CAUSES AND CONSEQUENCES OF BLUE-GREEN EGGSHELL COLOUR VARIATION IN MOUNTAIN BLUEBIRDS (*SIALIA CURRUCOIDES*)

by

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Abstract

The function and evolution of ornamental traits has been a major focus of evolutionary ecology. Despite this, female ornaments have received relatively little consideration, and are still poorly understood relative to those produced by males. However, presently, there is much interest in determining how sexual selection shapes female phenotypes. Blue-green eggshell colour, derived from the antioxidant pigment biliverdin, is one attribute produced by female birds that has come under scrutiny as a potentially sexually selected trait. Based on the possibility that biliverdin is limited and costly to produce, the sexually selected egg colour hypothesis predicts that blue-green egg colour has evolved in species with biparental care as an advertisement of female quality that elicits increased paternal effort from their social mate. I combined observations of naturally occurring patterns of eggshell colour variation and parental provisioning rates with an experimental approach to investigate the signalling function of blue-green eggshell colouration in mountain bluebirds (*Sialia currucoides*). I quantified patterns of within- and among-clutch colour variation and found that eggshell colour was repeatable for individual females, but declined later in the laying sequence within clutches, and between first and second breeding attempts within breeding seasons, suggesting pigments were limited. Using a food and nutrient (carotenoid) supplementation experiment, I determined that eggshell colour is sensitive to food, but not antioxidant availability in this species, and that supplementation did not affect patterns of pigmentation within clutches. Colour of eggshells was not consistently related to female traits such as clutch initiation date and plumage colour, but it did reflect investment in eggs, as eggshell colour was positively related to both egg mass and relative yolk volume. In addition, I conducted a cross-fostering experiment to test whether eggshell colour predicted nestling performance or rearing conditions. I found that eggshell colour was unrelated to nestling outcomes, as nestlings hatched from eggs with more saturated blue-green colour did not grow faster and were no more likely to fledge than those hatched from less saturated eggs. I detected relationships between the colour of the eggs in the nest that young were fostered in and their growth, but these associations were not consistent between years. Finally, I used the same cross-fostering design to test the effect of blue-green eggshell colour, separately from the potential influence of nestling phenotypes, on provisioning behaviour of males. I found that the provisioning rates of males during mid brood rearing were not related to the colour of the eggs that young hatched from, but were related to the colour of the eggs that were in their nest during incubation. However, contrary to my predictions if eggshell colour was sexually selected, males fed less at nests with more saturated eggshell colour. Together, my findings do not provide strong support for the sexually selected eggshell colour hypothesis. My results suggest that eggshell pigments are limited and sensitive to food availability and annual variation in conditions, but the inconsistent relationship with nestling performance, and the lack of a positive response by male birds, do not illustrate clear benefits for females to produce blue-green eggs.

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1. General introduction

1.1 Introduction

Ornamental traits of animals that are conspicuous and costly to produce and maintain are unlikely to be favoured by natural selection (Darwin 1871), as they may be detrimental to survival (Burk 1982). Instead, such attributes are thought to have evolved through sexual selection by improving an individuals' mating success (Darwin 1871; Andersson 1994). Sexual selection operates in two main ways; intrasexual competition favours weapons and characteristics that advertise fighting ability, while mate choice leads to the evolution of attributes that are attractive to prospective mates (Kodric-Brown and Brown 1984). Traits such as claws, horns, antlers, spurs, and badges of status are thought to principally improve performance in intra-sexual competition, while elaborate courtship displays, song, pheromones, and colourful skin and feathers are thought to function predominately in mate choice (Andersson 1994). Sexual selection has primarily focused on the evolution of male traits, because reproductive success of males often is highly variable and determined chiefly by their ability to gain access to mates, motivating males to compete for such access. In contrast, female reproductive success is mainly limited by their ability to produce gametes and raise young, resulting in females being selective when choosing mates (Trivers 1972). However, ornamental traits are widespread and common in females of many taxonomic groups (Amundsen 2000a), and although such characteristics have long been dismissed as non-functional genetic correlates of female preference (Lande 1980), evidence is mounting that males may also benefit from being choosy (Edward and Chapman 2011), and an increasing number of studies suggest that sexual selection also can shape the evolution of female traits (Clutton-Brock 2007, 2009).

Both males and females are thought to benefit from selecting a mate that expresses an elaborate ornamental trait, either through direct (increased access to resources) and/or indirect (favourable genes passed on to offspring) benefits (Kodric-Brown and Brown 1984; Andersson and Simmons 2006; Cornwallis and Uller 2010). Although it is generally assumed that choosing a mate with a particular phenotype confers advantages, the precise processes that maintain the information content and honesty of sexually selected traits are still the subject of debate (Kokko et al. 2003; Edward 2014). Historically, it has been assumed that ornaments must be costly to be honest advertisements of quality (Zahavi 1975), but recently theorists have proposed that the expression of adornments may be correlated with vital cellular processes, and that there need not be a cost for a trait to be informative (Hill 2011). One of the core mechanisms thought to maintain the information content of a sexually selected trait is that its expression is a function of the state (condition) of the individual expressing it (Andersson 1986; Morehouse 2014; but see Cotton et al. 2004). However, the multiple and ambiguous definitions of condition in the literature have complicated attempts to understand potentially condition-dependent traits. Hill (2011) defines condition as the ability of an individual to maintain cellular functions, suggesting that sexually selected ornaments should advertise this capacity. A significant threat to normal cellular function is the damage that can be caused by reactive oxidative species (ROS), which are toxic by-products of metabolism. Preventing and repairing oxidative damage, which results from an imbalance of ROS and antioxidant molecules, is an essential part of maintaining cellular integrity (von Schantz et al. 1999). Many compounds used in the production of colour, such as carotenoids, also protect organisms from oxidative damage (Kemp et al. 2012) and colourful traits have been found to advertise resistance to oxidative stress and antioxidant status (von Schantz et al. 1999; Pérez-Rodríguez 2009). Therefore, individuals with either low oxidative stress, or high levels of circulating antioxidants should be able to allocate more pigments to producing colour, while continuing to maintain cellular function without incurring extra oxidative damage. Because oxidative stress is high during reproduction (von Schantz et al. 1999; Wiersma et al. 2004; Fletcher et al. 2012), attributes produced in close proximity to breeding may be functionally linked to oxidative status and particularly informative signals of individual condition.

Eggshell colour is a trait that is produced by female birds concurrently with reproduction, and it has been proposed to function as an advertisement of female quality and condition. Two principal explanations for eggshell colouration, crypsis and defense against brood parasitism (Underwood and Sealy 2002), have not been supported for blue-green eggshell colour (Götmark 1992; Moreno and Oserno 2003). Based on this, and the fact that blue-green eggshell colour is the product of an antioxidant pigment, biliverdin, deposited in eggshells during egg laying (Stocker et al. 1990; Míšík et al. 1996), Moreno and Oserno (2003) proposed that blue-green egg colour may be a secondary trait that evolved to advertise female quality to males and elicit increased paternal care. Female birds experience high levels of oxidative stress during egg laying due to circulating progesterone (von Schantz et al. 1999); therefore, allocating biliverdin to eggshells is likely to be costly and should have some benefit to female fitness for the trait to be maintained. There is evidence that female condition (Moreno et al. 2006a; Siefferman et al. 2006; Krist and Grim 2007) and antioxidant status during laying (Hanley et al. 2008; Morales et al. 2011) correlates with a greater relative saturation of the blue-green portion of the visible spectrum (blue-green chroma) of eggshells. However, other studies found no relationship between female condition and blue-green egg colour (Cassey et al. 2008; Hargitai et al. 2008; Honza et al. 2012).There is also evidence that males invest differentially in the offspring of females that lay eggs that have more saturated blue-green colour (Moreno et al. 2004; Soler et al. 2008; Moreno et al. 2006b), but other studies have failed to find a change in male behaviour related to variation in egg colour (Krist and Grim 2007; Johnsen et al. 2011) or have had inconsistent results (English and Montgomery 2011). Overall, while there is some support for the function of blue-green egg colour as a sexually selected trait, more research is needed to confirm that it is an informative signal and also to separate confounding influences of female and nestling phenotypes on male behaviour from the potential effects of eggshell colour. Additionally, only a few species have been studied to date, so investigating a wider variety of species is necessary to determine whether sexual selection can be invoked as a general explanation for blue-green egg colour in birds.

1.2 Study area and species

I conducted my research on mountain bluebirds (*Sialia currucoides*) breeding in nest boxes at a study site southwest of Williams Lake, BC, Canada (51° N, 122° W), between April and August in 2011 and 2012. The habitat in the study area consisted of arid open grassland with scattered patches of Douglas-fir (*Pseudotsuga menziesii*), which is typical habitat for this species (Power and Lombardo 1996). There were 84 pairs of nest boxes, installed approximately 200 m apart to avoid intraspecific competition. Boxes were installed in pairs to avoid competition for nesting sites with other species, particularly tree swallows (*Tachycineta bicolor*). As mountain bluebirds typically defend territories that are at least 5 ha (Power and Lombardo 1996), only one box in each pair could be occupied by bluebirds. Mountain bluebirds are medium sized (~30 g) migratory thrushes that breed in western North America and winter in the southwestern United States. They are sexually dimorphic, socially monogamous passerines with biparental care. Males have structurally coloured cerulean blue feathers and females are dusky gray with structural blue colouring on their tails, wings, and rumps. Mountain bluebirds are generalist insectivores that feed on a wide variety of aerial and terrestrial insects (Herlugson 1982). They are secondary cavity nesters and will readily use nest boxes when they are provided. Females construct the nests and lay clutches that range from two to seven eggs in this population, with most clutches containing five or six eggs (O'Brien and Dawson 2013). The majority of eggs are blue-green in colour, ranging from pale blue to nearly turquoise, although white eggs also occur rarely (from 2 to 9% of clutches; Peak 2011). Mountain bluebirds are facultative double brooders and a portion of the population (up to 40 %) lay a second clutch of eggs after successfully raising a first brood (O'Brien and Dawson 2013). Females alone incubate eggs (~13 days), and brood young; but both parents sanitize nests and provision young during brood rearing (18-21 days) and after fledging (Power and Lombardo 1996).

1.3 Objectives

The potential for sexual selection to shape the evolution of female phenotypes as well as those of males has been acknowledged for some time (Amundsen 2000a; Clutton-Brock 2007; Clutton-Brock 2009). However, the information content of female ornaments and how they function in the context of sexual selection is still poorly understood compared to the ornamental traits of males. The focus of my thesis was to investigate the causes and consequences of variation in blue-green eggshell colour and to determine the information content of blue-green egg colour and whether it functions as a sexually selected signal. To accomplish this, I quantified eggshell colour of viable eggs late in incubation (see Appendix I) and explored the natural patterns of within- and among-clutch colour variation to determine if there was evidence that eggshell pigments are limited, and if pigmentation is related to other female characteristics such as clutch initiation date, feather colour, and investment in eggs. In addition, I evaluated the consistency of eggshell colour within clutches and between breeding attempts of individual females to determine if female and clutch identity were significant determinants of eggshell colouration. The results of these findings are presented in Chapter 2. In Chapter 3, I tested the condition dependence of blue-green eggshell colour by manipulating food and micronutrient (carotenoid) availability during pre-breeding and egg laying. I examined whether within-clutch patterns of pigmentation were different in nests where females were supplemented and whether the average colour of clutches were different among treatments. I also tested in Chapter 3 whether the relationship between female physical and reproductive characteristics and eggshell colour was altered by the supplementation experiment. Combining experimental and observational approaches allowed me to examine the natural patterns of egg colour variation, and separate potentially confounding influences of individual quality on eggshell colour from the effect of food and nutrient availability.

If eggshell colour is an informative signal that male birds respond to it should be related to nestling performance and to paternal investment (Moreno et al. 2003). In Chapter 4, I used a cross-fostering experiment, and measured nestling growth rates, to test whether nestling performance was related to either the colour of the eggs they hatched from or the colour produced by the resident female in the nest where they were raised. Finally, in Chapter 5, I quantified provisioning during brood rearing and compared both male and female provisioning rates to perceptually relevant measures of eggshell colour calculated using avian visual models (tetrahedral colour space; Stoddard and Prum 2008) to determine whether either males or females allocated more effort to broods hatched from bluer eggs. In addition to exploring natural associations between eggshell colour and provisioning, I used a cross-fostering experiment to decouple the potential influence of nestling phenotypes on provisioning rates from the effects of eggshell colour. Collectively, these studies permitted me to assess reproductive outcomes relative to blue-green eggshell colouration and the potential benefits to females of producing this pigmentation.

