



Pollutants and diet influence carotenoid levels and integument coloration in nestlings of an endangered raptor



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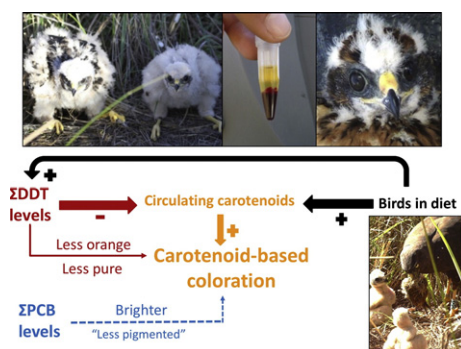
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HIGHLIGHTS

- PCBs and DDTs may affect the expression of bird carotenoid-based signals.
- Carotenoid-based coloration and plasma carotenoids increased with bird consumption.
- Carotenoids and coloration decreased in harrier nestlings with increasing DDT levels.
- Nestlings harriers with higher PCB levels had whiter, less pigmented integuments.
- Organochlorines may affect social communication based on carotenoid signals.

GRAPHICAL ABSTRACT



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ABSTRACT

Carotenoid-based traits or ornaments, such as yellow–red integuments (feathers, beaks, legs or eye-rings) displayed by birds, play key roles in social communication by reliably advertising an individual's quality or health. In some species, these traits are displayed not only by adults but also by nestlings, and function in parent-offspring communication or sibling competition by advertising an individual's physical or physiological condition. Pollutants such as organochlorine compounds (OCs) could have disruptive effects on the coloration of these traits, thereby interfering with communication processes. Such effects have been reported in adult birds, but are still largely unknown for nestlings. Here we investigated associations between polychlorinated biphenyl (PCB) and dichlorodiphenyltrichloroethane (DDT) blood-levels, circulating carotenoid levels and the yellow–orange coloration of the cere and tarsi of wild Black Harrier *Circus maurus* nestlings, a scarce raptor endemic to southern Africa. As carotenoid pigments must be acquired through the diet, we also tested for an effect of dietary composition. The orangeness–purity of cere and tarsi coloration positively correlated with circulating carotenoid levels, and increased with both nestling age and the proportion of birds consumed in the diet. Circulating carotenoid levels and the orangeness–purity of colored integuments were unrelated to blood PCB levels, although the brightness of integuments (i.e. lack of pigmentation) increased with PCB levels. Nestlings with more DDT had lower levels of circulating carotenoids and reduced carotenoid-based coloration (i.e. higher hue and lower saturation, reflecting a yellow rather than orange and less intense color, respectively). Together, our results are consistent with the hypothesis that OC contaminants, in particular DDT, may disrupt carotenoid-based signaling in exposed nestlings.

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1. Introduction

Carotenoids are pigments responsible for the red, orange and yellow coloration of integuments such as exposed skin, eye, cere, tarsus and plumages displayed by many avian species (Brush, 1990; Badyaev and Hill, 2000; Hill and McGraw, 2006). These carotenoid-based traits or ornaments play key roles in bird communication and in social interactions (Bortolotti et al., 2000; Costantini et al., 2007; Sternalsky et al., 2009) and are displayed by both adults and nestlings, sometimes from a very young age (Bortolotti et al., 2003; Sternalsky et al., 2009, 2011, 2012). In adults, colored traits are commonly used as an honest signal of individual quality such as a superior physical condition, foraging efficiency or ability to resist parasites (e.g. Hill and McGraw, 2006; Mougeot et al., 2007a). In the context of competition or mate choice, more colorful individuals are often dominant or more attractive (e.g. Goodwin, 1984; Webster et al., 2008; Lindsay et al., 2011). In nestlings, however, these colored displays have other functions, and likely play a role in parent-offspring communication, i.e. for parents to assess nestling condition and needs and so adjust their feeding and caring efforts (Lyon et al., 1994), or sibling competition, i.e. for sibs to assess each-other's competitive abilities and dominance (Biard et al., 2006; Sternalski et al., 2011, 2012). Understanding the factors that influence variation in the coloration of carotenoid-based traits is particularly relevant for understanding the functions and biological importance of these traits. In that respect, little is known about the potential effects that contaminants may have on the expression of carotenoid-based traits, and the broader implications for social communication.

Vertebrates cannot synthesize carotenoid pigments *de novo* but must ingest them; thus carotenoids may be a diet-limited resource (Goodwin, 1984). The total amount of carotenoid pigments available for an individual will depend on the quantity and the quality, in terms of carotenoid content, of the ingested food (Negro et al., 2002; Eeva et al., 2009). For predators, small mammal prey, for instance, are energy-rich but carotenoid-poor, while other prey such as birds, reptiles or insects have a greater carotenoid content (Goodwin, 1984). Dietary composition may, therefore, be important when studying variation in circulating carotenoid levels and the expression of carotenoid-based traits (e.g. Sternalsky et al., 2009), as well as interpreting the effect of persistent organic pollutants in birds (Mañosa et al., 2003). Carotenoids serve important health-related physiological functions: they can act as antioxidants and be used to limit oxidative damage (Pérez-Rodríguez, 2009). They can also boost the immune system through immuno-stimulation and immune-regulation functions (Favre et al., 2003; Blount et al., 2003). Carotenoid-based traits have been proposed to reliably signal an individual's healthiness, or for instance the ability to deal with parasites (e.g. Mougeot et al., 2007a). In this context, only healthy individuals could afford to deposit carotenoids to increase their ornamental coloration, rather than using them to limit oxidative damage or boost their immune defenses (Lozano, 1994; Møller and Mousseau, 2001; Mougeot et al., 2009; Biard et al., 2010; Pérez-Rodríguez et al., 2013). The carotenoid-based coloration may also vary with individual characteristics, including nestling age, sex (Sternalski et al., 2009, 2011, 2012), hatching order (i.e. rank within the brood) or condition (Senar et al., 2003; Sternalsky et al., 2009, 2012). It seems, therefore, important to consider these variables, as they may help explain variations in physiological aspects that may ultimately influence future reproductive outcomes of breeding birds (Eeva et al., 2012; Sassani et al., 2016).

