Integument colouration in relation to persistent organic pollutants and body condition in arctic breeding black-legged kittiwakes (Rissa tridactyla)

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Abstract

Vertebrates cannot synthetize carotenoids de novo but have to acquire them through their diet. In birds, carotenoids are responsible for the yellow to red colouration of many secondary sexual traits. They are also involved in physiological functions such as immunostimulation and immunoregulation. Consequently, carotenoid-based colouration is very often considered as a reliable signal for health and foraging abilities. Although a few studies have suggested that carotenoid-based coloured traits could be sensitive to environmental pollution such as persistent organic pollutants (POPs) contamination, the relationships between pollutants and colouration remain unclear. Here, we examined the relationships between the colouration of carotenoid-based integuments and individual POP levels in pre-laying female black-legged kittiwakes from very high latitudes. In this area, these arctic seabirds are exposed to high POPs contamination. Additionally, we investigated the relationships between colouration and body condition, a frequently used index of individual quality. We found a negative relationship between POP levels and several components of integument colouration: saturation of eye-ring, gapes and tongue, suggesting that POPs could disrupt colouration of labile integuments in female kittiwakes. In addition, we found that females in better body condition displayed more orange and brighter gapes and tongue than females in poor body condition. These results demonstrate that hue and brightness are sensitive to the current health and nutritional status of female kittiwakes. Overall, our study shows that carotenoid-based colour integuments can be affected by several environmental-driven variables.

Keywords

Arctic

Seabird

Carotenoid

Contamination

Pesticide

PCB

1. Introduction

Many animals exhibit elaborate ornamental traits such as colourful skin, feathers and cuticles that evolved as quality signals. Those signals can have an impact on the fitness of an individual by influencing the behaviour of mates or opponents (Andersson, 1994; Møller et al., 2000). Carotenoids represent one of the central components of colour signals used in animal communication, and thus are highly involved in social behaviours of many species (Møller et al., 2000; Olson and Owen, 1998). In birds, carotenoids are responsible for the yellow to red colouration of many secondary sexual traits (Brush, 1990). Mate choice studies have shown that the most preferred individuals are often those expressing greater carotenoid pigmentation in sexual signals (Amundsen and Forsgren, 2001; review in Hill, 2006). Although the antioxidant property of carotenoids appears to be controversial for birds (Costantini and Møller, 2007; Hartley and Kennedy, 2004; Krinsky, 2001), they are involved in other physiological functions such as immunostimulation and immunoregulation (Blount et al., 2003; Chew and Park, 2004; Faivre et al., 2003; review in Møller et al., 2000). Thereby, they enhance T- and B-lymphocyte proliferative responses, stimulate effector T-cell function, enhance macrophage and T-cell capacities, increase the population of specific lymphocyte subpopulations and stimulate the production of various cytokines and interleukins (Bendich, 1989; Chew, 1993). They also maintain the structural integrity of immune cells by removing free radical molecules that are produced through normal cellular activity, but also through environmental stressors (Chew, 1996). Consequently, carotenoids promote survival (immunity, antioxidant capacity) suggesting that a trade-off may exist between allocations of carotenoids to sexual ornaments signaling versus physiological functions for self-maintenance (Eraud et al., 2007; Pérez et al., 2010a;

Von Schantz et al., 1999). It is widely assumed that condition-dependence is a common feature of sexual displays (Kristiansen et al., 2006; Martinez-Padilla et al., 2007; Mougeot et al., 2006, 2007; Pérez-Rodríguez and Viñuela, 2008; Velando et al., 2006). This implies that healthy individuals should require fewer carotenoids for immune defenses and could therefore allocate more of this limited resource to enhance sexual signals, thereby indicating of a high-quality mate. Several studies have already highlighted some correlational evidences between carotenoid-based colouration and body condition, a frequently used index of individual quality (Birkhead et al., 1998; Bustnes et al., 2007; Massaro et al., 2003; Mougeot et al., 2006, 2007; Pérez-Rodríguez and Viñuela, 2008; Pérez et al., 2010b). As birds cannot synthetize carotenoids de novo, they have to acquire them through their diet and thus, carotenoid pigmentation depends on the quality and/ or quantity of food ingested (Goodwin, 1986). Consequently, carotenoid-based colouration can be considered as a reliable signal of health and foraging abilities (Olson and Owens, 1998).

