

Statistical analyses

Fed birds were captured in a more restricted time window than unfed birds (*t*-tests comparing capture date of fed and unfed males in each of the three years: all $P < 0.035$). To compare fed versus unfed birds, we therefore excluded all unfed birds that were caught outside this restricted time window, leading to a sample size of 47 unfed males in 2010, 57 unfed males in 2017 and 14 unfed males in 2018. Effects of food supplementation on body condition, levels of each carotenoid, and gape coloration (chroma and brightness) were

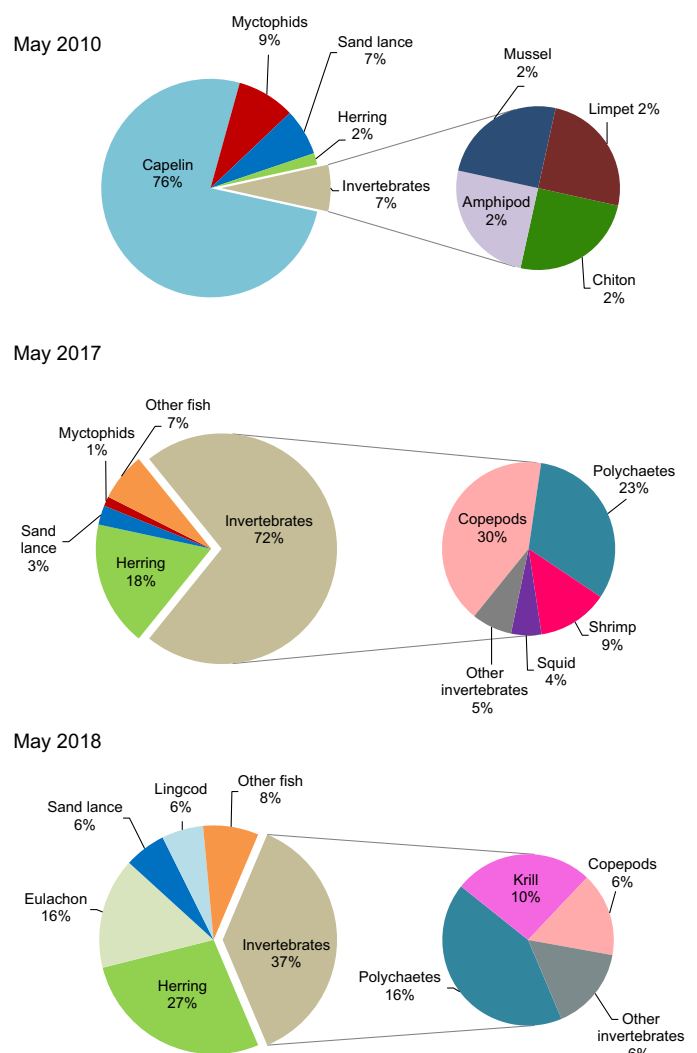


Fig. 1. Composition of the diet of unfed kittiwakes in May 2010, 2017 and 2018. Sample sizes are 51, 67 and 40 food samples, respectively.

assessed using linear mixed models (LMMs). Food-treatment, year, date and all two-way interactions were entered as fixed effects. Bird identity was entered as a random factor. Gape chroma, gape brightness and carotenoid levels were log transformed or Box–Cox transformed to meet the normality assumption in the residuals. Body condition was estimated as the residuals of a linear regression between body mass and head–bill length performed within each sex. We used head–bill length, as is known to correlate better with mass than other structural features (Golet and Irons, 1999; Jodice et al., 2000). β -cryptoxanthin and β -carotene levels did not meet the normality assumption despite transformation. We thus tested the effect of food supplementation and year on these two carotenoid levels using Wilcoxon rank-sum tests. To determine whether, in a given year, gape coloration in unfed males was a potential signal of condition and varied with carotenoid levels, we used LMMs with date, year and body condition as fixed variables and bird identity as a random effect. All statistical tests were performed with R (<https://www.r-project.org/>). Effects were tested using maximum likelihood ratio chi-square tests, and non-significant terms were backward dropped using a stepwise elimination procedure. For all LMMs, normality and homogeneity of variance were checked visually. When heterogeneity of variance was detected, we used a specific variance structure in the model (varIdent option in the ‘lme’ function of the ‘nlme’ package; Zuur et al., 2009).

RESULTS

Diet composition in unfed males

In 2010, capelin and other fish species dominated the diet of unfed birds (93% of the diet as estimated by relative occurrence; Hatch, 2013), while crustaceans (amphipods) represented only 2% of the diet (Fig. 1). In contrast, in 2017, crustaceans (copepods and shrimp) contributed substantially to the diet (39%; Fig. 1), while capelin was absent and other fish prey were in a low proportion (29%). In 2018, the diet of unfed birds was intermediate, and although no capelin was detected, fish represented 63% of the diet. Crustaceans represented 16% of the diet (Fig. 1).

Effects of food supplementation and year

Body condition varied with the interaction between treatment and year ($\chi^2=11.35$, $P=0.0034$; Fig. 2). In fed males, body condition did not vary with year ($\chi^2=1.38$, $P=0.50$); in unfed males, body condition was lowest in 2017 and highest in 2010 ($\chi^2=32.04$,

$P<0.0001$; Fig. 2). In 2017, fed males were in higher body condition than unfed males ($F_{1,76}=30.64$, $P<0.0001$), while we detected no difference in body condition between fed and unfed males in 2010 and 2018 ($F_{1,61}=2.43$, $P=0.12$ and $F_{1,28}=0.53$, $P=0.47$; Fig. 2).

Levels of astaxanthin and iso-astaxanthin (two ketocarotenoids) and levels of zeaxanthin, lutein, iso-lutein and anhydrolutein (four hydroxycarotenoids) in plasma were higher in unfed males compared with fed males (all $P<0.001$; Fig. 3 and Fig. S5). Astaxanthin levels were higher in 2017 than in 2010 ($\chi^2=9.28$, $P=0.0023$; Fig. 3A), while zeaxanthin, lutein and anhydrolutein levels in plasma were higher in 2010 than in 2017 (all $P<0.01$; Fig. 3B and Fig. S5). Iso-lutein and iso-astaxanthin levels did not vary with years ($\chi^2=0.05$, $P=0.82$ and $\chi^2=0.52$, $P=0.46$; Fig. S5). β -cryptoxanthin levels were higher in 2010 than in 2017 (fed: $\chi^2=12.26$, $P<0.001$; unfed: $\chi^2=26.35$, $P<0.001$) and higher in fed than unfed males in 2017 only (2017: $\chi^2=36.12$, $P<0.0001$; 2010: $\chi^2=0.36$, $P=0.55$; Fig. S5). Similarly, β -carotene levels were higher in fed males than unfed males in 2017 only (2017: $\chi^2=4.28$, $P=0.039$; 2010: $\chi^2=0.26$, $P=0.61$; Fig. S5).

