Ornamental colouration as an indicator of environmental pollution with application to the tree swallow (*Tachycineta bicolour*)

by

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Abstract

Anthropogenic pollution causes habitat degradation for many species, but its effects on individuals can be difficult to detect until they result in the decline or disappearance of populations. Current techniques to monitor the effects of pollution on wildlife usually include invasive sampling of expendable species, but measuring colouration of ornamental traits may provide an alternative that is non-invasive and suitable for species of conservation concern. As products of sexual selection, ornamental traits are sensitive to environmental conditions, thereby providing honest information about the health and condition of their bearers as ready-made biomarkers. The objectives of my thesis are (1) to review the literature on the effects of various pollutants on ornamental colouration of fish and birds; (2) determine whether colouration of wild tree swallows (*Tachycineta bicolour*) is sensitive to environmental metal pollution; and (3) compare colour metrics of tree swallows obtained via spectrophotometry and digital photography, while also determining if remote photography is precise enough to detect changes in colouration.

For objective 1, I conducted a literature search and reviewed the documented effects of several classes of pollutants on ornamental colouration of fish and birds. I found that several types of pollutants, including pharmaceuticals, pesticides, industry-related compounds and metals, impede the expression of carotenoid- and brown melanin-based ornamental colouration, enhance traits coloured by black melanin, and have opposing effects on different metrics of structural colouration. I concluded that by finding suitable model species and comparative colour metrics, it could be possible to lay a foundation for pollution monitoring that is less invasive and more generalizable than existing standards.

For objective 2, I studied tree swallows nesting in boxes at four sites surrounded by a different degree of urbanization. I estimated pollution exposure for each brood via feces produced by chicks and compared these values to the feather and health (measured via oxidative stress) and colouration of provisioning parents. I further compared parental colour to reproductive success (measured by number of young fledged). I found that plumage colour shifter from bluer to greener, while feather brightness increased, with increasing exposure to copper and zinc. Both patterns would be expected from changes in the microstructure of the feathers. Unexpectedly, increasing exposure to these metals correlated with increased apparent health (lower oxidative stress) in female swallows, but not males. Number of young fledged decreased slightly with exposure to metals but did not vary with the colour of parents.

For objective 3, I compared colour information obtained from digital photographs of birds in-hand to the differences in colour that I obtained using reflectance spectrophotometry on the same individuals. With different individuals, I calibrated digital photographs of tree swallows perched on their nest boxes with colour reference cards that had previously been attached to the boxes using three measures of colour: hue, saturation and brightness. I found that hue of tree swallows was highly correlated between techniques, and saturation and brightness were uncorrelated. Additionally, hue values measured using photography at a distance fell within the range of plumage hue reported for tree swallows, and the colour reference panels showed a very low variation between the photographs, even when taken on different days and under different environmental and illumination conditions.

In combination, my results suggest that ornamental colouration of birds can be a reliable tool for monitoring environmental pollution. For tree swallows, colouration seems to be sensitive to metal pollution, although more work will be needed to understand the complex relationships among ornamental colour, health, and fitness metrics. My results suggest that hue of iridescent plumage can be measured as effectively with photography as with spectrophotometry, and that calibrated remote photography may provide a reliable measure of it. These results support the suggestions by several previous authors that ornamental colouration could provide a non-invasive tool for assessing pollution exposure in birds while advancing tools for achieving that goal.

Preface

This thesis is an original work by Natalia Lifshitz. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Animal Care and Use Committee, Project Name "Development of non-invasive methods for using sexually-selected traits in birds as markers of aquatic pollutants", AUP00001501, May 25th, 2015.

Chapter 2 of this thesis has been published as N. Lifshitz and C.C. St. Clair, 2016. Coloured ornamental traits could be effective and non-invasive indicators of pollution exposure for wildlife. Conservation Physiology, 4(1). I was responsible for the data collection as well as the manuscript composition. C.C. St. Clair was the supervisory author and was involved with concept formation and manuscript composition.

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Chapter 1: General Introduction

Anthropogenic activities cause changes to the environment that contribute to population declines and extinctions of vulnerable species (Gibbons *et al.*, 2000; Kidd *et al.*, 2007). One of the consequences of anthropogenic activities with a negative impact on wildlife is pollution (Tyler *et al*., 1998; Zala and Penn, 2004; Isaksson, 2015; Trathan *et al.*, 2015), with indirect effects of habitat degradation and direct toxicological effects on many species. Chemical pollution is of special concern due to the constant use and deposition of environmental pollutants with deleterious effects on wildlife (Cowan *et al.*, 1995). Some of the reported negative effects of chemical pollution on animals include sub-optimal development (Colborn *et al*., 1993), impaired immune system (Desforges *et al.*, 2016), reproduction (Goutte *et al.*, 2014) and behaviour (Zala and Penn, 2004). By the time effects are detected, populations may already be declining (Gibbons *et al.*, 2000), which makes the underlying effects more difficult to reverse (Vickery *et al.*, 2004), or fatalities may have already occurred (for example after an oil-spill; Haney *et al.*, 2014).

Amongst vertebrates, birds were one of the first taxa to demonstrate pollution-related population declines (Carson, 1962). In addition, because of their abundance, sensitivity to pollution and important structural role as components of the ecosystem, birds have been recurrent models in studies of environmental monitoring, functioning as sentinels or indicators of environmental conditions (Burger and Gochfeld, 2004; Smits and Fernie, 2013; Plaza *et al.*, 2018). For example, blood samples of yellow-legged gulls were used to monitor oil pollution up to 17 months after the Prestige oil spill in Spain (Pérez *et al.*, 2008). In order to prevent the decline or disappearance of species due to pollution, the health of at-risk populations needs to be constantly monitored (Van der Oost, Beyer and Vermeulen, 2003). Traditional approaches to achieve this goal typically involve invasive sampling of indicator species (Golden and Rattner, 2003). Such techniques include blood sampling (e.g. to measure pollution-related stress hormones; Tartu et al. 2015), injection of immune trials (e.g. to evaluate the effects of pollutants in the response of the immune system; Cruz-Martinez *et al.,* 2015) and sacrifice of eggs or chicks (e.g. to measure pollutant concentration in yolk or bioaccumulation in tissue; Hoffman *et al.*, 1998; Snoeijs *et al.*, 2004). However, due to the invasive nature of these techniques, they can only be performed in expendable species, making them untenable for species of conservation concern. In addition, because these techniques need capture of individuals, their use is limited to species that live in locations that are accessible to researchers.

Given the current high rates of global pollution (Kabir *et al.*, 2012), there is an increasing necessity to monitor and detect more subtle and early effects of pollution on individuals, long before they can be seen as more catastrophic effects at the level of populations. The need for reliable and less-invasive monitoring protocols has promoted the development of some non-invasive techniques to assess exposure of wildlife to pollution (Kocagöz *et al.*, 2014; Richards *et al.*, 2014). However, most of these non-invasive techniques have been applied only to mammals (including humans; Alves *et al.*, 2014), as they include collection of feces in the wild (e.g. Lundin et al. 2016) and collection of hair samples using hair traps (e.g. Pollock *et al.* in press). For birds, collecting feces of small wild species without capture is a difficult task, and active collection of feathers requires capture, so it would have to be opportunistic to achieve less-invasiveness. A good and functional indicator of pollution exposure for wildlife populations should make it possible to correlate variation in exposure among individuals with measurable aspects of their physiological condition.

Physiological condition alone has long been associated with the expression of colourful ornamental traits, especially in birds (Andersson, 1982). The link between these traits and individual condition arises from the high costs entailed in the production and maintenance of such colourful ornaments (Zahavi, 1975). In the case of pigment-based colouration, the pigments required for colour production can be costly to produce, acquire, or allocate as ornaments; melanins are brown and black pigments that are produced by individuals (Jawor and Breitwisch, 2004). Carotenoids are yellow, orange and red pigments that must be acquired in the diet (McGraw 2006). Both types may be allocated into coloured ornaments at the expense of other bodily functions (Faivre *et al.*, 2003; Galván and Alonso-Alvarez, 2008). Structural colouration, including iridescence, is produced by the physical interaction of light waves and the nanostructure of feathers (Prum, 2006), which is also costly to produce and maintain (Lindström, *et al*., 1993; Eliason and Shawkey, 2010). Iridescence sometimes also requires melanin (Doucet *et al.*, 2006; Maia *et al.*, 2009), increasing its physiological cost and sensitivity to environmental conditions (McGraw, 2008). Because of these costs, only individuals in good health, with optimal functionality of body systems, can afford to produce and maintain colourful ornaments (Zahavi 1975). In turn, these ornaments become honest indicators of the health and condition of their bearers (Hamilton and Zuk, 1982), as ready-made biomarkers. Furthermore, as products of sexual selection, ornamental traits show high phenotypic plasticity (Cotton *et al.*, 2004), meaning that their expression is particularly sensitive to the environment and to the cascade of physiological mechanisms produced by stressful events (Hill, 1995; Buchanan, 2000), including low environmental quality and pollution.

As suggested by Hill (1995), decreased individual condition caused by an environmental stressor such as chemical pollution could be reflected in the susceptible condition-dependent ornamental traits before they are signaled by decreased health, reproduction or higher probability of mortality. Through effects on the physiology and condition of individuals, pollutants can potentially affect the production and maintenance of colourful ornaments either directly or indirectly. Directly, some metals can promote or prevent the mechanical processes of melanin production, although the results are contrasting; on the one hand, copper, zinc and calcium ions are cofactors of tyrosinase, which is a crucial component for melanin synthesis (Han *et al.*, 2007). On the other hand, in vitro studies have demonstrated that melanin production can be directly inhibited by exposure to the metals mercury, selenium and zinc as they can inhibit tyrosine hydroxylation (Lerner, 1952; Furumura *et al.*, 1998; Ahn *et al.*, 2006; Han *et al.*, 2007) and prevent melanogenesis potentially making it less available for feather colouration. Indirectly, pollutants could affect colouration through several non-exclusive mechanisms which depend on the type of colouration. Changes in colour can arise if pollutants: (a) disrupt the endocrine system (Tyler *et al*., 1998), (b) alter the oxidative status of individuals (Isaksson, 2010), (c) increase the production of stress glucocorticoids (Roulin *et al.*, 2008), (d) affect the production of the feather microstructure (D'Alba *et al.*, 2014), (e) alter the load or richness of feather-degrading bacteria (Gentes, Whitworth, *et al.*, 2007), and/or (f) affect the time that individuals spend caring for their ornaments (Chatelain *et al.*, 2015). By interfering with the normal function of the endocrine system, endocrine-disrupting pollutants can impact ornamental colouration if its expression is controlled by or related to sexual hormones (e.g. melanins; Bókony et al. 2008). Pollutants with pro-oxidant properties can affect ornamental colouration that depends upon pigments related to the antioxidant defenses of the organism (e.g. Galván and Alonso-Alvarez 2009; Pérez *et al.*, 2010). Additionally, by reducing condition, an increased oxidative status could deplete the individual's resources to produce good quality feathers. If pollution-related stress is causing the release of glucocorticoids, this could alter melanin-based colouration, as glucocorticoids can inhibit melanogenesis (Roulin *et al.*, 2008). Because feathers are costly to produce, decreased condition due to exposure to pollution can affect colouration that depends upon this microstructure, particularly structural and iridescent colourations (Doucet, 2006; Maia *et al.*, 2009). If pollution increases the load of feather-degrading bacteria by disturbing habitat characteristics, parasite predators and/or host resistance to parasites (Gentes, Whitworth, *et al.*, 2007), colouration would be affected by the mechanical damage bacteria can cause to the feathers (Shawkey *et al.*, 2007). And lastly, by decreasing condition, pollution could alter colouration if plumage colour depends on maintenance and preening oils (Eliason and Shawkey, 2010, 2011; Griggio *et al.*, 2010), and if affected individuals reduce the time allocated to preening (Chatelain *et al*., 2015) to invest it in other vital functions such as foraging. Because of the sensitivity of ornamental colouration to environmental conditions, it has been proposed to be a reliable indicator of overall environmental quality (Hill, 1995) and could function as an indicator that reflects individual exposure to pollution. Measuring changes in ornamental colouration could be a monitoring tool that is less-invasive and suitable for all species with coloured ornaments, including species of conservation concern.

If ornamental colouration is to be successfully used as an indicator of exposure to pollution, a first step is to measure and quantify it appropriately. Two traditional ways of rigorously quantifying bird colouration have been described in the literature. Measurements may be taken using reflectance spectrophotometry, where an objective measure of the properties of the colour signal is taken by measuring surface reflectance at a fine wavelength resolution (Montgomerie, 2006; Johnsen, 2016). Alternatively, measurements may be taken using calibrated digital photographs (Stevens *et al.*, 2007; Pike, 2011; Troscianko and Stevens, 2015; Johnsen, 2016) where individuals are held against a controlled, dark background with a calibration standard of known reflectances to permit subsequent calibration using photography-processing software. In published studies, however, researchers have only used one of the two methods to measure plumage colouration, assuming they both provide the same information. While using a spectrophotometer to measure colouration provides more spectral resolution, colour metrics calculated from calibrated digital photographs can be equally reliable (Stevens *et al.*, 2007; Johnsen, 2016). Furthermore, by using focusing optics, photography can eliminate the need for contact with the animal, offering a degree of non-invasive monitoring. This potential has been widely exploited in studies of remote sensing of vegetation, where cameras have become tools used to evaluate vegetation type, diversity, and health based on colour metrics (Fletcher *et al*. 2001; Newete *et al.*, 2014). However, the use of remote cameras to measure colour of animals from a distance remains highly unexplored even though a study with wild gelada baboons (*Theropithecus gelada*) has already demonstrated its high and accurate potential (Bergman and Beehner, 2008).

Tree swallows (*Tachycineta bicolour*) provide an excellent model species for examining colour in association with environmental quality. This small passerine occurs in a wide variety of aquatic habitats, including rivers, lakes, other palustrine wetlands and even disturbed landscapes and highly urbanized aquatic areas (Custer, 2011). Swallows are aerial insectivores that forage within a few hundred meters of their nest sites (Mengelkoch *et al*., 2004) so their exposure to air-, soil- and water-born pollutants is likely to bioaccumulate and represent local conditions (Jones, 2003; Custer, 2011). For this species, toxicological effects have been identified at the levels of populations (e.g., abundance) through to cellular and genetic biomarkers (Custer, 2011). As examples, exposure to polychlorinated biphenyls (PCBs) reduced hatching success (McCarty and Secord, 2009) and overwinter survival rates (Custer *et al.*, 2009), and exposure to pesticides increased immune system activity (reviewed in Custer, 2011).

Tree swallows exhibit dorsal blue-green iridescent colouration as adult males and older females after their second year, which is produced by the physical interaction of light waves and the nanostructure of the feathers (i.e. keratin and melanin; Eliason and Shawkey 2010). Females after their second year displaying plumage that is brighter, with greater blue and ultraviolet (UV) chroma, and reflecting light maximally at shorter wavelengths (bluer hue), are considered to be more ornamented because they have greater nanostructural organization of their feathers, lay heavier eggs and fledge more offspring (Bitton *et al*., 2008; Bentz and Siefferman, 2013). However, more ornamented females have also shown to suffer from greater nest parasitism, poorer immune defenses, and lower hematocrit levels, while also producing nestlings that are smaller or in poorer condition (Bentz and Siefferman, 2013), suggesting that producing and maintaining a high ornamentation is costly. Swallows also mate assortatively by plumage brightness (Bitton *et al*., 2008); for males, individuals with brighter plumage have greater success of extra-pair fertilization, resulting in greater reproductive success than duller males (Bitton *et al*., 2007; Whittingham and Dunn, 2016). For females, a recent study suggests that plumage brightness might signal intra-sexual competitive ability and influence the competition for access to nest boxes (Bitton *et al*., 2008; Berzins and Dawson, 2016).

Although the effects of environmental toxicants are well studied in this species and much is known about the production and information conveyed in its ornamental colouration, little is known about the effects of pollution on the colouration of their plumage, and on iridescent colouration in general. One recent study showed a decline in brightness and saturation of the iridescent plumage of urban pigeons exposed to higher concentrations of lead (Chatelain *et al.*, 2017). For tree swallows in particular, only one study has explored the effects of pollution on colouration and found that pollution by PCBs speeds the onset of females' plumage maturation (McCarty and Secord, 2000). However, no author has explored whether exposure to environmental pollutants has an effect on the quantifiable properties of colouration in tree swallows.

1.1 Summary of thesis objectives and methodologies

The goal of my thesis is to evaluate the potential of ornamental colouration as a non-invasive tool for monitoring the effects of environmental pollution on birds using tree swallows as a model. More specifically, I (1) reviewed the literature on the effects of various chemical pollutants on ornamental colouration of fish and birds; (2) investigated whether colouration of wild tree swallows is sensitive to environmental metal pollution in sites surrounded by different degrees of urbanization; and (3) compared colour metrics of tree swallows obtained via spectrophotometry and digital photography, and quantified variation in colour that could be measured with remote photography.

In Chapter 2, I reviewed the effects that different types of anthropogenic pollutants have on ornamental colouration of fish and birds. To do so, I reviewed the published literature on the reported effects of pharmaceuticals, pesticides, industry-related compounds and metals on melanin-, carotenoid-based and structural ornamental colouration of fish and birds. I also discuss the limitations to the use of colouration as an indicator of pollution exposure and propose some recommendations for future research.

In Chapter 3, I explored the effects of metal pollution on the iridescent colouration of tree swallows. To do so, I studied tree swallows nesting in boxes at four sites surrounded by a different degree of urbanization. I estimated pollution exposure for each brood via feces produced by the locally-fed chicks and compared these values to the feather colouration of provisioning parents. I further investigated if oxidative stress was the mediator of this relationship by exploring if metal pollution affected blood-based levels of the antioxidant glutathione. Finally, I assessed if pollution exposure had any impact on reproductive success (measured by number of young fledged) and if it influenced the relationship between parental colouration and reproductive success.

In Chapter 4, I evaluated if measurements of plumage colour of males obtained with calibrated photographs accurately reflect the values of colouration obtained using reflectance spectrophotometry and if they reveal a gradient of exposure to environmental metals measured via concentrations of metals in feces of chicks. I also evaluated if remote photographs of males provide colour measurements with a comparable range of values to those taken with birds in-hand and with spectrophotometer. To do so, I took calibrated photographs of birds in-hand and compared the colour information obtained to colour information obtained using reflectance spectrophotometry on plucked feathers of the same individuals. For different individuals, I compared colour metrics obtained from photographs of birds perched on their nest boxes to metrics obtained from photographs of birds in-hand and from spectrophotometry on plucked feathers. Additionally, I assessed the reliability of this remote technique by exploring the repeatability of colour metrics of the red and blue reference panels included in each photo.

I conclude my thesis by discussing and integrating my most salient findings and recommendations for using ornamental colouration as a non-invasive metric to monitor the effects of pollution on wild birds.

Chapter 2: Coloured ornamental traits could be effective and non-invasive indicators of pollution exposure for wildlife

2.1 Abstract

Growth in human populations causes habitat degradation for other species, which is usually gauged by physical changes to landscapes. Corresponding habitat degradation to air and water is also common, but its effects on individuals can be difficult to detect until they result in the decline or disappearance of populations. More proactive measures of pollution usually combine abiotic samples of soil, water or air with invasive sampling of expendable species, but this approach sometimes creates ethical dilemmas and has limited application for threatened species. Here, we describe the potential to measure the effects of pollution on many species of birds and fish by using ornamental traits that are expressed as coloured skin, feathers and scales. As products of sexual selection, these traits are sensitive to environmental conditions, thereby providing honest information about the condition of their bearers as ready-made biomarkers. We review the documented effects of several classes of pollutants, including pharmaceuticals, pesticides, industry-related compounds and metals, on two classes of colour pigments, namely melanins and carotenoids. We find that several pollutants impede the expression of both carotenoids and brown melanin, while enhancing traits coloured by black melanin. We also review some of the current limitations of using ornamental colour as an indicator of pollution exposure, suggest avenues for future research and speculate about how advances in robotics and remote imagery will soon make it possible to measure these traits remotely and in a non-invasive manner. Wider awareness of this potential by conservation managers could foster the development of suitable model species and comparative metrics and lay a foundation for pollution monitoring that is more generalizable and biologically relevant than existing standards.

2.2 Introduction

In the field of conservation biology, anthropogenic changes to the environment are widely known to contribute to population declines and extinctions of vulnerable species (reviewed by Gibbons *et al.*, 2000). In the field of behavioural ecology, ornamental traits are equally widely understood to be reliable indicators of individual condition and quality (Hamilton and Zuk, 1982;

Zahavi, 1975) that consistently reflect environmental conditions (Griffith, Owens and Burke, 1999; Monaghan, 2008). Despite extensive independent development and some appreciation of their combined explanatory potential (Hill, 1995; Sutherland, 1998), these two bodies of theory are almost never combined by conservation biologists and wildlife managers. As suggested by Hill (1995) better integration of these fields could provide a general, proactive, non-invasive, and effective diagnostic tool for detecting subtler anthropogenic effects on individuals before they are signaled by decreased reproduction or higher probability of mortality, which ultimate cause declining populations. In turn, those advantages could reduce the cost and increase the efficacy of mitigation efforts (Clout, Elliott and Robertson, 2002; Buchholz, 2007).

Estimating anthropogenic effects using ornamental traits requires mechanistic understanding of both anthropogenic effects and the expression of ornamental traits in wildlife. Separately, these mechanisms are particularly developed for one category of each component; chemical pollution as a type of anthropogenic effect, and the pigment-based colouration of the integument as a type of ornamental trait. In this review, we integrate these two specific categories to illustrate how they could be used by conservation biologists now, describe some limitations for expanding that use, and identify profitable directions for future research.

Chemical pollution can have acute negative effects on wildlife via development (Colborn *et al.*, 1993), physiology (de Swart *et al.*, 1994), behaviour (Zala and Penn, 2004), reproductive success (Goutte *et al.*, 2014), and survival (Martínez-Abraín *et al.*, 2006). It can also increase rates of hybridization (Ward and Blum 2012) and decrease genetic diversity (Bickham, 2000), ultimately reducing population viability and leading to extinction (Kidd *et al.*, 2007). All of these effects may be foretold by changes to the conspicuously coloured ornamental traits, which are prevalent in species with strong mate selection such as some birds and fish. As proposed by Darwin (1871), ornamental traits are morphological or behavioural traits that evolved via sexual selection to confer reproductive, rather than survival, advantages to the bearer through enhanced ability to attract mates. An example of this is the flamboyant tail of the male peacock. Zahavi (1975) expanded Darwin's theory to propose that such traits are so costly to their bearers that they can be produced and maintained as handicaps only by individuals of greater quality and condition. As such, ornamental traits are now widely recognized to reveal good genes, developmental conditions, local environments at the time when such traits are developed, or all three (von Schantz *et al.*, 1999).