In addition to this effect of body condition on carotenoid-based colouration, a few studies have suggested that environmental pollution could also affect, and more precisely disrupt, the expression of avian colouration (Eeva et al., 1998; Pérez et al., 2010a). For example, persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and pesticides appear to reduce the expression of carotenoid-based colouration. Thus, captive American kestrels (Falco sparverius) exposed to an enriched-PCB diet showed a disruption of both plasma carotenoid concentration and colouration of ceres and lores (Bortolotti et al., 2003). However, this effect of POPs on colouration does not seem equivocal since, Bustnes et al., (2007) did not find any relationships between POP levels and integuments' colouration in free-living great black-backed gulls (Larus marinus). Thereby, this discrepancy emphasizes the importance of conducting further studies on the potential deleterious impacts of POPs contamination on carotenoid-based colouration.

The black-legged kittiwake Rissa tridactyla is a long-lived and monogamous seabird. Males are bigger than females (Helfenstein et al., 2004; Jodice et al., 2010) but no sexual chromatic dimorphism are found (Doutrelant et al., 2013 in press; Leclaire et al., 2011a). Both sexes show intense carotenoid-based colouration during the breeding season (Doutrelant et al., 2013 in press; Leclaire et al., 2011a), including the red eye-ring, red/ orange gapes, orange tongue and yellow bill. Recent studies have shown that these integuments could reflect individual quality in both sexes (Doutrelant et al., 2013 in press; Leclaire, 2010; Leclaire et al., 2011a, 2011b). In the Arctic, black-legged kittiwakes are exposed to POPs which are known to act as endocrine disruptors and to have a negative impact on reproductive performances (Bustnes et al., 2003, 2008; Helberg et al., 2005; Nordstad et al., 2012). Black-legged kittiwakes are therefore excellent models to investigate the relationships between POPs and carotenoid-based colouration in free-living birds. In that context, the specific aims of the present study were to evaluate the potential correlates of individual POP levels on integument carotenoid-based colouration (eye-ring, gapes, tongue and bill) in pre-laying female kittiwakes from Svalbard. We predicted that females bearing high POP levels would show a reduced expression of integument colouration. In addition, we also examined the correlates between body condition and integument colouration since body condition could reflect individual quality in this species. According to previous studies (Doutrelant et al., 2013 in press; Leclaire, 2010; Leclaire et al., 2011a, 2011b), we predicted that females with a better body condition would display the most colourful integuments.

2. Materials and methods

2.1. Study area and sample collection

Fieldwork was carried out in 2011 from May 21st to June 7th in a colony of black-legged kittiwakes at Kongsfjorden, (Krykkjefjellet, 78°54’N, 12°13’E), Svalbard. POP analyses were conducted only for females, thus males were not included in this study. Individuals (n = 28) were caught on their nest with a noose at the end of a 5 m fishing rod during the pre-laying period (i.e. the courtship and mating period). Females were attending the colony, on their nest on cliffs at a height of 5-10 m during the pre-laying period (i.e. before egg-laying). Birds were individually marked with white PVC plastic bands engraved with a three-letter code and fixed to the bird’s tarsus. Thus, kittiwakes could be identified from a distance without perturbation. At capture, blood samples (2.5 mL) were collected from the alar vein using a heparinized syringe and a 25G needle for the determination of blood POP concentrations and molecular sexing. Then, birds were weighted to the nearest 2 g with a Pesola spring balance and skull length (head + bill) was measured with an accuracy of 0.1 mm using a calliper. Kittiwakes were marked with spots of dye on the forehead to distinguish them from their partner during subsequent observations and were released. Using a mirror at the end of an 8 m fishing rod, we checked the whole plot every two days to monitor the subsequent reproductive status of the sampled females (pre-laying breeders were the birds that laid at least one egg after the sampling period). Blood samples were stored at -20°C until subsequent analyses. Sex was determined at the Centre d’Etudes Biologiques de Chizé (CEBC), by polymerase chain reaction (PCR), as detailed in Weimerskirch (2005).