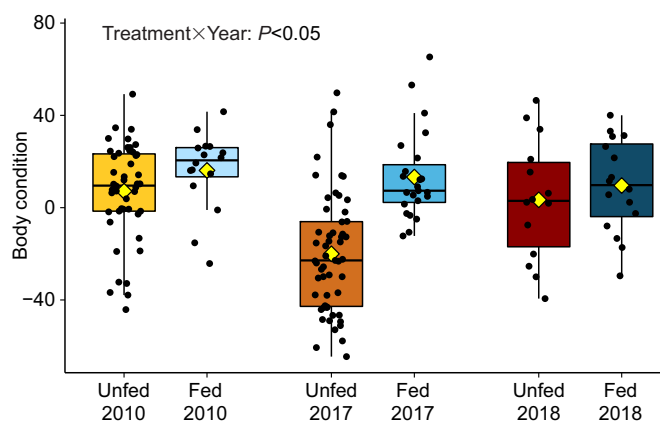


Fig. 2. Boxplots showing body condition in unfed and fed males in 2010, 2017 and 2018. For each group, points are slightly dispersed on the x-axis to avoid overlapping. The yellow diamonds correspond to the mean.

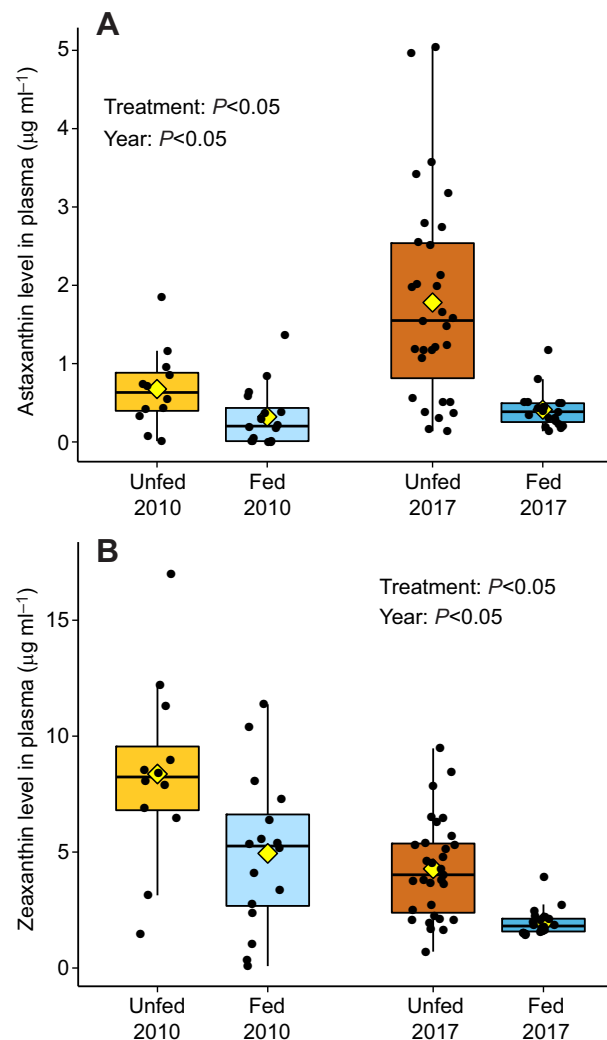


Fig. 3. Boxplots showing plasma levels of astaxanthin and zeaxanthin in unfed and fed males in May 2010 and May 2017. (A) Astaxanthin; (B) zeaxanthin. For each group, points are slightly dispersed on the x-axis to avoid overlapping. The yellow diamonds correspond to the mean.

Gape red chroma and gape brightness varied with the interaction between treatment and year ($\chi^2=13.16$, $P=0.0014$ and $\chi^2=13.62$, $P=0.0011$; Fig. 4 and Fig. S6). In 2010, fed males had higher gape red chroma and lower gape brightness than unfed males ($F_{1,59}=6.65$, $P=0.012$ and $F_{1,59}=7.83$, $P=0.007$; Fig. 4 and Fig. S6), while in 2017, they had higher brightness and lower red chroma than unfed birds ($F_{1,79}=9.22$, $P=0.003$ and $F_{1,79}=4.60$, $P=0.035$; Fig. 4 and Fig. S6). No differences in gape red chroma and brightness were detected between fed and unfed birds in 2018 ($F_{1,28}=0.02$, $P=0.89$ and $F_{1,28}=0.18$, $P=0.67$; Fig. 4 and Fig. S6). Gape red chroma varied with year in both fed and unfed birds ($\chi^2=6.40$, $P=0.041$ and $F_{1,115}=49.58$, $P<0.0001$), being highest in 2017 and lowest in 2010 (Fig. 4 and Fig. S6). In contrast, gape brightness varied with year in unfed males ($\chi^2=41.41$, $P<0.0001$) but not in fed males ($\chi^2=0.47$, $P=0.79$; Fig. 4 and Fig. S6).