Ornamental traits reveal quality and condition of their bearers partly because they have high phenotypic plasticity (Cotton *et al.*, 2004), meaning that their expression is particularly sensitive to the environment and to the cascade of physiological mechanisms produced by stressful events (Buchanan, 2000; Hill, 1995). Environmental conditions can be canalized during development (Naguib and Nemitz 2007) but they can also be reflected in a more recent or current fashion (e.g. Velando *et al.* 2006). This plasticity and honesty make ornamental traits especially useful for detecting both temporal and spatial variation in environmental conditions.

A prevalent type of ornamental trait in vertebrates is integument colouration (Hill and McGraw 2006b) which includes skin (e.g. Velando *et al*., 2006), scales (e.g. Plasman *et al.* 2015) hair (West and Packer 2002) and feathers (Safran and Mcgraw 2004). The appearance of the integument is dependent on its structure and the pigments deposited inside it as well as by the dirt, waxes, and abrasion applied to or acquired by it over time (Hill and McGraw 2006a). At least four kinds of costs can be revealed by these traits; first, the pigments involved in trait colouration may be physiologically costly to produce, such as melanin (Jawor and Breitwisch, 2003). Second, the pigments may be available only in some kinds of high-quality food, such as carotenoids (McGraw, 2006a). Third, the pigments may be needed for other functions, such as immune or antioxidant system support, and available for ornaments only when those functions have been met (Faivre *et al.*, 2003; Galván and Alonso-Alvarez, 2008). Finally, each of these costs may co-occur as additive or multiplicative effects. By any of these routes, only those individuals in good condition can afford to produce, acquire, or allocate pigments for trait colouration without compromising survival, making colouration an honest signal to observers (Grafen, 1990; Hamilton and Zuk, 1982).

The ubiquity, plasticity, and honesty of integument colouration make it a powerful indicator of the competing costs of environmental stressors, such as anthropogenic pollutants. The short temporal scale of environmental effects on the integument suggest the potential of using its colouration as a tool to detect negative environmental impacts on individuals long before they cascade through populations, communities, and ecosystems with effects that are more difficult and costly to reverse. These features give ornamental integument colouration enormous, but largely untapped, potential to diagnose many conservation problems at their most proximate stages to support solutions that are more proactive (e.g. Baruch-Mordo *et al*. 2013), generalizable (*sensu* Caughley 1994) and holistic (Caro and Sherman, 2013).

In the following sections we describe the two main types of pigment-based colouration found in ornamental integuments of vertebrates, melanins and carotenoids, and review the known effects on them of a variety of chemical pollutants (Fig 2.1 and Table 2.1). Existing research in this area has only focused on fish and birds, groups that frequently express quantifiable coloured integuments and so have well-developed literatures. Latin names for the species we refer to in the text are provided in Table 2.1. Following this review, we discuss the limitations to the use of colouration as an indicator of pollution exposure and propose some recommendations for future research.

2.3 Overview of pigment and pollutant types

Melanins are the most prevalent pigments in vertebrates, producing many yellow-brownish (pheomelanin) and grey-black (eumelanin) traits (reviewed by McGraw, 2005). They can also combine with keratin and air to produce structural colours such as blue, violet, green and ultraviolet hues, as well as iridescent colours (Prum, 2006). Because vertebrates can synthesize melanins de-novo from amino acid precursors (Lin and Fisher, 2007), they are frequently assumed to be an unlimited resource for ornamental trait building, strictly controlled by genes (Geoffrey E Hill and McGraw, 2006; Badyaev and Hill, 2008) . However, more recent studies have demonstrated that the expression of melanin-based ornaments can also be influenced by environmental factors such as rearing conditions, parasite infestation, and diet quality to give these ornaments a plastic and honest quality in vertebrates (Fargallo *et al.*, 2007; McGraw, 2008; Guindre-Parker and Love, 2014).

Despite the ubiquity of melanin in vertebrates, a second kind of pigments – the carotenoids – are more widely studied. They produce many yellow, orange, and red traits, but cannot be synthesized by vertebrates (Schiedt, 1989) so their acquisition through the diet makes them a limited resource. Moreover, carotenoids are thought to play key physiological functions, accepting free radicals to protect cells and tissues from oxidative damage and acting as immune system enhancers (reviewed by Lozano, 1994; Von Schantz *et al.*, 1999; but see Hartley and Kennedy, 2004; Pérez-Rodríguez, 2009). In combination, these properties provide information about animal foraging, carotenoid-uptake, and allocation efficiency that is both accurate and visible (McGraw, 2006a).

Both types of pigments are pertinent to the large literature addressing the negative physiological effects of pollution on wildlife either by disrupting the endocrine system (Tyler *et al.*, 1998) and/or altering the oxidative status of individuals (Isaksson, 2010). Endocrine disrupting compounds (hereafter EDCs) interfere with the normal function of the endocrine system in several ways, such as mimicking natural hormones, blocking or altering the binding of natural hormones to hormone receptors, altering production and breakdown of natural hormones, and altering the production and function of hormone receptors (Tyler *et al.*, 1998). These effects have the potential to impact ornamental colouration if its expression is controlled by or related to sexual hormones (e.g. Bókony *et al.*, 2008).

The effect of pollution on the oxidative status of individuals is subtler, but it may also be more ubiquitously and generally expressed. Metabolism produces reactive oxygen species (ROS) as a by-product and when this production exceeds the capacity of the antioxidant defense and repair mechanisms, an imbalance called oxidative stress arises which leads to oxidative damage to biomolecules (i.e. proteins, lipids and DNA) (Halliwell and Gutteridge, 2007). This imbalance is known to be a key component in the life-history trade-offs between growth, reproduction and self-maintenance, or survival (Monaghan *et al*. 2009). Therefore, animals exposed to pollutants with pro-oxidant properties and whose ornamental colouration depends upon pigments related to antioxidant defenses are faced with a trade-off between using antioxidants to combat oxidative stress or to signaling functions and ultimately may have to sacrifice their investment in signaling to support survival.

The categorization of pollutants is confusing because it sometimes refers to generalized effects on natural systems (e.g. EDCs), sometimes on the chemical nature of compounds (e.g. polycyclic aromatic hydrocarbons or PAHs) and sometimes by the originating industries (e.g. petroleum products). In this review, we simplify that categorization by referring consistently to the anthropogenic context of pollutants and review examples of effects on ornament colouration for each of pharmaceuticals and active ingredients in personal care products, pesticides, industrial pollutants that are prevalent, but not restricted to petroleum production, and heavy metals that frequently result from mining and manufacturing. Within each of these source categories, we review the known effects on the production and expression of melanins and on the uptake and use of carotenoids for ornament colouration (Table 2.1 and Fig. 2.1).

2.4 Pharmaceuticals and active ingredients in personal care products (PPCPs)

This category conventionally includes prescription and nonprescription drugs, oral contraceptives, fragrances, and cosmetics (Daughton and Ternes, 1999). These ubiquitous products are usually disposed or discharged into the environment on a continual basis via domestic and industrial sewage systems and wet-weather runoff, where they enter water courses and come into direct contact with aquatic organisms. Relative to other pollutants, the emission of pharmaceutical products tends to be chronic and concentrated in areas with high human density (Kolpin *et al.*, 2002). Because some PPCPs –such as oral contraceptives – are specifically designed to target and modulate the endocrine system, they are likely to affect any form of ornamental colouration that is controlled by it.

Despite the high potential for PPCPs to influence ornamental colouration, this has only been addressed in fish and no study has targeted birds. To date, there is no evidence of negative effects of PPCPs on melanin-based colouration, where likely effects include ornamental traits that are mediated and stimulated by hormones, such as androgens or thyroid hormones (reviewed by McGraw, 2006b).

The anticipated effects of exogenous estrogens on aquatic species have been robustly demonstrated with fish. In a laboratory study, exposure to the artificial estrogen 17α-ethinylestradiol (EE2) reduced the area of carotenoid-orange colouration as a percentage of the total body area of male guppies (Kristensen *et al*. 2005) character known to function as a sexually-selected signal to females (Kodric-Brown and Nicoletto, 2001). In addition, exposed male guppies showed a significant reduction in courtship behaviour, sperm count, and paternity when competing for fertilizations with unexposed males (Kristensen *et al*., 2005). Similar effects occurred in male guppies, zebra fish and red shiners that were exposed in the lab to the natural estrogen 17β-estradiol (E2), EE2, and E2 respectively and then expressed dampened mating colouration (Toft and Baatrup, 2001; Larsen *et al.*, 2008; McGree *et al.*, 2010). Treated male guppies produced fewer offspring and displayed paler ornaments even after 3 months of recovery in clean water (Toft and Baatrup, 2001). Male zebra fish also showed a significant reduction in courtship behaviours, failing to induce spawning in females (Larsen *et al*., 2008). Similarly, courtship behaviours of male shiners were significantly reduced as well as their fertilization success, causing null hatching success of the fertilized eggs. Importantly, when exposure ceased, other reproductive endpoints, but not colouration, of the shiners improved significantly, demonstrating that the dulling effects were longer-lasting (McGree *et al*., 2010).

2.5 Pesticides

Pesticides are a broad category of compounds that are commonly used to protect crops, livestock, domestic animals, and humans from damage and diseases caused by fungi (fungicides), insects (insecticides), rodents (rodenticides), competition with unwanted plants (herbicides), and other so-called pests. Pesticides can also be categorized according to their chemical structure into inorganic (compounds that contain arsenic, copper, lead or mercury) and organic (artificially synthesized chemicals such as DDT; reviewed by Freedman, 2004). Animals may be exposed to pesticides through consumption of contaminated food or water, as well as through inhalation of contaminated air. Although pesticides have been used for millenniums, their use has increased greatly during the past half century because of their economic benefits and increased worldwide availability (Pimentel *et al.*, 1992). This has increased the geographical and temporal risk of exposure for wide-ranging and migratory birds (Stutchbury, 2009) among other species. Pesticides primarily cause damage to organisms by producing free radicals which overwhelm the antioxidant system (Abdollahi *et al.*, 2004), but some compounds also alter the endocrine system (Colborn *et al*., 1993).

Effects on melanin-based colouration can be complex and counterintuitive, as has been shown in red- legged partridges exposed to the contact herbicide, diquat. In these birds, the eumelanic (black) plumage of adults was unexpectedly enlarged following exposure to the pesticide during development, to produce larger black-spotted bibs and black flank bands (Galván and Alonso-Alvarez, 2009), which signal higher quality individuals of both sexes (Bortolotti *et al.*, 2006). However, exposure to the herbicide simultaneously reduced expression of pheomelanin to cause the brown flank bands to be smaller (Galván and Alonso-Alvarez, 2009). The authors speculated that the pesticide increased oxidation, which depleted the intracellular antioxidant glutathione and lessened the amount available to produce brown pigment in the flank bands. Because circulating glutathione blocks eumelanin synthesis, its depletion also caused the larger black bibs and flank bands (Galván and Alonso-Alvarez, 2009). This study underscores the need to understand underlying physiological processes to interpret the effects on colouration of pollution exposure.

The negative effects of pesticides on carotenoid-based ornamental colouration have been robustly demonstrated in fish. For example, the consistent preference by female guppies for males with larger and more intense orange spots (above) clearly predicts a disadvantage to males exposed to the fungicide vinclozolin and to the principal metabolite of the insecticide DDT (*p,p´*-DDE) because they reduced both the size and intensity of the spots (Baatrup and Junge, 2001). These morphological changes also occurred when guppies were exposed to the herbicide atrazine with corresponding reductions in courtship displays and aggressive behaviour toward other males during competition for mates (Shenoy, 2012). The effects of pollutants on adult colouration can also result from exposure during development. The sexually-selected yellow colouration of adult, male Amarillo fish (Macias Garcia, 1990) was duller in males exposed as embryos to the insecticide methyl parathion (MeP) and resulted in lower rates of female visitation and copulation attempts during courtship (Arellano-Aguilar and Macías Garcia, 2008). The authors speculated that these effects in adults could have been caused by one or both of long-lasting damage to the physiological systems that process and deposit carotenoids, or permanent damage to their antioxidant system with resulting increases in the use of dietary carotenoids to combat oxidative stress during adulthood.

In some cases, the interacting and opposing effects of pollutants on different colourful ornaments may help to identify basic physiological pathways and reveal the detrimental mechanisms of pollution exposure. An example of this potential is provided by the red-legged partridges (described above) in which the diquat-exposed birds with enhanced eumelanic traits also had paler carotenoid-based ornaments in the form of red beak and eye rings (Alonso-Alvarez and Galván, 2011). Because the red colouration in the head integument is positively correlated with the health status of individuals (Mougeot *et al.*, 2009) and positively affects female reproductive investment (Pérez-Rodríguez and Viñuela, 2008), researchers could see that the net effects of pesticide exposure were negative, despite the increase in eumelanin expression (Alonso-Alvarez and Galván, 2011). These negative effects on the carotenoid-based colouration of red-legged partridges were later confirmed for two fungicides (thiram and difenoconazole) and an insecticide (imidacloprid) via reductions in the percentage of carotenoid pigmentation in the eye ring. As additional evidence of net negative effects, all three pesticides reduced the size of eggs, imidacloprid and difenoconazole reduced the fertilization rate, and thiram and imidacloprid reduced chick survival (Lopez-Antia *et al.*, 2013). Similar effects have been detected in free-living birds. Female black-legged kittiwakes with higher levels of various pesticides and PCBs in their blood samples exhibited duller orange-red labile integuments (i.e. eye-ring, gapes and tongue) and these ornaments are believed to reflect individual quality in both sexes also (Blévin *et al.*, 2014).

2.6 Industry-related compounds

Many kinds of industrial pollutants potentially affect the colouration of ornamental traits in vertebrates, but only three have been studied in this context; Polychlorinated biphenyls (hereafter PCBs), phenols (particularly bisphenol–A, hereafter BPA, and octylphenol) and polycyclic aromatic hydrocarbons (hereafter PAHs). PCBs are stable, human-made organic compounds that were commonly used in electrical applications, hydraulic equipment, plasticizers, paints, plastics, rubber production, and as an insulating agent (reviewed by Blocker and Ophir, 2013). Production of PCBs ceased in 1972 (Dunlap, 2014) after their long-lasting toxic effects were realized, but the compounds persist in the environment and are capable of bio-accumulating with trophic position in lipid tissues. PCB's are well known to disrupt endocrine function by agonizing and antagonizing natural estrogens (Colborn *et al*., 1993) and negatively affecting thyroid function (Boas *et al.*, 2006). Similar endocrine-disrupting and estrogenic effects occur from exposure to bisphenol–A (BPA) and octylphenol (Bonefeld-Jørgensen *et al.*, 2007). These industrial pollutants typically reach wildlife through discharge to water courses, often via sewage treatment effluent (Kolpin *et al*., 2002). Despite its detrimental effects, BPA production is increasing owing to desirable commercial qualities for the manufacture of polycarbonate, epoxy and polyester resins (Crain *et al.*, 2007). Octylphenol is a degradation by-product of chemicals used in the manufacture of detergents and in agricultural and industrial products (Ying *et al.* 2002).

A third class of industrial pollutants that has been studied in the context of colouration is PAHs, which are the most toxic components of liquid petroleum products, but also occur as airborne pollutants (Eisler, 1987). Airborne PAHs typically result from combustion involving both anthropogenic sources (e.g. motor vehicles and many industrial processes) and natural ones (e.g. forest and prairie fires). PAH's can reach aquatic environments via condensation in the atmosphere as well as via discharge of liquid waste through domestic and industrial sewage effluents, surface runoff from land, and spillage of petroleum products into water bodies (reviewed by Eisler, 1987). The acute toxicity of PAHs is mainly attributed to the oxidative stress they generate in exposed organisms, who adaptively activate their antioxidant system in order to survive in polluted environments (Pérez *et al.*, 2010).

Among the few studies that have addressed the negative effects of PCBs on vertebrate colouration, only one examined melanic pigmentation. McCarty and Secord (2000) showed that subadult female tree swallows breeding along a river with high levels of PCB pollution displayed the blue-green iridescent plumage that is characteristic of adult birds of both sexes, rather than the brown upperparts that usually characterize subadult females. They speculated that the early expression of adult traits was caused by the estrogenic properties of PCBs (McCarty and Secord, 2000). If delayed plumage maturation in females of this species is an adaptation to signal subordinate status, thereby reducing conspecific aggression (Coady and Dawson, 2013), earlier development of adult plumage may disadvantage these birds.

Surprisingly few studies have addressed the effects of PCB's on carotenoid-based colouration in fish and birds, given the enormous literatures that address their effects for more conventional physiological metrics (e.g. Eisler, 1987; Colborn *et al*., 1993; Flint *et al.*, 2012), but they suggest consistently negative effects. For example, breeding male American kestrels that were exposed to a mix of PCB Aroclors lost brightness of the facial skin with yellow-orange pigments, which otherwise signals individual quality (Bortolotti *et al.*, 2003). Similarly, black-legged kittiwakes showed dampening of the orange-red colouration in their faces with increasing concentrations of pesticides and PCBs in their blood (Blévin *et al.*, 2014).

As a class of industrial chemicals, the effects of phenols on colouration have been studied only in fish. Guppies exposed to octylphenol produced fewer offspring while displaying orange spots that were smaller (due to inhibited growth of the spot) and less brightly coloured (Toft and Baatrup, 2001). Interestingly, cessation of exposure caused the colour, but not size, of the orange spots to recover, which may reflect functional differences in information content of the two colour signals. A similar effect occurred when male red shiners were exposed to the phenol BPA for a period as short as 14 days wherein there was a loss in the intensity of red breeding colouration in heads and fins, and blue iridescence in bodies (Ward and Blum, 2012). As an additional consequence of these colour changes, both sexes were less able to discriminate between conspecific and heterospecific partners during mate choice trials (Ward and Blum, 2012).

The effects of PAHs have been extensively studied in birds (Eisler, 1987), but their effects on ornament colouration have been explored only recently and in relation to the Prestige oil spill (González *et al.*, 2006). Researchers working with yellow-legged gulls breeding in colonies located in the pathway of the spill focused on the carotenoid-based red bill spot, which is exhibited by both sexes and appears to reliably reflect the antioxidant status and capacity for parental investment of the bearer (Pérez *et al*., 2008; Morales *et al.*, 2009). They found that the size of the red spot was negatively correlated with blood-based measures of AST (aspartate aminotransferase), an enzyme that is commonly used as an indicator of hepatic damage in birds (Pérez *et al.*, 2010). Additionally, gulls that were experimentally fed heavy fuel oil from the same spill had higher levels of PAHs, vitamin E, and carotenoids in blood plasma, suggesting that the latter two had been mobilized for antioxidant defenses to cause the observed reductions in the size of the red bill spot (Pérez *et al*., 2010). As for the studies examining orange spots in guppies following exposure to pesticides (above), the work following the *Prestige* spill suggests that (a) sexually-selected, carotenoid-based traits respond very rapidly to environmental pollutants and (b) might be particularly suited as indicators following acute events.

2.7 Metals

Several heavy metals, metalloids and trace elements are natural constituents of the Earth's crust, but they can also accumulate as a function of anthropogenic activity to become persistent environmental contaminants (Freedman, 2001). Because metals cannot usually be degraded or destroyed, organisms that are exposed to metals via inhalation, absorption, and ingestion often experience cumulative effects via one or both of bioaccumulation over time and biomagnification over trophic levels (Duruibe *et al.* 2007). Some elements, such as lead, mercury and arsenic, are toxic even at very low concentrations, but others, such as copper, selenium, iron and zinc, are essential to many biological processes and become toxic only at elevated concentrations (Ercal *et al*., 2001). Exposure to detrimental concentrations of either type can cause a cascade of effects that include decreased immune responsiveness (Kakuschke and Prange, 2007), increased oxidative stress (Valko *et al*., 2005), and ultimately, lower survival or reproductive performance (Dauwe *et al.*, 2004). The effects of metals on ornamental traits have been demonstrated for both melanins and carotenoids, but the direction of these effects differs.

For melanins, exposure to pollution from a lead smelter that included cadmium, zinc and copper resulted in larger eumelanic (black) breast stripes in great tits (Dauwe and Eens, 2008). This effect was surprising because larger stripes are preferred by females during mate choice (Norris, 1990). A similar positive trend was apparent in the brighter melanin-based plumage of belted kingfishers nesting close to a mercury-contaminated river and with higher levels of mercury in feathers and blood (White and Cristol, 2014). Like pesticides, metals seem to increase the expression of eumelanin-based ornamental traits, potentially disrupting the honesty of information they convey about bearer condition.

Also aligning with the evidences on ornamental traits for pesticides, metals appear to have a dampening effect on carotenoid-based ornaments. In great tits, pollution from metal smelters has been robustly demonstrated to reduce the carotenoid-based, yellow colouration in the breast feathers of both nestlings and adults to signal a loss in individual quality and condition (Eeva *et al*., 1998; Dauwe and Eens, 2008; Geens *et al*., 2009a). The authors of these correlative studies emphasized different proximate effects of metal pollution on ornaments, which could include reduced access to carotenoid sources (Eeva *et al*., 1998), metal-induced oxidative stress (Geens *et al*., 2009), of both effects (Dauwe and Eens, 2008). It is already known that metal pollution reduces carotenoid synthesis in plants (Rai *et al.*, 2005) which, in turn, could also reduce carotenoid concentrations in herbivores (e.g. caterpillars; Isaksson and Andersson, 2007). Whether they are predators or prey, individuals living in polluted sites appear generally to have reduced access to carotenoids, which may increase levels of oxidative stress to reduce condition in organisms with, as well as those without, carotenoid-based ornaments.

2.8 Limitations to the use of colouration as an indicator of pollution exposure

Several factors that we have not much addressed in our review potentially limit the use of sexually-selected ornamental traits as indicators of exposure to pollution or other stressors. Most importantly, there must be visible and meaningful variation in ornaments among individuals that experience different environmental conditions over tractable scales of space and / or time. Nonetheless, the importance and meaning of traits based on a given kind of pigment can by highly variable between species. For example, in great tits (*Parus major*), the melanin-based breast stripe is condition dependent, while in American goldfinches (*Carduelis tristis*), the melanin-based black cap is not (Mcgraw and Hill, 2000; Fitze and Richner, 2002). Furthermore, there could even be variation in trait information content within a species. For example, in common yellowthroats (*Geothlypis trichas*), the melanin-based mask appears to be the target of sexual selection in one population, while the carotenoid-based bib conveys greater selective advantage in another population (Dunn *et al.*, 2010). These examples demonstrate the need to examine, rather than assume, what information is conveyed by ornaments in a species- and even population-specific way before studying anthropogenic effects on those traits. This will be challenging because the rapidity of anthropogenic changes to environments may often exert strong selection pressures of their own (e.g. Candolin *et al*., 2007) that constrain, nullify, or otherwise change the information contained in the variation that remains visible as the colour of ornaments.