2. Egg colour in mountain bluebirds (*Sialia currucoides*): patterns of variation and relationships with female quality and investment in eggs

2.1 Abstract

Determining the functional significance of ornamental traits and whether they are shaped by sexual selection requires insight into how they vary among individuals and their relationship with other measures of quality. Conspicuous eggshell colouration is a trait produced by female birds that has been suggested to have evolved through sexual selection as a signal of female quality and reproductive investment. This hypothesis is based on the possibility that the pigment used to colour blue-green eggs (biliverdin) is limited and potentially costly to allocate to eggshells. To investigate the signalling potential of blue-green eggshell colour, I quantified patterns of within- and among-clutch colour (brightness, hue, blue-green and ultraviolet chroma) variation in mountain bluebirds (*Sialia currucoides*) to determine if there was evidence that eggshell pigments are limited and if the intensity of eggshell colouration was repeatable within individual females. I also explored the relationship between eggshell colour and other female traits (feather colour and clutch initiation date) and investment in eggs (egg mass and relative yolk volume) over two breeding seasons. I found that eggshell colour was highly repeatable within clutches for most measures and also relatively consistent between breeding events in different years, but not consistent between first and second clutches within years. These findings suggest that eggshell colour is intrinsic to individuals, but may also be influenced by environmental conditions. In addition, eggs laid in second clutches had less saturated blue-green colour compared to first clutches, and within clutches blue-green colour generally decreased after the first-laid egg, suggesting that eggshell pigments may be limited. There were also some

relationships between measures of female investment in eggs and eggshell colour. Eggshell brightness (suggesting lower pigment levels) was negatively related to egg mass, but egg mass did not vary with other aspects of eggshell colour. Relative yolk mass was higher for eggs with higher blue-green chroma and lower UV chroma and brightness, but was not related to hue. The relationship between female traits and eggshell colour was not consistent among years; in one season females with bluer and brighter rump feathers laid eggs with higher blue-green chroma, while in the other season eggshell brightness increased with clutch initiation date, indicating a decline in eggshell pigmentation. Together, these findings suggest that there is potential for blue-green egg colour to function as a signal of female quality, but considering the lack of strong correlations between female traits and egg colour further study is needed to determine if egg colour is a truly informative signal.

2.2 Introduction

Conspicuous ornamental traits of animals, such as colourful plumage, coloured patches of skin, song, and complex display behaviours, are generally thought to have evolved through sexual selection (Darwin 1871; Andersson 1994). Such traits are often costly to produce and maintain (Zahavi 1975), and are not likely to be favoured by natural selection. Instead, ornamental traits evolve because they confer a reproductive advantage through increased access to mates (Andersson 1994). The two principal mechanisms of sexual selection through which ornaments evolve are intrasexual competition and mate choice (Kodric-Brown and Brown 1984; Andersson 1994). Ornamental traits can advertise hormone levels and competitive ability, thereby functioning as badges of status that provide valuable information to opponents (Berglund et al. 1996). These characteristics also influence mate choice by signalling good genes and current condition to potential mates (Trivers 1972;

Møller and Alatalo 1999; Hill 2011). Individuals that select highly ornamented mates are thought to gain advantages either through direct benefits such as resources or indirect benefits by acquiring superior genes for their offspring (Cornwallis and Uller 2010).

The evolution of ornamental traits has primarily been investigated in males because of the assumed asymmetry in reproductive investment between males and females (Bateman 1948; Trivers 1972); females are expected to be more selective as their reproductive success is often limited energetically, rather than by access to mates, as is generally the case for males (Trivers 1972). However, more recently it has been predicted that male mate choice should occur when male investment in mating effort (e.g., competition, courtship, nuptial gifts, and paternal care) is such that they cannot invest equally in mating with all available females (Edward and Chapman 2011). Indeed, the females of many taxa produce ornamental traits that may have evolved through sexual selection (e.g., nuptial colouration in fish [Bernet 1998; Amundsen and Forsgren 2001], throat colour in lizards [Weiss 2006; Baldauf et al. 2011], and plumage colour and female song in birds [Roulin et al. 2000; Siefferman and Hill 2005a; Karubian 2013]). Such traits have long been dismissed as non-functional genetic correlates (Lande 1980), but there is mounting evidence that female ornaments are functional (e.g., Amundsen et al. 1997; Rosvall 2011), and that they can advertise quality (Roulin et al. 2000) and fecundity (Amundsen and Forsgren 2001).

One trait, only produced by females, that has been proposed as a potentially sexually selected ornament, is blue-green eggshell colour. The vibrant blue-green eggshell colour produced by many species of birds, which has long eluded other functional explanations, may advertise female quality to their social mates (Moreno and Oserno 2003). Blue-green eggshell colour is the result of biliverdin deposited in the surface layer of eggshells during laying (Kennedy and Vevers 1976; Míšík et al. 1996; Sparks 2011). Biliverdin is a metabolically produced antioxidant pigment with the potential to provide important health benefits by scavenging free radicals (Stocker et al. 1990; Stocker et al. 1990). As oxidative stress is high during egg laying (von Schantz et al. 1999), female birds may be limited in the amount of biliverdin they can allocate to eggshells rather than use endogenously. If biliverdin is limited, and eggshell colour accurately reflects pigment levels as some studies suggest (Moreno et al. 2006a; López-Rull et al. 2008; but see Cassey et al. 2012), then eggshell colour should be less saturated for eggs laid later within clutches and for second breeding attempts in the same season by an individual female. Several studies have shown the predicted decrease in the blue-green colour of later-laid eggs in comparison to eggs laid earlier in the laying sequence (Moreno et al. 2005; Krist and Grim 2007; Morales et al. 2011; López de Hierro and De Neve 2010), but these findings are not universal (Siefferman et al. 2006; Hargitai et al. 2008; Hanley and Doucet 2009) and more research is needed to confirm that pigment depletion occurs. Studies that directly compared subsequent breeding attempts within seasons also provide evidence for pigment limitation, showing a decline in the pigmentation of successive clutches (López de Hierro and De Neve 2010; Honza et al. 2012).

For a trait to be a sexually selected signal of intrinsic quality, it should be repeatable within individuals, but if the characteristic is condition-dependent, it is also likely to be sensitive to resource availability and affected by environmental conditions, and show variation within an individual among years or breeding attempts (Vitousek et al. 2012). Numerous studies have found that egg colour is more consistent within clutches than among clutches (Moreno et al. 2004; Seifferman et al. 2006; Krist and Grim 2007; Soler et al. 2008; Hanley and Doucet 2009; Morales et al. 2011, Honza et al. 2012). However, only a few studies have investigated the repeatability of egg colour between breeding attempts of individual females, and these studies have generally focused on egg recognition as a mechanism for preventing brood parasitism (Honza et al. 2011) or have studied domestic fowl which are not subject to the same array of environmental pressures and lay eggs continuously rather than in discrete clutches (Dearborn et al. 2012).

For eggshell colour to be a meaningful signal of female quality it should co-vary with other female traits associated with quality (e.g., clutch initiation date, female age, size, and plumage colour in some species) and investment in reproduction (e.g., clutch size, egg mass and size, and yolk components such as carotenoids, hormones and immunoglobulins). There is evidence for several of these predictions, but they are far from universally supported. Females that initiate clutches earlier in the season have been found to lay more highly pigmented eggs (Moreno et al. 2006a; Honza et al. 2012), providing support for eggshell colour as a quality signal as older, higher quality females are known to initiate clutches earlier in the breeding season (Perrins 1970; Murphy 2004), but other studies have found no relationship (Siefferman et al. 2006; Hargitai et al. 2008). Direct evidence for age-related differences in colour is also equivocal; some studies have found that older females lay more pigmented eggs (Siefferman et al. 2006), but opposite trends have also been reported (Moreno et al. 2005), and still others have found no effect of female age on eggshell colour (Hargitai et al. 2008; Morales et al. 2011). Interpretation of age-related effects are complicated by lack of accuracy in age estimates and the fact that the effect of age is not likely to be linear. Avian reproductive performance often improves as an individual ages, particularly between the first year of breeding and subsequent breeding seasons (Curio 1983), but this trend levels off and performance begins to decline as an individual senesces (Møller and De Lope 1999; Nussey et al. 2008). Measures of structural size and condition have also been found to both be positively related to egg colour (Moreno et al. 2005; Siefferman et al. 2006; Krist and Grim 2007: but see Cassey et al. 2008; Hargitai et al. 2008; López-Rull et al.

2008; Johnsen et al. 2011), but few studies have investigated correlations between egg colour and other ornamental traits. The only study to my knowledge that investigated relationships between female plumage ornaments and egg colour found that the size of white wing patches of female collared flycatchers (*Ficedula albicollis*) was not related to the colour of their eggs (Hargitai et al. 2008). Support for correlations between maternal investment pre-hatch and eggshell colour are similarly mixed. Some studies have found that egg colour was positively related to clutch size (López de Hierro and De Neve 2010), egg mass (Moreno et al. 2006a; Siefferman et al. 2006), and also indicative of maternal investment in yolk components such as antibodies (Morales et al. 2006) and carotenoids (Navarro et al. 2011), but other studies have not found these relationships (Cassey et al. 2008; Hargitai et al. 2008; Krištofík et al. 2013).

I examined the natural patterns of variation in the colour of eggs laid by female mountain bluebirds (*Sialia currucoides*) to determine whether there is support in this species for the possibility that blue-green eggshell colour is a sexually selected trait. I quantified eggshell colour and examined variation within and among clutches. If pigments were limited I expected eggshell colour to decline with laying order within clutches and between subsequent breeding attempts of individual females in the same year. However, if eggshell colour is intrinsic to individual females I expected that, although pigmentation may decline through laying, clutches laid by the same female should be more similar to each other than to clutches laid by different females. In addition, I investigated the relationship between eggshell colour and a suite of female (clutch initiation date and plumage colour) and egg traits (average egg mass and relative yolk volume). If eggshell colour is a signal of female quality and investment, then I expect females that initiate clutches earlier in the season and those that have bluer plumage will lay clutches of eggs that are more saturated blue-green in colour and that the colour of these clutches will be positively related to the mass and yolk volume of eggs laid.

2.3 Methods

2.3.1 Study site, species, and general field procedures

I studied mountain bluebirds breeding in nest boxes (84 pairs) southwest of Williams Lake, BC, Canada (51°N, 122°W), from mid-April to early August in 2011 and 2012. The habitat in the study area consists of arid open grassland with scattered stands of Douglas-fir (*Psuedotsuga menziesii*). Mountain bluebirds are migratory, medium-sized (~30 g), socially monogamous passerine birds with biparental care. At this site, they return from the wintering grounds in early March and begin initiating first clutches in late April. A portion of this population also double broods and lays a second clutch of eggs after successfully raising the young from their first nest (up to 40%; O'Brien and Dawson 2013). Clutch sizes range from two to seven eggs, but clutches of five or six eggs are most common (O'Brien and Dawson 2013). Eggshells of mountain bluebird eggs range in colour from pale blue to nearly turquoise, although white eggs have been documented (from 2 to 9% of clutches; Peak 2011).

I began monitoring nest boxes early in the breeding season to determine the start of nest construction and subsequent clutch initiation. I weighed each egg using a portable balance (nearest 0.01g) on the day they were laid. In addition, I quantified the yolk volume of a subset of clutches in 2012 from images taken using an ovolux and a digital camera (see Ardia et al. 2006). I used ImageJ software to measure the yolk and egg dimensions and I then calculated relative yolk volume by dividing yolk volume ($4/3*\pi*\text{radius}^3$) by the total egg volume which I calculated using the equation found in Hoyt (1979).

After clutches were complete (>24h with no new eggs laid), nests were not disturbed until day 9-10 of incubation (incubation period is ~13 days; Power and Lombardo 1996) when I measured the colour of complete clutches of eggs by quantifying their reflective properties using a spectrometer (see section 2.3.2 for details). I then returned on day 12 of incubation to check for hatch. Once nestlings had hatched, for most broods I captured adult females while they were feeding young. For identification I banded all females with a unique combination of a numbered aluminum band and three coloured plastic bands. Additionally, I collected rump feathers (8-12) and measured their spectral properties (see section 2.3.2 for details).

2.3.2 Colour quantification

I measured the colour of eggs on day 9-10 of the incubation period over the range of wavelengths visible to songbirds (300-700nm) using an Ocean Optics USB2000 spectrometer (Dunedin, FL) with a deuterium tungsten halogen light source (Avantes, Broomfield, CO). Three measurements of reflectance of each egg, relative to a white standard, were taken in an enclosed dark environment to eliminate ambient light. From these reflectance spectra, I calculated metrics for brightness (total light reflectance from 300-700 nm), hue (the wavelength of maximal reflectance), blue-green chroma (relative saturation from 400-605 nm), and ultraviolet (UV) chroma (relative saturation from 300-400 nm) using the package pavo (Maia et al. 2013) in R 3.1.2 (R Core Team, 2014) and then averaged the values for each egg. Eggshell blue-green chroma was negatively correlated with UV chroma ($n = 68$, $r = -0.82$, $P < 0.001$) and brightness ($n = 68$, $r = -0.77$, $P < 0.001$), which were positively related ($n = 68$, $r = 0.58$, $P < 0.001$). Eggshell hue was negatively correlated with UV chroma ($n = 68$, $r = -0.31$, $P = 0.01$), but was not related to blue-green chroma ($n = 68$, $r = -0.07$, $P = 0.58$) or brightness ($n = 68$, $r = 0.06$, $P = 0.63$). I averaged the above colour metrics of each clutch, for developed eggs only (see Appendix I), and used these averages in all among-clutch comparisons.