Relationships between color, body condition and carotenoid levels in unfed males

In unfed males, gape red chroma increased with body condition in the three years studied ($\chi^2=4.13$, $P=0.042$; interaction between year and body condition: $\chi^2=1.15$, $P=0.56$; Fig. 5), while gape brightness did not vary with body condition ($\chi^2=2.05$, $P=0.15$). In addition, gape red chroma increased with circulating lutein levels ($\chi^2=5.37$, $P=0.021$; Fig. 6A), circulating iso-lutein levels ($\chi^2=7.35$, $P=0.007$) and tended to increase with zeaxanthin levels ($\chi^2=3.47$, $P=0.063$). It tended also to vary with the interaction between astaxanthin level and year ($\chi^2=3.64$, $P=0.056$). Gape red chroma increased with astaxanthin in 2010 ($F_{1,15}=4.63$, $P=0.048$), while it did not vary with astaxanthin levels in 2017 ($F_{1,31}=0.08$,

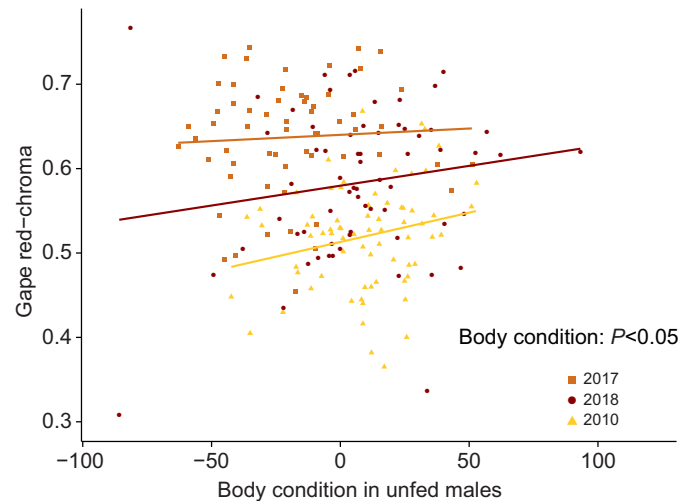


Fig. 5. Gape red chroma in relation to body condition in unfed males in May 2010, 2017 and 2018.

$P=0.77$; Fig. 6B). Gape brightness did not vary with carotenoid levels (all $P>0.05$).

DISCUSSION

To shed some light on the main proximate mechanisms maintaining the honesty of gape coloration, we investigated the factors that shape this color signal in black-legged kittiwake males. First, in line with previous studies in this species (Blévin et al., 2014; Doutrelant et al., 2013; Leclaire et al., 2013, 2011a), we found that within a year, unsupplemented males in better condition had redder gape than unsupplemented males in poorer condition, thereby confirming the honesty of this color signal in male breeders (Fig. 6). Then, using both correlational and experimental observations, we detected a complex mechanism shaping gape coloration.

Dietary ketocarotenoids as drivers of gape coloration?

The three study years varied in environmental food composition. In May 2010, the diet of black-legged kittiwakes was mainly composed of lipid-rich capelin, while in May 2017 there was no capelin in the diet, and crustaceans such as shrimp and copepods represented a high proportion. Crustacean prey of seabirds contain high levels of astaxanthin (a ketocarotenoid), while different fish prey contain low or very low astaxanthin levels (Hipfner et al., 2010). Consequently, unsupplemented males had higher astaxanthin levels in blood in the 2017 crustacean-rich year than in the 2010 fish-rich year. This result adds evidence to the suggestion that, in contrast to terrestrial granivorous birds (García-de Blas et al., 2016), aquatic birds feeding on fish and crustaceans have the capacity to assimilate astaxanthin directly from the diet (Juola et al., 2008; McGraw and Hardy, 2006; Negro and Garrido-Fernández, 2000).

As the red-orange coloration of kittiwake integuments is mainly due to red ketocarotenoids (Leclaire et al., 2015), kittiwake gape were redder in the crustacean-rich year than in the fish-rich year (i.e. higher red chroma and lower brightness in gape in 2017 than in 2010). Consistently, in 2018, when the kittiwake diet contained an intermediate percentage of fish and crustaceans compared with 2010 and 2017, birds had intermediate gape coloration. These results suggest that dietary ketocarotenoids, which are assimilated and present in plasma, can be deposited in the integument. They further suggest that large inter-annual variations in dietary availability of

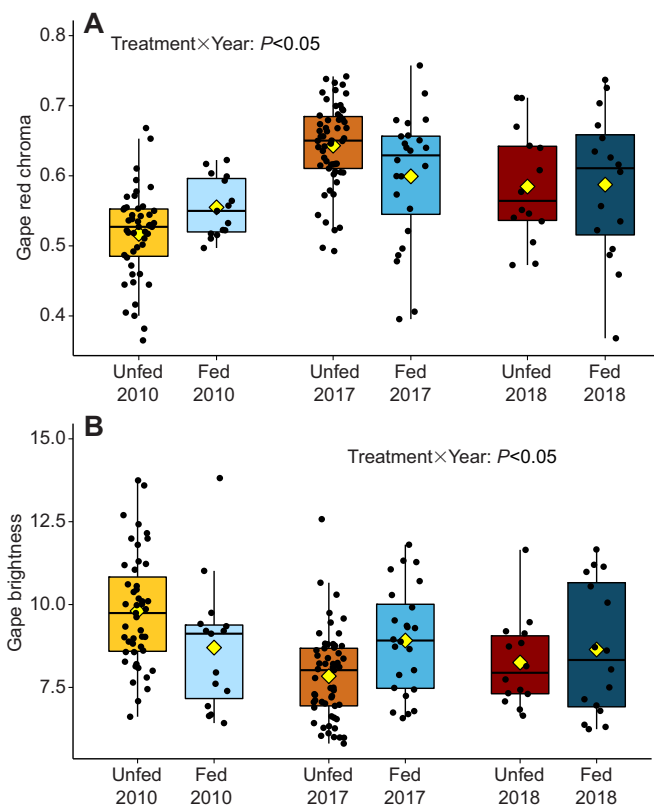


Fig. 4. Boxplots showing gape red chroma and gape brightness in unfed and fed males in May 2010, 2017 and 2018. (A) Gape red chroma; (B) gape brightness. For each group, points are slightly dispersed on the x-axis to avoid overlapping. The yellow diamonds correspond to the mean.