2.2. POPs analyses

POPs were analysed from whole blood at the Norwegian Institute for Air Research (NILU) in Tromsø. The following compounds were analysed: the PCBs (CB-28, -52, -99, -101, -105, -118, -128, -138, -153, -180, -183, -187 and -194), and the pesticides (p,p-DDE, α-, β-, γ-HCH, HCB, oxychlordane, trans-, cis-chlordane, trans-, cis-nonachlor). Congeners detected in less than 70% of the samples were removed from the data set (Noël et al., 2009). Thereby, those remaining for further investigations were the PCBs (CB-99, -105, -118, -128, -138, -153, -180, -183, -187 and -194), and the pesticides (p,p-DDE, HCB, oxychlordane, trans-chlordane, trans-, cis-nonachlor). To a blood total sample of 0.5 to 1.5 mL, a 100 µL internal standard solution was added (^{13}C-labelled compounds from Cambridge Isotope Laboratories: Woburn, MA, USA). The sample was extracted twice with 6 mL of n-hexane, after denaturation with ethanol and a saturated solution of ammonium sulphate in water. Matrix removal on florisil columns, separation on an Agilent Technology 7890 GC and detection on an Agilent Technology 5975C MSD were performed as described by Herzke et al. (2009). The limit for detection was threefold the signal-to-noise ratio, and for the compounds investigated the limit ranged from 0.4 to 122 $pg \cdot g^{-1}$wet weights (ww). For validation of the results, blanks (clean and empty glass tubes treated like a sample, 3 in total) were run for every 10 samples, while standard reference material (3 in total, 1589a human serum from NIST) was run for every 10 samples. The accuracy of the method was within the 70 and 108% range.

2.3. Colour measurements

Integument colouration was measured from digital photographs as detailed in Montgomerie (2006). Pictures were taken at a standard distance of approximately 40 cm using a digital camera (Olympus U770sw, s770sw) with flash. For each photograph, the same colour swatch was placed next to the bird to standardize subsequent measurements. Prior to photographs analysis, low quality pictures (due to ambient lighting variations) were removed from the data set, thus individuals used for one given integument can partially be different for another one. All pictures were analysed using Adobe Photoshop v 12.0. The average components of red (R), green (G) and blue (B) were recorded within the whole area of the eye-ring and in a standardized selected area for the gapes, tongue and bill. Each component was assessed 3 times to ensure a good repeatability of the measurement (relative standard deviation $< 5\%$, in all cases). RGB system was then converted into hue (H), saturation (S) and brightness (B). The HSB values of each integument were corrected according to the HSB values of the colour swatch. This system is by far the most commonly reported tristimulus colour variables measured in the study of bird colouration and is extensively commented in literature (Montgomerie, 2006).

Such human-oriented model presents some inaccuracies and is only an approximation since gulls and other birds have a tetrachromatic vision and can perceive UV light (Cuthill et al., 2006; Hastad et al., 2009). However, this method has already been investigated on black-legged kittiwakes (Leclaire, 2010; Leclaire et al., 2011a, 2011b) and information obtained from digital pictures is still very useful as it reveals patterns and effects of biological meaning (Alonzo-Alvarez et al., 2004; Bortolotti et al., 2003; Kilner, 1997; Leclaire, 2010; Leclaire et al., 2011a, 2011b; Massaro et al., 2003; Mougeot et al., 2007; Pérez-Rodríguez and Viñuela, 2008; Pérez et al., 2010b).

Furthermore, using photography rather than spectrometry might be advantageous because digital photography is a much easier method for quantifying colouration of wet, hard to reach, and irregular surfaces such as gapes, irises and bills (Montgomerie, 2006).

2.4. Statistical analyses

Statistical tests were performed using R 2.14.1 (R Core Team, 2012). ΣPCB and ΣPesticides were highly significant and positively related (Pearson correlation, $r = 0.907$, $t = 10.756$, $P < 0.001$, $n = 27$). Consequently, continuous explanatory variables were defined as follow: body condition (i.e. the residuals of the regression of body mass against skull length) and ΣPOP concentrations (the sum of PCB and pesticides). We first tested the relationship between these two variables using a Pearson correlation. Then, the influence of POPs contamination and body condition on colouration parameters was investigated with General Linear Models (GLMs). HSB values of each integument as independent variables were log transformed and models were constructed with a normal distribution and an identity link function. Explanatory variables were both included simultaneously in each model. Diagnostic plots were then assessed to test whether the data sufficiently met the assumption of the linear model. A significance level of $\alpha < 0.05$ was used for all tests.