Another important challenge for the use of ornaments as indicators of environmental pollutants will be to unravel the separate effects on multiple traits and their associated impacts on individual quality and fitness. Many species exhibit more than one ornamental trait that may function as redundancy (e.g. 'back up signals; Møller and Pomiankowski, 1993), as multiple, or reinforcing, messages (Johnstone, 1996; Candolin, 2003), or even as evidence of trade-offs with each other (Andersson *et al.*, 2002). Our review of the currently available literature suggests that ornaments coloured by different pigments may respond to a given pollutant in opposite directions (e.g. red-legged partridge [Alonso-Alvarez and Galván, 2011] and great tit [Dauwe and Eens, 2008]), but also that different ornaments formed with the same pigment respond similarly (e.g. various carotenoid-based ornaments in the amarillo fish; Arellano-Aguilar and Macías Garcia, 2008).

A third challenge for the use of ornamental traits as indicators of pollution exposure is the inability for humans to see ornaments as other species might. A well-known example of this limitation is provided by the tetrachromatic vision of both birds (reviewed by Cuthill, 2006) and fish (Bowmaker, 1990), which affords detection of the ultraviolet portion of the colour spectrum. UV colouration is already known to be used in mate choice and to reflect the condition and quality of the bearer (reviewed by Prum, 2006), and could provide another indicator of pollution exposure if it could be measured accurately. Although conventional digital cameras can be fitted with UV sensors (e.g. lifepixel.com), their use in the field has been restricted so far to tame and accessible species (e.g. Meyer-Rochow *et al*., 2008). Even the melanic and carotenoid-based traits we measure more confidently could actually look quite different to other species (reviewed by Cuthill, 2006).

2.9 Recommendations for future research

Despite the limitations we acknowledge above, we see enormous potential for using ornamental traits that resulted from sexual selection to signal present-day exposure to anthropogenic pollutants. We suggest that in order to achieve this potential we will require: a) the study and establishment of baselines of trait colour in the populations of interest, b) conducting broad-scale colour monitoring along different gradients of exposure to pollution, and c) an expansion in the existing techniques used to measure colouration, which are not much less invasive than the physiological metrics they might replace. Typically, quantifying colour still involves the capture and extensive handling of individuals and those requirements potentially exclude many of the species that are actually most threatened by anthropogenic pollutants.

The first recommendation, establishing colour baselines, is necessary because of the enormous variability in the responses of ornamental traits to the wide variety of anthropogenic pollutants. Until more is known about how trait responses can be generalized among traits, species, pollutants, locations, etc., baseline metrics of trait colouration should be limited to small temporal and spatial scales with comparable environments. For example, bird feathers might be measured annually for a set of species in relation to standardized moult or breeding schedules and within a designated area. Once appropriate baselines are established, more robust comparisons could occur over space, time, and anthropogenic conditions. Establishment of such baseline levels could create opportunities to assess predicted effects in such changes as land use practices, water treatment standards, industrial development, and even the cumulative effects of adjacent human populations.

A second recommendation that could complement the development of the aforementioned baselines, is a broad-scale monitoring of ornamental colour. For this, the colour of ornamental traits of many species could be measured along known gradients of exposure to pollution to get a general picture of sensitivity of such traits. This could be done repeatedly along several similar gradients to generalize the conclusions and along gradients of different sources of pollution. In addition to this, researchers or managers already monitoring populations and capturing individuals for other purposes, could take colour measurements and/or photos to expand our knowledge on colour variation in wild populations.

Because the difficulty and invasiveness of capturing and taking samples from wild animals is a core motivator for our review paper, we see a primary research need as the development of remote sensing tools for measuring the colouration of ornamental traits. By remote sensing, we mean the use of cameras that can collect multi-spectral imagery while operating autonomously, similarly to the way wildlife cameras are increasingly used for animal detection and surveillance (Turner *et al.*, 2003; Swann *et al.*, 2004; Bolton *et al.*, 2007) and to the way vegetation type and phenological stage have long been assessed via multi-spectral sensors contained in satellites or airplanes. Already it is possible to combine these techniques via multi-spectral sensors in unmanned vehicles that can operate in each of air, land, and water as media (reviewed by Linchant *et al.*, 2015). The revolutionary potential of these vehicles has already been appreciated in many other conservation domains and more applications are revealed steadily (reviewed by conservationdrones.org). So far, the use of these tools for monitoring wildlife populations has been restricted mostly to detecting the presence of individuals or specific behaviours (e.g. Claridge *et al*., 2004; Grenzdörffer, 2013; Swann *et al*., 2004), but they are already being used to monitor the health of plants through changes in colouration that include the infrared portion of the colour spectrum (Fletcher et al., 2001; Newete *et al*., 2014).

Even if drones can be brought rapidly to the service of quantifying ornament colour for conservationist purposes, a related challenge is to create methods for calibrating photos taken outdoors under varying conditions. Just as it is important to standardize assessments of coloured ornaments based on human observation (Montgomerie, 2006) and digital photography (Stevens *et al*., 2007), it will be important to develop methods to standardize images collected remotely. Although the same principles apply, much greater variation in light intensity and colour balance will apply to remote imagery collected outdoors. Variation in distance to subjects, resolution, and orientation will make it harder to quantify relatively simple traits, like ornament size. Overcoming the challenge of standardization will not be a trivial task and it is likely best approached for this purpose within multidisciplinary teams that could include engineers, computing scientists, and others who have solved similar problems for other purposes.

Equally important to standardizing the appearance of sexually-selected ornaments by remotely-collected photos will be to continue the excellent pioneering work others have done to relate ornament colouration to physiological condition (e.g. Hamilton and Zuk, 1982; Kodric-Brown, 1985) and then to relate both metrics to environmental pollutants (e.g. Hill *et al*., 2002; Table 2.1). Although we focused our review on birds and fish because they have received all the attention to date, other taxonomic groups may offer advantages for the extension of these metrics via remote sensing. For example, amphibians are well-known to be highly sensitive to environmental pollutants (Gibbons *et al*., 2000). Both amphibians and reptiles are typically terrestrial, which may make study via hand-held sensors in the wild easier than it would be for either of birds or fish. The subjects of this work are not restricted to vertebrates; any taxonomic group that exhibits colourful integument, such as insects and crustaceans, is potentially relevant to advancing a general understanding of the way colouration could signal the detrimental presence or effects of anthropogenic pollution. With such general knowledge, conservation practitioners might be able to both detect and mitigate pollution effects long before they cause local population declines or extirpation.

A final target of future research is to identify generalizations from studies of sexually-selected indicators and use them to predict which species, populations and individuals are most likely to adapt to vs. deteriorate from changing environments. One example of this proactive approach would be to target the closely-related, sympatric species that are more likely to use colourful ornamental traits for premating reproductive barriers (Ritchie, 2007; Price, 2008). Without those barriers, hybridization and genetic introgression could cause reductions in offspring survival (Pryke and Griffith, 2009) or the loss of local adaptations (Bourret *et al.*, 2011), ultimately contributing to extinctions (Rhymer and Simberloff, 1996). A well-known example of this sequence occurred with cichlid fishes in Lake Victoria. There, intensified deforestation and agricultural practices increased water turbidity and reduced colour perception by fish, which increased rates of hybridization to cause cascading losses of fish diversity (Seehausen *et al*., 1997).

Another proactive research target would be to determine the extent and implications of increased expression of melanic traits in individuals that have been exposed to pollution. The ability of melanin to bind with metal ions means that animals with melanic ornaments can eliminate excess metals – which are essential in small quantities but toxic in higher concentrations – by sequestering them in hair of feathers (McGraw, 2003). Recent work suggests that this mechanism can cause directional selection for more melanic phenotypes in polluted environments (Chatelain *et al.*, 2014), but the reverse may also occur (Senar *et al.*, 2014). If melanin-based sequestration of toxicants is widespread in animals, it could have big implications for changing patterns of biodiversity, especially in urban areas. For example, McKinney's (2002) categorization of bird species as urban avoiders, adapters, and exploiters was originally based on correlations between life-history traits and abundance, but a systematic review may reveal the same categories correlate with the prevalence of melanic traits. The intriguing relationships among metal pollution and melanins have been most explored in birds, but they deserve investigation in mammals, especially in invasive urban-adapting species that exhibit variation in both black and reddish colouration caused by melanins (e.g. coyotes, *Canis latrans*; Anderson *et al.*, 2009). Clearly, much more research is needed to address how melanins interact with a variety of anthropogenic effects.

2.10 Conclusions

Exponential growth in human populations and associated habitat degradation increasingly threaten the retention of biodiversity on Earth. Stemming this loss will require efficient conservation action that identifies the mechanisms of population decline, acts proactively to reduce the cost and increase the efficacy of mitigation, and operates holistically to generalize efforts across ecological units and scales. When these three things can be achieved, the results are heartening. For example, the identification of volatile CFC's in aerosol sprays as the cause of ozone depletion resulted in rapid regulatory change, widespread compliance, the recovery of an essential ecosystem service, and benefits to hundreds of species (Noakes, 1995).

Unfortunately, that critical first step – identifying the mechanism of population decline – is surprisingly difficult, especially when it involves anthropogenic pollutants with nebulous components, sources, and effects. Millions of chemicals are produced annually and released into the environment (Postel, 1987), but only a small fraction of those are tested for their environmental impacts (Tolba, 1992). There is an enormous need to develop and apply more methods to monitor the health of wildlife exposed to new and existing anthropogenic pollutants, but current methods are invasive, sometimes lethal (e.g. Farombi *et al.*, 2007), often limited to blood-based or reproductive parameters (e.g. Fernie *et al.*, 2001), or are reliant on opportunistic observations of mortality (e.g. Piatt *et al.*, 1990). Additionally, none of these methods is suitable for estimating effects of pollutants in wild, but sensitive populations that inhabit locations that are remote, vulnerable to disturbance, difficult to access, or endangered. Alas, those are precisely the species, populations, and locations where such assessments are most needed to support proactive and effective conservation action.

In this review we champion an idea presented by Hill (1995) over 20 years ago, that the ornamental traits of many organisms could be used to fill this void, revealing the quality and condition of environments that are also occupied by many other species. Since then, many researchers have explored species- and trait-specific effects of particular pollutants to accumulate a substantial literature. We synthesized and reviewed that literature in relation to two types of pigments that are prevalent in ornaments in vertebrate classes; melanins and carotenoids. We also assessed their known interactions with several environmental pollutants. Despite the number of relevant studies, most of this literature describes laboratory or case studies and is usually specific to taxonomic groups or particular toxicological effects. These characteristics impede the emergence of generalizable mechanisms that could reveal wider conservation problems and suggest appropriate mitigations. Consequently, the potential for ornamental traits to comprise a versatile tool for conservation biology remains unmet. We see seminal roles for development of this theory using pollution as an anthropogenic effect, ornamented vertebrates as study subjects, and pigments as mechanistic intermediaries. These targets are illustrative because pollution often interferes with the biochemical creation and physiological maintenance of ornamental traits, which are prevalently expressed with pigments. Moreover, pigments are costly to synthesize or acquire, are fairly well-understood in the contexts of biochemistry, physiology, toxicology, and sexual selection, and should be readily visible to both human observers and their automated devices. With time, we expect that ornamental traits will become familiar tools for diagnosing and addressing a wide variety of anthropogenic effects with tractable applications for hundreds of species and ecosystems worldwide.

We attempted to advance Hill's (1995) insight by reviewing that literature and using it to pose ideas for future research that could help generalize the use of this method, while acknowledging some important limitations. We hope this review encourages more researchers and wildlife managers to include measures of ornamental colour whenever possible and, especially, when it can easily be added to existing monitoring or research protocols. We see enormous potential for these traits to provide a much-needed, non-invasive tool for detecting subtle and early effects of pollution long before they can be seen as more catastrophic effects at

the level of populations. Species that exhibit carotenoid-based ornaments appear to offer particular promise as highly responsive indicators that might reveal pollution exposure that is not easily detected, but actually occurs, in other species and even whole communities.
Despite the high potential we see in the utility of ornamental traits as environmental indicators, we also emphasize the importance of unraveling the specific physiological mechanisms of pollutants on colouration before generalizing their interpretations, particularly when anthropogenic stressors cause opposing or multiplicative effects. A striking contradiction is evident in the enhancing effects of both pesticides and heavy metals on eumelanic (dark) traits, with simultaneous negative effects on pheomelanic (lighter) and carotenoid-based traits that are undoubtedly detrimental or organisms.

More systematic work on the specific sources, mechanisms, and mitigations of pollutant effects on sexually-selected ornaments could provide conservation biologists with a suite of context-specific canaries for the diverse coal mines of anthropogenic effects. At the same time, such work may provide unanticipated insights relevant to more basic questions in physiology, ecology, and evolution.

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location and on-site security and safety restrictions for data collection by people. Funding for that project resulted from a creative sentence in the ruling of *R. v. Syncrude Canada Ltd. 2010 ABPC 229*.

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2.14 Tables

Table 2.1: Summary of the effects of chemical pollutants on coloured ornamental traits of fish and birds.

Product, use and concentration	Suggested pathway	Pigment	Affected trait and direction of effect	Age class, sex and latin name	Reference
Pharmaceuticals and active ingredients in personal care products (PPCPs)					
E2 / natural estrogen / AN	ED	Car	Area and colour of body orange spots (-)	Adult male guppies (*Poecilia reticulata*)	Toft and Baatrup (2001)
EE2 / artificial estrogen / AN	ED	Car	Area of body orange spots (-)	Adult male guppies (*Poecilia reticulata*)	Kristensen *et al*., (2005)
EE2 / artificial estrogen / WN	ED	Car	Reddish body colouration (-)	Adult male zebrafish (*Danio rerio*)	Larsen *et al*., (2008)
E2 / natural estrogen / WN	ED	Car	Colouration of pectoral and caudal fins (-)	Adult male red shiners (*Cyprinella lutrensis*)	McGree *et al.,* (2010)
Pesticides					
Vinclozolin / fungicide and p,p´-DDE / principal metabolite of the insecticide DDT	ED	Car	Area and colour of body orange spots (-)	Adult male guppies (*Poecilia reticulata*)	Baatrup and Junge (2001)
Methyl parathion / insecticide / WN	Damage to embryo's physiology	Car	Colour of yellow fins and body (-)	Adult male amarillo fish (*Girardinichthys multiradiatus*)	Arellano-Aguilar and Macías Garcia (2008)

Atrazine / herbicide / WN	ED	Car	Area of orange spots (-)	Adult male guppies (*Poecilia reticulata*)	Shenoy (2012)
Diquat / contact herbicide	OS	Mel	Area of black (+) and brown (-) plumage patches	Adult male red- legged partridges (*Alectoris rufa*)	Galván and Alonso-Alvarez (2009)
Diquat / contact herbicide	OS	Car	Colour of red beak and eye rings (-)	Adult male red- legged partridges (*Alectoris rufa*)	Alonso-Alvarez and Galván (2011)
Thiram and difenoconazole / fungicides and imidacloprid / insecticide / WN	OS	Car	Area of red eye ring (-)	Adult male red- legged partridges (*Alectoris rufa*)	Lopez-Antia *et al*., (2013)
Mix of pesticides and PCBs	OS (potentially)	Car	Colour of orange-red eye-ring, gapes and tongue (-)	Adult female black-legged kittiwakes (*Rissa tridactyla*)	Blévin *et al*., (2014)
Industry-related compounds					
Octylphenol / AN	ED	Car	Colour and size of orange spots (-)	Adult male guppies (*Poecilia reticulata*)	Toft and Baatrup (2001)
BPA	ED	Car	Body colour intensity (-)	Adult male red shiners (*Cyprinella lutrensis*)	Ward and Blum (2012)
PCBs (mix)	ED	Mel	Onset of plumage maturation (+)	Subadult female tree swallows (*Tachycineta bicolour*)	McCarty and Secord (2000)

Aroclor (PCB) / WN	ED	Car	Colour of yellow facial skin (-)	Adult male American kestrels (*Falco sparverius*)	Bortolotti *et al*., (2003)
PAHs (mix) / WN	OS	Car	Size of red bill spot (-)	Adult male and female yellow-legged gulls (*Larus michahellis*)	Pérez *et al*., (2010)
Metals					
Lead, cadmium, zinc and copper	OS / carotenoid sources	Mel	Area of black breast stripes (+) and colour of yellow breast feathers (-)	Young and adult male and female great tits (*Parus major*)	Dauwe and Eens (2008)
Mercury	OS, health and/or pigment production	Mel	Brightness of blue chest feathers (+)	Adult male and female belted kingfishers (*Megaceryle alcyon*)	White and Cristol (2014)
Sulphuric oxides, copper, zinc, nickel and lead	OS / carotenoid sources	Car	Intensity of yellow breast feathers (-)	Nestlings of great tit (*Parus major*)	Eeva *et al*., (1998)
Cadmium, lead, arsenic, copper and zinc	OS / carotenoid sources	Car	Colour of yellow breast feathers (-)	Young and adult male and female great tits (*Parus major*)	Geens *et al*., (2009)

For experimental studies, the concentration is indicated when available; within natural levels (WN) or above natural levels (AN). The pollutants included act via two main suggested physiological effects; oxidative stress (OS) and endocrine disruption (ED); other possible causes are indicated. Effects of pollutants on ornamental traits are further categorized by type of pigment; melanins (Mel) and carotenoids (Car), and their expression in coloured, integumentary traits of fish and birds. Each example also names the product and use, the affected trait, age class, sex, and scientific name of the studied species, and the associated reference.

2.15 Figures

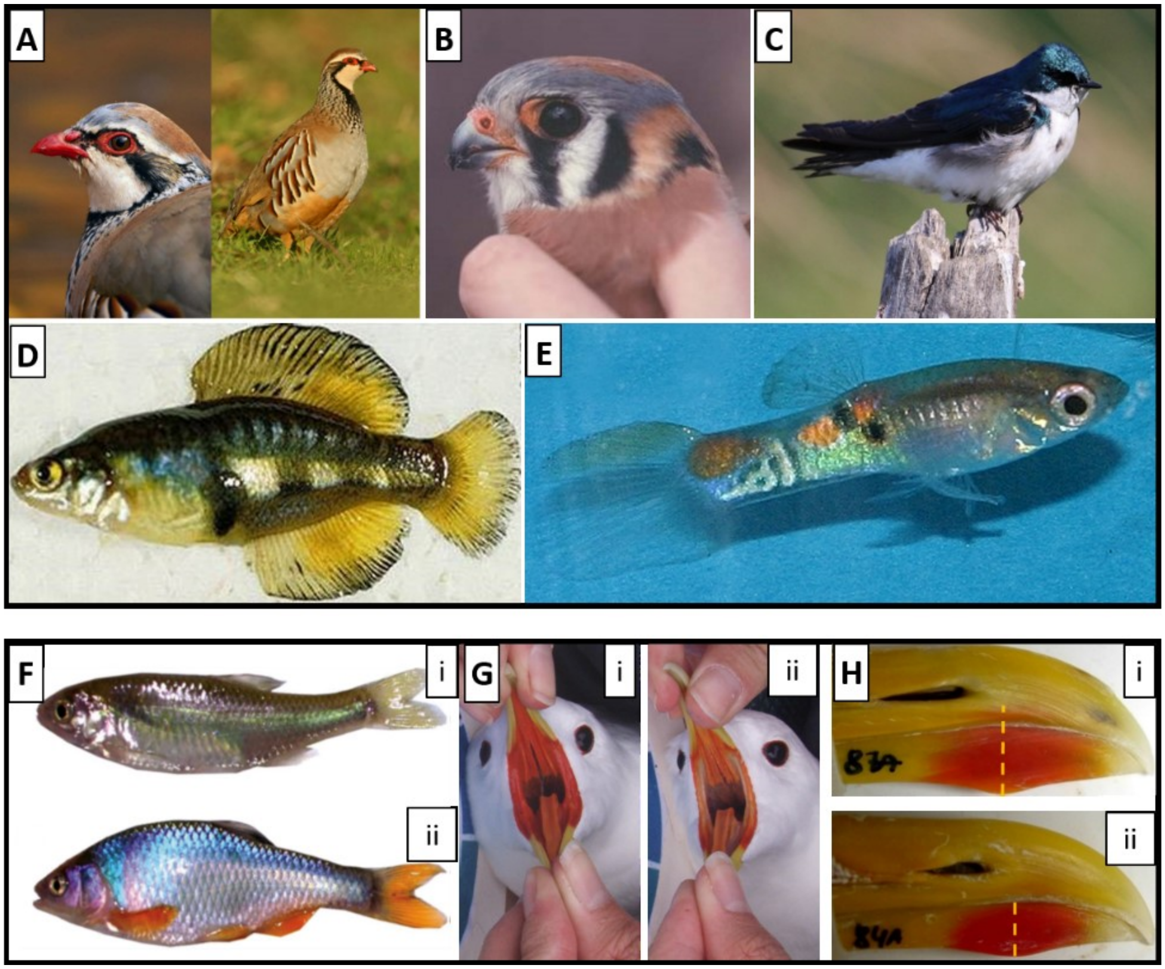

Figure 2.1: Upper panel; examples of coloured ornamental traits that show evidence of pollution. (**A**) Red colouration of the facial skin, brown and black flank feather bands and black feather bib of red-legged partridges (Alonso-Alvarez and Galván, 2011; Galván and Alonso-Alvarez, 2009) Photography from Alonso-Alvarez and Galván, 2011. (**B**) Yellow colouration of the facial skin of American kestrels (Bortolotti *et al*., 2003). Photographs by Gary Bortolotti and Russell D. Dawson, courtesy of Kim Fernie. (**C**) Iridescent blue-green plumage of tree swallows (McCarty and Secord, 2000). Photograph by Natalia Lifshitz. (**D**) Yellow colouration of fins and tale of amarillo fish (Arellano-Aguilar and Macías Garcia, 2008). Photograph by Shane Webb, courtesy of Constantino Macías García. (**E**) Body yellow-orange spots of guppies (Kristensen *et al*., 2005; Shenoy, 2012; Toft and Baatrup, 2001). Photograph by Erik Baatrup. **Lower panel**; Examples of changes in ornamental colouration of fish and birds exposed to anthropogenic pollutants. (**F**) Ornamental body colouration of adult male red shiner (*Cyprinella lutrensis*) i)

exposed to the estrogen 17β-estradiol and ii) control water. (McGree *et al*., 2010). (**G**) Gape and tongue colouration of female black-legged kittiwakes (*Rissa tridactyla*) with i) low vs ii) high concentrations of pesticides and PCBs in blood (Blevin *et al*., 2014). Photographs by Olivier Chastel. (**H**) Red bill spot (controlled by bill size) of yellow legged male gulls (*Larus michahellis*) i) experimentally fed with oil from the *Prestige* oil spill and ii) control sunflower oil (Pérez *et al*., 2010). Photographs by Cristobal Pérez.