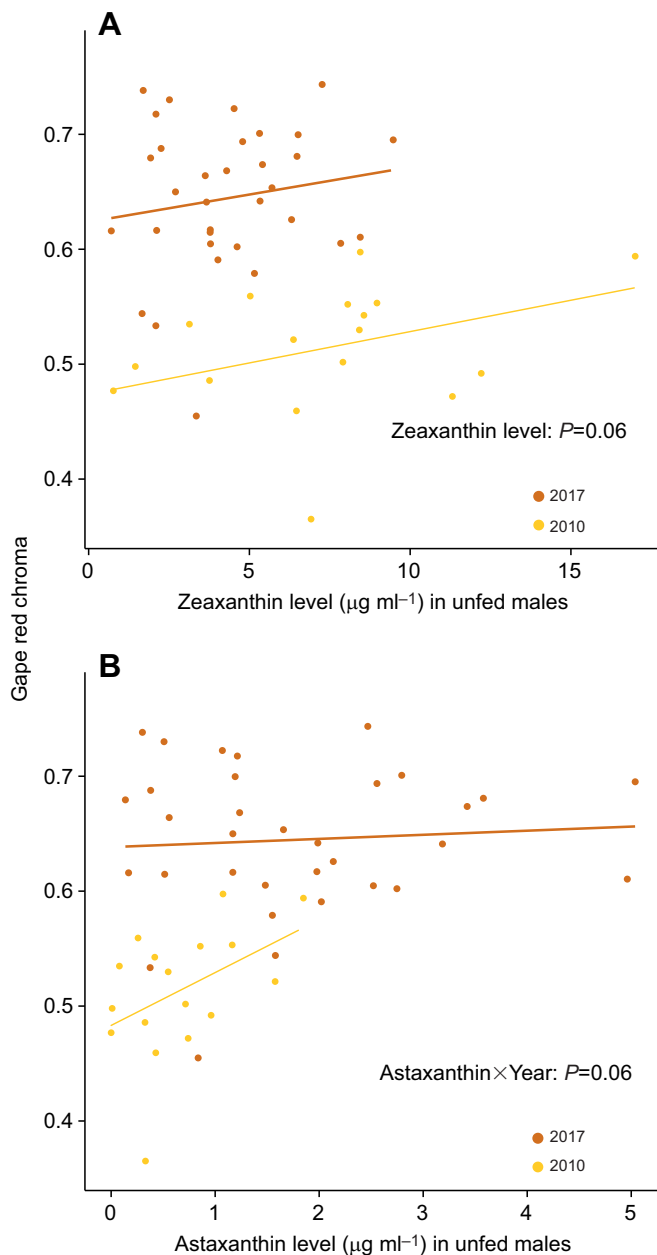


Fig. 6. Gape red chroma in relation to zeaxanthin and astaxanthin plasma levels in unfed males in May 2010 and May 2017. (A) Zeaxanthin; (B) astaxanthin.

ketocarotenoids contained in crustaceans can drive inter-annual variations in gape coloration in this population of kittiwakes. Similar inter-annual relationship between dietary carotenoids and coloration in wild species have been shown, for instance, in American redstarts (*Setophaga ruticilla*), who develop redder feathers during rainy molting periods, when insect abundance and dietary carotenoid availability are higher (Reudink et al., 2015), and in Montagu's harrier (*Circus pygargus*) nestlings, which develop duller bare parts in years when voles, a carotenoid-poor prey, are abundant (Sternalski et al., 2010).

Food-supplemented males had lower ketocarotenoid levels in plasma than unsupplemented males in both 2010 and 2017. However, they developed redder gape than unsupplemented males in 2010. Their redder gape – despite lower ketocarotenoid levels in plasma – in 2010, and the lack of correlation between plasma

astaxanthin levels and gape coloration within individuals observed in 2017 support the suggestion that dietary astaxanthin levels is not the main factor limiting the expression of gape coloration.

Dietary hydroxycarotenoids as drivers of gape coloration?

Within a year, males with higher plasma levels of zeaxanthin, lutein and iso-lutein (three hydroxycarotenoids) displayed a redder gape. In many taxa including birds, zeaxanthin and lutein are precursors of red ketocarotenoids deposited in bare parts or plumage (García-de Blas et al., 2016; LaFountain et al., 2015; McGraw, 2006; McGraw et al., 2001). These three hydroxycarotenoids were in lower levels in plasma in 2017 than in 2010, suggesting that they are more abundant in fish than in crustaceans. Accordingly, lutein and zeaxanthin are detected in fish prey such as sand lance, capelin and herring, whereas they are not detected in crustaceans such as krill and copepods (Hipfner et al., 2010; Slifka et al., 2013). It is possible that males that develop redder coloration might have better foraging abilities and thus be able to acquire higher quantities of hydroxycarotenoid-rich fish. However, a previous study in kittiwakes showed that an experimental supplementation with lutein+zeaxanthin, causing a decrease in blood astaxanthin levels, does not increase gape coloration in males (Leclaire et al., 2015). Thus, variation in dietary levels of lutein+zeaxanthin alone does not necessarily explain variation in gape coloration.

Although under natural conditions, a fish-based diet seems richer in hydroxycarotenoids than a crustacean-based diet, capelin-supplemented males had lower hydroxycarotenoid plasma levels than unsupplemented males. This difference between natural and experimental conditions might stem from supplemental capelins being less rich in carotenoids than other natural fish prey. In addition, in our study, supplemental capelins came from Iceland and Newfoundland, and were thus likely to have been caught in winter (International Council for the Exploration of the Sea, 2017), when capelin carotenoid contents are low compared with during the bird breeding period (Bragadóttir et al., 2002). Freezing might also have decreased carotenoid quantity in supplemental capelins. Despite lower hydroxycarotenoid levels in plasma, capelin-supplemented males developed redder gape than unsupplemented males in 2010. This result further suggests that dietary acquisition of hydroxycarotenoids is not the main limiting factor for gape coloration.

Internal processes as drivers of gape coloration?

The redder gape, despite lower carotenoid levels in plasma, of food-supplemented males compared with unsupplemented males in 2010 suggests that internal factors acting somewhere between the blood and integuments may also influence gape coloration in black-legged kittiwakes. Capelin-supplemented birds, which are probably in better physiological condition (albeit not in higher body condition, which might be explained by the lean-and-fit hypothesis; Schultner et al., 2013), may have lower demands for immunity than unfed males, as shown in fed kittiwake chicks (Gasparini et al., 2006), and thus they may be able to allocate higher levels of the immunostimulating hydroxycarotenoids (Leclaire et al., 2015) into ketocarotenoid formation rather than into immunity (trade-off hypothesis; Blount et al., 2003; Lozano, 1994; Moller et al., 2000). Another, not mutually exclusive hypothesis is that fed males, being in better physiological condition overall than unfed males, may have higher mitochondrial performance, which seems to be required for efficient carotenoid metabolic conversion (shared pathway hypothesis) (Hill, 2011; Weaver et al., 2018b). Disentangling these two hypotheses is challenging, but some avenues have been suggested, including experimental manipulations of mitochondrial

functions and the use of radiolabeled carotenoids to track carotenoid movement through the body (Bhosale et al., 2007; Koch and Hill, 2018; McGraw, 2009; Weaver et al., 2017)

