Science of the Total Environment 470–471 (2014) 248–254

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

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HIGHLIGHTS

- We studied the relationships between POPs burden and integument colouration in an artic seabird.
- Saturation of eye-ring, gapes and tongue was negatively related to POPs' burden.
- Individuals with a better body condition displayed more orange gapes and tongue.
- POPs could affect the amount of carotenoid coloration in this arctic seabird.

ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form 15 September 2013
Accepted 16 September 2013
Available online 18 October 2013

Editor: Frank Riget

Keywords:
Arctic
Seabird
Carotenoid
Contamination
Pesticide
PCB

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.

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

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http://dx.doi.org/10.1016/j.scitotenv.2013.09.049

(Costantini and Møller, 2008; 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, and also through environmental stressors (Chew, 1996). Consequently, carotenoids promote survival (immunity, antioxidant capacity) suggesting that a trade-off may exist between allocations of carotenoids towards sexual ornaments signalling 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 defences 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., 2000) but no sexual chromatic dimorphisms are found (Doutrelant et al., 2013; Leclaire et al., 2011a). Both sexes show intense carotenoid-based colouration during the breeding season (Doutrelant et al., 2013; 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; Leclaire, 2010; Leclaire et al., 2011a,b). 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; Leclaire, 2010; Leclaire et al., 2011a,b), 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 et al. (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