I quantified female feather colour by mounting eight feathers on a non-reflective black background in an overlapping position to mimic their natural positioning on the body, and measuring the spectral characteristics of the feathers from 300-700 nm following O'Brien and Dawson (2011). I took three measurements from random locations at the distal tip of the feather sample from each individual. As with the quantification of eggshell colour, I extracted colour metrics from the raw spectral data using pavo (Maia et al. 2013) and averaged these values. For feathers, I calculated brightness, hue, and UV-blue chroma (relative reflectance between 300-510 nm). These measures were correlated, and a previous study has found that dimension reduction yields a biologically meaningful measure of feather colour in this species (O'Brien and Dawson 2011); therefore, I used principal components analysis to collapse these metrics into a single measure of colour. The first principal component (PC1) explained 69.6% of the variation in female feather colour. Brightness loaded positively (0.62) with PC1 as did UV-blue chroma (0.96), while the loading value for hue was negative (-0.89). Feathers with high PC1 scores therefore were bright with high UV-blue chroma and maximum reflectance at shorter wavelengths (more blue).

2.3.3 Statistical analyses

I used repeated measures analysis of variance (ANOVA) to assess if egg colour varied predictably within clutches as a function of position in the laying sequence. I used Mauchly's test to determine if the assumption of sphericity was met and in cases where it was not ($P < 0.05$) I used a Greenhouse-Geisser correction. I included only complete first clutches that did not contain any undeveloped eggs (n = 18 in 2011, n = 23 in 2012), and analyzed clutches of different sizes separately (n = 28 five egg clutches, n = 13 six egg clutches). I only examined five and six egg clutches because other clutch sizes were rare. I included clutch initiation date as a covariate, year as a fixed factor, and egg position in the laying sequence as the repeated measure. Initial models included all covariates and first-order interactions and I used a backwards stepwise approach to remove terms from the model that did not approach significance ($P > 0.10$). In no case was clutch initiation date, year, or interaction terms found to be significant so final models included only egg position as an explanatory variable for eggshell colour.

I used repeatability analyses (Lessels and Boag 1987) to assess the consistency of egg colour within the clutches (n = 29 in 2011, n = 30 in 2012) relative to among-clutch variability, and whether average egg colour was consistent within individual females between first and second clutches (n = 9) laid in the same year, and between first clutches laid in different years (n = 12). Finally, I compared the average colour of first and second clutches laid in the same year using a paired *t*-test to determine if eggshell colour differed between first and second clutches of individual females.

I examined the relationship between the average colour of first clutches (n = 29 in 2011, n = 30 in 2012) and female traits and investment in eggs using linear mixed-effects models. I included clutch initiation date as a proxy for female age and quality (Ardia 2005; O'Brien and Dawson 2013), female feather colour (PC1), and average egg mass as a measure of investment in eggs. Sample sizes were smaller for models that included feather colour as not all females were captured (n = 24 in 2011, n = 24 in 2012). The reproductive patterns for first clutches were different between the two years that egg colour data were collected, likely due to different spring temperature and rainfall patterns. In 2011, clutch initiation dates were earlier (3.9 days ± 0.8 SE, $t_{165} = -4.9$, $P < 0.001$) and clutch sizes were larger (0.29 eggs ±

0.11 SE, $t_{152} = 2.62$, $P = 0.01$). Also, the relationship between clutch initiation date and clutch size was different between the two years (negatively correlated in 2011: n = 79, $r_s = -0.31$, $P = 0.006$, and unrelated in 2012: n = 75, $r_s = -0.09$, $P = 0.45$). Therefore, I performed analyses of egg colour and reproductive performance separately for each year. Data from one female were collected in both years, but as years were not pooled, both measures were included. I also used linear mixed-effects models to assess the relationship between eggshell colour and relative yolk volume for a subset of later-laid clutches (CID: June 17th to June 30th) in 2012 (n = 12). Some of these nests were second nests (n = 7), some were renests after a failed first clutch (n = 3) and some were late first clutches (n = 2); therefore, the nest attempt type was included as a fixed factor as well as clutch initiation date as a covariate. For both analyses, I included clutch identity as a random factor and egg number nested in clutch identity as a repeated measure to account for the non-independence and non-random distribution of eggs within nests, and estimated degrees of freedom using the containment method (Schaalje et al. 2001). Initial models included all covariates, and I used a backwards stepwise approach to remove terms that did not approach significance ($P > 0.10$).

Linear mixed-effects models were performed using SAS 9.3 (SAS Institute Inc.) and all other analyses were completed using SPSS version 20 (IBM Corp 2011), I present means ± 1 standard error, and considered results significant at the $P \leq 0.05$ level.

2.4 Results

Eggshell colour generally changed both within clutches through the laying sequence and between first and second nests (Fig. 2.1 & 2.2). For within-clutch colour variation, I found that the position an egg occurred in the laying sequence had a significant effect on the blue-green chroma (5 egg clutches: $F_{2.87,74.7} = 5.10$, $P = 0.003$; 6 egg clutches: $F_{5,60} = 5.86$, P

< 0.0001) and brightness (5 egg clutches: $F_{4,104} = 7.47$, $P < 0.0001$; 6 egg clutches: $F_{2.77,54.6} = 4.87$, $P = 0.008$) of the eggshell. First-laid eggs had the highest blue-green chroma, eggs laid in the middle of the sequence had the lowest, and last-laid eggs showed a slight increase in blue-green chroma relative to mid-sequence eggs (Fig. 2.1C). Brightness of eggshells, which was negatively correlated with blue-green chroma, followed a similar but inverse trend (Fig. 2.1A). In six-egg clutches there was both a significant effect of laying sequence on eggshell UV-chroma ($F_{5,60} = 2.36$, $P = 0.05$), with mid-sequence eggs having higher values (Fig. 2.1D), and a trend for eggs at the beginning and end of the laying sequence to have longer (more green) hues relative to mid-sequence eggs ($F_{5,60} = 2.13$, $P = 0.07$; Fig. 2.1B), but neither of these patterns were found for five egg clutches (UV-chroma: $F_{4,104} = 1.14$, $P = 0.34$; hue: $F_{2.10,54.6} = 0.44$, $P = 0.66$; Fig. 2.1B & D). In addition, I found a significant difference between the average colour of first and second nests for all chromatic colour measures (Fig. 2.2). Eggs from first clutches had significantly longer wavelength (more green) hue values ($t_9 = 3.06$, $P = 0.01$; Fig. 2.2B), higher blue-green chroma ($t_9 = 3.82$, $P = 0.004$; Fig. 2.2C), and lower UV chroma ($t_9 = -2.59$, $P = 0.03$; Fig. 2.2D) than second clutches, but there was no significant difference in the brightness of eggshells between first and second clutches ($t_9 = -0.41$, $P = 0.69$; Fig. 2.2A).

Eggshell blue-green chroma and brightness were consistent within clutches and between the first nests of individual females in different years, but were not repeatable between first and second clutches in the same year (Table 2.1). Ultraviolet chroma and hue were also consistent within clutches, but were not as repeatable as blue-green chroma and brightness based on r values, and were not repeatable between first and second nests or clutches laid by the same female in two different years (all P-values > 0.14; Table 2.1). Overall, within-clutch colour was more consistent than among-clutch colour, and eggshell

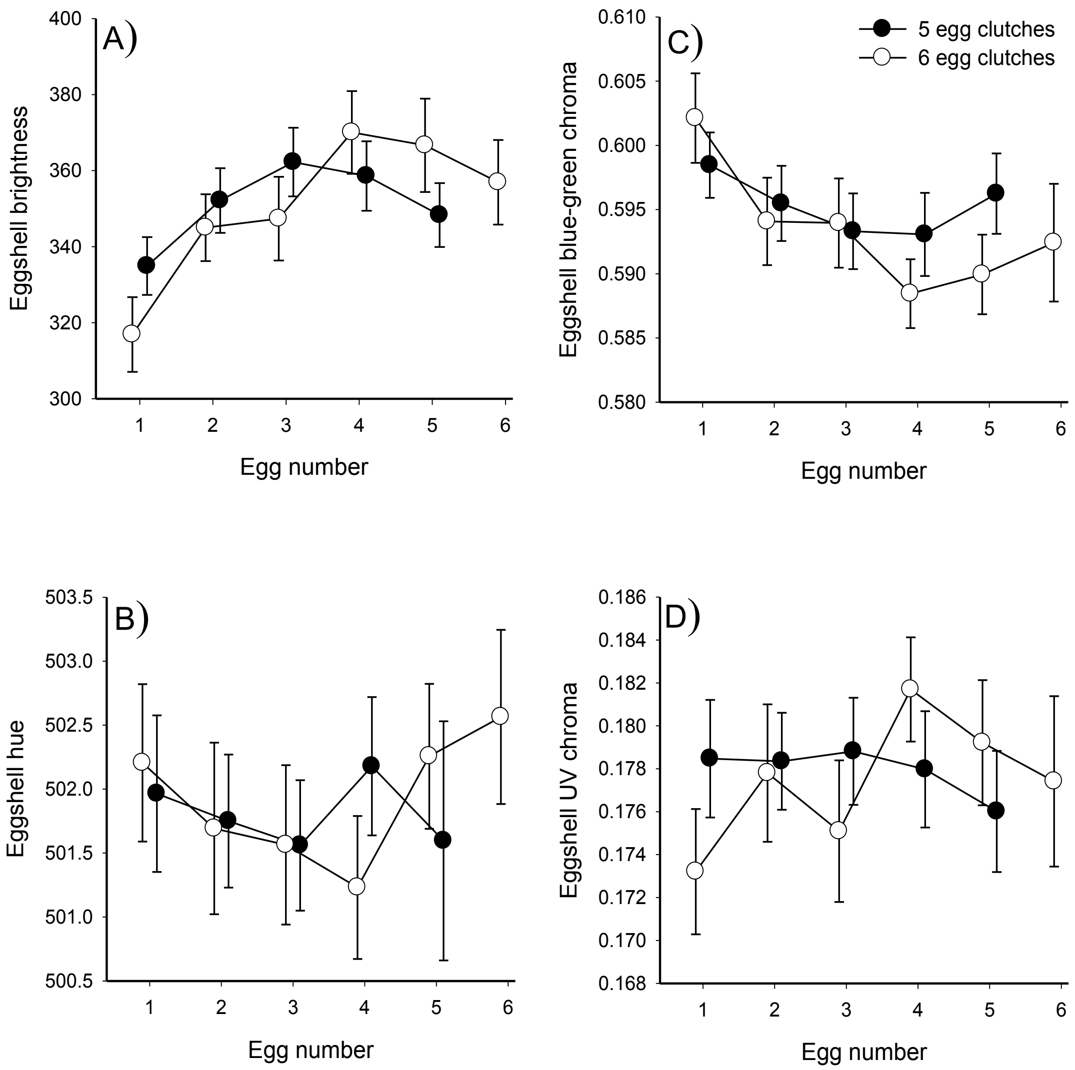

Figure 2.1. The mean (± SE) A) brightness, B) hue, C) blue-green chroma, and D) ultraviolet (UV) chroma of eggshells through the laying sequence for mountain bluebirds. Sample sizes were 28 five-egg clutches and 13 six-egg clutches. See Methods for details of the calculation of eggshell colour variables.