3. Results

Individual POPs concentration ranged from 1.21×10^4 to 1.01×10^5 pg•g^{-1} ww in whole blood. Body condition was not related to ΣPOP levels (Pearson correlation: $r = 0.06$, $t = 0.306$, $P = 0.766$, $n = 27$, Fig. 1). Saturation of eye-ring and gapes decreased significantly with ΣPOPs and a similar relationship, although not statistically significant was found between tongue's saturation and ΣPOPs (Fig. 2; Table 1), i.e. the most contaminated individuals were those displaying a reduced saturation of their labile integuments. Hue of tongue was negatively related to ΣPOPs but the relation seems to be driven by the presence of an outlier (Fig. 2; Table 1). Besides, hue and brightness were not related to ΣPOPs for all integuments (GLMs: all p-values > 0.315, Fig. 2; Table 1). Hue of gapes and tongue significantly increased with increasing body condition, i.e. individuals with a better condition displayed more orange gapes and tongue (Fig. 3; Table 1). We found a significant increase of brightness of the gapes with increasing body condition, and a similar trend, although not statistically significant was found between the brightness of the tongue and body condition i.e. individuals in better body condition displayed brighter gapes and tongue (Fig. 3; Table 1). By contrast, brightness of the bill decreased in birds with higher body condition (Fig. 3; Table 1). No significant relationships were found between body condition and saturation for all integuments (GLMs: all p-values > 0.116) (Fig. 3; Table 1).

4. Discussion

The results of this study first indicated a negative relationship between POP levels and saturation of labile integuments (i.e. eye-ring, gapes and tongue). This suggests that POPs could affect integument carotenoid-based colouration of kittiwakes. Secondly, body condition was positively related to hue and brightness for gapes and tongue implying that these colour parameters are sensitive to current nutritional conditions and health of individuals, as previously found in this species (Doutrelant et al., 2013 in press; Leclaire, 2010; Leclaire et al., 2011a, 2011b).

4.1. POPs and integument colouration

We found that POP levels negatively affected saturation of labile integuments. This colour parameter is usually assumed as a proxy of the amount of carotenoids present in tissues (Montgomerie, 2006) when colour is produced by only one pigment. However, integuments colouration of kittiwakes results from a mix of different carotenoid species (Doutrelant et al., 2013 in press) and, therefore, a same saturation can be obtained from mix of carotenoid species at different concentrations. This also means that equivalent amounts of carotenoids may produce different saturations depending on the exact composition of the mix of carotenoids. Consequently, POP levels could affect saturation either by decreasing the amount of pigments and/ or by modifying the carotenoid species composition present in integuments. Thereby, our study provides evidences that POPs contamination can affect integument carotenoid-based colouration. This is consistent with previous work since Pérez et al. (2010a) showed that organic compounds negatively influence the red bill spot size of adults yellow-legged gulls (Larus michahellis) during the courtship period. Moreover, Bortolotti et al. (2003) found that colouration of ceres and lores were disrupted by an enriched-PCB diet in captive American kestrels: exposed males were duller than controls, and juveniles of both sexes were brighter in winter. By contrast, Bustnes et al. (2007) did not find any relationship between POP levels and integuments' colouration in adult breeding great black-backed gulls (Larus marinus). Consequently, relationships between POPs and colouration seem to be complex and future studies including further parameters such as period, sex or stage might provide clearer information.

Carotenoids are thought to promote survival (immunity, antioxidant capacity) and might be mobilized to overcome the harmful effects of POPs ingestion on immunity (Bustnes et al., 2004; e.g. Pérez et al., 2010a; Sagerup et al., 2009) at the expense of coloured sexual signals. Under this scenario, female kittiwakes with the highest POP levels could allocate preferentially their carotenoids towards protective physiological functions (immunity, antioxidant capacity) whereas female kittiwakes with the lowest POP levels could allocate preferentially the available carotenoids towards sexual signaling. These results are thus consistent with the existence of a trade-off between allocations of carotenoids to sexual ornaments signaling versus physiological functions for detoxification processes (Pérez et al., 2010a). However, we did not perform any physiological analysis in our study and, thus, the existence of this trade-off could only be confirmed by coupling integument and plasma carotenoid measurements and POP levels in future studies.

4.2. POPs and body condition

Inter-individual variations in POP levels potentially originated from different foraging behaviours. Indeed, variations in POP levels between birds could be related to the foraging areas used by kittiwakes, i.e. during the pre-laying period, birds forage in oceanic and coastal areas (GPS tracking: Goutte et al., unpublished data). It could also be related to the type of prey ingested, i.e. contaminant levels increase with trophic position according to the biomagnification process (Kelly et al., 2007). Besides, POPs contamination is usually negatively related to the body condition (Bustnes et al., 2010; Henriksen et al., 1996; Kenntner et al., 2003; Nordstad et al., 2012); organic pollutants are lipophilic and if body fat reserves are low, POPs can be redistributed in internal tissues through the bloodstream (Fuglei et al., 2007). The lack of relationship between POP levels and body condition in our study may be related to an overall sufficient body condition of pre-laying females avoiding a redistribution of POPs in internal tissues.