Chapter 3: Iridescent colouration of tree swallows relates to environmental metal pollution.

3.1 Abstract

Ornamental colouration in birds has been identified as a powerful, non-invasive tool for identifying exposure to metal pollution. Despite this potential, few studies have examined the effects of metals on iridescent colouration or assessed related impacts on bird fitness. Iridescent colouration is likely to be sensitive to metal pollution because it is already known to affect melanin production and this form of colouration is produced when light is refracted through layers of keratin and melanin inside feather barbules. In this study, we measured variation in plumage colouration, health (via oxidative stress) and reproductive success (via number of young fledged) of tree swallows (Tachycineta bicolour) nesting adjacent to water bodies containing different levels of metal pollution. Plumage hue shifted from bluer to greener and feather brightness increased, with increasing exposure to copper and zinc. Both patterns would be expected from changes in the microstructure of the feathers. Unexpectedly, increasing exposure to these metals correlated with increased apparent health (lower oxidative stress) in female swallows, but not males. Number of young fledged decreased slightly with exposure to metals but did not vary with the colour of parents. Our results suggest the relationships between ornamental colour, including iridescence, and fitness metrics are complex and much more work will be needed before colour of iridescent feathers can provide a proactive, non-invasive and effective diagnostic tool for detecting subtle effects of pollution on birds.

3.2 Introduction

Elemental metals are naturally found in the environment (Bradl 2005) and some are a critical part in the diet of vertebrates (Bogden and Klevay 2000, Valko et al. 2005). However, environmental concentrations of many metals have increased due to anthropogenic activities (Azimi et al. 2005, Roux and Marra 2007), reaching levels that become harmful instead of helpful for exposed individuals (Ercal et al. 2001). Normally, industrial areas are most affected by this kind of pollution (Hsu et al. 2006), but metal levels are high in urban areas as well, raising concerns for the health of both humans and wildlife that reside there (Roux and Marra

2007, Wei and Yang 2010). It has become common to estimate potential detrimental effects of many environmental contaminants by studying their effects on sentinel wildlife species (Rabinowitz et al. 2005), including several species of wild birds (e.g. Burger and Gochfeld 2004, Smits and Fernie 2013).

Known negative effects of metal pollution on birds include depressed immune function (Snoeijs et al. 2004), increased oxidative stress (Koivula and Eeva 2010), and impaired reproduction (Brasso and Cristol 2008). Determining these effects has usually required invasive methods, such as blood sampling to measure health metrics (e.g., Koivula et al. 2011), injection of immune-stimulating agents to assess individual responsiveness (e.g., Cruz-Martínez et al. 2015), and sacrifice of eggs and/or individuals to sample tissues for presence of metals (e.g. Hoffman and Heinz 1998; Snoeijs et al. 2004). Such invasive techniques are often not justifiable on ethical grounds unless pollution levels are already believed to be significant, which may not occur until environmental degradation is difficult to reverse and population declines have already begun (Vasseur and Cossu-Leguille 2006). A familiar example of this sequence is provided by the historic egg-shell thinning of raptorial birds, which was suspected (Carson 1962) decades before it was confirmed and corrected (Freedman 1989).

Ornamental colouration of birds provides a reliable and non-invasive alternative to these invasive approaches, because it reveals physical condition, in turn indicating early effects of environmental pollution (reviewed in Lifshitz and St. Clair 2016). Because production and maintenance of coloured ornaments is costly and directly linked to individual condition, these traits are extremely sensitive to environmental quality relative to traits maintained by natural selection (Moller and Pomiankowski 1993, Hill 1995), and reveal the sum of all environmental pressures. This link means that a localized reduction in ornament colouration could signal the first subtle decline in environmental quality, occurring when there is still time to identify and reverse the source of degradation (Hill 1995). Several studies have already explored the link between bird colouration and metal pollution by focusing on carotenoid- and melanin-based colouration (e.g. Eeva et al. 1998, Dauwe and Eens 2008, Geens et al. 2009, Giraudeau et al. 2015), but the effects of metals on structural colouration, especially iridescence, remain largely unexplored (McCarty and Seccord 2000, White and Cristol 2014, Chatelain et al. 2017).

One possible reason for the relative lack of attention linking metal pollution to iridescent colouration is the complexity of the underlying physical and chemical mechanisms leading to its production. Iridescent colouration is produced by the refraction of light through the specifically-arranged nanostructure of the feather, which consists of layers of keratin and melanin (Doucet et al. 2006). Each of these layers is responsible for different characteristics of the colour produced; hue (or colour) is determined by the thickness of the keratin cortex of barbules, such that birds with thicker barbule cortexes have plumage that reflects light maximally at longer wavelengths (Doucet et al. 2006). Colour saturation and UV reflectance are determined by the concentration and type of melanin granules deposited under the keratin cortex, such that birds with greater densities of melanin in their barbules have plumage that looks more saturated and reflects proportionally more in UV wavelengths (Doucet et al, 2006; Maia et al. 2009). Environmental metals could potentially affect the production of iridescent colours through several, non-exclusive mechanisms. First, some metals can directly influence the biochemical pathway of melanin production, either by promoting it (Prota 1993, Jawor and Breitwisch 2003) or preventing it (Lerner 1952), and can also alter production of the keratin-based structure of the feather (Crewther et al. 1965). Second, metals have endocrine-disruptive properties (Iavicoli et al. 2009), which could cause changes to the production of sexual hormones, also known to regulate melanin production (Bokony et al. 2008). Additionally, metals could alter feathers indirectly by degrading the condition of individuals which could, in turn, increase the production of corticosterone, a stress hormone known to prevent melanogenesis (Slominski 2004, Roulin et al. 2008), deplete the resources individuals can allocate to production of the keratin-based feather structure (DesRochers et al. 2009), or even by reducing the time birds spend preening and caring for their feathers (Chatelain et al. 2016).

Here, we explored the effects of metal pollution across an urban gradient on iridescent plumage colouration of Tree Swallows (*Tachycineta bicolour*). Tree Swallows are assumed to reveal nearby water pollution because they regularly forage within a few hundred meters of their nest sites (Mengelkoch et al. 2004) on emergent insects from which many pollutants, including metals, bioaccumulate (Bishop et al. 1995, McCarty 2001, Custer 2011). They readily nest in boxes that can be installed adjacent to sites of interest, exhibit high site fidelity (Winkler et al. 2004), have low rates of breeding dispersal (i.e. movements between successive breeding sites; Shutler and Clark 2003), and produce altricial young that they feed for at least 20 days. These features make Tree Swallows particularly effective as bioindicators of pollution (McCarty 2001, Custer 2011, Smits and Fernie 2013), but past studies have emphasized metrics from swallow blood (e.g., Gentes et al. 2007a), eggs (e.g., Custer et al. 2005) or tissue (e.g., Custer et al. 2006). Almost nothing is known about the effects of pollutants on colouration of Tree Swallows, but pollution from polychlorinated biphenyls (PCBs) caused earlier acquisition of adult plumage in females (McCarty and Seccord 2000), suggesting a disruption in the endocrine system that affected colour production.

We expected iridescent blue plumage of Tree Swallows to be particularly revealing of environmental stressors during breeding for three reasons. First, structural colours may be costly to produce; iridescent plumage of Cowbirds (*Molothrus ater*) was affected by nutritional stress during feather production (McGraw et al 2002). Second, the blue colouration of Tree Swallows is an ornamental trait under mutual sexual selection in males and females, which increases the likelihood of being an honest indicator of condition (sensu Hamilton and Zuk 1982). Both sexes appear to prefer brighter partners (Bitton et al. 2008) and bluer plumage correlates with higher reproductive success of females (Bitton et al. 2007, 2008, Bentz and Siefferman 2013). Third, environmental effects on colouration of Tree Swallows likely occur via conditions on natal and breeding territories. Tree Swallows begin molting new feathers on their breeding range following each breeding season (Stutchbury and Rohwer 1990). Adding this to the fact that they show high rates of nest-site fidelity (Shutler and Clark 2003), adult plumage in a given year has been assumed to reveal and relate to pollution levels of the same breeding sites in the previous year (McCarty and Seccord 2000). Additionally, breeding season stressors are known to have carry-over effects that extend through migration (Catry et al. 2013, Legagneux et al. 2013, Schultner et al. 2014) potentially to completion of molt, although stressors, including pollution, may also occur during migration to affect feather colour.

The objective of this study was to determine if higher concentrations of environmental metals correlated with variation in colouration of Tree Swallow feathers, thereby demonstrating the potential for this ornamental trait of Tree Swallows, already a recognized bioindicator species (Custer 2011), to be a non-invasive indicator of metal pollution. To verify the expected negative effects of pollution on bird health that could be reflected in colouration, we had two subsidiary objectives to determine whether increased metal concentrations reduced the health of breeding adults, as measured via oxidative stress, and reduced reproductive success, as measured by the number of young fledged. Oxidative stress refers to the ratio in blood between oxidized and reduced glutathione (GSSG:GSH), the latter serving as the main intracellular antioxidant, such that higher ratio values signal poorer health (Isaksson et al. 2005). In addition to assessing the presumed causal effects of metal pollution on each of these three types of variables, we also explored the relationship between colour of parents and number of young fledged, as a proxy of reproductive success.

3.3 Methods

3.3.1 Study area

The study was conducted in the summer of 2016. Based on existing information about heavy metal concentrations in water (A. Liu personal communication, St. Albert 2012) and on surrounding habitat and land use type, we chose 4 sites along apparent gradients of built density and distance to downtown Edmonton, Alberta. Two urban sites occurred in constructed wetlands designed to collect and clean storm water in the city of Edmonton; Roper Pond (53.4979°N, 113.4375°W; 6.3 km to downtown Edmonton) and Fulton Marsh (53.4813°N, 113.3594°W; 11.5 km to downtown Edmonton). One suburban site was a natural wetland, Big Lake, in Lois Hole Centennial Provincial Park on the outskirts of the city of St. Albert (53.6136°N, 113.6582°W; 13.3 km from downtown Edmonton). The fourth site was located at the Beaverhill Lake, a provincially-protected area (53.3824°N, 112.5277°W; 67 km from downtown Edmonton) that is the site of a long-term monitoring program for Tree Swallows (Beaverhill Bird Observatory; http://beaverhillbirds.com/). Each site contained 22-50 boxes that were built following the Golondrinas design from Cornell University (http://golondrinas.cornell.edu), erected on wooden poles, and protected from ground predators using metal sheeting. Boxes were positioned in transects around the ponds in Roper Pond and Fulton Marsh and in a grid in Big Lake and Beaverhill Lake (which was erected years previously by the staff of the Beaverhill Bird Observatory). Boxes were always positioned approximately 15-20 m apart. Boxes at Beaverhill Lake were installed prior to 2001 and some boxes at Roper Pond and Fulton Marsh were established between 2006 and 2016. We added 15 boxes at Roper Pond in 2015, 20 boxes at Fulton Marsh in 2016 and all the boxes at Big Lake in early spring 2016, augmenting an existing grid in the nearby area (installation time unknown) that was recently removed, and based on prior observations of nesting swallows in the area. As a coarse measure of water contaminants, we collected 4 water subsamples from each site at the end of the 2016 breeding season and pooled them by site (~60 ml total per site) that we sent for analyses of metals by a commercial lab (ALS Environmental Laboratory in Edmonton, AB.). Water samples were collected on July 9th at Roper Pond and Fulton Marsh, July 12th at Big Lake and July 16th at Beaverhill Lake.

3.3.2 Bird monitoring and measurements

Starting in May and until the breeding season ended in mid-July, we monitored clutches every 3rd day and determined clutch initiation date by the presence of a fresh egg. Tree Swallows lay one egg per day (Robertson et al. 1992) early in the morning so we performed nest checks after 10 AM, which made it possible to assign a clutch of 1 to that day, a clutch of 2 to the previous day, and a clutch of 3 to the day before that. The initiation date of the first clutch in the entire study area was denoted as Study Day 1 in analyses (16 May). We recorded maximum clutch size and number of fledglings that left the nest. At day 12 ± 1 day post-hatch we captured breeding pairs using nest-box traps (G. Holroyd, personal communication). Both adults were banded with metal government-issued bands, weighed with a digital balance (± 0.1 g), and measured with a metallic wing ruler (head-bill, wing, and tail length; ± 0.1 mm). We collected a sample of 5 mantle feathers and stored them in opaque paper envelopes for subsequent colour analyses. We drew a blood sample of 150 µl via venipuncture of the cutaneous ulnar vein. Blood was immediately placed in liquid nitrogen until permanent storage at -80C. Using the delayed plumage maturation of females characteristic of Tree Swallows (Hussell 1983), we classified adult females as second-year (SY) or after second-year (ASY). We did not assign males to age classes because they cannot be aged by plumage and only Beaverhill Lake had enough birds that were banded previously.

On a single day, when the oldest nestling was estimated to be 12 days old, we banded and weighed (± 0.1 g) all the chicks in the nest. We did not handle chicks at older ages because disturbing them can cause premature fledging. When handling nestlings for banding, we collected fecal sacs from defecating chicks into glass containers and pooled these by brood owing to the small mass of each individual fecal sac. We stored these samples in ice in the field and sent them to a commercial lab (ALS Environmental Laboratory in Edmonton, AB.) for a finer-scale, brood-specific measure of metal concentrations. We collected data from a total of 34 nest boxes, 63 adults, and 196 chicks. Number of adults does not correspond to number of boxes because in some occasions, we could not trap both adults of a box.

3.3.3 Plumage colouration

To measure plumage colouration quantitatively, we taped feathers onto matte black cardboard and arranged them in an overlapping fashion to approximate their usual configuration on a bird's body. We measured reflectance of these feathers using an S2000 spectrophotometer and deuterium tungsten-halogen light source (Ocean Optics, Dunedin, FL, USA). All measurements were taken with unpolarized light. We took readings using a bifurcated fibre-optic cable mounted in a metal-encased probe that transmitted incident light to the measurement area and reflected light to the spectrometer. The probe was mounted in a metal base that excluded ambient light and maintained the probe at a fixed angle perpendicular to the feather surface. Using OOIBase32 software (Ocean Optics), we took five readings from each feather sample, with each reading comprising an average of 20 spectra measured sequentially. All measurements were expressed as percent reflectance relative to a Spectralon white standard (WS-1; Ocean Optics). We used the function 'peakshape' in the R package pavo (Maia et al. 2013) to summarize our reflectance data by calculating three independent colour variables that approximate three dimensions of colour: hue (peak wavelength or wavelength of maximum reflectance), saturation (full width at half maximum; wavelength bandwidth of the interval of wavelengths at which the reflectance is half that at the peak), and brightness (intensity; maximum relative reflectance or reflectance at wavelength of maximum reflectance; Montgomerie, 2006). We described the spectral profile of iridescent plumage at only one angle (90 degrees to the reading surface), but the general height of the curve for this plumage region would be expected to vary with the angle at which the feather is analyzed. Although we did not measure repeatability of our colour metrics, our procedure was similar to that used in other studies (e.g. Whittingham and Dunn 2016) and no consistent bias or confound is known to result from it. We did not characterize the brown plumage of SY females because it is achromatic (Bentz and Siefferman 2013).

3.3.4 Oxidative stress measurements

To measure total glutathione (tGSH) and the ratio of reduced and oxidized glutathione (GSSG:GSH; hereafter oxidative stress) we used the DetectX Glutathione Fluorescent Detection Kit (Arbor Assays, Ann Arbor, MI, USA) following the manufacturer's protocol (K006-F1, Arbor Assays). The detection method uses ThioStar glutathione detection reagent, which is a fluorescent reagent that binds to GSH. All measurements were done in duplicate using a 96 microplate with FLUOstar OPTIMA plate reader (BMG Labtechnologies) and 2 control samples were used on each plate with a detection limit of 38 nM in the free GSH and 42 nM in the total GSH assays.

3.3.5 Statistical analyses

All statistical analyses were performed in R 3. 4. 3 (R Core Team, 2017). Because of the relatively strong correlations among multiple measures for each of metal levels, we used principal component analyses (PCA) with oblimin rotation to produce synthetic variables for further analyses using the R function princomp. The original variables were first log-transformed to normalize their distributions. We used the PCA to consolidate variation among fecal samples in heavy metal concentrations of aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), lead (Pb), antimony (Sb), selenium (Se), zinc (Zn), and vanadium (V). The PCA resulted in two components; $PC1_{MET}$ that explained 70 % of the variation and had positive loadings for Al (0.78), As (0.83), Cr (0.79), Fe (0.91), Se (0.93), and V (0.76) and $PC2_{MET}$ that explained 10 % of the variation and had positive loadings for Cu (0.94) and Zn (0.88).

We addressed our objectives (above) by constructing linear models. Our dependent variables did not depart significantly from normality for either sex (Shapiro Wilk tests: all $P > 0.05$). Thus, we used models with Gaussian error distributions and the identity link, and we verified that the residuals for fitted models were normally distributed in every case. We evaluated model fit using an information-theoretic approach (Burnham and Anderson 2003) and standardized all variables before analysis to support interpretation of relative effect sizes and interaction estimates (Grueber et al. 2011). We compared all models (Appendix 1) using Akaike's Information Criterion corrected for small sample size (AICc).We considered models with $\Delta AICc<2$ to be equivalent and averaged their coefficients (i.e., multi model inference; Harrison et al. 2018) using the R library MuMIn (Barton, 2017); otherwise we identified the single best-fit model. We report standardized regression coefficients and their confidence intervals to evaluate effect sizes for predictors. Sample sizes varied among the analyses described below because data were not available for every individual and brood. We omitted from these analyses two outliers that appeared to result from measurement error or contamination; one fecal sample from Roper Pond with metal concentrations 6-times higher than the rest and one colour measurement with a blueness value 53.3% above the average.

To determine whether higher concentrations of metals influenced the colouration of males and females, we constructed 3 models; one for each colour metric (hue, saturation, and brightness). For each colour variable, we considered 12 candidate models that included, as explanatory variables, a combination of $PC1_{MET}$, $PC2_{MET}$ sex, and their interaction, and a model with only the intercept (Appendix 1, Table A1.1, A1.2, and A1.3 respectively for hue, saturation, and brightness). Because site was strongly correlated with the concentration of both metal principal components, it was not included in the final models. Sexes were analyzed together because preliminary analyses showed no significant differences between males and females (all $P > 0.05$). The interactions of metals with sex were included to test whether metals affect the colour of males and females differently.

We determined whether heightened concentrations of heavy metals increased oxidative stress, by constructing separate models for males and females after preliminary analyses showed that oxidative stress of males was higher than that of females (two-sample t-test: $t_{47} = -2.43$, $P = 0.02$). For females, we also ran separate models for ASY and SY females because they comprised different proportions of individuals among our four sites. For each sex and age category, we considered 7 candidate models that included, as explanatory variables, a combination of $PC1_{MET}$, $PC2_{MET}$, and laying date, and a model with only the intercept (Appendix 1, Table A1.4).

To measure the effects of metals on the number of chicks that fledged, we used all nests that produced at least 1 chick (n = 34). We considered 20 candidate models that included, as explanatory variables, a combination of $PC1_{MET}$, $PC2_{MET}$, laying date, age of the mother, and the interactions between age and metals. We included laying date because of its known influence on reproductive parameters of birds (Verhulst and Nilsson 2008) and age due to its strong influence on reproductive success in this species (Robertson and Rendell 2001; Appendix 1, Table A1.5). In addition to exploring the hypothesized causal effects of metals on each of colour, oxidative stress, and number of chicks that fledged, we explored the relationship between feather colour of both parents and number of chicks that fledged. To do this, we considered all pairs with an ASY female and evaluated all possible combinations of the 3 colour metrics of each parent, with laying date and site as fixed factors. Means are presented ± SE, unless stated otherwise.

3.4 Results

We collected data from the clutches of 7 to 9 pairs from each of four sites with a proportion of older (ASY) females that ranged from 63-100% (Roper Pond, 7 males, 7 ASY females, and 2 SY females; Fulton Marsh, 8 males, 5 ASY, and 3 SY females; Big Lake, 8 males, no ASY females, and 8 SY females; Beaverhill Lake, 7 males and 7 ASY females). Water samples from our sites did not exhibit the gradient of metal concentrations we expected along an urban gradient. Instead, metal concentrations were highest in the site farthest from downtown (Beaverhill Lake), followed by the stormwater pond closest to downtown (Roper Pond), followed by the suburban site (Big Lake), and the more peripheral stormwater pond, Fulton Marsh (Figure 3.1A and B). A similar pattern of metal concentrations occurred for our finer-scale samples from chick feces, but only for $PC2_{MET}$ (Cu and Zn; Figure 3.2B); $PC1_{MET}$ (Al, As, Cr, Fe, Se, and V) in chick feces increased with proximity to downtown Edmonton (Figure 3.2A).

We found some support for our prediction that higher concentrations of metals, as measured in the fecal samples of chicks, would correlate with differences in feather colouration of adults. Increased concentrations of $PC2_{MET}$ in chick feces, was marginally correlated with increased plumage hue (greener colour; Table 3.1 and Figure 3.3A). Concentrations of metals had no effect on saturation of feathers of adult swallows (Table 3.1). Greater concentrations of $PC2_{MET}$ in chick feces was more strongly correlated with brightness of adult feathers (Table 3.1 and Figure 3.3B).

If metal pollution is detrimental to swallows, we predicted that adult birds whose chicks had higher fecal metal concentrations, would also exhibit higher levels of oxidative stress (Isaksson et al. 2005, Koivula and Eeva 2010). We found no evidence of this relationship in males, and an unexpected positive relationship in ASY females (Table 3.1). For males, the best-fit model showed that oxidative stress declined with advancing laying date but with confidence intervals that overlapped zero (Table 3.1). For ASY females, higher concentrations of $PC2_{MET}$ were negatively correlated with oxidative stress (Table 3.1 and Figure 3.4). For SY females, oxidative stress declined with increasing laying date, although this relationship was weak (Table 3.1).