A complex interaction between internal processes and dietary carotenoid access

In the 2017 crustacean-rich year, supplemented males were not able to develop redder gape than unsupplemented males, despite being in much better condition. When ketocarotenoids are abundant in the environment (such as in crustacean-rich years), higher efficiency in internal processes by fed birds might thus not compensate for the direct deposition of unmodified dietary ketocarotenoids into integuments by unfed males. Therefore, although differences in internal processes seem sufficient to maintain the honesty of gape coloration when the diet is poor in astaxanthin, they are not when large inter-individual variations in dietary astaxanthin levels occur. Yet, in the 2017 crustacean-rich year, gape coloration was related to individual condition in unsupplemented males. Further studies investigating, for instance, how gape coloration depends on foraging abilities and diet composition are needed to evaluate whether, under a crustacean-rich diet, the honesty of gape coloration may be mainly maintained by dietary carotenoid access. Because crustaceans are richer in ketocarotenoids but poorer in energy than fish, this mechanism of honesty might have evolved only if the quantity of food acquired varies among individuals whilst the diet composition is relatively stable. However, during the pre-laying period, diet composition of kittiwakes can change drastically within a few days (Hatch, 2013). For instance, in May 2018, the diet went from being mainly based on crustaceans to being mainly based on fish, and we observed males regurgitating fish, and others regurgitating crustaceans on the same days (S.L., personal observation). If good-quality individuals were the first to forage on fish, they may have acquired less dietary astaxanthin, and thereby developed a less-red gape. Thus the dynamics of red coloration needs to be studied to determine whether, during this short period of time, when the type of carotenoid ingested varies greatly among individuals, gape coloration honestly reveals the condition of the bearer.

Concluding remarks

We found that food supplementation, despite leading to elevated reproductive success (Gill and Hatch, 2002; Gill et al., 2002), might disrupt the honesty of integument coloration in kittiwakes. At our study site, fed birds are surrounded by unfed neighbors, and during crustacean-rich years, they may display duller gape than their neighbors despite being in better condition, and thus potentially good-quality partners. In such circumstances, females might breed with a suboptimal partner if they use gape coloration to choose their mate. More generally, while wildlife feeding is a common activity that provides an energy source to animals, it can cause deficiency in essential nutrients such as carotenoids (Tauler-Ametller et al., 2019), and potentially change selective pressures on phenotypic traits.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.L.; Methodology: S.L., S.A.H.; Formal analysis: S.L.; Investigation: S.L., M.P.; Writing - original draft: S.L.; Writing - review & editing: V.B., M.P., P.B., E.D., S.A.H.; Supervision: S.L.; Funding acquisition: S.L., V.B., E.D., S.A.H.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.210237.supplemental>

References

- Andersson, S. and Prager, M. (2006). Quantifying colors. In *Bird Coloration. I. Mechanisms and Measurements* (ed. G. E. Hill and K. J. McGraw), pp. 41-89. London: Harvard University Press.
- Arnold, K. E., Ramsay, S. L., Henderson, L. and Larcombe, S. D. (2010). Seasonal variation in diet quality: antioxidants, invertebrates and blue tits *Cyanistes caeruleus*. *Biol. J. Linn. Soc.* **99**, 708-717. doi:10.1111/j.1095-8312.2010.01377.x
- Barrett, R. T., Camphuysen, K. C. J., Anker-Nilssen, T., Chardine, J. W., Furness, R. W., Garthe, S., Hüppop, O., Leopold, M. F., Montevecchi, W. A. and Veit, R. R. (2007). Diet studies of seabirds: a review and recommendations. *ICES J. Mar. Sci.* **64**, 1675-1691. doi:10.1093/icesjms/fsm152
- Blévin, P., Tartu, S., Angelier, F., Leclaire, S., Bustnes, J. O., Moe, B., Herzke, D., Gabrielsen, G. W. and Chastel, O. (2014). Integument colouration in relation to persistent organic pollutants and body condition in arctic breeding black-legged kittiwakes (*Rissa tridactyla*). *Sci. Total Environ.* **470-471**, 248-254. doi:10.1016/j.scitotenv.2013.09.049
- Bhosale, P., Serban, B., Zhao, D. Y. and Bernstein, P. S. (2007). Identification and metabolic transformations of carotenoids in ocular tissues of the Japanese quail *Coturnix japonica*. *Biochemistry* **46**, 9050-9057. doi:10.1021/bi700558f
- Blount, J. D., Metcalfe, N. B., Birkhead, T. R. and Surai, P. F. (2003). Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* **300**, 125-127. doi:10.1126/science.1082142
- Bragadóttir, M., Pálmadóttir, H. and Kristbergsson, K. (2002). Seasonal changes in chemical composition and quality parameters in capelin (*Mallotus villosus*). *J. Aquat. Food Prod. Technol.* **11**, 87-103. doi:10.1300/J030v11n03_08
- Butler, M. W., Toomey, M. B. and McGraw, K. J. (2011). How many color metrics do we need? Evaluating how different color-scoring procedures explain carotenoid pigment content in avian bare-part and plumage ornaments. *Behav. Ecol. Sociobiol.* **65**, 401-413. doi:10.1007/s00265-010-1074-1
- Chew, B. P. (1993). Role of carotenoids in the immune-response. *J. Dairy Sci.* **76**, 2804-2811. doi:10.3168/jds.S0022-0302(93)77619-5
- Costantini, D. and Møller, A. P. (2008). Carotenoids are minor antioxidants for birds. *Funct. Ecol.* **22**, 367-370. doi:10.1111/j.1365-2435.2007.01366.x
- Doutrelant, C., Grégoire, A., Gomez, D., Staszewski, V., Arnoux, E., Tveraa, T., Faivre, B. and Boulinier, T. (2013). Colouration in Atlantic puffins and black-legged kittiwakes: monochromatism and links to body condition in both sexes. *J. Avian Biol.* **44**, 451-460. doi:10.1111/j.1600-048x.2013.00098.x
- Duffy, D. C. and Jackson, S. (1986). Diet studies of seabirds: a review of methods. *Colonial waterbirds* **9**, 1-17. doi:10.2307/1521138
- Endler, J. A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**, 76-91. doi:10.1111/j.1558-5646.1980.tb04790.x
- García-de Blas, E., Mateo, R. and Alonso-Alvarez, C. (2016). Specific carotenoid pigments in the diet and a bit of oxidative stress in the recipe for producing red carotenoid-based signals. *PeerJ* **4**, e2237. doi:10.7717/peerj.2237
- García-Navas, V., Ferrer, E. S. and Sanz, J. J. (2012). Plumage yellowness predicts foraging ability in the blue tit *Cyanistes caeruleus*. *Biol. J. Linn. Soc.* **106**, 418-429. doi:10.1111/j.1095-8312.2012.01865.x
- Gasparini, J., Roulin, A., Gill, V. A., Hatch, S. A. and Boulinier, T. (2006). In kittiwakes food availability partially explains the seasonal decline in humoral immunocompetence. *Funct. Ecol.* **20**, 457-463. doi:10.1111/j.1365-2435.2006.01130.x
- Gill, V. A. and Hatch, S. A. (2002). Components of productivity in black-legged kittiwakes *Rissa tridactyla*: response to supplemental feeding. *J. Avian Biol.* **33**, 113-126. doi:10.1034/j.1600-048X.2002.330201.x
- Gill, V. A., Hatch, S. A. and Lanctot, R. B. (2002). Sensitivity of breeding parameters to food supply in black-legged kittiwakes *Rissa tridactyla*. *Ibis* **144**, 268-283. doi:10.1046/j.1474-919X.2002.00043.x
- Golet, G. H. and Irons, D. B. (1999). Raising young reduces body condition and fat stores in black-legged kittiwakes. *Oecologia* **120**, 530-538. doi:10.1007/s004420050887
- Hatch, S. A. (2013). Kittiwake diets and chick production signal a 2008 regime shift in the Northeast Pacific. *Mar. Ecol. Prog. Ser.* **477**, 271-284. doi:10.3354/meps10161
- Hill, G. E. (1992). Proximate basis of variation in carotenoid pigmentation in male house finches. *Auk* **109**, 1-12. doi:10.2307/4088262
- Hill, G. E. (1996). Redness as a measure of the production cost of ornamental coloration. *Ethol. Ecol. Evol.* **8**, 157-175. doi:10.1080/08927014.1996.9522926