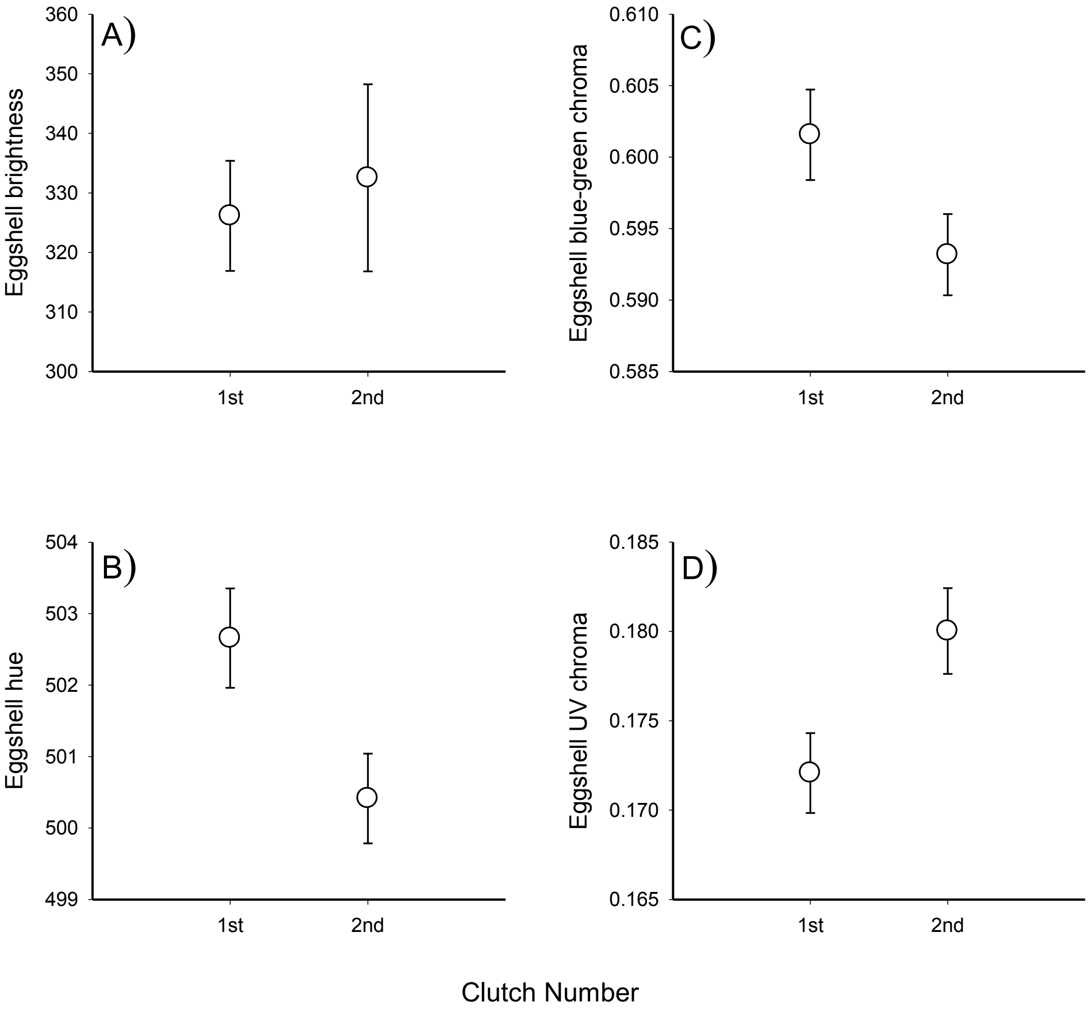

Figure 2.2. Mean (± SE) A) brightness, B) hue, C) blue-green chroma, D) and ultraviolet (UV) chroma of eggs within clutches of mountain bluebirds in first and second breeding attempts within the same season (n = 10 females). See Methods for details of the calculation of eggshell colour variables.

Table 2.1. Results of repeatability analyses of eggshell colour within clutches, average colour of first and second clutches in the same year, and first breeding attempts between years (2011 and 2012) for individual female mountain bluebirds. See Methods for details of the calculation of colour variables and repeatability analyses.

Comparison	**Variable**	**Repeatability**	***F***	***df***	***P***
Within Clutches (n = 59)	Brightness	0.68	12.6	58,238	<0.001
	Blue-green Chroma	0.81	23.8	58,238	<0.001
	UV Chroma	0.33	3.66	58,238	<0.001
	Hue	0.51	6.61	58,238	<0.001
Between First and Second Clutches (n = 10)	Brightness	0.29	1.83	9,10	0.18
	Blue-green Chroma	0.47	1.57	9,10	0.07
	UV Chroma	-0.10	0.82	9,10	0.62
	Hue	0.10	1.23	9,10	0.37
Between Years (n = 14)	Brightness	0.81	9.45	11,12	<0.001
	Blue-green Chroma	0.69	5.51	11,12	0.003
	UV Chroma	0.32	1.92	11,12	0.14
	Hue	0.08	1.17	11,12	0.39

colour was repeatable for most measures of colour for first clutches between years, but did not show high similarity between first and second clutches of individual females in the same year. Natural variation in eggshell colour was not consistently related to timing of breeding, investment in eggs, or female traits. In 2012, clutch brightness increased with later clutch initiation dates ($F_{1,114} = 6.13$, $P = 0.01$; Fig. 2.3A), and in 2011 females with brighter, more UV-blue feathers (high PC1) laid clutches of eggs with higher blue-green chroma ($F_{1,98} = 4.11$, $P = 0.04$; Fig. 2.4C), but neither clutch initiation date nor female feather colour were related to any other colour variable (all P-values > 0.18; Fig 2.3 & 2.4). Additionally, egg mass was negatively related to brightness in both years (2011: $F_{1,121} = 4.42$, $P = 0.04$; 2012: $F_{1,114} = 4.15$, $P = 0.04$; Fig. 2.5 A) and there was a trend in 2012 for heavier eggs to have lower UV chroma ($F_{1,115} = 3.34$, $P = 0.07$; Fig. 2.5 D). Egg mass was not related to UV chroma in 2011, or to blue-green chroma and hue in either year (all P-values > 0.20; Fig 2.5).

Eggs with lower brightness ($F_{1,34} = 7.05$, $P = 0.01$; Fig. 2.6 A) and UV chroma ($F_{1,34} = 7.18$, $P = 0.01$; Fig. 2.6 D) and higher blue-green chroma ($F_{1,34} = 10.33$, $P = 0.003$; Fig. 2.6 C) had larger relative yolk volumes, but relative yolk volume was not related to eggshell hue ($F_{1,34} = 0.66$, $P = 0.42$; Fig 2.6 B). Relative yolk volume was also significantly related to clutch initiation date in all models (all P-values < 0.03), decreasing with later clutch initiation dates (Fig. 2.7). The nest attempt type (first, second, renest) did not have an effect on relative yolk volume in any model (all P-values > 0.79).

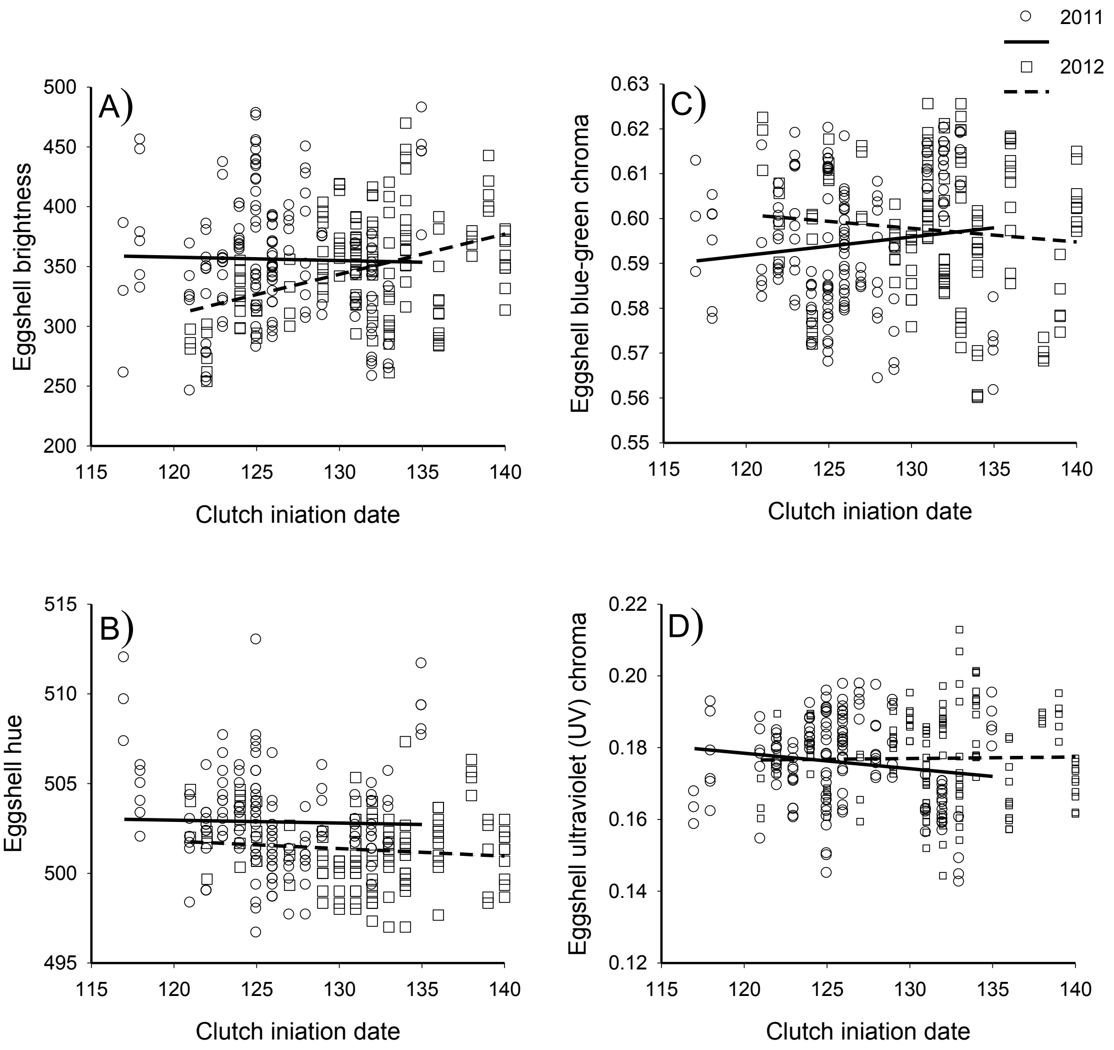

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Figure 2.3. Relationship between clutch initiation date and eggshell A) brightness, B) hue, C) blue-green chroma, and D) ultraviolet (UV) chroma of mountain bluebirds in 2011 (n = 151 eggs from 29 clutches) and 2012 (n = 146 eggs from 30 clutches). See Methods for details of the calculation of eggshell colour variables.

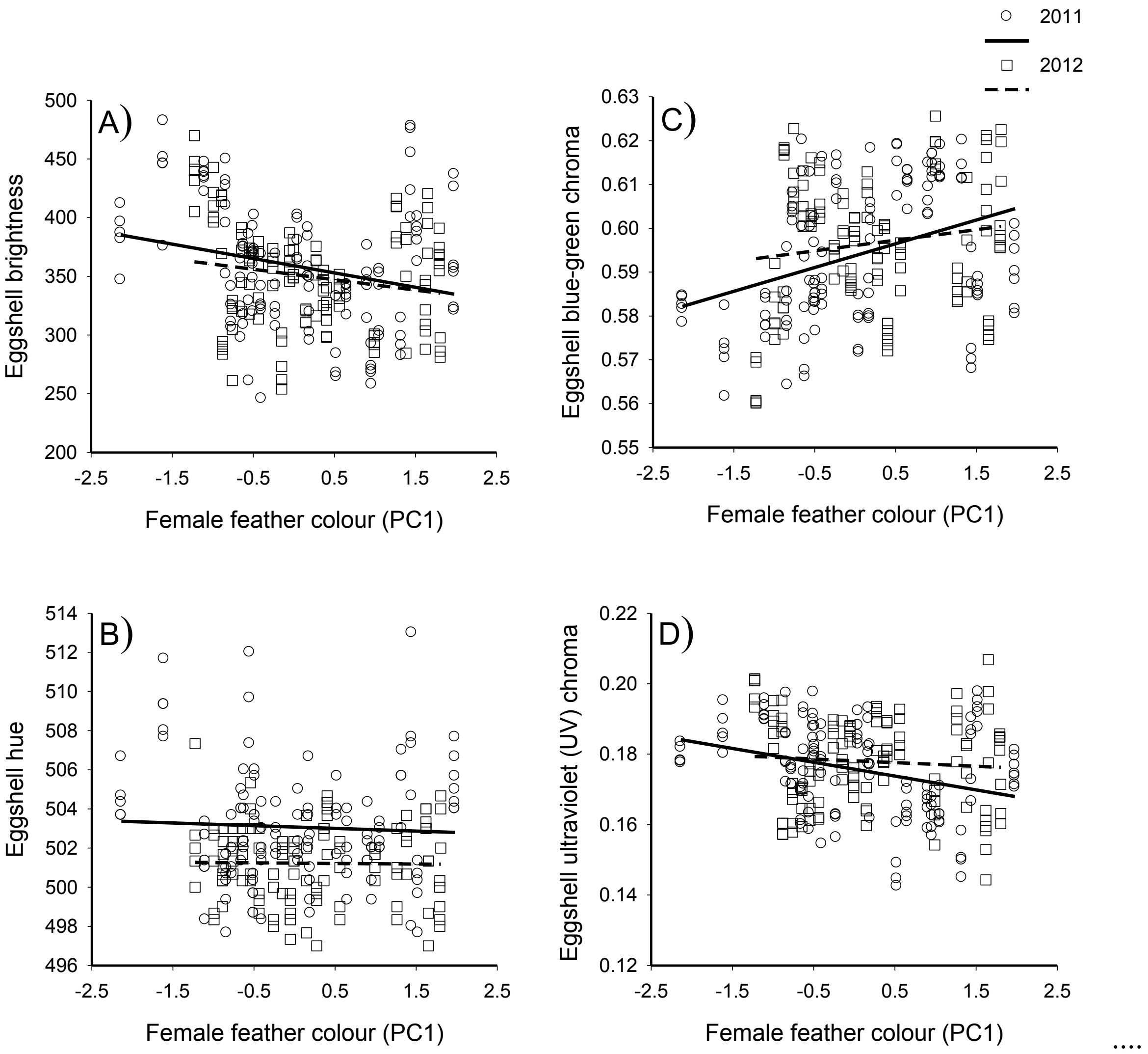

Figure 2.4. Relationship between female feather colour (PC1) and eggshell A) brightness, B) hue, C) blue-green chroma, and D) ultraviolet (UV) chroma of mountain bluebirds in 2011 (n = 122 eggs from 24 clutches) and 2012 (n = 117 eggs from 24 clutches). See Methods for details of the calculation of colour variables for eggshells and female feathers.