Our third objective was to determine if increased concentrations of metals reduced the reproductive success of pairs, which we measured as the number of young fledged, for all pairs that produced chicks, and did so separately for pairs with SY and ASY females. We found no effect of concentrations of metals on the number of young that fledged the nest of pairs with SY females (Table 3.1). However, ASY females fledged fewer young when exposed to greater concentrations of $PC1_{MET}$, although the confidence intervals for this coefficient overlapped zero (Table 3.1). We found a weak negative effect on number of young fledged of both male hue and laying date, and a weak positive effect of male brightness, but all confidence intervals overlapped zero (Table 3.1).

3.5 Discussion

In this study, we aimed to assess the impact of environmental metals on plumage colouration, health and reproductive success of Tree Swallows with a goal of determining the extent to which pollution levels might be revealed non-invasively in the feather colour of nesting birds. To do so, we measured metal concentrations via chick feces of swallow pairs breeding in 2 urban sites, one suburban, and one rural site and compared these concentrations to three common metrics of avian feather colour, hue, saturation, and brightness. We compared metal concentrations to oxidative stress, and both metal concentrations, and feather colour to number of young fledged. In support of our predictions, ornamental colour was affected by metal concentrations, but the effects differed for $PC1_{MET}$ (Al, As, Cr, Fe, Se, and V) and $PC2_{MET}$ (Cu and Zn), and among our three colour metrics (below). Contrary to our predictions, we found no evidence of an increase in oxidative stress in males exposed to more metals and the effect of $PC2_{MET}$ on females was positive; opposite to what we predicted. As expected, there was a tendency for pairs exposed to higher concentrations of $PC1_{MET}$ to fledge fewer young, but we found no effect of plumage colouration of males or females on the number of young fledged. These results suggest that the effects of metals on birds are complex and will require both more study and nuanced interpretation.

Our results provided limited support for the hypothesis that environmental metals would alter the feather colour in Tree Swallows (McGraw et al. 2002, Hill et al. 2005, Chatelain et al.

2017), but with opposing effects among our three colour metrics and with only partial congruence with other studies. Whereas adult swallows whose chicks exhibited higher concentrations of $PC2_{MET}$ in feces had higher values of plumage hue (a shift from bluer to greener colour), they also had higher values of plumage brightness. By contrast, feral pigeons (*Columba livia*) that were exposed to lead, exhibited declines in both blue iridescence and brightness but only in the absence of zinc (Chatelain et al. 2017). These contrasting results suggest that relationships among various metals and iridescent colouration are complex and may act on different metrics in opposing ways (Chatelain et al. 2017).

The effects of metals on iridescent colouration may be especially difficult to determine because of the complex nanostructure of the feather barbules, which consists of keratin and melanosomes (Eliason and Shawkey 2012; Xiao et al. 2014). Variation exists in the width of the keratin cortex (Maia et al. 2011) with wider values increasing reflectance in longer wavelengths (Doucet et al. 2006), and the number and condition of barbules (Shawkey et al. 2003, Doucet et al. 2006), including tilt, influencing feather brightness (Van Wijk et al. 2016). Stress during feather growth, including that caused by metals, could affect both of these microstructural characteristics of feathers as well as melanin deposition in growing feathers (Griffith et al. 2006, Maia et al. 2011, Roulin 2016), which helps delimit the width of the keratin cortex (Maia et al. 2011). Once formed, feather colour could be further affected by bacteria (Shawkey et al. 2007), which might be particularly important for iridescent feathers infected with keratynolitic bacteria that affects brightness in pigeons (Leclaire et al. 2014). Pollution is known to affect susceptibility to parasites in Tree Swallows (Gentes et al. 2007b), which could extend to bacteria that alters the feather structure responsible for iridescence. Thus, while iridescent feathers may be revealing of environmental metals (Chatelain et al. 2017), much work will be required to unravel the particular mechanisms and effects.

Additionally, the physiological costs, stress, and metal accumulation derived from reproducing in polluted sites could cause carry-over effects that impact individuals long after the breeding season (Catry et al 2013, Legagneux et al 2013, Schultner et al 2014), including the completion of molt, which happens during migration (Knight et al 2018). Evidence of these type of carry-over effects in birds include male Bluebirds (*Sialia sialis*) that invested more in parental care by raising experimentally enlarged broods and subsequently expressed plumage colour that was duller than in the year of the manipulation (Siefferman and Hill 2005). Also, in breeding

Tree Swallows, reproductive effort is known to reduce long-term immune function (Ardia et al. 2003). Therefore, because the growth of feathers is a costly process (Lindström et al. 1993, Romero et al. 2005), any physiological wear of breeding in a polluted environment may decrease the ability of birds to produce good-quality feathers during molt (DesRochers et al. 2009, Lattin et al. 2011; Maia et al. 2011). However, there is still an unexplored potential for metals acquired elsewhere to affect plumage colour, either during completion of molt, or if some of the birds disperse between seasons. The limitation imposed by potential breeding-site dispersion is lessened by the inclusion of birds at Beaverhill Lake, most of which were recaptures from previous banding seasons at the Beaverhill Bird Observatory.

High variation in metal sources at our study sites and the complex ways environmental stressors, including metals, may have influenced feather colour may have obscured the positive relationship we expected between metal concentrations and oxidative stress. We were surprised that oxidative stress was unaffected by metals for males, and negatively associated for older females, because positive correlations have been reported for other bird species (Koivula and Eeva 2010, Martinez-Haro et al. 2011). One explanation for this discrepancy with prediction is that $PC2_{MET}$ concentrations were low enough in our study area that greater concentrations actually boosted immune function for ASY females. This possibility is supported by other studies of nestling passerines in which low metal concentrations did not cause oxidative stress (Koivula et al. 2011, Rainio et al. 2013). Our measures of oxidative stress (via the ratio of oxidized to free glutathione as 0.3 – 2.8) were much lower than a study that showed a positive correlation between urban pollution and oxidative stress (i.e. the ratio of oxidized to free glutathione was 2 – 4; Isaksson et al. 2005).

In contrast to oxidative stress, the negative effect of metals we predicted was apparent in a slight decline in the number of young fledged, our measure of reproductive success, with increasing concentrations of $PC1_{MET}$. We could not readily relate this effect of $PC1_{MET}$ on reproductive success via the proxy of feather colour we had hoped to reveal; colour was affected only by $PC2_{MET}$ (above). Nonetheless, reproductive success was not unrelated to the colour of male swallows, showing a weak positive effect of brightness and a weak negative effect of hue, but with sources we cannot attribute to variation in the metals we measured. Meanwhile, the negative effect on reproductive success we found of $PC1_{MET}$, which includes several metals of environmental concern (Environmental quality guidelines for Alberta surface waters, Government of Alberta 2018), is consistent with their greater concentrations in urban areas (Lee et al. 1994, Davis et al. 2001, Wei and Yang 2010), and expected toxicological effects (e.g. Eeva et al. 2009).

The broader interpretations of our results are limited by several factors including unanticipated sources of metal pollution and the constraints of a correlative study. When we predicted metal pollution to increase with proximity to an urban centre (Davis et al. 2001), partly owing to traffic volume (Wei and Yang 2010), we did not appreciate how both our suburban and rural sites were embedded in agricultural areas, which are also well-known sources of metals, including copper (Carnelo et al. 1997, Wightwick et al. 2008). We took and pooled water samples from our sites on a single day, but repeated samples might have increased our ability to associate metal concentrations to bird colouration, fecal measurements, adult health, and reproductive success. Such limitations are exacerbated for correlational studies like ours because confounding sources of variation are often unexplored. For example, we lacked information on the quality and history of birds that nested at our four sites. One example of these effects is bird age, which influences each of bird colouration (Bitton and Dawson 2008), oxidative stress (Alonso-Alvarez et al. 2009), and reproductive success (Robertson and Rendell 2001), but we had only a coarse measure of age (SY and ASY) and only for females. On the other hand, experimental studies that manipulate only one variable often contradict the results of studies in the wild (Caudill et al. 2015, Ruuskanen et al.2015) and field-relevant work will be needed to understand how anthropogenic pollutants affect the colouration of ornamental features in wildlife (Lifshitz and St. Clair 2016). There may be particular promise in greater exploration of the nanostructure of iridescent feathers for which metal pollution may exert numerous effects.

In summary, we showed that increased concentrations of copper and zinc, metals that were abundant in feces closer to downtown and in our rural site, slightly correlated with increased feather greenness and substantially correlated with increased brightness of adult Tree Swallows. Despite evidence that these metals affected swallow colour, we found no support for our prediction that concentrations of metals would increase oxidative stress of adults. Although we found a slight negative relationship between metals and reproductive success, the effects of metals on colour did not predict the effects of colour on reproductive success. Despite its apparent complexity, we encourage more exploration of feather colouration as an early, non-invasive signal of subsequent detrimental effects of anthropogenic pollutants on bird populations

(Hill 1995, Lifshitz and St. Clair 2016). In Tree Swallows, brightness may be especially revealing; brighter males are more likely to achieve extra-pair paternity (Bitton et al. 2007), which is especially prevalent in this species (Barber et al. 1996) and pairs exhibit positive assortative mating for plumage brightness (Bitton et al. 2008). Intriguingly, the metal-induced brightness we observed might cause metal-exposed birds to gain attractiveness to potential mates which, if actually detrimental, could create a signal-based evolutionary trap (sensu Schlaepfer et al. 2002). Such subtle mechanisms are both tractable and worthy of study in Tree Swallows, which are among the migratory aerial insectivores that have been declining across North America since the mid 1980's (Nebel et al. 2010, Shutler et al. 2012) and may advance methods for species of even greater conservation concern.

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3.8 Tables

Table 3.1. Plumage colour, oxidative stress and number of young fledged in relation to metals in feces of tree swallow chicks. These model-averaged estimates were obtained from linear models. Bold predictors have 95% confidence intervals (CI) that do not include zero.

Response	Model set	Predictor	Beta	95% CI
Hue	Males and ASY females	$PC2_{MET}$	5.7	-0.01, 11.42
		Sex	-8.4	-18.21, 1.41
Saturation	Males and ASY females	$PC2_{MET}$	1.01	-1.47, 3.48
		$PC1_{MET}$	-0.51	-1.51, 0.49
		Sex	-1.09	-5.34, 3.16
Brightness	Males and ASY females	**$PC2_{MET}$**	4.70	3.00, 6.40
		Sex	2.04	-0.85, 4.92
Oxidative stress	Males	Laying date	-0.008	-0.01, 0.001
	Females ASY	**$PC2_{MET}$**	-0.13	-0.22, -0.04
	Females SY	Laying date	0.01	-0.004, 0.04
Number of young fledged	Males and females (in relation to metals)	$PC1_{MET}$	-0.19	-0.43, 0.04
Number of young fledged	Males and ASY females (in relation to colour)	Laying date	-0.65	-1.72, 0.42
		Hue of male	-0.64	-1.71, 0.42
		Brightness of male	0.62	-0.46, 1.69

Standardized coefficient estimates are given for the average of supported models within 2 AICc units of the best-fit model.

3.9 Figures

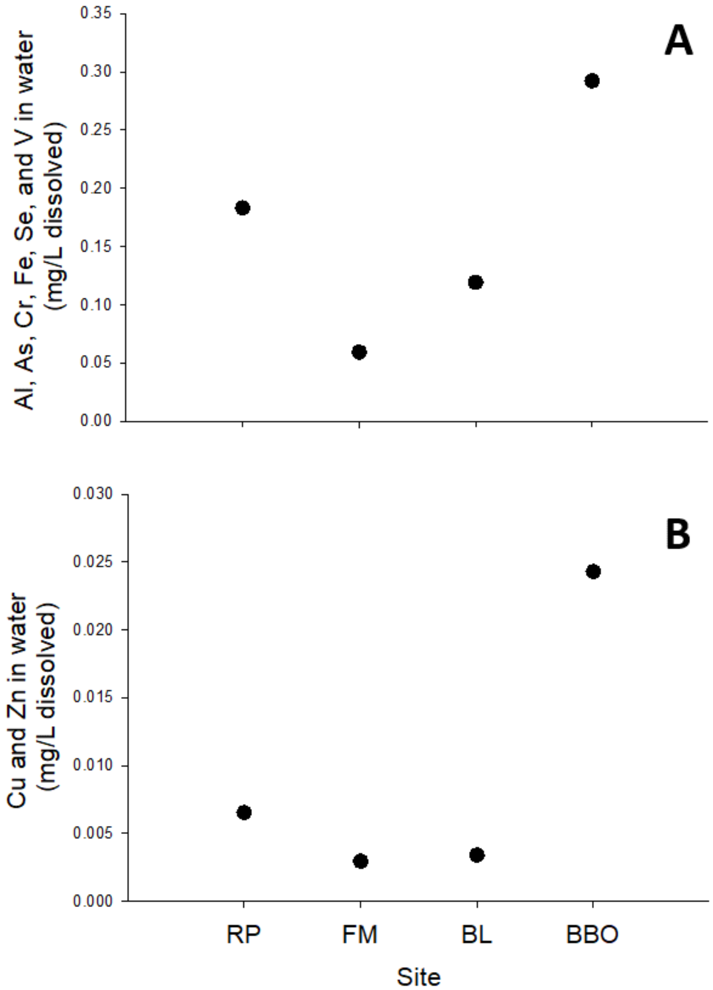

Figure 3.1. Single-sample water concentrations of (A) non-essential and (B) essential metals from our 4 sites. On the same day, we collected 4 samples on different locations of each site and pooled them for analysis. Note that these are not means. RP = Roper Pond, FM = Fulton Marsh, BL = Big Lake, BBO = Beaverhill Lake. Sites are presented in order of proximity to downtown Edmonton.

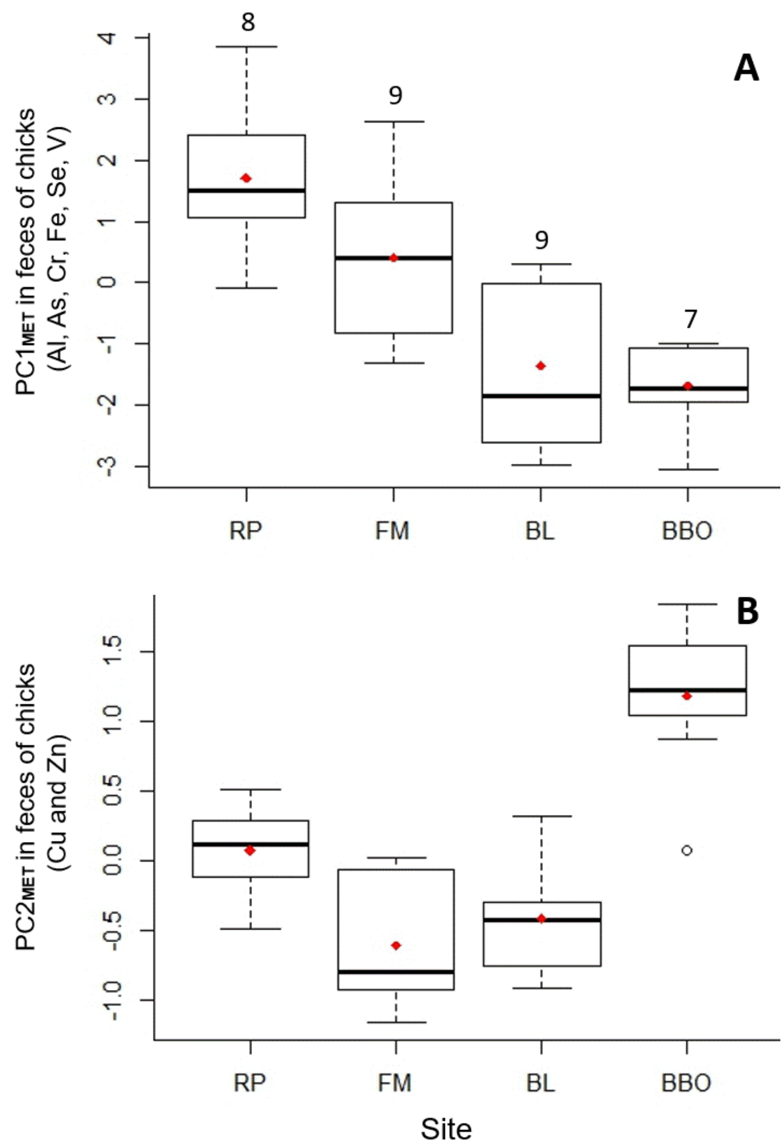

Figure 3.2. Box-and-whisker plot showing outliers (circles), minimum and maximum values (whiskers), inner and outer quartile ranges (boxes), the mean (red dot), and the median value (heavy bar) of (A) $PC1_{MET}$ (Al, As, Cr, Fe, Se and V) and (B) $PC2_{MET}$ (Cu and Zn) in feces of nestling tree swallows separated by site. Sample sizes are indicated over the whiskers. RP = Roper Pond, FM = Fulton Marsh, BL = Big Lake, BBO = Beaverhill Lake. Sites are presented in order of proximity to downtown Edmonton.

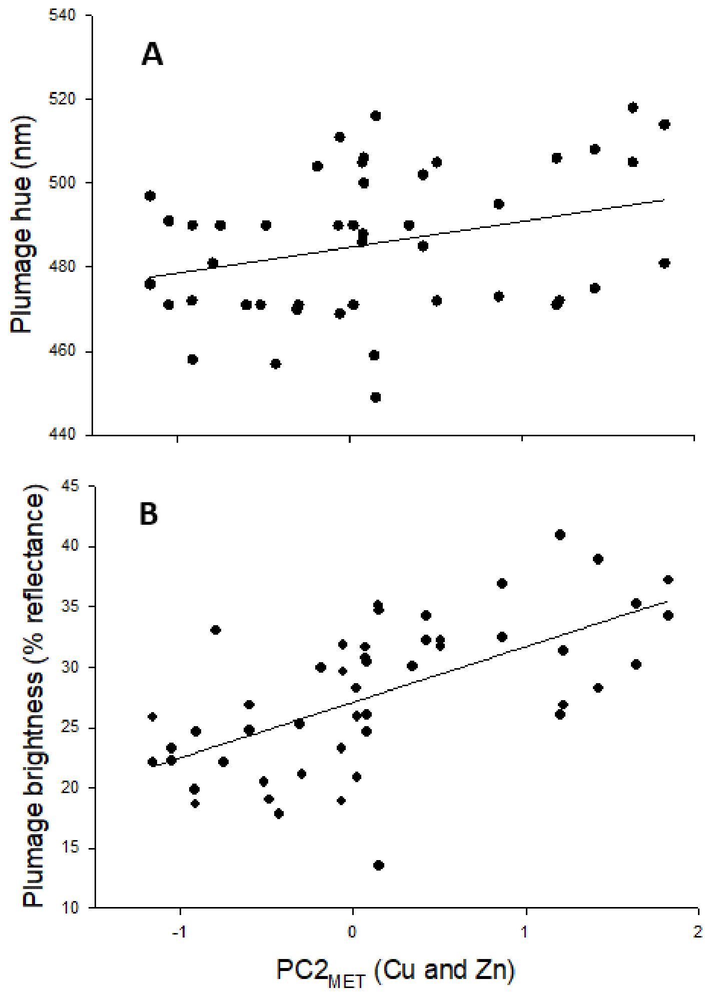

Figure 3.3. Relationship between (A) plumage hue and (B) plumage brightness of adult tree swallows and the concentration of PC2 $_{MET}$ in the feces of their chicks. Original data is plotted.

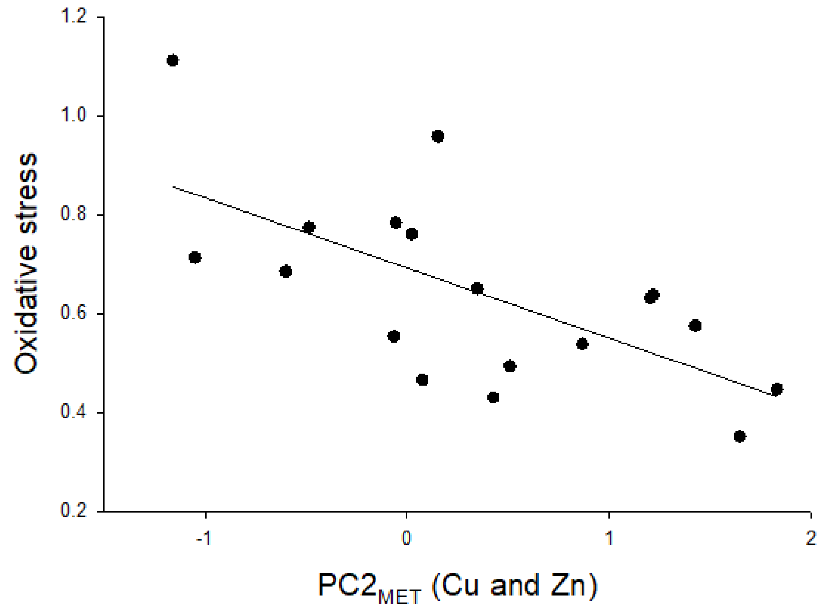

Figure 3.4. Relationship between oxidative stress of ASY females and the concentration of $PC2_{MET}$ in the feces of their chicks.

3.9 Appendix

Appendix 1 - Candidate linear models of adult plumage colour, oxidative stress and reproductive success in relation to concentrations of metals in feces of chicks of tree swallows.

Table A1.1

Candidate linear models of adult plumage hue in relation to fecal metals of tree swallow chicks.

Predictors	logL	AICc	ΔAICc	wi
$PC2_{MET}$ + Sex	-204.13	417.2	0.00	0.241
PC2met	-205.51	417.6	0.38	0.199
Sex	-205.97	418.5	1.30	0.125
$PC1_{MET}$ + $PC2_{MET}$	-205.14	419.2	2.02	0.088
$PC2_{MET}$ * Sex	-203.98	419.4	2.18	0.081
$PC1_{MET}$ * Sex + $PC2_{MET}$	-202.96	419.9	2.74	0.061
Intercept only	-207.89	420.0	2.87	0.057
$PC1_{MET}$ + Sex	-205.90	420.7	3.53	0.041
$PC1_{MET}$ * Sex	-204.72	420.8	3.67	0.038
$PC2_{MET}$ * Sex + $PC1_{MET}$	-203.75	421.5	4.32	0.028
$PC1_{MET}$	-207.68	421.9	4.71	0.023
$PC1_{MET}$ * Sex + $PC2_{MET}$ * Sex	-202.85	422.4	5.26	0.017

logL, log-likelihood; AICc, size-corrected Akaike information criterion; ΔAICc, difference in AICc value with highest supported model; w_i , Akaike weight.

Table A1.2

Candidate linear models of adult plumage saturation in relation to fecal metals of tree swallow chicks.