- Hill, G. E. (2000). Energetic constraints on expression of carotenoid-based plumage coloration. *J. Avian Biol.* **31**, 559–566. doi:10.1034/j.1600-048X.2000.310415.x
- Hill, G. E. (2006a). Environmental regulation of ornamental coloration. *Bird Coloration* **1**, 507–560.
- Hill, G. E. (2006b). Female mate choice for ornamental coloration. In *Bird Coloration. II. Function and Evolution*, (ed. G. E. Hill and K. J. McGraw), pp. 137–200. London: Harvard University Press.
- Hill, G. E. (2011). Condition-dependent traits as signals of the functionality of vital cellular processes. *Ecol. Lett.* **14**, 625–634. doi:10.1111/j.1461-0248.2011.01622.x
- Hill, G. E. (2014). Cellular respiration: the nexus of stress, condition, and ornamentation. *Integr. Comp. Biol.* **54**, 645–657. doi:10.1093/icb/ctu029
- Hipfner, J. M., Hobson, K. A., Dale, J. and McGraw, K. J. (2010). Stable isotopes link diet to avian yolk carotenoid allocation: a comparative study of five auk species (Charadriiformes: Alcidae). *Physiol. Biochem. Zool.* **83**, 481–489. doi:10.1086/651515
- Hudon, J. (1994). Showiness, carotenoids, and captivity: a comment on Hill (1992). *Auk* **111**, 218–221. doi:10.2307/4088529
- Ilyina, T., Kerimov, A., Zagubizhenko, M. and Maksimov, G. (2013). Seasonal dynamics of leaf-eating insects biomass and its influence on carotenoid content in feathers of Great Tit nestlings. *Russ. J. Ecol.* **44**, 507–514. doi:10.1134/S1067413613060076
- International Council for the Exploration of the Sea (2017). Capelin in the Iceland East Greenland Jan Mayen area. In *Report of the North Western Working Group (NWWG)*, pp. 300–321. Copenhagen: ICES.
- Jodice, P. G. R., Lanctot, R. B., Gill, V. A., Roby, D. D. and Hatch, S. A. (2000). Sexing adult black-legged kittiwakes by DNA, behavior, and morphology. *Waterbirds* **23**, 405–415. doi:10.2307/1522177
- Jodice, P. G. R., Roby, D. D., Hatch, S. A., Gill, V. A., Lanctot, R. B. and Visser, G. H. (2002). Does food availability affect energy expenditure rates of nesting seabirds? A supplemental-feeding experiment with black-legged kittiwakes (*Rissa tridactyla*). *Can. J. Zool.* **80**, 214–222. doi:10.1139/z01-221
- Johnson, J. D. and Hill, G. E. (2013). Is carotenoid ornamentation linked to the inner mitochondria membrane potential? A hypothesis for the maintenance of signal honesty. *Biochimie* **95**, 436–444. doi:10.1016/j.biochi.2012.10.021
- Juola, F. A., McGraw, K. and Dearborn, D. C. (2008). Carotenoids and throat pouch coloration in the great frigatebird (*Fregata minor*). *Comp. Biochem. Physiol. B* **149**, 370–377. doi:10.1016/j.cbpb.2007.10.010
- Karu, U., Saks, L. and Hõrak, P. (2007). Carotenoid coloration in greenfinches is individually consistent irrespective of foraging ability. *Physiol. Biochem. Zool.* **80**, 663–670. doi:10.1086/521084
- Koch, R. E. and Hill, G. E. (2018). Do carotenoid-based ornaments entail resource trade-offs? An evaluation of theory and data. *Funct. Ecol.* **32**, 1908–1920. doi:10.1111/1365-2435.13122
- Koch, R. E., Wilson, A. E. and Hill, G. E. (2016). The importance of carotenoid dose in supplementation studies with songbirds. *Physiol. Biochem. Zool.* **89**, 61–71. doi:10.1086/684485
- Koch, R. E., Josefson, C. C. and Hill, G. E. (2017). Mitochondrial function, ornamentation, and immunocompetence. *Biol. Rev.* **92**, 1459–1474. doi:10.1111/brv.12291
- LaFountain, A. M., Prum, R. O. and Frank, H. A. (2015). Diversity, physiology, and evolution of avian plumage carotenoids and the role of carotenoid–protein interactions in plumage color appearance. *Arch. Biochem. Biophys.* **572**, 201–212. doi:10.1016/j.abb.2015.01.016
- Leclaire, S., Bourret, V., Wagner, R. H., Hatch, S. A., Helfenstein, F., Chastel, O. and Danchin, E. (2011a). Behavioral and physiological responses to male handicap in chick-rearing black-legged kittiwakes. *Behav. Ecol.* **22**, 1156–1165. doi:10.1093/behecol/arr149
- Leclaire, S., White, J., Arnoux, E., Faivre, B., Vetter, N., Hatch, S. and Danchin, É. (2011b). Integument coloration signals reproductive success, heterozygosity, and antioxidant levels in chick-rearing black-legged kittiwakes. *Naturwissenschaften* **98**, 773–782. doi:10.1007/s00114-011-0827-7
- Leclaire, S., Blanchard, P., White, J., Hatch, S. A. and Danchin, É. (2013). Symmetry of black wingtips is related to clutch size and integument coloration in black-legged kittiwakes (*Rissa tridactyla*). *Auk* **130**, 541–547. doi:10.1525/auk.2013.13044
- Leclaire, S., Bourret, V., Blanchard, P., de Franceschi, C., Merklung, T., Hatch, S. A. and Danchin, É. (2015). Carotenoids increase immunity and sex specifically affect color and redox homeostasis in a monochromatic seabird. *Behav. Ecol. Sociobiol.* **69**, 1–15. doi:10.1007/s00265-015-1922-0
- Linville, S. U. and Breitwisch, R. (1997). Carotenoid availability and plumage coloration in a wild population of northern cardinals. *Auk* **114**, 796–800. doi:10.2307/4089305
- Lozano, G. A. (1994). Carotenoids, parasites, and sexual selection. *Oikos* **70**, 309–311. doi:10.2307/3545643
- Maia, R., Eliason, C. M., Bitton, P.-P., Doucet, S. M. and Shawkey, M. D. (2013). pavo: an R package for the analysis, visualization and organization of spectral data. *Methods Ecol. Evol.* **4**, 906–913. doi:10.1111/2041-210X.12069
- McGraw, K. J. (2006). Mechanics of carotenoid-based coloration. In *Bird coloration. I. Mechanisms and Measurements* (ed. G. E. Hill and K. J. McGraw), pp. 177–242. Cambridge, MA: Harvard University Press.