Carotenoid-based coloration and circulating carotenoids have been shown to be affected by both non-organic contaminants such as heavy metals (Camplani et al., 1999; Møller and Mousseau, 2001; Eeva et al., 2008, 2009; Giraudeau et al., 2015; Vallverdú-Coll et al., 2015, 2016a) and organic contaminants such as persistent organic pollutants (POPs) (McCarty and Secord, 2000; Bortolotti et al., 2003; López-Antia et al., 2013; Blévin et al., 2014; López-Antia et al., 2015a, 2015b) or fuel oil pollution (Pérez et al., 2010). Pollutants could thereby potentially interfere with communication processes that rely on carotenoid-colored traits

(see Marasco and Costantini, 2016). For example, the carotenoid-based coloration of eye-rings, gapes and tongues of adult female Kittiwakes *Rissa tridactyla* decreased with increasing levels of POPs in the blood (Blévin et al., 2014). By contrast, no association was found between POPs contamination and the carotenoid-based coloration of integuments of breeding Great Black-Backed Gulls *Larus marinus* (Bustnes et al., 2007). Several studies have related exposure to pollutants to both circulating carotenoids and carotenoid-based coloration (Lopez-Antia et al., 2013, 2015a, 2015b; Vallverdú-Coll et al., 2015, 2016a; García-de Blas et al., 2016), but few of them have been performed with birds in the wild (Bortolotti et al., 2003; Eeva et al., 2008; Vallverdú-Coll et al., 2016b). In addition, most studies have been conducted on adult birds, but relatively few have been undertaken with nestlings and the knowledge and understanding of how these contaminants affect them is particularly lacking.

The main goal of the present study was to investigate whether OCs affect the circulating carotenoid levels and the carotenoid-based coloration of integuments (yellow-orange cere and tarsi) developed by Black Harrier *Circus maurus* nestlings. Unlike feathers, the carotenoid-based coloration of fleshy integuments is dynamic, with the potential for rapid change (Velando et al., 2006; Pérez-Rodríguez, 2008). Therefore, changes in coloration of integuments such as cere and tarsi and in circulating carotenoid levels may reflect a more recent intake of carotenoids and recent changes in individual condition (Favre et al., 2003; López et al., 2011).

The Black Harrier is a scarce medium-sized raptor, endemic to southern Africa, and classed as Endangered in South Africa, Namibia and Lesotho (Simmons et al., 2015; Taylor, 2015). This ground-nesting bird of prey breeds in indigenous vegetation of south western South Africa, preferentially along the coast within the Fynbos biome, and also inland within the Karoo biome (Curtis et al., 2004; Curtis, 2005; García-Heras et al., 2016). Like most raptors, Black Harriers display carotenoid-based coloration from a young age, i.e. a few days after birth. Black Harrier broods include generally 2 to 4 nestlings (Simmons, 2000; Simmons et al., 2005) and competition for food can be intense in harsh conditions, sometimes leading to chick mortality (Simmons, 2000; MSGH, *personal observations*). A recent study revealed the presence of OCs in both adults and nestlings (García-Heras et al., *under review*). PCBs (PCBs 52, 101, 138, 153, 180) and DDTs (p,p'-DDT and its metabolite p,p'-DDE) were detected in the blood plasma of 82% and 81% of nestlings, respectively. Detected concentrations were high enough to induce physiological effects, such as increased heterophil to lymphocyte ratio in PCB-exposed individuals and increased white blood cell counts in DDT-exposed individuals, but had no effect on nestling's body condition (García-Heras et al., *under review*). Black Harriers prey mostly on small mammals, but also on birds such as Common Quails (*Coturnix coturnix*) as alternative prey (García-Heras et al., 2017a, b). Birds are generally known to bio-accumulate more OCs than small mammals (Fossi et al., 1995; Mañosa et al. 2003; van Drooge et al. 2008), and García-Heras et al. (*under review*) found a positive association between blood-p,p'-DDE levels and the proportion of bird biomass in Black Harriers diet. In this context, diet may affect carotenoid availability directly because small mammals are carotenoid-poor prey compared with birds, as well as indirectly because, in this region, individuals eating more birds are also more exposed to DDTs (García-Heras et al., *under review*).

We first tested whether carotenoid levels and carotenoid-based coloration varied with nestling age, sex, rank, physical condition and dietary composition. We expected well-fed nestlings in better condition (i.e. mass corrected for age) to be more colored, and individuals eating more birds to be less carotenoid-limited and more colored. Second, we tested whether circulating carotenoid and coloration varied with DDT and PCB levels, and expected exposed nestlings to display less colored integuments, because they may allocate available carotenoids towards detoxification to counter adverse physiological effects of OCs.

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