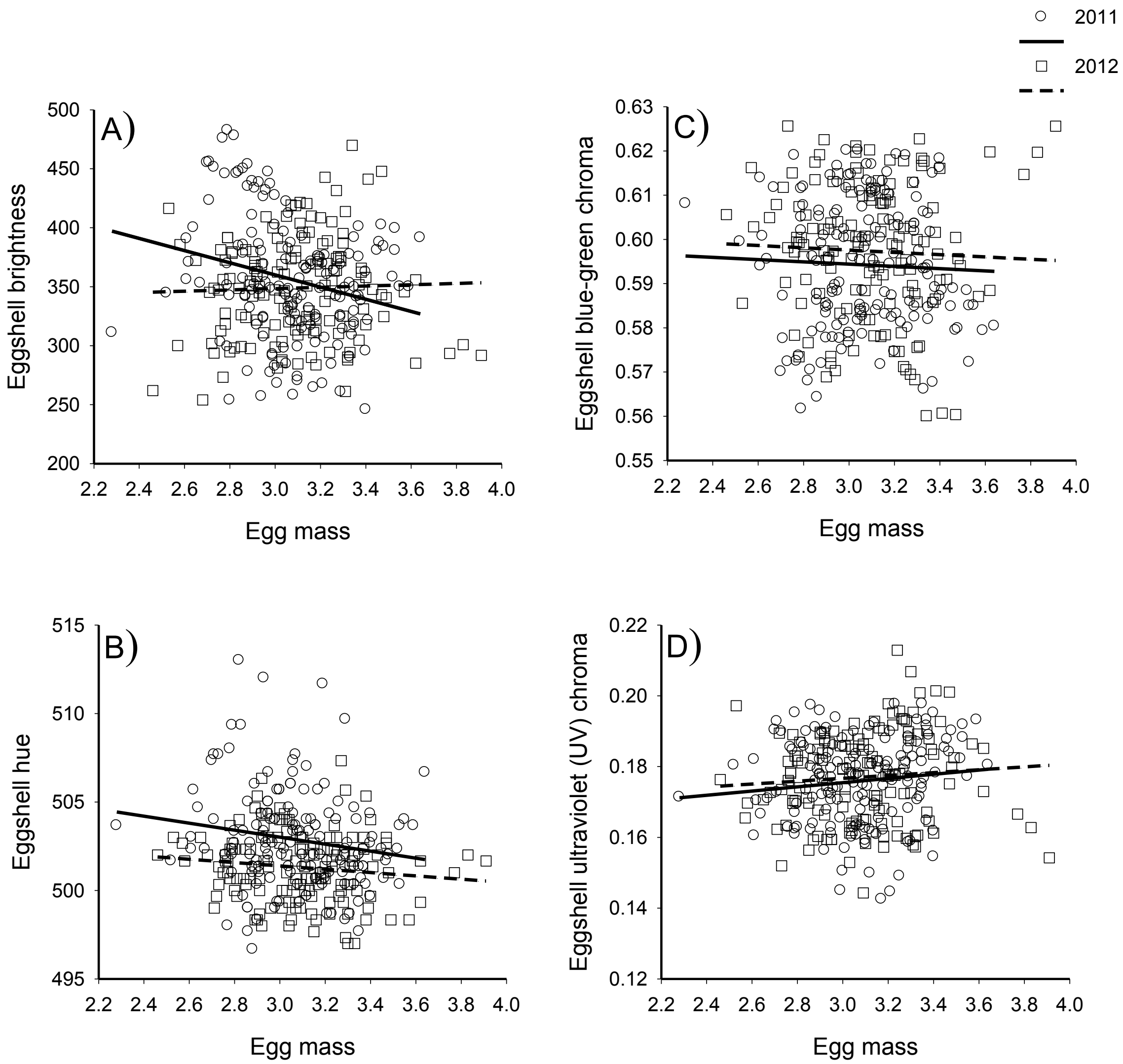

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Figure 2.5. Relationship between egg mass and eggshell A) brightness, B) hue, C) blue-green chroma, and D) ultraviolet (UV) chroma of mountain bluebirds in 2011 (n = 151 eggs from 29 clutches) and 2012 (n = 146 eggs from 30 clutches). See Methods for details of the calculation of eggshell colour variables.

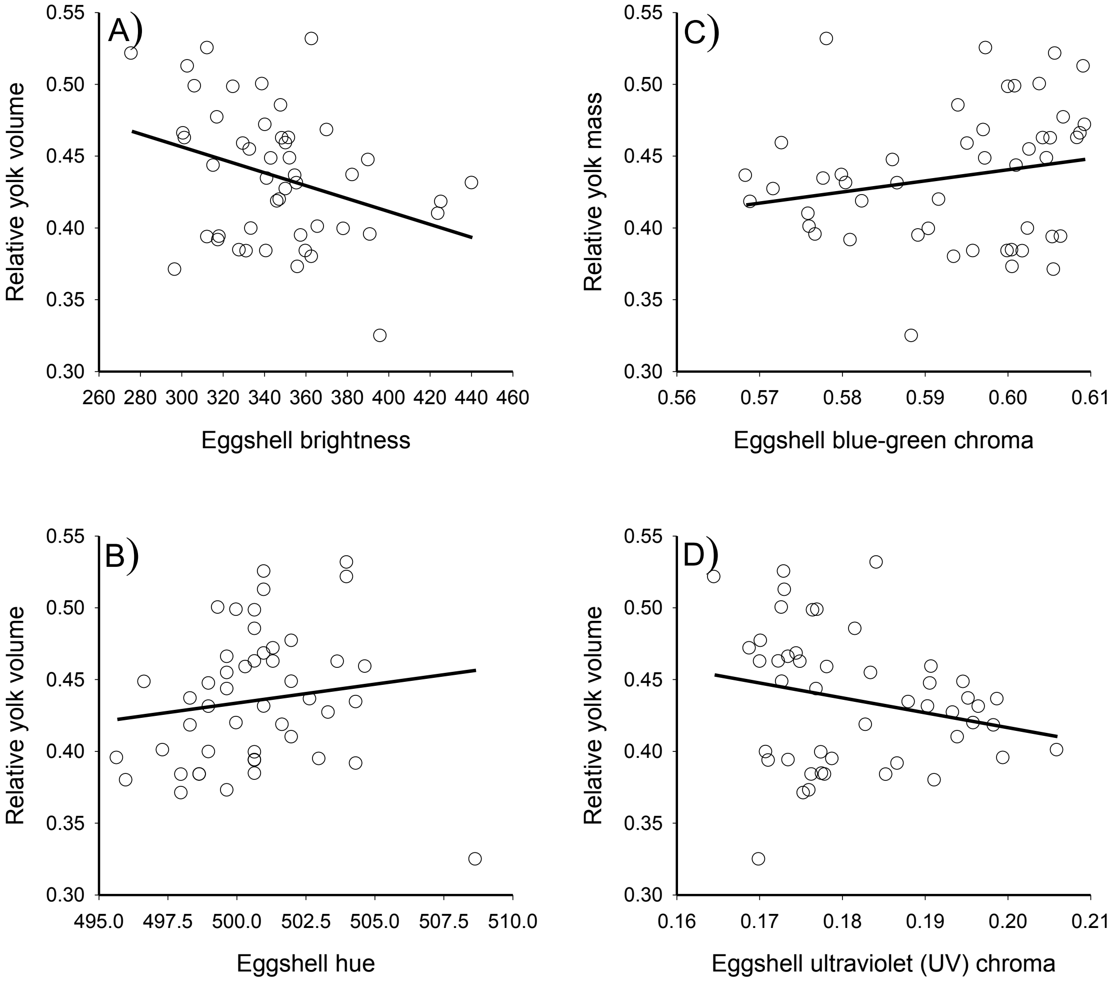

Figure 2.6. Relationship between relative yolk volume and eggshell A) brightness, B) hue, C) blue-green chroma, and D) ultraviolet (UV) chroma of mountain bluebirds measured in 2012 (n = 48 eggs from 12 clutches). See Methods for details of the calculation of eggshell colour variables and relative yolk volume.

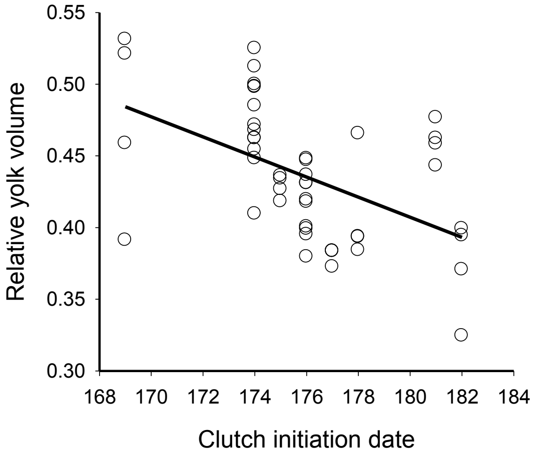

Figure 2.7. Relationship between clutch initiation date and the relative yolk volume of the eggs of mountain bluebirds measured in 2012 (n = 48 eggs from12 clutches). See Methods for details of the calculation of relative yolk volume.

2.5 Discussion

The patterns of variation I observed within clutches, and between first and second nests within a season, suggest that biliverdin, the pigment used to colour eggshells of mountain bluebirds, may be limited. Within-clutches, blue-green chroma decreased in a non-linear fashion after the first-laid egg, indicating a reduction in the pigmentation of later laid eggs if eggshell colour accurately reflects the quantity of pigments deposited in eggshells (Moreno et al. 2006a; López-Rull et al. 2008: but see Cassey et al. 2012). Mid-sequence eggs were the least saturated in colour but there was a slight, though not statistically significant, increase in the blue-green chroma of the last-laid egg (Fig. 2.1). A similar non-linear decrease in the blue-green chroma of eggshells was found in both pied (*Ficedula hypoleuca*; Moreno et al. 2005) and collared flycatchers (Krist and Grim 2007). A decrease in pigmentation was also found between the first and second eggs of blue-footed boobies (*Sula nebouxii*; Morales et al. 2011), a seabird which generally lays only two eggs. However, these results contradict the findings of three other studies that have found an increase in the pigmentation of later laid eggs in eastern bluebirds (*Sialia sialis*; Siefferman et al. 2006), collared flycatchers (Hargitai et al. 2008), and ring-billed gulls (*Larus delawarensis*; Hanley and Doucet. 2009). Furthermore, a clear pattern was not found for all colour metrics. Brightness of eggshells followed an inverse pattern to blue-green chroma, with the brightest eggs occurring mid-sequence as would be expected if pigments are limited, given that brightness is negatively correlated with blue-green chroma. However, the UV chroma and hue of eggshells were only related to laying sequence in six-egg clutches, where mid-sequence eggs had higher UV-chroma and reflected maximally at shorter wavelengths.

The results of comparisons between first and second nests also indicated that eggshell pigments may be limited. Average clutch blue-green chroma was significantly lower in second nests compared to first nests, UV chroma was higher, and average hue values were shorter (blue-shifted) (Fig. 2.2). However, average clutch brightness did not change between first and second clutches (Fig. 2.2). The lower levels of pigments could be the result of pigment depletion from having laid a first clutch of eggs, stress from having incubated and fed nestlings, changing environmental conditions later in the breeding season, or a combination of these factors. Replacement clutches of great reed warblers (*Acrocephalus arundinaceus*) contained brighter eggs (lower pigmentation) than first clutches (Honza et al. 2012), suggesting that laying more eggs alone without the stress of brood rearing is enough to cause pigmentation to decline. Another possibility is that the later initiation dates associated with replacement clutches and second nests result in lower levels of investment due to the declining reproductive value of the offspring (Drent and Daan 1980).

Eggshell colour, particularly blue-green chroma, was consistent both within clutches and between first clutches of the same female laid in different years. The repeatability of eggshell colouration indicates that there may be a genetic basis for eggshell colouration, as found in previous studies (Collias 1993; Morales et al. 2010a) and also suggested by observations that female mountain bluebirds that lay white eggs will do so consistently throughout their lifetime (O'Brien and Dawson, unpublished data). However, I found that repeatability of eggshell colour was higher (for all metrics) within-clutches than between breeding attempts (Table 2.1), with eggshell colour being particularly inconsistent between first and second clutches in the same breeding season. These findings, along with results showing that colour is more consistent within than among clutches (Moreno et al. 2004; Seifferman et al. 2006; Krist and Grim 2007; Soler et al. 2008; Hanley and Doucet 2009; Morales et al. 2011; Honza et al. 2012), suggest that variation among clutches is influenced both by female identity (e.g., Dearborn et al. 2012) and by environmental conditions during a given breeding attempt, such as food availability and individual condition (Honza et al. 2012).