Predictors	logL	AICc	ΔAICc	wi
Intercept only	-165.39	335.1	0.00	0.309
$PC1_{MET}$	-164.86	336.3	1.21	0.169
$PC2_{MET}$	-165.05	336.6	1.58	0.140
Sex	-165.26	337.0	1.99	0.114
$PC1_{MET}$ + $PC2_{MET}$	-164.57	338.0	3.00	0.069
$PC1_{MET}$ + Sex	-164.62	338.1	3.10	0.066
$PC2_{MET}$ + Sex	-164.97	338.9	3.81	0.046
$PC1_{MET}$ * Sex	-163.89	339.2	4.12	0.039
$PC2_{MET}$ * Sex	-164.45	340.3	5.25	0.022
$PC1_{MET}$ * Sex + $PC2_{MET}$	-163.74	341.5	6.43	0.012
$PC2_{MET}$ * Sex + $PC1_{MET}$	-163.99	342.0	6.95	0.010
$PC1_{MET}$ * Sex + $PC2_{MET}$ * Sex	-163.44	343.6	8.56	0.004

logL, log-likelihood; AICc, size-corrected Akaike information criterion; ΔAICc, difference in AICc value with highest supported model; w_i , Akaike weight.

Table A1.3

Candidate linear models of adult plumage brightness in relation to fecal metals of tree swallow chicks.

Predictors	logL	AICc	ΔAICc	wi
$PC2_{MET}$	-145.99	298.5	0.00	0.350
$PC2_{MET}$ + Sex	-144.94	298.8	0.27	0.306
$PC1_{MET}$ + $PC2_{MET}$	-145.96	300.8	2.32	0.110
$PC2_{MET}$ * Sex	-144.77	300.9	2.43	0.104
$PC1_{MET}$ * Sex + $PC2_{MET}$	-143.79	301.6	3.08	0.075
$PC2_{MET}$ * Sex + $PC1_{MET}$	-144.68	303.4	4.86	0.031
$PC1_{MET}$ * Sex + $PC2_{MET}$ * Sex	-143.54	303.8	5.31	0.025
Intercept only	-158.18	320.6	22.11	0.000
Sex	-158.13	322.8	24.29	0.000
$PC1_{MET}$	-158.17	322.9	24.36	0.000
$PC1_{MET}$ + Sex	-158.13	325.2	26.65	0.000
$PC1_{MET}$ * Sex	-156.97	325.3	26.84	0.000

logL, log-likelihood; AICc, size-corrected Akaike information criterion; ΔAICc, difference in AICc value with highest supported model; w_i, Akaike weight.

Table A1.4

Candidate linear models of oxidative stress of adult males, ASY females and SY females in relation to fecal metals of tree swallow chicks.

Predictors	logL	AICc	ΔAICc	wi
Males				
LDATE	25.54	-44.0	0.00	0.35
Intercept only	23.90	-43.3	0.73	0.24
$PC1_{MET}$ + LDATE	25.83	-41.0	2.19	0.12
$PC1_{MET}$	24.33	-41.6	2.41	0.10
$PC2_{MET}$ + LDATE	25.56	-41.3	2.74	0.09
$PC2_{MET}$	23.95	-40.9	3.17	0.07
$PC1_{MET}$ + $PC2_{MET}$	24.38	-38.9	5.10	0.03
ASY Females				
$PC2_{MET}$	9.77	-11.8	0.00	0.45
$PC2_{MET}$ + LDATE	11.30	-11.5	0.30	0.39
$PC1_{MET}$ + $PC2_{MET}$	9.78	-8.5	3.34	0.08
LDATE	7.63	-7.5	4.28	0.05
Intercept only	4.74	-4.7	7.14	0.01
$PC1_{MET}$ + LDATE	7.65	-4.2	7.59	0.01
$PC1_{MET}$	4.99	-2.3	9.55	0.00
SY females				
LDATE	8.37	-8.1	0.00	0.39
Intercept only	6.09	-7.0	1.09	0.23
$PC1_{MET}$	7.40	-6.1	1.93	0.15
$PC1_{MET}$ + LDATE	9.03	-5.1	3.01	0.09

$PC2_{MET}$ + LDATE	8.76	-4.5	3.55	0.07
$PC2_{MET}$	6.11	-3.6	4.52	0.04
$PC1_{MET}$ + $PC2_{MET}$	8.07	-3.2	4.92	0.03

logL, log-likelihood; AICc, size-corrected Akaike information criterion; ΔAICc, difference in AICc value with highest supported model; w_i, Akaike weight; LDATE, laying date.

Table A1.5

Candidate linear models of number of young fledged in relation to fecal metals of tree swallow chicks.

Predictors	logL	AICc	ΔAICc	wi
$PC1_{MET}$	-46.00	98.9	0.00	0.33
$PC1_{MET}$ + AGEf	-45.88	101.3	2.42	0.09
$PC1_{MET}$ + $PC2_{MET}$	-45.98	101.5	2.61	0.09
$PC1_{MET}$ + LDATE	-46.00	101.5	2.65	0.09
AGEf	-47.39	101.7	2.78	0.08
$PC2_{MET}$	-47.42	101.7	2.85	0.08
LDATE	-47.42	101.7	2.85	0.08
$PC2_{MET}$ * AGEf	-45.75	103.9	5.02	0.03
$PC1_{MET}$ * AGEf	-45.82	104.0	5.15	0.02
$PC2_{MET}$ + AGEf	-47.36	104.3	5.37	0.02
LDATE + AGEf	-47.39	104.3	5.43	0.02
$PC2_{MET}$ + LDATE	-47.42	104.4	5.49	0.02
$PC2_{MET}$ * AGEf + $PC1_{MET}$	-44.93	105.4	6.46	0.01
$PC2_{MET}$ * AGEf + LDATE	-45.73	107.0	8.08	0.00
$PC1_{MET}$ * AGEf + $PC2_{MET}$	-45.74	107.0	8.09	0.00
$PC1_{MET}$ * AGEf + LDATE	-45.82	107.1	8.25	0.00
$PC1_{MET}$ * AGEf + $PC2_{MET}$*AGEf	-44.90	108.7	9.79	0.00
$PC2_{MET}$ * AGEf + $PC1_{MET}$ + LDATE	-44.91	108.7	9.81	0.00
$PC1_{MET}$ * AGEf + $PC2_{MET}$ + LDATE	-45.73	110.3	11.45	0.00
$PC1_{MET}$ * AGEf + $PC2_{MET}$ * AGEf + LDATE	-44.90	112.3	13.45	0.00

logL, log-likelihood; AICc, size-corrected Akaike information criterion; ΔAICc, difference in AICc value with highest supported model; w_i, Akaike weight; LDATE, laying date; AGEf, age of the female.

Chapter 4: Photographs can emulate spectrophotometric measures of plumage hue of tree swallows as a non-invasive metric of pollution exposure*

4.1 Abstract

Anthropogenic pollution causes habitat degradation for many species, but its effects on individuals can be difficult to detect until they result in the decline or disappearance of populations. Techniques to monitor the effects of pollution on wildlife usually include invasive sampling of expendable species, but a close link between individual condition and colouration of ornamental traits could make photography an alternative, non-invasive method that is suitable for species of conservation concern. We advanced such a technique by comparing colour metrics (hue, saturation, and brightness) of the same tree swallows (*Tachycineta bicolour*) obtained via spectrophotometry and in-hand photographs, and, for different birds, determined whether a similar range of colour variation could be obtained with remote photography alone. We found that hue of tree swallows was moderately to highly correlated between techniques. On the contrary, saturation and brightness were uncorrelated, probably due to the structural properties of iridescent plumage and its dependence on the angle of light incidence. Plumage hue calculated from in-hand photographs was weakly related to exposure to environmental metals. Additionally, hue values measured using remote photography fell within the range of plumage hue obtained using spectrophotometry, while the colour references showed very low variation between photographs, even when taken on different days and under different illumination conditions. These results suggest that hue of iridescent plumage can be measured effectively with photography and potentially reflects metal pollution in bird diets. Remote photography offers similar promise for consistent measurement of hue provided photographs can be properly calibrated. Our study extends the suggestions by several previous authors that ornamental colouration could provide a non-invasive tool for assessing pollution exposure in birds while advancing tools for achieving that goal using remote cameras.

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4.2 Introduction

Urban expansion and related increases in anthropogenic activity in human-dominated landscapes cause numerous stressors for wildlife (Wilcove *et al.*, 1998; Donald *et al.*, 2006; Pollack, Ondrasek and Calisi, 2017; Renthlei, Borah and Trivedi, 2017), one of which is the ingestion of chemical pollutants (Tyler, Jobling and Sumpter, 1998; Zala and Penn, 2004; Isaksson, 2015; Trathan *et al.*, 2015). Birds appear to be especially sensitive to multiple kinds of chemical pollutants (Burger and Gochfeld, 2004; Smits and Fernie, 2013; Plaza *et al.*, 2018), which may be severe enough to cause population declines (Laurance and Useche, 2009; Xu *et al.*, 2016; Sanderfoot and Holloway, 2017). Unfortunately, these deleterious effects may not be recognized in time to prevent widespread damage to ecosystems. Well-known examples of this problem occurred when songbirds declined suddenly amid widespread use of pesticides after the Second World War (Carson, 1962) and birds of prey exhibited reduced hatching success because of eggshell thinning caused by exposure to DDT (Ratcliffe, 1967). Avoiding these population-level consequences requires constant monitoring, to achieve early detection of deleterious effects of chemical pollutants on individuals, which is the reason that several physiological techniques are used for this purpose. Most often, such physiology-based biomarkers evaluate the impact or concentrations of pollutants in blood or tissue physiology, and then determine correlates between these measures and the reproductive success or survival of individuals across a gradient of exposure (e.g. Hallinger et al. 2011; Burger & Gochfeld 2004). For example, levels of circulating stress hormones in the blood of snow petrels (*Pagodroma nivea*) indicated concentrations of persistent organic pollutants in blood (Tartu *et al.*, 2015). Alternatively, immune trials that involve the injection of the protein phytohaemagglutinin can evaluate the effects of air pollutants on the immune response of tree swallows (*Tachycineta bicolour*) breeding in the oil sands of Alberta (Cruz-Martinez et al. 2015). Such techniques typically require that birds be captured, manipulated, and sampled, which involve invasive methods as they are either blood- or tissue-based, and at times, they even require the sacrifice of eggs, chicks or adults to measure pollutant concentration in yolk or bioaccumulation in tissue (Hoffman *et al.*, 1998; Snoeijs *et al.*, 2004). Because of their invasiveness, these techniques are unsuitable for many species that are difficult to catch or generate conservation concern.

An alternative, but seldom used metric to evaluate the effects of pollution on individuals and populations is ornamental colouration (Hill 1995; reviewed by Lifshitz and St Clair 2016).

This is because the production and maintenance of an attractive colouration is physiologically costly and it is also dependent upon the condition of the bearer (Hamilton and Zuk, 1982). These ornaments show higher phenotypic plasticity than other traits (Cotton *et al*., 2004) and are also more sensitive to environmental conditions (Moller and Pomiankowski, 1993; Hill, 1995). Several forms of ornamental traits formed by feathers and skin are sensitive to environmental pollutants, which has been demonstrated in both the wild and in controlled laboratory experiments (reviewed by Lifshitz and St. Clair, 2016). As examples, wild great tits (*Parus major*) nesting near a metal smelter produced paler carotenoid-based yellow colouration (Geens *et al*., 2009), red-legged partridges that were experimentally exposed to a pesticide had smaller melanin-based brown flank bands (Galván and Alonso-Alvarez, 2009) and wild belted kingfishers (*Megaceryle alcyon*) exposed to environmental mercury had less saturated structural blue back feathers (White and Cristol, 2014).

Decades ago, bird plumage was compared by eye to colour standards (Endler, 1990) but subtle changes in the intensity of plumage colouration are better measured with reflectance spectrophotometry or photography. A spectrophotometer can be placed directly on the bird or on its collected feathers to measure the properties of the colour signal via surface reflectance at a fine wavelength resolution (Montgomerie, 2006). This technique compares the amount of light reflected from an object in each wavelength bin with the amount of light reflected from a standard object, usually a flat white surface (Johnsen, 2016). Alternatively, measurements may be obtained using calibrated digital photographs (e.g. Simpson and McGraw 2018) where after capture, individuals are held against a controlled, dark background and adjacent to a calibration standard of known reflectance for further calibration using photography-processing software (Stevens *et al.*, 2007; Pike, 2011; Troscianko and Stevens, 2015; Johnsen, 2016).

Despite the popularity of spectrophotometers and photography for colour assessment and the growing number of studies on bird colouration, to our knowledge, published studies use either spectrophotometry or photography assuming they provide comparable measurements. One study compared these two techniques to investigate colour change in veiled chameleons (*Chamaeleo calyptratus*) and found no difference between values of stimulation of chameleon photoreceptors obtained from photographs to values obtained with spectrophotometry (Ligon and McGraw, 2013). However, we found no paper that assessed the correlation between the two techniques when quantifying colouration of the same individuals. Such a comparison could be important because even though spectrophotometers generally provide more resolution (Johnsen, 2016), the greater focal length of photography reduces the need to capture individuals, offering a degree of non-invasive remote sensing (Bergman and Beehner, 2008). These properties of photography have already turned cameras into useful tools of remote sensing for evaluating health and characteristics of vegetation based on colouration patterns (Fletcher *et al.,* 2001; Newete *et al.*, 2014). Exploiting these same characteristics of cameras, a study used remote photographs to measure colour of the chest patch of wild male gelada baboons (*Theropithecus gelada*) in their natural habitat, and successfully placed males in their age group based on the redness of their chest (Bergman and Beehner, 2008). Surprisingly, we found no study that used remote cameras to quantify ornamental colouration of birds from a distance, even though they are routinely used to monitor other aspects of bird populations, such as the presence of ultra violet ornaments in gentoo (*Pygoscelis papua*) and king penguins (*Aptenodytes patagonicus*; Meyer-Rochow and Shimoyama 2008), the size of Adélie penguin colonies (*Pygoscelis adeliae*; LaRue *et al.* 2014) and predation events on nests of Lapwings (*Vanellus vanellus*) and Spotted Flycatchers (*Muscicapa striata*; Bolton *et al*. 2007). Expanding the use of remote cameras to monitor ornamental colouration would enhance the set of tools for non-invasive monitoring of sensitive species and remote locations.

The objectives of this study were to (a) evaluate if measurements of plumage colouration obtained with calibrated photographs of birds in-hand accurately reflect the values of colouration obtained using reflectance spectrophotometry on feathers plucked from the same individuals; (b) determine whether photographed measures of plumage colour revealed a gradient of exposure to environmental metals; and (c) determine whether remote photographs supported with colour reference cards provide colour measurements with a comparable range of values to those taken with birds in-hand and with spectrophotometer. As a model species, we used the tree swallow (*Tachycineta bicolour*), a small passerine among the many migratory aerial insectivores that have been declining across North America since the mid 1980's (Nebel *et al.*, 2010; Shutler *et al.*, 2012). This species is commonly used in toxicology studies as a sentinel of environmental pollution (Custer, 2011; Smits and Fernie, 2013). They present dorsal iridescent colouration that reflects individual quality and is sexually selected (Bitton and Dawson, 2008; Bitton *et al*., 2008; Bentz and Siefferman, 2013) and somewhat sensitive to metal pollution (Lifshitz and St. Clair *in review*). Tree swallows are obligate cavity nesters that readily colonize man-made nest boxes, which they aggressively defend from neighbors during reproduction (Winkler *et al*., 2011), making them easy to spot and monitor during this period.

4.3 Methods

4.3.1 Fieldwork and data collection

Collection of feathers and digital photographs for objective 1 (compare methods) took place in June 2015 and 2016. In 2015 sampling was carried out in two sites with different proximity to downtown Edmonton, Alberta and with different surrounding land use. The urban site occurred in a constructed wetland designed to collect and clean storm water in the city of Edmonton; Roper Pond (RP; 53.4979°N, 113.4375°W; 6.3 km to downtown Edmonton). The rural site was located at the Beaverhill Lake, a provincially-protected area (BBO; 53.3824°N, 112.5277°W) is 67 km from downtown Edmonton and is the site of a long-term monitoring program for tree swallows (Beaverhill Bird Observatory; http://beaverhillbirds.com/). In 2016, we added two more sites to create a gradient of exposure to pollution and distances to downtown, which were expected to have intermediate concentrations of heavy metals relative to the two sites used in 2015 (A. Liu personal communication, St. Albert 2012). These sites included another constructed wetland; Fulton Marsh (FM; 53.4813°N, 113.3594°W; 11.5 km to downtown Edmonton) and one suburban site in a natural wetland, Big Lake, in Lois Hole Centennial Provincial Park on the outskirts of the city of St. Albert (BL; 53.6136°N, 113.6582°W; 13.3 km from downtown Edmonton).

From the beginning of each breeding season, we monitored clutches every 3rd day and determined clutch initiation date by the presence of a fresh egg. At day 12 ± 1 day post-hatch, we collected fecal sacs from defecating chicks into glass containers and pooled these by brood owing to their small mass. We stored these samples in ice in the field and sent them to a commercial lab (ALS Environmental Laboratory in Edmonton, AB.) for a fine-scale, brood-specific measure of metal exposure. This procedure was performed for the 2016 field season only and these fecal samples supported our second objective, to compare variation in feather colour for adults measured with photographs to metal exposure in their diets, as expressed by metals in fecal sacs of their chicks. On the same 12 day post-hatch, and for both field seasons, we captured adult male tree swallows using nest-box traps, banded them, collected 5 mantle iridescent feathers for subsequent assessment in the lab and took a digital photograph. After collection of feather samples, we placed them into opaque paper envelopes, which were sealed and stored in the dark, to minimize colour degradation, until the end of the field season. Colour quantification of feather samples in the lab using spectrophotometry took place in September 2015 and 2016 respectively. For the photographs, individuals were held against a black background with a grayscale standard series of known reflectances and three series of increasing intensities of red, green and blue (Figure 4.1a). They were photographed outdoors at the same distance from the objective and at approximately the same time of day. We used a Nikon D700 camera and photographs were taken in RAW format, which includes the data from the sensors prior to any white-point balancing or other non-linear transformation inside the camera (Stevens *et al.*, 2007; Johnsen, 2016).

For colour assessment using spectrophotometry in the lab, we taped feathers onto matte black cardboard and arranged them in an overlapping fashion to approximate their usual configuration on a bird's body. We measured the reflectance of these feathers using an S2000 spectrophotometer and deuterium tungsten-halogen light source (Ocean Optics, Dunedin, FL, USA). All measurements were taken with unpolarized light. We took readings using a bifurcated fibre-optic cable mounted in a metal-encased probe that transmitted incident light to the measurement area and reflected light to the spectrometer. The probe was mounted in a metal base that excluded ambient light and maintained the probe at a fixed angle perpendicular to the feather surface. Following the recommendations of Johnsen (2016), we used this geometry and technique to measure colour of tree swallows to support comparison of our data with previous work (Bitton and Dawson, 2008; Bitton *et al*., 2008; Bentz and Siefferman, 2013).

Using OOIBase32 software (Ocean Optics), we took five readings from each feather sample, by removing the reflection probe and light source and placing them again on the colour patch between readings. Each reading comprised an average of 20 spectra measured sequentially. N. Lifshitz took all the measurements, which were expressed as percent reflectance relative to a Spectralon white standard (WS-1; Ocean Optics). We summarized our reflectance data by averaging the 5 readings per bird and calculating three tristimulus colour variables to approximate three dimensions of colour: brightness, hue, and blue chroma as a measure of spectral purity (Montgomerie, 2006). We calculated average brightness as the average percent reflectance between 400 and 700 nm (Doucet, 2002). As an index of hue, we used the wavelength of maximum reflectance in the visible spectrum, from 400 to 700 nm. Blue chroma was calculated as the relative contribution of the blue range as a percentage of the overall brightness (400-512 nm/400-700 nm). We described the spectral profile of iridescent plumage at only one angle (90 degrees to the reading surface), but the general height of the curve for this plumage region would be expected to vary with the angle at which the feather is analyzed. Although we recognized that variation in feather angle would contribute to noise variation in our measurements, we did not anticipate a consistent bias or confound from this procedure.

We accomplished our third objective, to assess variation in colour metrics from remote photographs, by collecting photographs of swallows at the same four sites in June of 2017. From the beginning of the breeding season, we monitored pairs every 3rd day and determined clutch initiation date by the presence of a fresh egg. During the incubation period, we taped a colour reference card on all sides of the nest-boxes and allowed the birds to return to their boxes. Once the pair returned, we took digital photographs at an approximate distance of 3-7 meters depending on the proximity tolerance of each individual (Figure 4.1b). We always photographed the male perched outside the box, while the female was incubating inside. Photographs were taken in RAW format using a Canon Rebel T5i camera with a Canon zoom lens.

To obtain the colour of the swallows' plumage from the digital photographs, we extracted the average value of each RGB channel. On each digital photograph, we created five sampling windows of three per three pixels using ENVI 5.1 (Exelis Visual Information Solutions, Boulder, Colourado, v5.1). Specifically, the sampling windows extracted the raw pixel values from the head of the bird, the back of the bird, the grey reference, the black reference and the white reference panels. For the assessment of remote photographs, we used Adobe® Photoshop® software CC 2019 (Adobe Systems Incorporated, San Jose, CA, USA) and created two additional sampling windows, one for the red reference and one for the blue reference panels in order to compare their repeatability between photos. Then, the values from the head, back, red and blue reference panels were transformed to radiance ($W \cdot m^{-2} \cdot sr^{-1} \cdot nm^{-1}$) and reflectance (relation of the radiance of the object and the irradiance, derived from a white reference panel). We did this by using a calibration method employed by others (Smith and Milton, 1999; Del Pozo *et al.*, 2014; Johnsen, 2016) based on the use of adjacent white, grey and black reference panels of known reflectance. Finally, we converted the RGB values obtained for each bird body part and colour reference panel into HSB space (Hue, Saturation and Brightness; Montgomerie 2006) to make them comparable to the values obtained with the spectrophotometer.

4.3.2 Statistical analyses

To compare colour metrics derived from spectrophotometry and photography, we extracted the spectral data from feathers and digital photographs and calculated the colour variables (HSB) of back of each bird for spectrophotometry, and both, back and head for photographs. Following the procedures of others that have compared methodologies for colour measurement (Quesada and Senar, 2006; Vaquero-Alba *et al.*, 2016), we performed Pearson's product-moment correlations between colour metrics of the back plumage obtained with spectrophotometry and colour metrics of back and head plumage obtained with photographs; we did this separately for the 3 head colour metrics and the 3 back colour metrics obtained from photographs. High correlation values would indicate that the two procedures measured colour in a consistent way, regardless of whether absolute values were similar. Because neither head nor back colour metrics differed between years (all t-tests, $P > 0.05$) we pooled the data from both years for the analyses.