- McGraw, K. J. (2009). Identifying anatomical sites of carotenoid metabolism in birds. *Naturwissenschaften* **96**, 987–988. doi:10.1007/s00114-009-0544-7
- McGraw, K. and Hill, G. (2001). Carotenoid access and intraspecific variation in plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). *Funct. Ecol.* **15**, 732–739. doi:10.1046/j.0269-8463.2001.00574.x
- McGraw, K. J. and Ardia, D. R. (2003). Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. *Am. Nat.* **162**, 704–712. doi:10.1086/378904
- McGraw, K. J. and Hardy, L. S. (2006). Astaxanthin is responsible for the pink plumage flush in Franklin's and Ring-billed gulls. *J. Field Ornithol.* **77**, 29–33. doi:10.1111/j.1557-9263.2006.00008.x
- McGraw, K. J., Hill, G. E., Stradi, R. and Parker, R. S. (2001). The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and Northern cardinals (*Cardinalis cardinalis*). *Physiol. Biochem. Zool.* **74**, 843–852. doi:10.1086/323797
- McGraw, K. J., Tourville, E. A. and Butler, M. W. (2008). A quantitative comparison of the commonly used methods for extracting carotenoids from avian plasma. *Behav. Ecol. Sociobiol.* **62**, 1991–2002. doi:10.1007/s00265-008-0622-4
- Merklung, T., Leclaire, S., Danchin, E., Lhuillier, E., Wagner, R. H., White, J., Hatch, S. A. and Blanchard, P. (2012). Food availability and offspring sex in a monogamous seabird: insights from an experimental approach. *Behav. Ecol.* **23**, 751–758. doi:10.1093/behecol/ars023
- Michael, N. P., Torres, R., Welch, A. J., Adams, J., Bonillas-Monge, M. E., Felis, J., Lopez-Marquez, L., Martínez-Flores, A. and Wiley, A. E. (2018). Carotenoid-based skin ornaments reflect foraging propensity in a seabird, *Sula leucogaster*. *Biol. Lett.* **14**, 20180398. doi:10.1098/rsbl.2018.0398
- Moller, A. P., Biard, C., Blount, J. D., Houston, D. C., Ninni, P., Saino, N. and Surai, P. F. (2000). Carotenoid-dependent signals: Indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian Poult. Biol. Rev.* **11**, 137–159.
- Montgomerie, R. (2006). Analyzing colors. In *Bird Coloration. I. Mechanisms and Measurements* (ed. G. E. Hill and K. J. McGraw), pp. 90–147. Cambridge, MA: Harvard University Press.
- Mundy, N. I., Stapley, J., Bennison, C., Tucker, R., Twyman, H., Kim, K.-W., Burke, T., Birkhead, T. R., Andersson, S. and Slate, J. (2016). Red carotenoid coloration in the zebra finch is controlled by a cytochrome P450 gene cluster. *Curr. Biol.* **26**, 1435–1440. doi:10.1016/j.cub.2016.04.047
- Negro, J. J. and Garrido-Fernández, J. (2000). Astaxanthin is the major carotenoid in tissues of white storks (*Ciconia ciconia*) feeding on introduced crayfish (*Procambarus clarkii*). *Comp. Biochem. Physiol. B* **126**, 347–352. doi:10.1016/S0305-0491(00)00180-2
- Pérez-Rodríguez, L., Martínez-Padilla, J. and Mougeot, F. (2013). Carotenoid-based ornaments as signals of health status in birds: evidences from two galliform species, the red-legged partridge (*Alectoris rufa*) and the red grouse (*Lagopus lagopus scoticus*). In *Carotenoids: Food Sources, Production and Health Benefits* (ed. M. Yamaguchi), pp. 173–198. Hauppauge, NY: Nova Science Publishers.
- Reudink, M. W., McKellar, A. E., Marini, K. L., McArthur, S. L., Marra, P. P. and Ratcliffe, L. M. (2015). Inter-annual variation in American redstart (*Setophaga ruticilla*) plumage colour is associated with rainfall and temperature during moult: an 11-year study. *Oecologia* **178**, 161–173. doi:10.1007/s00442-014-3167-4
- Salin, K., Roussel, D., Rey, B. and Voituron, Y. (2012). David and Goliath: a mitochondrial coupling problem? *J. Exp. Zool. A Ecol. Genet. Physiol.* **317**, 283–293. doi:10.1002/jez.1722
- Salin, K., Auer, S. K., Rey, B., Selman, C. and Metcalfe, N. B. (2015). Variation in the link between oxygen consumption and ATP production, and its relevance for animal performance. *Proc. R. Soc. B* **282**, 20151028. doi:10.1098/rspb.2015.1028
- Schultner, J., Kitaysky, A. S., Welcker, J. and Hatch, S. (2013). Fat or lean: adjustment of endogenous energy stores to predictable and unpredictable changes in allostatic load. *Funct. Ecol.* **27**, 45–55. doi:10.1111/j.1365-2435.2012.02058.x
- Senar, J. C. and Escobar, D. (2002). Carotenoid derived plumage coloration in the siskin *Carduelis spinus* is related to foraging ability. *Avian Science* **2**, 19–24.
- Simons, M. J. P., Cohen, A. A. and Verhulst, S. (2012). What does carotenoid-dependent coloration tell? Plasma carotenoid level signals immunocompetence and oxidative stress state in birds – a meta-analysis. *PLoS ONE* **7**, e43088. doi:10.1371/journal.pone.0043088
- Slifka, K. A., Wells, R. S., Ardente, A. J. and Crissey, S. (2013). Comparative diet analysis of fish species commonly consumed by managed and freeranging bottlenose dolphins (*Tursiops truncatus*). *Internet J. Vet. Med.* **10**, 1582. http://ispub.com/IJVM/10/1/1582
- Stanley, W. C., Khairallah, R. J. and Dabkowski, E. R. (2012). Update on lipids and mitochondrial function: impact of dietary n-3 polyunsaturated fatty acids. *Curr. Opin. Clin. Nutr. Metab. Care* **15**, 122. doi:10.1097/MCO.0b013e32834fdaf7
- Sternalski, A., Mougeot, F., Eraud, C., Gangloff, B., Villers, A. and Bretagnolle, V. (2010). Carotenoids in nestling Montagu's harriers: variations according to age, sex, body condition and evidence for diet-related limitations. *J. Comp. Physiol. B* **180**, 33–43. doi:10.1007/s00360-009-0384-y
- Svensson, P. and Wong, B. (2011). Carotenoid-based signals in behavioural ecology: a review. *Behaviour* **148**, 131–189. doi:10.1163/000579510X548673