I found that eggshell colour was related to female traits, but not consistently. In 2012, clutch brightness increased with later clutch initiation dates (Fig. 2.3), suggesting a decrease in eggshell pigmentation with later clutch initiation dates, as would be predicted if eggshell colour is a signal of female quality. A similar seasonal pattern was found in the eggs of great reed warblers which also lay brighter eggs later in the season (Honza et al. 2012), but other studies have shown either no relationship between egg colour and clutch initiation date (Siefferman et al. 2006) or an increase in blue-green chroma later in the season (Hargitai et al. 2008). I also found that in 2011 female birds with bluer rump feathers laid eggs with higher blue-green chroma. These results are intriguing, because there is evidence that structural plumage is condition dependent (McGraw 2002) and there is some evidence that female plumage colour may be sexually selected in mountain bluebirds (Morrison et al. 2014; but see Balenger et al. 2007), and a closely related species, eastern bluebirds (Siefferman and Hill 2005a). A relationship between feather colour and egg colour could represent a meaningful multicomponent signal, as the traits are produced at different stages of the annual cycle.

There was a relationship between several measures of eggshell colour and egg mass, which is often used as a measure of investment in eggs (Bernardo 1996), particularly in birds (Wiebe and Bortolotti 1995; Rutkowska and Cichoń 2002), as well as relative yolk volume, which is also an established measure of egg quality (Ardia et al. 2006). In both study years eggs with brighter shells weighed less (Fig. 2.5). Egg mass was not significantly related to other measures of eggshell colour in either year, but there was a trend in 2012 for eggs with higher UV chroma to be lighter. Additionally, relative yolk volume was strongly positively related to eggshell blue-green chroma and negatively related to brightness and UV chroma (Fig. 2.6) as would be predicted if eggshell colour saturation advertised female investment in eggs. Moreno et al. (2006a) found that food supplementation resulted in eggs that were both heavier and had higher blue-green chroma, but two other studies of passerines found that egg mass correlated very weakly (Cassey et al. 2008), or not at all, with eggshell colour (Hargitai et al. 2008). More convincing relationships have been found between the colour of eggshells and the composition of eggs such as concentrations of antibodies (Morales et al. 2006) and carotenoids of yolks (Navarro et al. 2011; but see Cassey et al. 2008), suggesting that egg colour may be more specifically related to maternal investment in yolk than overall egg mass and egg size, which is often relatively consistent within individuals (Christians 2002).

2.6 Conclusion

The patterns of natural egg colour variation in mountain bluebirds suggest that blue-green egg colour has the potential to function as a signal of female quality. The within-clutch decline in pigment-associated colour through the laying sequence and the change in colour between first and second nests imply that the pigments responsible for blue-green eggshell colouration are limited. In addition, the relative repeatability of eggshell colour within and between clutches of individual females suggests that eggshell colour has both an intrinsic aspect and is influenced by current conditions. However, the information content of egg colour is still unclear. Females that initiated clutches earlier and had bluer rump feathers laid eggs with more saturated colour, but these findings were not consistent between seasons or among colour metrics. Nevertheless, eggshell colour does seem to reflect investment in eggs. Egg mass was negatively related to eggshell brightness, and relative yolk volume was positively related to eggshell blue-green saturation. Further work is needed to determine if egg colour is merely a by-product of physiological processes or an informative signal shaped by sexual selection.

3. An experimental test of the effect of food and antioxidants on blue-green eggshell colouration in mountain bluebirds (*Sialia currucoides*).

3.1 Abstract

Evolutionary biologists have long debated the functional relevance of the ornamental traits that occur in many animal taxa; however, female ornaments have received relatively little attention compared to those produced by males. A greater understanding of female traits, particularly those that are not analogous to male traits, may shed light on the potential for sexual selection to shape female phenotypes. Recently, the blue-green eggshell colour derived from the antioxidant pigment, biliverdin, has been proposed as a candidate trait that advertises female quality to males in species of birds with biparental care. However, studies have been equivocal in their support for blue-green eggshell colour as an informative signal, and correlations between eggshell colour and other female characteristics have been inconsistent. I conducted a supplementation experiment to test if improving the access of female birds to food resources and micronutrients, thereby improving their condition prior to egg laying, would intensify the blue-green colouration of the eggs they laid. I provided mountain bluebirds (*Sialia currucoides*) with supplemental food and carotenoids during nest building and egg laying in two breeding seasons, and assessed both within- and among-clutch variation in colour. Supplementation did not affect patterns of within-clutch variation, but did result in differences in colour among clutches. Specifically, I found that food, but not carotenoid, supplementation resulted in higher colour saturation, and decreased the brightness of blue-green eggshells. Although this trend was observed in both years, the effect was statistically significant only in one year. My results suggest that food availability influences eggshell colour variation among clutches and that conditions, such as weather and

food availability, which vary between years, may also determine how female birds allocate pigment to eggshells.

3.2 Introduction

Sexually selected traits are thought to function as honest advertisements of quality, in part because they are costly to produce and maintain (Zahavi 1975). The expression of sexually selected ornaments is thought to depend on individual condition (Andersson 1986; Morehouse 2014; but see Cotton et al. 2004) and cyclically produced ornaments advertise the state of the organism when the trait was produced (e.g., Fairhurst et al. 2014). Recently, the blue-green eggshell colouration of some avian species has been proposed as a sexually selected trait that reflects quality and condition of females during egg laying (Moreno and Oserno 2003) and it may be linked to health and oxidative condition. Blue-green egg colour is the result of the deposition of biliverdin into eggshells during laying (Kennedy and Vevers 1976; Míšík et al. 1996). Biliverdin is a metabolically produced antioxidant pigment that functions as an important cytoprotectant (Stocker et al. 1990; Barañano et al. 2002), and given its dual role as both antioxidant and pigment, there is a strong theoretical basis for the possibility that blue-green eggshell colour is an indicator of female condition. If eggshell colour reflects female state, then it should be positively related to female condition and antioxidant status prior to egg laying, but support for this hypothesis has been inconsistent. Some observational and experimental studies have detected positive associations between blue-green eggshell colour and the antioxidant status of female birds (Hanley et al. 2008; Morales et al. 2011), while others have found dietary antioxidants to be relatively unimportant determinants of eggshell colour (Dearborn et al. 2012). In addition, eggshell pigmentation has been found to be related to female condition (Moreno et al. 2005;

Siefferman et al. 2006; Krist and Grim 2007), but none of these studies measured condition prior to egg laying and such findings have not been consistently supported even in closely related species (Cassey et al. 2008; Hargitai et al. 2008; López-Rull et al. 2008; Johnsen et al. 2011). Experimental manipulations of either resource availability or workload have proved more decisive; resource enhancement increased eggshell pigmentation and increased workload decreased pigmentation (Moreno et al. 2006a; Soler et al. 2008). Nonetheless, further research, particularly experimental studies, are needed to confirm the relevance of sexual selection as a general explanation of blue-green eggshell colour in birds.

I investigated the effect of food and micronutrient supplementation on both within- and among-clutch variation in colour of eggshells of mountain bluebirds (*Sialia currucoides*). To separate the effects of food and micronutrients, I supplemented one treatment group with food only, and a second group with both food and carotenoids, which are dietary antioxidants that have been shown to play an important role in the production of ornaments (McGraw and Ardia 2003). Both groups were supplemented prior to laying until clutch completion. I predicted that food supplementation would improve female nutritional state and lower oxidative stress prior to egg laying (Fletcher et al. 2012; Giordano et al. 2015). I expected that if eggshell colour is condition dependent, and the saturation of blue-green eggshell colour accurately reflects biliverdin concentration in eggshells, as some studies suggest (Moreno et al. 2006a; but see Cassey et al. 2008), food-supplemented females would lay clutches that were more saturated in blue-green colour than unsupplemented females. I expected that carotenoid supplementation, in addition to food, would increase levels of circulating antioxidants and that individuals who received the combined treatment of food and carotenoids would lay clutches containing the most highly pigmented eggs. I also expected that supplementation of both types would mitigate the within-clutch decline in pigmentation that is observed in natural clutches (see Chapter 2).

3.3 Methods

3.3.1 Study site, species, and general field procedures

I studied mountain bluebirds breeding in nest boxes (84 pairs) southwest of Williams Lake, BC, Canada (51°N, 122°W) from mid-April to early August in 2011 and 2012. The habitat was arid open grassland with scattered patches of Douglas-fir (*Psuedostuga menziesii*). The study population returns to this site in early to mid-March and begins laying eggs in late April. Mountain bluebirds are medium-sized (~30 g), sexually dimorphic, socially monogamous passerines with biparental care (Power and Lombardo 1996). Females construct the nests and incubate the eggs (~13 days), and both males and females provision young during brood rearing (Power and Lombardo 1996). Clutch sizes range between two and seven eggs, but clutches of five or six eggs are most common at this site (O'Brien and Dawson 2013). Mountain bluebirds are facultatively double brooded, with a portion of the population initiating a second clutch after raising a successful first brood (up to 40%; O'Brien and Dawson 2013). Most clutches contain blue-green eggs, ranging from pale blue to nearly turquoise, although white eggs also occur rarely (from 2 to 9%; Peak 2011).

Starting in mid-April, I checked nest boxes daily to monitor nest building, begin the supplementation experiment (see section 3.3.2 for details), and determine date of clutch initiation. I weighed each egg using a portable balance (nearest 0.01g) on the day it was laid. I measured the colour of complete clutches of eggs (see section 3.3.3 for details) in late incubation (day 9-10) and then returned to check for hatch on day 12 of incubation. I captured adult females (n = 34 in 2011, n = 41 in 2012) while they were feeding offspring to collect rump feathers for spectral analysis (see section 3.3.3 for details) and measure physical characteristics. I weighed birds using a spring balance (nearest 0.25 g), measured the lengths of the wing and 8th primary flight feather with a ruler (nearest 0.5 mm), and measured length of tarsus and the combined head and bill length using digital calipers (nearest 0.1 mm). I banded all captured females with an individual-specific combination of a numbered aluminum band and three coloured plastic leg bands for identification.

3.3.2 Experimental design

When females had accumulated sufficient material in nest boxes and began to form the nest cup, I installed a feeding tray near the nest box, and sequentially assigned each female to one of three treatment groups (Fed, Fed/Carotenoid, and Control) after determining the treatment order randomly. To test how the quantity and quality of food available during pre-breeding and egg laying affected eggshell colour, I provided supplemental food until the start of incubation to one group of birds (Fed), food and carotenoids to a second group (Fed/Carotenoid), and a third unsupplemented group acted as controls. Fed treatment pairs (n = 13 in 2011, n = 14 in 2012) received 10 grams of mealworms (*Tenebrio molitor*) and 2 grams of waxworms (*Galleria mellonella*) daily (approximately 50% of an adult mountain bluebird's nutritional requirement during incubation and brood rearing; Mock 1991; Merkle and Barclay 1996), while birds in the carotenoid treatment (n = 8 in 2011, n = 16 in 2012) received the same amount of food plus 2.6 mg of carotenoid powder (see O'Brien and Dawson 2008). For the carotenoid treatment, commercially available carotenoid supplements containing 5% lutein and 0.2% zeaxanthin (Lutein-Force, Prairie Naturals) were crushed into a fine powder, then mixed with a small amount of liquid gelatin and coated onto five waxworms. To control for potential effects of the gelatin, I also coated five waxworms in the fed treatment with gelatin. I installed feeding trays at control nests (n = 19 in 2011, n = 23 in

2012) and visited them daily but did not provide them with any supplemental food. I monitored supplemented nests from a distance to confirm that resident pairs were consuming the food and carotenoids. I used an open feeding tray in 2011 and excluded 13 supplemented nests from the study because common ravens (*Corvus corax*) also were observed consuming the food. In 2012, I used covered feeders designed to keep out larger birds, which effectively excluded ravens while still allowing resident pairs access to the food.

3.3.3 Colour quantification

I measured the eggshell colour of complete clutches across the range of wavelengths visible to songbirds (300-700 nm) using an Ocean Optics USB2000 spectrometer (Dunedin, FL) with a deuterium tungsten halogen light source (Avantes, Broomfield, CO). I took three measurements of reflectance for each egg, relative to a white standard, in a dark environment to eliminate ambient light. I calculated from each individual measure the values for brightness (total light reflectance from 300-700 nm), hue (peak wavelength), and blue-green (relative reflectance from 400-605 nm) and ultraviolet (UV) chroma (relative reflectance from 300-400 nm), using the package pavo (Maia et al. 2013) in R 3.1.2 (R Core Team, 2014), and then averaged the values for each egg. Blue-green chroma corresponds with the region of least absorbance, and therefore maximal reflectance by biliverdin, making it the measure most directly linked to eggshell pigmentation (Falchuk et al. 2002). Eggshell blue-green chroma was negatively correlated with UV chroma ($n = 63$, $r = -0.84$, $P < 0.001$) and brightness ($n = 63$, $r = -0.77$, $P < 0.001$), while UV chroma and brightness were positively related ($n = 63$, $r = 0.63$, $P < 0.001$). Eggshell hue was negatively correlated with UV chroma ($n = 63$, $r_s = 0.02$, $P < 0.01$), but unrelated to blue-green chroma ($n = 63$, $r_s = 0.02$, $P = 0.88$) or brightness ($n = 63$, $r_s = -0.14$, $P = 0.27$). I chose to perform analyses on all colour metrics rather than a single metric, such as the first component from a principal components analysis, as eggshell colour is the focus of this work and I wished to have a complete understanding of which measures were potentially affected by food rather than using a summary composite measure.