4.3. Body condition and integument colouration

We reported that body condition was positively related to gapes and tongue hue and brightness, suggesting a beneficial effect of the current condition of kittiwakes on colouration. These results are consistent with the literature since Doutrelant et al. (2013, in press) have shown that kittiwakes displayed brighter and more orange gapes when in better body condition. Animals are thought to absorb carotenoids and other dietary lipids (e.g. fats, oil) through the gut lining via passive diffusion (Parker, 1996; but see During et al., 2002). By doing so, they mix carotenoids with bile salts and fatty acids to form micelles that migrate through the intestinal mucosa and are incorporated into chylomicrons to be secreted into lymph. Then, these micelles enter into the blood where they are transported via lipoproteins (Furr and Clack, 1997). During poor nutritional conditions, the amount of lipids and lipoproteins is reduced (Alonzo-Alvarez and Ferrer, 2001) and this may reduce the extraction yield of carotenoids from food (Solomons and Bulux, 1993), which in turn results in a reduction of circulating carotenoids and, ultimately, in a reduced transfer of carotenoids into integuments. Consequently lower hue and brightness may be related either to poor individual foraging efficiencies (birds in poor body condition ingesting less and/or low quality food), either to poor environmental quality and thus, to nutritional conditions (e.g. Leclaire, 2010). In addition, metabolic pathways may also be condition-dependent. Before being deposited into integuments, ingested carotenoids may be reduced through the activation of metabolic pathways that may depend on the birds' body condition (Hill, 2000; McGraw et al., 2005). Finally, the relationship between body condition and integument colouration might be related to the current physiological condition of individuals, i.e. the most colourful kittiwakes are those with the best immunological status. This implies that healthy individuals should require fewer carotenoids for immune defenses and could therefore allocate more carotenoids to enhance colouration (Pérez et al., 2010a; Pérez-Rodríguez and Viñuela, 2008). However, this hypothesis could only be confirmed by measuring carotenoids in integuments and plasma coupled to the measure of immunological parameters.

Each relationship between POP levels and saturation was very similar among labile integuments. Similarly, relationships between body condition and colouration parameters (hue and brightness) for gapes and tongue suggest that POPs and body condition affect in the same way each labile integuments colouration. Contrary to fleshy integuments with rapid colour changes, e.g. 48 h for the skin of the blue-footed booby Sulax nebouxi (Velando et al., 2006), the bill is a keratinized structure and the turnover of carotenoids deposited in the bill is obviously slower (Pérez-Rodríguez and Viñuela, 2008). Moreover, body condition and POP levels of kittiwakes from the studied colony are known to vary rapidly through the breeding season (Moe et al., 2002; Nordstad et al., 2012). Therefore, the faster turnover of eye-ring, gapes and tongue colouration compared to that of bill may explain why we only observed relationships between the current condition of birds (POPs level and body condition) and labile integuments colourations.

4.4. Conclusion

The present study provides the first evidence of a potential effect of individual POP levels on integument carotenoid-based colouration of black-legged kittiwakes. In addition, it also shows that body condition can explain integument colouration in this species. However, and importantly, body condition and POPs burden do not seem to act on the same component of integument colouration. Consequently, our results suggest that, in female black-legged kittiwakes, carotenoid-based colour integuments may be sensitive to several independent pressures, such as POPs contamination, nutritional conditions and current health of individuals.

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Figures caption

Fig. 1: Relationship between ΣPOPs (pg•g^{-1} ww) in blood and body condition of pre-laying female black-legged kittiwakes.

Fig. 2: Relationships between colouration parameters (hue, saturation, brightness) and ΣPOPs (pg•g^{-1} ww) in blood of pre-laying female black-legged kittiwakes for all integuments. Solid line represents significant relationship ($P < 0.05$) and dashed line represents marginally significant relationship ($P < 0.1$).

Fig. 3: Relationships between colouration parameters (hue, saturation, brightness) and body condition (residuals) of pre-laying female black-legged kittiwakes for all integuments. Solid line represents significant relationship ($P < 0.05$) and dashed line represents marginally significant relationship ($P < 0.1$).