- Tauler-Ametlller, H., Pretus, J. L., Hernández-Matías, A., Ortiz-Santaliestra, M. E., Mateo, R. and Real, J.** (2019). Domestic waste disposal sites secure food availability but diminish plasma antioxidants in Egyptian vulture. *Sci. Total Environ.* **650**, 1382-1391. doi:10.1016/j.scitotenv.2018.09.069
- Weaver, R. J., Koch, R. E. and Hill, G. E.** (2017). What maintains signal honesty in animal colour displays used in mate choice? *Phil. Trans. R. Soc. B* **372**, 20160343. doi:10.1098/rstb.2016.0343
- Weaver, R. J., Cobine, P. A. and Hill, G. E.** (2018a). On the bioconversion of dietary carotenoids to astaxanthin in the marine copepod, *Tigriopus californicus*. *J. Plankton Res.* **40**, 142-150. doi:10.1093/plankt/fbx072
- Weaver, R. J., Santos, E. S., Tucker, A. M., Wilson, A. E. and Hill, G. E.** (2018b). Carotenoid metabolism strengthens the link between feather coloration and individual quality. *Nat. Commun.* **9**, 73. doi:10.1038/s41467-017-02649-z
- Welcker, J., Speakman, J. R., Elliott, K. H., Hatch, S. A. and Kitaysky, A. S.** (2015). Resting and daily energy expenditures during reproduction are adjusted in opposite directions in free-living birds. *Funct. Ecol.* **29**, 250-258. doi:10.1111/1365-2435.12321
- White, J., Leclaire, S., Kriloff, M., Mulard, H., Hatch, S. A. and Danchin, E.** (2010). Sustained increase in food supplies reduces broodmate aggression in black-legged kittiwakes. *Anim. Behav.* **79**, 1095-1100. doi:10.1016/j.anbehav.2010.02.003
- Young, A. J. and Lowe, G. M.** (2001). Antioxidant and prooxidant properties of carotenoids. *Arch. Biochem. Biophys.* **385**, 20-27. doi:10.1006/abbi.2000.2149
- Young, R. C., Welcker, J., Barger, C. P., Hatch, S. A., Merklings, T., Kitayskaia, E. V., Haussmann, M. F. and Kitaysky, A. S.** (2017). Effects of developmental conditions on growth, stress and telomeres in black-legged kittiwake chicks. *Mol. Ecol.* **26**, 3572-3584. doi:10.1111/mec.14121
- Yu, L., Fink, B. D., Herlein, J. A., Oltman, C. L., Lamping, K. G. and Sivitz, W. I.** (2014). Dietary fat, fatty acid saturation and mitochondrial bioenergetics. *J. Bioenerg. Biomembr.* **46**, 33-44. doi:10.1007/s10863-013-9530-z
- Zuur, A., Ieno, E., Walker, N., Saveliev, A. and Smith, G.** (2009). Dealing with heterogeneity. In *Mixed Effects Models and Extensions in Ecology with R* (ed. M. Gail, K. Krickeberg, J. M. Samet, A. Tsiatis and W. Wong), pp. 71-100. New York: Springer Science and Business Media.