To quantify plumage colour of females, I mounted eight feathers on a non-reflective black background in an overlapping position to mimic their natural positioning on the body, and measured their spectral properties (reflectance) from 300-700 nm (see O'Brien and Dawson 2011). I took measurements in three randomly selected places at the distal tip of each feather, calculated brightness, hue, and UV-blue chroma (relative reflectance from 300-510 nm) for each reading using the package pavo (Maia et al. 2013) in R 3.1.2 (R Core Team, 2014). I then averaged the colour metrics from the three readings for each bird. I quantified UV-blue chroma because the feathers of mountain bluebirds reflect maximally in this region (Balenger and Johnson 2009; O'Brien and Dawson 2011). I used principal components analysis to reduce the three feather colour variables to a smaller set of components, as the individual colour metrics were moderately to highly correlated. The first component (PC1) explained 69.6% of the variation in feather colour. Brightness loaded positively (0.62) with PC1 as did UV-blue chroma (0.96), while the loading value for hue was negative (-0.89). Feathers with high PC1 scores were therefore bright with high UV-blue chroma and maximum reflectance at shorter wavelengths (more blue).

3.3.4 Statistical Analyses

I used linear mixed-effects models to assess if the colour of eggshells was affected by the position of eggs within the laying sequence. I predicted that egg colour would decline as a function of laying order in clutches laid by unsupplemented females (see Chapter 2), but that food supplementation would alleviate this decline. For these analyses, I used only complete clutches where colour was measured for all eggs (control nests: n = 11 in 2011, n = 15 in 2012; food supplemented: n = 12 in 2011, n = 13 in 2012; food and carotenoid supplemented: n = 6 in 2011, n = 8 in 2012). Clutch colour changes between first and second clutches laid by the same female (see Chapter 2), so I only included first clutches in analyses. I also only analyzed clutches that contained five or six eggs, as other clutch sizes were uncommon in this population. To permit data from five and six egg clutches to be analyzed together, I grouped eggs by their relative laying order (see Krist and Grim 2007). I classified eggs as occurring either early (egg 1 in 5 egg clutches, egg 1-2 in 6 egg clutches), mid (egg 2-3 in 5 egg clutches, egg 3-4 in 6 egg clutches), or late (egg 4-5 in 5 egg clutches, egg 5-6 in 6 egg clutches) in egg laying. Initial models included clutch identity as a random factor to control for the non-independence of eggs within a nest, relative position of eggs within the laying sequence, treatment, and clutch size as fixed factors, clutch initiation date as a covariate, and all two-way interactions between egg position and other explanatory variables in the model. I used a backwards stepwise approach to remove terms that did not approach significance ($P > 0.10$), except egg position, treatment, and the interaction of treatment and egg position which were retained in final models as they were the variables of interest.

I also investigated the effect of the food and carotenoid supplementation experiment on the average shell colour of eggs laid in first clutches using analysis of covariance. I started with a model that included treatment as a fixed factor, with clutch initiation date, average egg mass, and female feather colour (PC1) as covariates, and all first order interactions between treatment and the covariates. Sample sizes for analyses that included female feather colour were reduced, as not all females were captured (fed: n = 11 in 2011, n= 12 in 2012; carotenoid: n = 6 in 2011, n = 12 in 2012; control: n = 17 in 2011, n = 17 in 2012). I removed terms that did not approach significance ($P < 0.1$) in a backward stepwise fashion, but treatment was always retained in the final model as it was the variable of interest. In addition to testing the effect of food supplementation on average eggshell colour, I also investigated the effect of treatment on other reproductive variables including clutch initiation date, clutch size, and average egg mass using one-way analysis of variance.

All analyses were performed using SPSS version 20 (IBM Corp 2011), I present means ± 1 standard error, and considered results significant at the $P \leq 0.05$ level. Post-hoc tests were conducted when appropriate using a Bonferroni correction for multiple comparisons. As reproductive patterns were different between the two study years (see Chapter 2), I conducted all analyses of the effects of treatment on egg colour and reproduction separately by year. Eight females were measured in both years, but as years were not pooled, data from both years were analyzed.

3.4 Results

Position of eggs in the laying sequence had a significant effect on brightness (2011: $F_{2,131.1} = 20.4$, $P < 0.001$; 2012: $F_{2,161.1} = 14.3$, $P < 0.001$) and blue-green chroma (2011: $F_{2,131.1} = 19.4$, $P < 0.001$; 2012: $F_{2,160.0} = 5.79$, $P = 0.004$) in both study years, and the hue of eggshells in 2011 ($F_{2,131.1} = 3.76$, $P = 0.03$), but not 2012 ($F_{2,161.2} = 0.61$, $P = 0.55$). Post-hoc comparisons showed that early-laid eggs were less bright and had higher blue-green chroma than those laid mid and late in the laying sequence, but there was no difference between mid and late-laid eggs (Fig. 3.1A & C). In 2011, the hue of late-laid eggs was greater (i.e., more green) than eggs that occurred in the middle of the laying sequence, but there was no difference between early laid eggs and those laid either midway or late in the laying sequence (Fig. 3.1B). There was no significant effect of position in the laying sequence on the UV

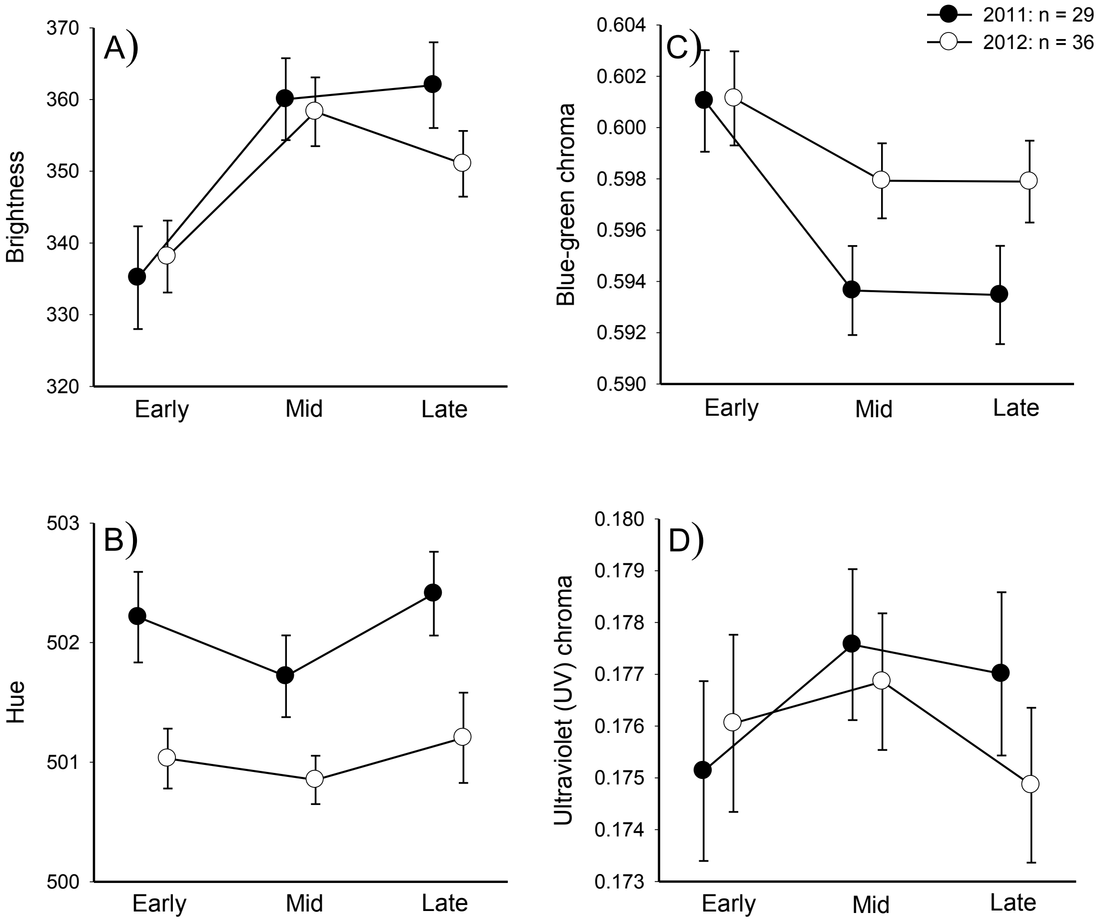

Figure 3.1. Variation (± SE) in the A) brightness, B) hue, C) blue-green chroma, and D) ultraviolet (UV) chroma of the eggshells of mountain bluebirds according to position in the laying sequence. Early eggs were egg one for five-egg clutches, and egg one and two for six-egg clutches. Mid-sequence eggs are egg two and three in five-egg clutches, and three and four in six-egg clutches. Late eggs were egg five in five-egg clutches, and egg five and six in six-egg clutches. See Methods for calculation of colour variables and details of the supplementation experiment.

chroma of eggshells in either year (2011: $F_{2,130.4} = 1.52$, $P = 0.22$; 2012: $F_{2,160.4} = 2.04$, $P = 0.13$). In general there was no effect of treatment on the relationship between egg position and eggshell colour; however, the interaction between treatment and egg position on eggshell brightness approached significance in 2012 ($F_{4,157.1} = 2.35$, $P = 0.06$); therefore, I conducted further analyses of brightness separately by treatment. I found that egg position had a significant effect on brightness for clutches laid by control ($F_{2,62.0} = 8.12$, $P = 0.001$) and food-supplemented ($F_{2,58.0} = 12.56$, $P < 0.001$) females. For control females, mid-laid eggs were brighter than early and late-laid eggs, but there was no difference in the brightness of eggs laid early and late in the laying sequence (Fig. 3.2). For clutches laid by food-supplemented females both mid- and late-laid eggs were brighter than early laid eggs, but there was no difference in the brightness of mid- and late-laid eggs (Fig. 3.2). Trends for brightness were similar for clutches laid by females that were supplemented with both food and carotenoids, but only egg position approached significance ($F_{2,37.0} = 2.85$, $P = 0.07$; Fig. 3.2).

Treatment overall had a significant effect on both the average brightness ($F_{2,37} = 8.82$, $P = 0.03$) and blue-green chroma ($F_{2,37} = 4.37$, $P = 0.02$) of clutches in 2011, but not 2012 (brightness: $F_{2,50} = 0.55$, $P = 0.58$; blue-green chroma: $F_{2,49} = 1.88$, $P = 0.16$). Post-hoc comparisons revealed that clutches laid by food-supplemented females had lower average brightness and higher blue-green chroma than those laid by control females (Fig. 3.3A & C). However, there was no difference in either brightness or blue-green chroma between the eggs laid by food and carotenoid supplemented females and those laid by controls (Fig. 3.3A & C). Treatment did not have a significant effect on UV chroma (2011: $F_{2,36} = 2.81$, $P = 0.07$; 2012: $F_{2,48} = 1.58$, $P = 0.22$) or hue (2011: $F_{2,37} = 2.31$, $P = 0.11$; 2012: $F_{2,35} = 1.06$, $P = 0.36$) in either year, although there was a trend in 2011 for clutches of eggs laid by

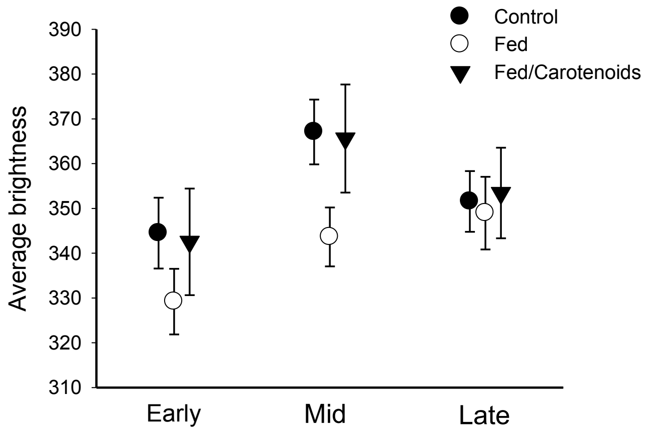

Figure 3.2. Brightness (± SE) of early, mid-, and late-laid eggs within clutches of mountain bluebirds in 2012 according to whether they received supplemental food (Fed), supplemental food and carotenoids (Fed/Carotenoids), or acted as controls. Early eggs were egg one for five-egg clutches, and egg one and two for six-egg clutches. Mid-sequence eggs were egg two and three in five-egg clutches, and three and four in six-egg clutches. Late eggs were egg five in five-egg clutches, and egg five and six in six-egg clutches. See Methods for calculation of brightness and details of the supplementation experiment.