To investigate if the colour metrics we extracted from photographs taken in 2016 were sensitive to metal pollution, and because of the relatively strong correlations among multiple measures of metals in feces, we first conducted a principal component analysis (PCA) with oblimin rotation, using the R function *princomp* to produce synthetic variables of metals for further analyses (Lifshitz and St. Clair *in review*). The original variables were first log-transformed to normalize their distributions. We used the PCA to consolidate variation among fecal samples in heavy metal concentrations of aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), lead (Pb), antimony (Sb), selenium (Se), zinc (Zn) and vanadium (V). The PCA resulted in two components; PC1MET that explained 70 % of the variation and had positive loadings for Al (0.78), As (0.83), Cr (0.79), Fe (0.91), Se (0.93) and V (0.76); hereafter non-essential metals, and PC2MET that explained 10 % of the variation and had positive loadings for Cu (0.94) and Zn (0.88); hereafter essential metals (Mudipalli and Zelikoff, 2017). With these principal components, we constructed generalized linear mixed effects models (GLMMs) only for the colour metrics that showed correspondence with spectrophotometry, and included site as random effect. We fitted one model for hue of the head plumage and one for hue of the back plumage. We considered 5 candidate models that included a combination of essential metals and non-essential metals, and a model with only the intercept. Our dependent variables did not depart significantly from normality for either sex (Shapiro Wilk tests: all $P > 0.05$). Thus, we used models with Gaussian error distributions and the identity link, and we verified that the residuals for fitted models were normally distributed in every case. We evaluated model fit using an information-theoretic approach (Burnham and Anderson, 2003) and standardized all variables before analysis to support interpretation of relative effect sizes and interaction estimates (Grueber *et al.*, 2011). We compared models using Akaike's Information Criterion corrected for small sample size (AICc). We considered models with ΔAICc<2 to be equivalent and averaged their coefficients (i.e., multi model inference; Harrison et al. 2018) using the R library MuMIn (Barton, 2017); otherwise we identified the single best-fitting model.

For the assessment of colour data obtained from calibrated remote photographs, we calculated the coefficient of variation of plumage colour metrics. We did this only for the swallows' head, because without controlling for the exact angle of light incidence on the body, the back plumage looked very dark in several photos. On the contrary, the birds always turned their heads to face the photographer. To evaluate the reliability of the remote photographs, we also calculated the coefficients of variation of the red and blue panels in the colour reference cards. We carried out our statistical analyses using R (Crawley 2007, R Development Core Team 2010).

4.4 Results

We collected colour data from 18 males in 2015, 29 males in 2016, and 59 males in 2017, with the following distribution among sites: 2015; Roper Pond: 7 males, Beaverhill Lake: 11 males. 2016; Roper Pond: 7 males, Fulton Marsh: 8 males, Big Lake: 7 males and Beaverhill Lake: 7 males. 2017; Roper Pond: 12 males, Fulton Marsh: 16 males, Big Lake: 14 males and Beaverhill Lake: 17 males. Regarding the pattern of heavy metals in feces, we found that non-essential metals in chick feces increased with proximity to downtown Edmonton, while the concentrations of essential metals were highest in the site farthest from downtown (Beaverhill Lake), followed by the urban wetland closest to downtown (Roper Pond), followed by the suburban site (Big Lake) and the more peripheral stormwater pond, Fulton Marsh (for more details see: Lifshitz and St Clair, *in review*).

Our first objective was to evaluate if measurements of plumage colouration obtained using calibrated photographs of tree swallows in-hand accurately reflect the values obtained using reflectance spectrophotometry on feathers plucked from the same individuals. Correlations between these methods for the three colour metrics (hue, saturation and brightness) showed a significantly positive values only for the hue of head (r_{44} = 0.31, *P* = 0.04) and back (r_{42} = 0.56, *P* < 0.001; Table 4.1, Figure 4.2). Plumage saturation and brightness showed very low correlations (all r < 17; all P > 0.05) between the measurement techniques (Table 4.1).

Our second objective was to investigate if the colour metrics of head and back plumage that we calculated from calibrated photographs could be related to exposure to environmental metals. Because we had previously demonstrated such relationships using spectrophotometry (Lifshitz and St. Clair *in review*), we used only the photographic metric, hue, that exhibited a positive correlation. We performed generalized linear mixed effects models that explored the relationship between exposure to metals (measured via fecal samples of the nestlings) and the hue of head and back plumage of the father. The hue of back plumage exhibited a weak positive correlation with essential metals (β = 0.92 ± 0.88, C.I. = -0.92 – 2.76; Figure 4.3) indicating that greener plumage occurred in birds whose chicks exhibited higher levels of these metals in their feces. Although this was the best-fitting model, the confidence intervals for the effect of essential metals on hue of back colour overlapped zero, which invites a cautious interpretation. Head colouration of males was not explained by the concentration of essential metals in chick feces (Akaike weights ≤ 0.16).

Our third objective was to determine if remote photographs of bird heads**,** calibrated using colour reference cards, exhibit ranges of variation in plumage colouration of tree swallows that are comparable to those obtained from photographs of hand-held birds and from spectrophotometry. We performed this comparison only for head plumage because in most of the remote photographs the back plumage looked very dark due to the positioning of the bird in respect to the camera. Also, we only assessed the variation of plumage hue as it was the only photograph-based colour metric that correlated with the colour metric obtained with the spectrophotometer. In general, hue values of head plumage from remote photographs taken in 2017 (mean = 490 nm, S.E. = 1.13; range: 477-518 nm) were similar in mean and with greater variation than those taken via photographs of birds in hand in 2015 and 2016 (mean = 488.9, S.E. = 0.67; range: 481.8- 499.6 nm). Additionally, hue values of head plumage from remote photographs had a larger mean and smaller variation than those taken via reflectance spectrophotometry in 2015 and 2016 (mean = 483.9, S.E. = 2.3; range: 457-518 nm), but fell within the range of the latter (Figure 4.4). Finally, the coefficients of variation for bird hue were about 10 times larger than they were for the red and blue panels on the colour reference cards (CV of bird hue from spectrophotometry = 0.03; CV of bird hue from in-hand photographs = 0.01; CV of bird hue from remote photographs = 0.04; CV of hue of red and blue panels = 0.002).

4.5 Discussion

Ornamental colouration could replace some of the invasive techniques currently used to monitor the effects of chemical pollution on wild birds because it is closely linked to individual condition and health (Hamilton and Zuk, 1982). By exploiting the focusing optics of cameras, colouration could be measured remotely, thus eliminating the need to capture individuals (Johnsen, 2016). In order to expand on this possibility, we assessed the reliability of colour metrics of tree swallows obtained from photographs, compared to colour metrics of the same individuals obtained from spectrophotometry, and explored if these photograph-based colour metrics reflect individual exposure to metals. Additionally, we evaluated the potential of using remote photographs to detect and quantify variations in swallow colour, by using colour reference cards to calibrate such photographs.

The outcome for our first objective, to determine the correlation of paired measurements of bird colouration taken by spectrophotometry and digital camera, differed among colour metrics. For the metric of hue (comparable to what we perceive as colour), this correlation was moderate for head feathers and high for back feathers. This finding supports the contention by others that digital photography provides a good and reliable tool to measure bird plumage (Stevens et al 2007; Johnsen 2016). However, we found no similar correlations to support the metrics of saturation and brightness, whether measured for head or back feathers, which counters those claims. One reason for this discrepancy could be an inconsistency in measurements caused by the directionality that is characteristic of iridescent plumage, where movements of the animal in direct sunlight create changes in the brightness of the ornament (Osorio and Ham 2002; Van Wijk *et al*. 2016; Simpson and McGraw 2018). This directionality property has a strong influence in the brightness and saturation of colour, because at larger angles of light incidence a glare is produced, causing an increase in brightness and a consequent decrease in saturation (Doucet *et al.,* 2006). In contrast, when we measured plumage brightness using spectrophotometry, light was always shone on the same 90-degree angle and under highly controlled conditions. These conditions were difficult or impossible to replicate using digital photographs in the field, particularly for measurements of brightness, because of greater variation in the angle of natural illumination relative to both the subject and the photographer. In the future, this problem may be overcome by increasing control of the angle of light incidence when taking photographs of birds in the field, particularly when working with iridescent species (Simpson and McGraw, 2018). Additionally, photographs of iridescent species could be taken at various angles of light incidence to capture plumage directionality, which could result in more comprehensive colour measurements. A similar methodology has been suggested for spectrophotometry by recent studies that used a goniometer to measure iridescent colouration (Meadows *et al.*, 2011; Van Wijk *et al*., 2016). This apparatus provides a more robust quantification of the variation in iridescent colours by allowing the probe of the spectrophotometer to rotate at different angular positions and thus, taking viewing geometry into consideration.

Based on the finding that only hue offered a correlation between techniques, we used this single metric to explore our second objective that related plumage colouration to metal exposure. We found a weak relationship for hue of back plumage, measured with in-hand photographs to increase (revealing greener colour) when exposed to higher concentrations of essential metals. This result supports a previous finding, based exclusively on spectrophotometry measurements, wherein increased exposure to essential metals was related to greener plumages (Lifshitz and St. Clair, *in review*). In male tree swallows, bluer plumage is considered to be more ornamented as it signals higher quality and greater probability of survival than green plumage (Bitton and Dawson, 2008). Thin-film optical modelling has shown that in iridescent feathers, hue is determined by the thickness of the keratin cortex, such that birds with thicker barbule cortexes have plumage that reflects light maximally at longer wavelengths *(*greater hue values; Doucet *et al,.* 2006). In accordance with this, a study with tree swallows showed that iridescent feathers exposed to high levels of humidity change colouration from blue (shorter wavelengths) to green (longer wavelengths) as a response to the thickening of the keratin cortex caused by absorption of water (Eliason and Shawkey, 2010). This high sensitivity of iridescent feathers to environmental humidity could be avoided through the deposition of hydrophobic oils onto the feathers during preening (Giraudeau *et al.*, 2010) and through the constant care and maintenance of the plumage (Walther and Clayton, 2005; Eliason and Shawkey, 2011). If metal pollution creates sites with less or poorer quality food, birds may reduce the frequency of preening behaviour (Chatelain *et al.*, 2015) in order to invest in other activities such as foraging of chick rearing. Without enough preening feathers may lose their water repellency, causing the keratin cortex to thicken and the feather's hue to shift towards the observed, greener wavelengths. Similar results have been reported for the feral pigeon (*Columbia livia*), for which exposure to environmental lead decreased preening behaviours (Chatelain *et al*., 2015) and the blue colouration of iridescent feathers (Chatelain *et al.*, 2017). The (partial) relationship between metals and back plumage, but not with head plumage could be due to the fact that in tree swallows, crown and back colouration are associated to different fitness components (Van Wijk *et al.* 2016), therefore, their production and vulnerability to metals could differ.

In order to explore the potential of photographs to function as a remote, non-invasive tool to monitor colouration, we assessed the capacity of remote photography to capture the range of variation in plumage colouration of tree swallows and its capacity to obtain reliable measurements of colour standards. First, we found that, measurements of plumage hue obtained from remote photographs of male tree swallows perched on their nest-boxes were similar to the measurements obtained from in-hand photographs and fell within the range of values obtained with spectrophotometry, although with a smaller amount of variation. Second, we found that this method demonstrated accuracy and precision, even across varying light conditions. Hue measurements taken on the red and blue reference panels across different light conditions and different times of day did not differ between photographs. These results suggest that remote photograph, when calibrated, could accurately capture variation and changes in plumage hue from a distance without the need for capturing the birds. Cameras are already used to measure colour remotely, but this application has mostly been exploited in studies of vegetation (Fletcher, Escobar and Everitt, 2001; Newete *et al.*, 2014), where cameras have demonstrated to be reliable tools to detect differences and changes in plant colouration from the air. One study with gelada baboons (*Theropithecus gelada*) showed that the chest colour of wild males can be accurately measured using calibrated photographs (Bergman and Beehner, 2008), and the same application should be functional for bird colouration.

Several things limit the generalizations that might stem from our study. First, our results were correlative so even for the colour metric of hue, we cannot be sure that the relationship between green colouration and metal exposure was caused by metals. Because our goal is to use this colour-based technique to monitoring the effects of pollution, a necessary first step is to demonstrate a proximate mechanism by which metals affect the hue of iridescent plumage of tree swallows. In order to achieve this, further studies should investigate the effects of metal exposure on both the microstructure of feathers (particularly the width of the keratin cortex) and on preening behaviours. Once such link has been described, a photographic protocol could help establish a baseline of ornamental colour in a population of interest to help detect any departures from it caused by pollution. Second, we measured structural iridescent colouration, and quite different results might result from measurements of carotenoid-based colouration or melanin-based ornaments that are not iridescent. The appearance of pigment-based ornaments does not depend on the angle of light incidence. By eliminating the variation caused by directionality (or glare; Doucet et al. 2006), measurements of brightness and saturation of pigmentary ornaments should be as reliable as measurements of hue, showing high correlations with measurements taken with spectrophotometry. And third, our method was limited to measuring colour variation within the spectral range of the camera and thus, excluded the ultraviolet range of the spectrum. In the context of sexual selection, the purpose of colouration is to signal information to a receiver, and birds often rely on the ultraviolet range of the spectrum to convey this information through their ornaments (Cuthill *et al.*, 2000). Spectrophotometers already measure colouration in the ultraviolet range of the colour spectrum, so in order to obtain comparable measurements using photography, the use of multispectral imaging that includes ultraviolet light should be implemented (Troscianko and Stevens, 2015).

Taken together, the results of our study suggest that photography, including remote photographs, can provide a reliable measure of colouration of iridescent plumage that may reveal exposure to pollutants comparable to what has previously been revealed with spectrophotometry (e.g., Chatelain *et al*., 2017). This capacity provides an important advance for non-invasive monitoring of bird populations, although more work will be needed to interpret differences quantitatively. So far, it appears that using photography to assess the effects of metal pollution on iridescent ornamental colouration may be limited to the metric of hue, but future refinements in photographic techniques may make it possible to include measures of saturation and brightness. The capacity for ornamental colouration to provide an indicator of pollution exposure (Lifshitz and St. Clair 2016) encourages development of these techniques, which would allow for non-invasive monitoring of species of conservation concern and also species that are inaccessible to researchers. This approach might also provide efficiencies with other research goals, such as applying nest-cameras used to monitor behavior and reproduction of American kestrels (*Falco sparverius*; Dawson and Bortolotti 2000; Dawson and Bortolotti 2002) to the assessment of carotenoid-based facial colouration, which is highly sensitive to environmental pollution (Bortolotti *et al*., 2003). Researchers could anticipate this potential application by applying a colour reference card inside, or adjacent to nests when cameras are installed. This technique might also be expanded to measure the ultraviolet section of the colour spectrum using digital cameras fitted with ultraviolet sensors (Troscianko and Stevens, 2015). Overcoming the challenges of using this technique, will be best approached within multidisciplinary teams that could include physiologists, photographers, engineers, computing scientists and other scientists who work with coloured ornaments and have solved similar problems.

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4.8 Tables

Table 4.1. Correlation values between spectrophotometer and photograph measurements of plumage colouration of tree Swallows, pooled from measurements taken in 2015 and 2016. Photograph-based measurements were taken on both head and back and they were both independently correlated to spectrophotometer-based measurements taken on back feathers. Colour values from photographs were calculated after calibration with three reference colours (white, gray and black).

	Head		**Back**	
	r	**P**	**r**	**P**
Correlation spectro-photo (both years)				
Hue	*0.31*	*0.04*	*0.56*	*<0.001*
Saturation	0.17	0.23	0.12	0.40
Brightness	0.05	0.73	-0.05	0.71

4.9 Figures

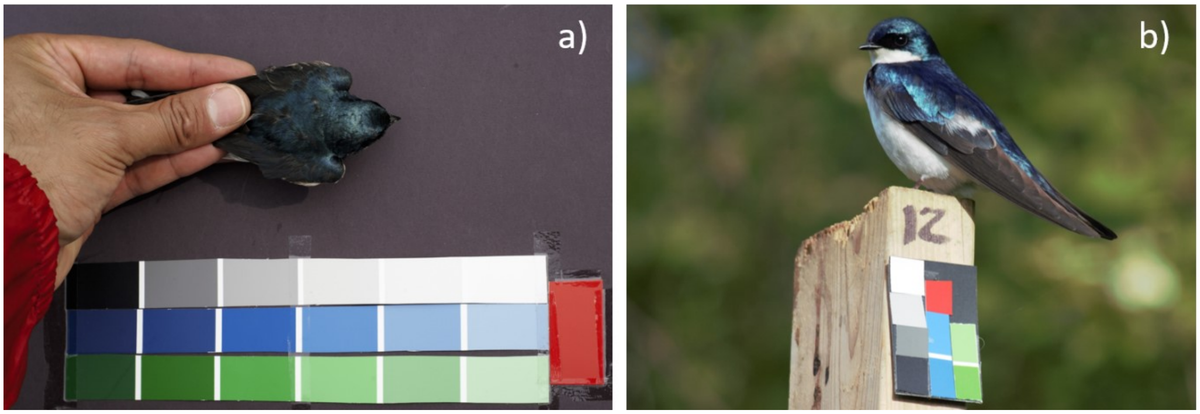

Figure 4.1. Photographs taken in the field to measure ornamental colouration of tree swallows a) in hand, and b) remotely. Card with colour reference panels used to calibrate photographs for different illumination conditions is shown in both photographs.

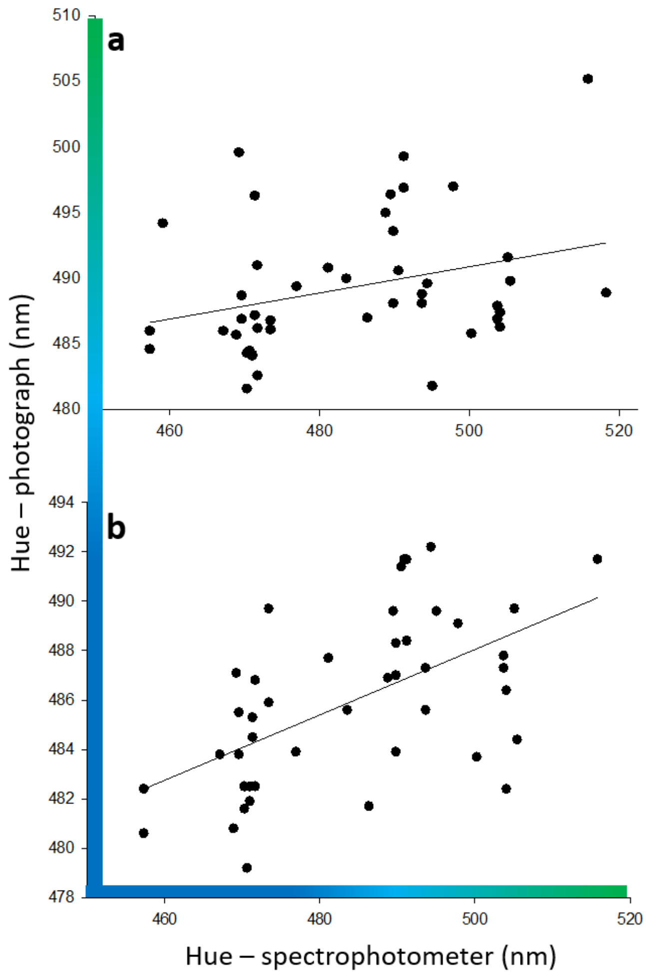

Figure 4.2. Relationships between hue of tree swallows' a) head and b) back plumage calculated from digital photographs and hue of the same individuals calculated using reflectance spectrophotometry on plucked back feathers. Because hue from photographs is calculated as degrees in a 360° circle, we converted them to dominant lambda (wavelength of maximum reflectance) to match the scale of hue measurements obtained with spectrophotometry. All measurements are in nanometers (nm).

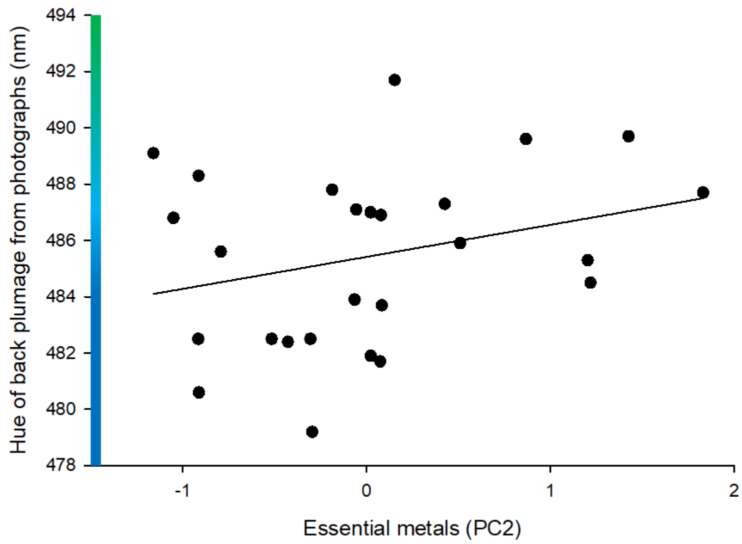

Figure 4.3. Relationship between the hue of the back plumage of tree swallows calculated from calibrated photographs, and exposure to essential metals via the feces of their chicks. Higher values of essential metals (PC2) indicate greater concentrations of copper and zinc.

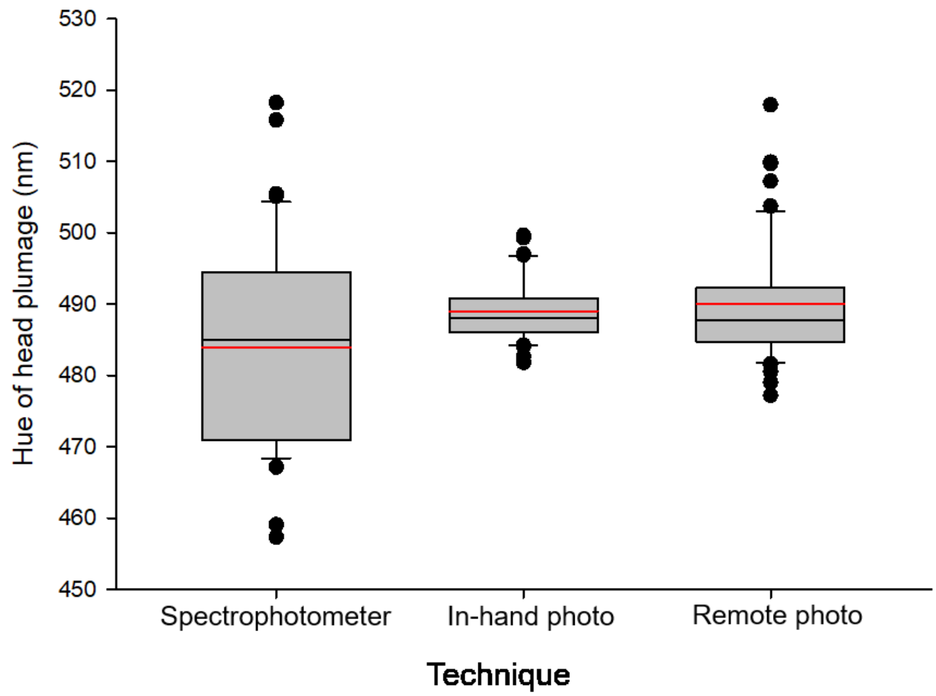

Figure 4.4. Comparison of the range of values of hue of head plumage of male tree swallows perched on their nest-boxes, calculated using spectrophotometer (n = 46) and in-hand photographs (n = 43) in 2015 and 2016, and remote photographs (n = 51) in 2017 in our 4 sites pooled together. Means are indicated with the red lines.

Chapter 5: General discussion

Chemical pollution is one of the consequences of anthropogenic activity with negative effects on wildlife and its habitat (Tyler *et al*., 1998; Zala and Penn, 2004; Trathan *et al.*, 2015). Understanding these effects requires quantitative evaluation of the effects of pollution on animals, which researchers and managers have traditionally achieved using a wide variety of physiological metrics. Usually, such measurements require blood and tissue samples, injection of immune-activating substances, and even sacrifice of individuals (reviewed by Golden and Rattner 2003). However, the invasive nature of most of these techniques makes them untenable for species of conservation concern and, because they require capture, they cannot be used on species that live in inaccessible locations. More than two decades ago, Hill (1995) proposed the use of ornamental traits as reliable indicators of environmental quality due to their close link to the condition of the bearer (Andersson, 1982; Hamilton and Zuk, 1982). In fact, several subsequent studies have shown that ornamental colouration in both fish and birds is sensitive to environmental pollution (reviewed by Lifshitz and St Clair 2016), but these traits are rarely used to monitor pollution exposure. In this dissertation, I explored the functionality of ornamental colouration as an indicator of environmental pollution and with three main objectives to: (1) review the literature on the effects of various chemical pollutants on ornamental colouration of fish and birds (Chapter 2); (2) investigate whether metal pollution over a gradient of urbanization is correlated with measurable variation in the iridescent colouration of tree swallows, additionally measuring correlations with breeding success and oxidative stress (Chapter 3); and (3) assess whether digital photography provides measures of plumage colour variation comparable to those obtained from reflectance spectrophotometry, that might be achieved with remote photography in future (Chapter 4). In the following paragraphs, I summarize the main results and interpretation of each of these chapters.

Chapter 2 investigated the logical basis and supporting literature for my intention to use ornamental colouration and develop photography-based methods for assessing pollution exposure. Because the production and maintenance of ornamental traits is highly costly for individuals, such traits are considered to be honest signals of individual quality and condition (Zahavi, 1975; Hamilton and Zuk, 1982; Zahavi and Zahavi, 1997). Relative to traits that evolved through natural selection, the dependence of ornamental traits on individual condition produces high phenotypic plasticity (Cotton *et al*., 2004), which makes their expression particularly sensitive to the environment and to the series of physiological mechanisms produced by stressful events (Hill, 1995; Buchanan, 2000). The capacity to reflect environmental stressors suggests that ornamental traits could be reliable indicators of overall environmental quality that reflect the sum of environmental pressures on an organism (Hill 1995), including chemical pollution. In my review I found that there are several types of anthropogenic pollutants with the capacity to affect ornamental colouration of fish and birds (Chapter 2), which I grouped into four categories by source; pharmaceuticals and active ingredients in personal care products; pesticides; industrial pollutants that are prevalent but not restricted to petroleum production; and heavy metals that frequently result from mining and manufacturing. These pollutants showed effects on several kinds of coloured ornaments via two main pathways; either by disrupting the endocrine system (Tyler *et al*. 1998) and/or by altering the oxidative status of individuals (Isaksson, 2010; Table 2.1; Figure 2.1).

Through the first pathway, endocrine disrupting compounds (EDC's) can potentially impact ornamental colouration if its expression is controlled by or related to sexual hormones. Examples of this mechanism are the dulling of the carotenoid-based orange colouration of the pectoral and caudal fins of male red shiners (*Cyprinella lutrensis*) exposed to a natural estrogen (McGree *et al.*, 2010), and the change in the onset of plumage maturation of female tree swallows exposed to polychlorinated biphenyls (PCBs) from the Hudson River (McCarty and Secord, 2000). Through the second pathway, alteration of the oxidative status, colouration of animals exposed to pollutants with pro-oxidant properties might be affected if the ornament depends upon pigments that are directly (i.e. carotenoids; Alonso-Alvarez *et al*. 2004) or indirectly (i.e. melanins; Matsuki *et al*. 2008) related to antioxidant defenses. Examples of this mechanism are the decrease in intensity of the carotenoid-based yellow colouration of young and adult great tits (*Parus major*) exposed to metals from a smelter (Geens *et al.*, 2009), and the increased area of black and decreased area of brown melanin-based plumage in red-legged partridges (*Alectoris Rufa*) experimentally exposed to diquat, a contact herbicide (Galván and Alonso-Alvarez, 2009).

The variety of examples in my review demonstrate the multiple ways chemical pollutants might alter ornament expression. In the partridge example, different responses to one chemical for ornaments composed of brown vs. black melanin highlights the complex relationships between ornaments and the properties of each particular pigment type and pollutant.

Nonetheless, my review supported the suggestion by Hill (1995), that coloured ornamental traits could be used to evaluate pollution exposure, potentially providing a generalizable, proactive, less-invasive diagnostic tool for detecting subtle anthropogenic effects on individuals before they are signalled by decreased reproduction or higher mortality, which ultimately cause population declines. In turn, those advantages might reduce the cost and increase the efficacy of mitigation efforts to increase conservation success.

In Chapter 3, I explored the potential to link anthropogenic pollution caused by heavy metals to the colouration of tree swallows. Earlier (Chapter 2), I showed that heavy metals are one of the groups of anthropogenic pollutants that can alter ornamental colouration, although the direction of effects seems to depend on the type of colouration and pigment involved. At the time I collected data for Chapter 3, no study had investigated the effects of metal pollution on iridescent colouration. Iridescent colouration of tree swallows is produced by the heterostructure of feather barbules consisting of keratin and melanin (Eliason and Shawkey, 2010) and studies with iridescent species have shown that each of keratin and melanin, are responsible for different characteristics of the colour produced; while hue is determined by the thickness of the keratin cortex, such that birds with thicker barbule cortexes have plumage that reflects light maximally at longer wavelengths (Doucet *et al.*, 2006), saturation and UV reflectance are determined by the concentration and type of melanin granules deposited under the keratin cortex, such that birds with greater densities of melanin in their barbules have plumage that looks more saturated and reflects proportionally more in the UV (Doucet *et al.*, 2006; Maia *et al.*, 2009). By affecting any of these components of the feather heterostructure, metals could potentially affect the appearance of iridescent colours.

Results from Chapter 3 add to this literature by showing that ornamental colour of tree swallows relates to exposure to metals, although the effects differed for $PC2_{MET}$ (Cu and Zn) and $PC1_{MET}$ (Al, As, Cr, Fe, Se, V), and among the 3 colour metrics I calculated. In more detail, I found that increased exposure to $PC2_{MET}$ (Cu and Zn), measured in the feces of their chicks, shifted the plumage hue of adult swallows from bluer to greener (Table 3.1 and Figure 3.3A), while increasing their brightness (Table 3.1 and Figure 3.3B). Because I did not find an increase in oxidative stress of adults exposed to higher concentrations of metals, other mechanisms should be mediating the relationship between metals and ornamental colouration of swallows. Somewhat consistent with our findings, a recent study with feral pigeons (*Columba livia*) found that reflectance in blue-wavelengths declined in pigeons that were naturally and experimentally exposed to lead, except when simultaneously exposed to high levels of zinc, suggesting that zinc exposure seems to offset the negative effects of lead exposure. However, Chatelain et al. (2017) also found a decline in the brightness of pigeons exposed to lead. These contrasting results show the complexity of relationships among various metals and iridescent colouration. Individual metals might reduce some components of iridescent colouration while others can enhance them, effects may differ across concentrations, and some metals may offset the negative effects of others, highlighting the importance of assessing the effects of pollution in natural and realistic conditions, where individuals are simultaneously exposed to various stressors.

As opposed to oxidative stress, the negative effect of metals we predicted was apparent in a slight decline in the number of young fledged, our measure of reproductive success, with increasing concentrations of $PC1_{MET}$. However, we could not readily relate this effect of $PC1_{MET}$ on reproductive success via the proxy of feather colour we had hoped to reveal, as colour was affected only by $PC2_{MET}$. Nonetheless, reproductive success was not unrelated to the colour of male swallows, showing a weak positive effect of brightness and a weak negative effect of hue, but with sources we cannot attribute to variation in the metals we measured. Meanwhile, the negative effect on reproductive success we found of $PC1_{MET}$, which includes several metals of environmental concern (Environmental quality guidelines for Alberta surface waters, Government of Alberta 2018), is consistent with their greater concentrations in urban areas (Lee et al. 1994, Davis et al. 2001, Wei and Yang 2010), and expected toxicological effects (e.g. Eeva et al. 2009).

Tree swallows begin molting in their breeding grounds and molt is completed during migration (Knight *et al.*, 2018). Therefore, one of the shortcomings of my study is the assumption that metal pollution in the breeding range affects feather production. One way for pollution in breeding grounds to affect plumage production throughout molt is through carry-over effects from the physiological costs and stress derived from reproduction (Harrison *et al.*, 2011). Carry-over effects can have impacts on individuals long after the breeding season (Catry *et al.*, 2013; Legagneux *et al.*, 2013; Schultner *et al.*, 2014), including the completion of molt (Knight et al 2018). Evidence of these type of carry-over effects in birds include male bluebirds (*Sialia sialis*) that invest more in parental care by raising experimentally enlarged broods and subsequently expressed plumage colour that was duller than in the year of the manipulation

(Siefferman and Hill, 2005). Also, in breeding tree swallows, reproductive effort can reduce long-term immune function (Ardia *et al*., 2003). Therefore, because the growth of feathers is a costly process, (Lindström *et al*., 1993; Romero *et al*., 2005) sensitive to stress (DesRochers *et al.*, 2009; Lattin *et al.*, 2011), any physiological wear of breeding in a polluted environment may decrease the ability of birds to produce good-quality feathers during molt.

Overall, my results and the results of previous studies show that, although promising, much more research is needed to understand the effects of metals on ornamental colouration, and particularly on iridescent colouration (Chatelain *et al.*, 2017), because different types of metals can affect colour in opposing or complimentary directions. Additionally, other metrics such as feather microstructure, levels of sexual hormones and preening behaviours need to be taken into consideration in order to distinguish between the several non-exclusive mechanisms that can mediate the effects of metals on ornamental colouration.

In Chapter 4, I explored the potential to use information that might be gained and generalized in studies like the one I conducted in Chapter 3 by monitoring pollution exposure in free-living birds with remote cameras. I approached this goal with three steps by first determining whether digital photography could produce results similar to those obtained from spectrophotometry, then to explore is these photography-based colour metrics reflect exposure to metal pollution and finally to determine whether photographs taken remotely (i.e., without birds in hand) generated similar variation in colour metrics. If quantifying ornamental colouration is to be used as an indicator of environmental pollution (Chapter 2 and 3), it is of great importance to measure it using the right tools and techniques (e.g. Quesada and Senar 2006; Montgomerie 2006). Reflectance spectrophotometry and calibrated digital photography are both used to measure plumage colouration (Johnsen, 2016), but other researchers have used one or the other exclusively, which limits the ability to compare results among studies. I believe mine if the first study to assess the comparability between these two techniques by using them both to quantify the colouration of the same individuals.

In this chapter, I found medium to high correlations between measurements of plumage hue obtained using each of digital photographs and spectrophotometry, suggesting that using calibrated photographs to measure hue of iridescent feathers can produce accurate and consistent measurements. However, I found no correlations between these methods for measures of saturation or brightness, suggesting that for these two parameters of iridescent feathers, photography does not provide measurements that are comparable to measurements obtained with spectrophotometry. Previous studies with tree swallows have found significant relationships among colour, body condition, and reproductive variables, when measuring iridescent colour using spectrophotometry at a consistent angle of 90 degrees (Bitton and Dawson, 2008; Bentz and Siefferman, 2013). The appearance of iridescent plumages is highly dependent on the angle of light incidence, which can create changes in the perceived colour and on the brightness of the ornament, also called directionality (Osorio and Ham 2002; Doucet *et al*., 2006; Van Wijk *et al*., 2016). These properties of iridescence might have caused a variation between photographs that was too high to detect significant correlations with the more consistent measurements taken with spectrophotometry.

Because hue was the only colour metric that showed a correlation between techniques, I explored this metric's potential to reflect exposure to metal pollution. I found a weak relationship between hue of back plumage, obtained from in-hand photographs, and exposure to essential metals. This result supports my previous finding where using exclusively spectrophotometry, exposure to essential metals was related to greener plumages (Chapter 3). In iridescent feathers, hue is normally determined by the thickness of the keratin cortex of the barbules, such that feathers with thicker cortexes reflect light at longer wavelengths, or greater hue values (Doucet *et al.,* 2006). In accordance with this, the feathers of tree swallows change from short (blue) to longer (green) wavelengths when exposed to humidity as a response to the thickening of the highly hydrophilic keratin cortex (Eliason and Shawkey 2010). By reducing environmental quality and individual condition, metal pollution may cause plumage to shift towards greener hues through its effect in decreasing the time spent preening (Chatelain *et al.*, 2015), as this behaviour helps maintain the water repellency of feathers (Walther and Clayton 2005; Giraudeau *et al.*, 2010; Eliason and Shawkey, 2011).

A positive quality of photography is that it can operate autonomously. Because of this, remote cameras are widely used in studies of wildlife detection and surveillance (Turner *et al.*, 2003; Swann *et al.*, 2004; Bolton *et al.*, 2007; Grenzdörffer, 2013; Linchant *et al.*, 2015). In addition, the focusing optics of cameras allow for remote sensing, a quality highly exploited by studies that use them to assess type, phenological stage and even colour-based health of vegetation from airborne devices (Fletcher *et al.*, 2001; Newete *et al.*, 2014). Only one study has used remotes cameras to measure ornamental colouration of animals and they successfully placed wild male gelada baboons (*Theropithecus gelada*) in their age group based on the redness of their chest patch (Bergman and Beehner, 2008). However, to my knowledge, my study is the first one to attempt to combine these attributes of cameras to measure ornamental colouration of birds remotely. Despite variation in ambient light for photographs taken on different days and times of day, calibrating the photographs with colour reference cards made it possible to obtain, with remote photography, values of plumage hue that fell within the range of plumage hue that has been obtained for this species using spectrophotometry. This result suggests that it will be possible to use remote photography to quantify pollution exposure, as well as other sources of variation in plumage hue for free-living birds.

I also wanted to evaluate the precision and repeatability of colour measurements obtained from calibrated remote photos. For this, I included the same colour reference card on all the remote photographs and found very little variation in hue of the red and blue panels between photographs. This indicates that as I proposed in Chapter 2, it is possible to obtain reliable and repeatable measurements of colour using calibrated remote photography in the field to control for different illumination conditions. Because this was the first attempt at using remote photography to measure iridescence of wild birds, much more research is needed to explore its functionality and improve the technique. Overcoming the challenges of using remote photography will be best approached within multidisciplinary teams that could include engineers, computing scientists, photographers and other scientists who have solved similar problems. The advantages of using cameras is that they could be used as a non-invasive tool to monitor health of populations through changes in ornamental colouration. Furthermore, cameras are more available and affordable than reflectance spectrophotometers. Future research should focus on species with pigment-based colouration, because brightness and saturation of iridescence seemed to vary too much for this technique. This technique could be applied to the various projects that use nest-cameras to monitor behaviour and reproduction of birds with condition-dependent ornaments. Therefore, by introducing colour reference cards into the nests along with the camera, it could be possible to assess and monitor the colouration of those birds non-invasively in order to detect early effects of pollution before they become more evident in other traits such as health or reproduction.

5.1 Summary and future recommendations

At the end of my review in Chapter 2, I highlighted some limitations and made several suggestions in order to successfully use ornamental colouration as an indicator of pollution. I attempted to follow those recommendations in the subsequent chapters. I believe I succeeded in finding visible and meaningful variation in iridescent colouration among tree swallows that experience exposure to different concentrations of metals over a tractable scale of space (Chapter 3). With this work, I also established a colour baseline for my study population from which it would be possible to make comparisons and detect fluctuations over time. Also, while investigating the effects of metal pollution on coloured traits, I (3) attempted to unravel their associated impacts on individual quality and fitness. And finally, I (4) took a first step into implementing the use of remote cameras to quantify colouration of tree swallows non-invasively.

I believe my work demonstrated each of these steps as proofs of concept, but much more work will be needed before these techniques can be implemented as a tool for monitoring pollution exposure in populations of wild birds. Like many studies, my work ended with more questions than I posed at the beginning. For example, I was surprised that levels of oxidative stress in tree swallows did not increase with metal exposure, potentially because essential metals actually enhance antioxidant capacity for swallows in urban areas. But even if that mechanism occurs, other effects of metals that I did not measure may exert negative effects on health or longevity. Similarly, although I was able to quantify differences in colour with metal exposure, these only slightly correlated with reproductive success of the social pair. However, many other factors, including extra-pair paternity, may have obscured relationships between colouration and body condition and reproductive success. More research will also be needed to unravel the pathways by which anthropogenic pollutants potentially affect colourful ornaments as well as the meaning of those changes for associated metrics of fitness. Despite all this unresolved complexity, my final chapter offered promise that remote photography can capture variation in ornament colouration, at least for the metric of hue in iridescent feathers. I believe this finding will spark additional work by others to explore non-invasive, remote photography as a means of quantifying variation in the condition of ornamented individuals. Identifying and solving the limitations of my study are made more urgent by the increasing necessity to monitor endangered species non-invasively and by the constant creation and discovery of new anthropogenic pollutants in the environment. Monitoring these early effects of anthropogenic disturbance is an important step in preventing population declines of vulnerable species and ornamental colouration seems to be a promising tool to achieve this task.

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Appendix

Table A1. Total concentrations of metals dissolved in water samples from the four sites of our study. Concentrations are in mg/L.

Site	Al	As	Cr	Cu	Fe	Se	V	Zn
Roper Pond	0.0048	0.00242	0.0002	0.00144	0.175	0.000145	0.00067	0.0051
Fulton Marsh	0.0062	0.00284	0.00017	0.00196	0.049	0.000242	0.00099	0.001
Big Lake	0.0028	0.00178	0.00013	0.00141	0.114	0.000109	0.0005	0.002
Beaverhill Lake	0.0955	0.0024	0.00056	0.0043	0.191	0.00025	0.0025	0.02

Table A2. Total concentrations of metals in fecal samples from all the nests in the four sites of our study. Concentrations are in mg/kg wet weight.

Site	Nest	Al	As	Cr	Cu	Fe	Se	V	Zn
RP	1	49.7	0.076	0.134	10.6	221	0.435	0.145	59.3
RP	3	30.5	0.092	0.081	11.2	164	0.568	0.09	67
RP	8	81.3	0.137	0.169	16.9	152	0.819	0.225	86.7
RP	10	36.4	0.109	0.14	17.2	131	0.577	0.123	98.6
RP	11	29.7	0.06	0.097	7.6	269	0.513	0.096	45.9
RP	14	11.8	0.074	0.055	11	134	0.513	0.047	73.2
RP	16	31	0.091	0.089	10.8	160	0.438	0.098	71.9
RP	18	115	0.184	0.227	20.2	247	1.24	0.31	111
RP	22	456	0.427	0.743	27	640	1.22	1.14	154
FM	2	14.2	0.063	0.04	8.15	257	0.506	0.042	45.3
FM	3	88.1	0.093	0.167	13.4	222	0.802	0.21	79.6
FM	4	50.5	0.049	0.079	7.43	150	0.389	0.125	34.8
FM	6	8	0.034	0.04	7.8	139	0.477	0.026	46.5
FM	8	54.3	0.13	0.086	12.9	91.6	0.994	0.123	58.6
FM	9	41.9	0.088	0.079	8.37	349	0.68	0.101	44.1
FM	10	22.6	0.08	0.058	8.35	220	0.667	0.066	51.2
FM	14	6.6	0.071	0.04	8.31	162	0.86	0.031	49.1
FM	17	5.9	0.084	0.04	8.26	179	0.702	0.026	44.9
BL	2	14.7	0.065	0.04	8.45	174	0.299	0.054	48.1
BL	4	5.4	0.0035	0.04	5.82	116	0.232	0.023	42.9
BL	7	19.8	0.057	0.04	9.72	311	0.369	0.085	65.1
BL	8	4.1	0.037	0.04	5.21	132	0.302	0.014	43.9
BL	9	29.3	0.055	0.045	6.74	177	0.348	0.085	41.1
BL	13	4.3	0.031	0.04	7.34	212	0.383	0.021	49.2

BL	14	2.3	0.041	0.04	7.87	190	0.278	0.01	49.9
BL	16	2	0.028	0.04	6.66	109	0.208	0.01	39
BL	24	7.5	0.045	0.04	8.78	168	0.24	0.025	51.4
BBO	6	9.4	0.02	0.04	10.9	41.5	0.092	0.03	85.8
BBO	12	5.9	0.029	0.04	10.6	45	0.098	0.034	56.9
BBO	13	5.2	0.038	0.04	7.58	148	0.125	0.029	75.2
BBO	14	11.5	0.031	0.04	37.1	52.4	0.135	0.043	132
BBO	24	15.2	0.04	0.04	19.9	43.7	0.104	0.052	78.8
BBO	26	2	0.021	0.04	14.4	25.9	0.11	0.02	45.9
BBO	37	10.2	0.048	0.04	25	78.9	0.191	0.036	92.3

RP = Roper Pond, FM = Fulton Marsh, BL = Big Lake, BBO = Beaverhill Lake