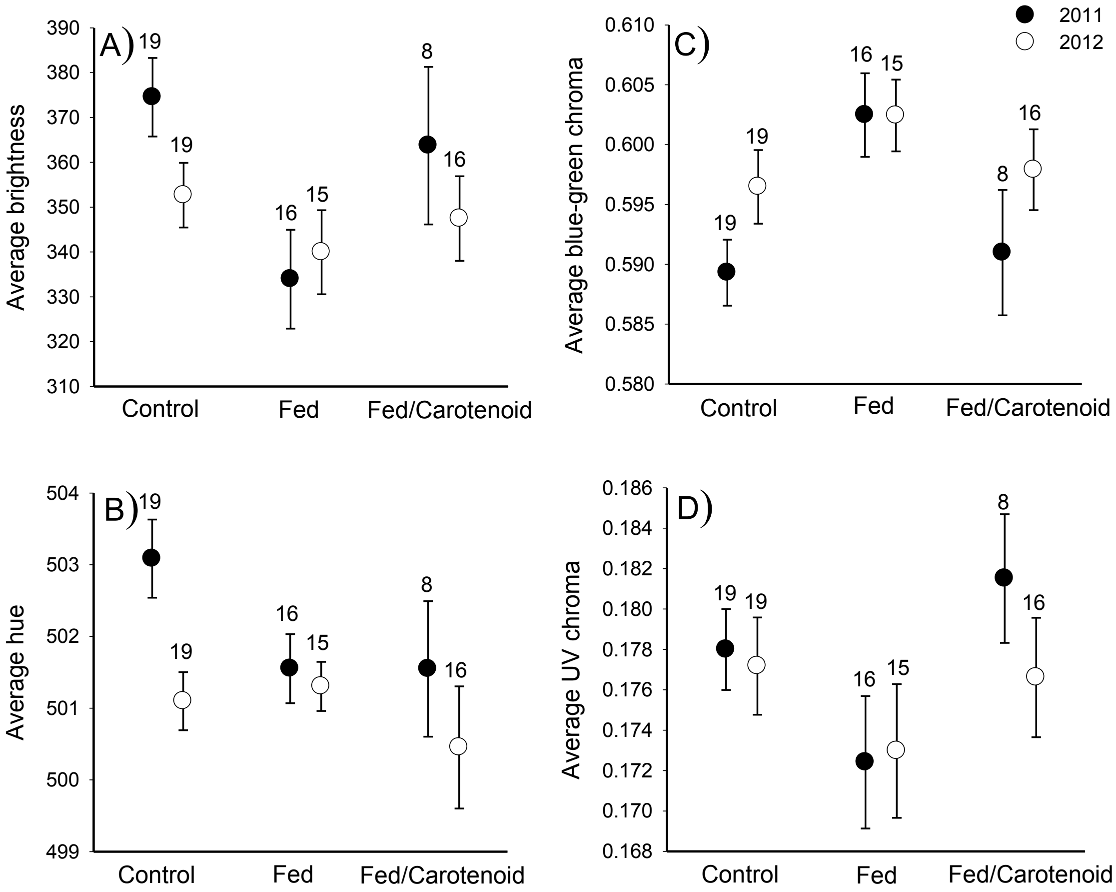

Figure 3.3. Average (± SE) A) brightness, B) hue, C) blue-green chroma, and D) ultraviolet chroma of the eggshells of mountain bluebirds according to whether they received supplemental food (Fed), supplemental food and carotenoids (Fed/Carotenoid), or acted as controls. See Methods for calculation of colour variables and details of the supplementation experiment.

food-supplemented females to have reduced UV chroma compared to those laid by controls (Fig. 3.3D). In 2012, the average blue-green chroma of clutches increased ($F_{1,48} = 4.26$, $P = 0.04$) and UV chroma decreased ($F_{1,48} = 5.26$, $P = 0.03$) with later initiation dates. There were also trends for females with higher feather PC1 scores (more saturated UV-blue feathers) to lay eggs with longer (more green) hue values ($F_{1,35} = 3.64$, $P = 0.07$), and for clutches with heavier eggs to have shorter (more blue) hue values ($F_{1,35} = 3.46$, $P = 0.07$).

Food supplementation increased average egg mass ($F_{2,49} = 4.20$, $P = 0.021$) and clutch size ($F_{2,49} = 4.31$, $P = 0.019$) in 2012 but did not have a significant effect on either trait in 2011 ($F_{2,37} < 1.66$, $P > 0.20$), or affect clutch initiation date in either year (2011: $F_{2,37} = 1.68$, $P = 0.35$; 2012: $F_{2,49} = 2.42$, $P = 0.10$). In 2012, food-supplemented females laid larger clutches than control females (0.54 eggs ± 0.19 eggs, $P = 0.02$) that contained heavier eggs (0.19g ± 0.07g heavier, $P = 0.03$). There were no significant differences between the number and mass of eggs laid by females that received carotenoids in addition to supplemental food and controls (all *P*-values > 0.10).

3.5 Discussion

I found that food and/or carotenoids supplementation had little effect on changes in colour through the laying sequence within clutches. There was a significant decrease in blue-green chroma and an increase in the brightness of later-laid eggs (Fig. 3.1), regardless of treatment. If shell colour reflects pigment concentration (Moreno et al. 2006a; but see Cassey et al. 2008), then these findings indicate that biliverdin deposition declines through egg laying and that neither food nor micronutrients consistently alters this pattern. This finding is consistent with natural patterns of egg colour variation (see Chapter 2) and supports the supposition that shell pigments are limited (Moreno and Oserno 2003), but contrasts with previous findings in other species. In blue-footed boobies (*Sula nebouxii*), blue-green chroma declines naturally after the first-laid egg, but when females are supplemented with carotenoids after laying their first egg, the colour of the second-laid egg is increased (Morales et al. 2011). Different results for boobies compared to mountain bluebirds may be due to differences in supplementation protocols between studies. Rather than supplementing females after clutches were initiated, as in Morales et al. (2011), I started supplementation during nest building. Therefore, any additional resources due to treatment would be available when the first egg was laid. My findings suggest that the colour of early-laid eggs may represent a maximal investment in eggshell pigmentation for females and consistently higher food and antioxidant availability does not have a strong influence on the relative amount of pigments allocated to later eggs. Instead, the difference in allocation is seen in a shift in the colour of all eggs in the clutch.

Food supplementation had a significant effect on average eggshell colour (Fig. 3.3), and positively affected both average egg mass and clutch size, but these effects were context dependent, since treatment effects differed between years. In 2011, food supplementation significantly increased eggshell pigmentation but not clutch size or average egg mass. In 2012, food-supplemented females laid larger clutches of heavier eggs than those that did not receive supplemental food, but there was no significant effect on eggshell colour. There were also differences in the relationship between eggshell colour and measures of breeding phenology. In 2012 only, eggshell blue-green chroma increased with later clutch initiation dates and UV chroma decreased. Demographic data indicated that 2012 was a less productive year than 2011, and that environmental and climate conditions early in the season, while females were nest-building and laying eggs, were poorer (Randall and Dawson, unpublished data). In 2012, the average clutch size was smaller, clutch initiation dates were later, and early-initiating females did not have larger clutches of eggs than later-initiating females (Randall and Dawson, unpublished data) as is usually the case in passerines (Drent and Daan 1980). This indicates that although food supplementation during pre-breeding has the potential to increase eggshell pigmentation, this effect is mediated by environmental conditions. Eggshell pigments may represent an optional advertisement on the part of females, and when conditions are challenging they may allocate resources to more essential aspects of reproduction such as increasing mass of eggs and clutch size.

Contrary to my prediction, I did not find that carotenoid supplementation enhanced eggshell colour (Fig. 3.3). If eggshell colour advertises antioxidant capacity (Hanley et al. 2008; Giordano et al. 2015) and levels of circulating antioxidants (Morales et al. 2008), increasing the quantity of antioxidants consumed by females should enhance the effects of food supplementation on egg production in addition to increasing eggshell pigmentation. Indeed, other studies have found that supplementing female birds with antioxidants had a positive effect on eggshell pigmentation (Morales et al. 2011, Dearborn et al. 2012). There are several possible explanations for why my study does not support those findings. It is possible that the carotenoids enhanced the resident male's perception of the value of the supplemental food and that they consumed a disproportionate amount of the food provided, reducing the quantity of the supplemental food that females actually consumed. Alternatively, supplemental carotenoids may have induced some unforeseen, potentially detrimental effects on females. Although carotenoids are generally thought to be beneficial to an organisms health (Chew and Park 2004; Constantini and Møller 2008; but see Pérez-Rodríguez 2009), carotenoid supplementation has been found to produce neutral and sometimes deleterious effects (Olson and Owens 1998), such as failure to decrease the occurrence of major cancers and cardiovascular disease in human subjects (Mayne 1996; Bjelakovic et al 2004; Seifried et al. 2007). The immune-stimulating function of carotenoids in birds is well established (Blount et al. 2003; Saino et al. 2003; O'Brien and Dawson 2009), but their function *in vivo* actively preventing oxidative damage is far from clear (Garratt and Brooks 2012) and the actions of carotenoids in living organisms are likely to be highly influenced by interactions with other molecules and intracellular conditions (Britton 1995; Garratt and Brooks 2012). In addition, reactive oxygen species, in low concentrations within cells, may have important physiological functions (Garratt and Brooks 2012). My experiment tested for effects of increased food in addition to carotenoid-enhanced food, and food supplementation alone may be sufficient to reduce oxidative stress (Fletcher et al. 2012; Giordano et al. 2015). The combination of food and carotenoid supplementation may have lowered reactive oxygen species to suboptimal levels, counteracting any positive effects of increased food availability and resulting in eggshell colour and investment in eggs that were not significantly different from clutches of eggs laid by control females.

3.6 Conclusion

The positive effect of food supplementation on average clutch colour and the lack of influence on changes in colour within clutches in mountain bluebirds indicates that the pigmentation of early-laid eggs represents a maximal investment that reflects current resource availabilities, and that the total amount of pigment available for a breeding attempt is limited. However, the relationship between antioxidant availability and egg colour is still unclear, as supplemental carotenoids did not enhance eggshell colour as expected. Given these unexpected findings, and that the *in vivo* antioxidant function of carotenoids is still poorly understood, future studies should consider using different types of supplementary antioxidants and also combine supplementation with measures of oxidative stress prior to egg laying. In addition, researchers may wish to consider alternate delivery methods to avoid potentially confounding effects of combining food and micronutrient treatments. Finally, the inconsistent effects of treatment between a “good” and a “poor” breeding season indicate that the results of single-year studies should be interpreted with caution and that longer term studies may be needed to unravel the true function and informative value of blue-green eggshell colour.

4. Blue-green eggshell colour does not predict nestling performance and may negatively relate to rearing conditions in mountain bluebirds (*Sialia currucoides*)

4.1 Abstract

The colourful ornamental traits of animals and their evolution through sexual selection has been a major focus of evolutionary ecology. Despite this, the functional significance of many ornamental traits, particularly female traits, are still poorly understood. The colourful eggshells of birds are one such attribute. Theory suggests conspicuous eggshell colours, such as blue-green, may be a sexually selected signal that advertises female quality and investment in reproduction to male birds. If eggshell colour is an honest advertisement then it should be positively related to nestling performance and outcomes (i.e., growth rates and fledging success). I conducted a cross-fostering experiment in mountain bluebirds (*Sialia currucoides*) to separate the influence of brood rearing conditions from eggshell colour, and used an information theoretic approach to evaluate the relative importance of eggshell colour as a predictor of nestling growth rates. I also investigated the relationship between blue-green eggshell colour and the fledging success (percentage of young and total number of young fledged) of nestling birds, and the intensity of infestation by a common nest-dwelling ectoparasite (*Protocalliphora* spp). Eggshell colour did not predict that a nest would experience brood reduction, but overall fewer young fledged from nests with eggs that were more saturated blue-green in colour. In one study year the rate of mass gain and primary feather growth of cross-fostered young was positively related to the colour of the eggs in the foster nest, but the opposite trends were found in the other study year. Furthermore, the performance of cross-fostered young was not related to the colour of the eggshells in the nest where they hatched. Mass and primary growth rates also were not related to eggshell colour

for young that were raised in their natal nests. These findings indicate that eggshell colour may advertise some aspects of the natal environment but that this signal is not strong or consistent. Additionally, eggshell colour does not seem to signal pre-hatching maternal investment that has important consequences for nestling growth and success, so it may not be an honest signal and alternate explanations for its variation should be pursued.

4.2 Introduction

Sexual selection is thought to be responsible for the evolution of ornamental traits that advertise the quality and condition of an individual to prospective mates (Kodric-Brown and Brown 1984; Andersson 1994). Sexually selected traits can influence differential allocation, where investment in offspring is contingent on the ornamentation of the mate the offspring were produced with (Burley 1986; Sheldon 2000), as well as mate choice (Andersson 1994). Because the reproductive success of females is thought to be limited by their ability to produce gametes and raise young, rather than access to mates, as is often assumed to be the case for males, mate choice and differential allocation by males has been investigated infrequently (Trivers 1972; Sheldon 2000; Kiss et al. 2013). However, ornamental traits are also widespread among females (Amundsen 2000a & b; Amundsen and Pärn 2006). Despite early dismissal of female ornaments as non-functional genetic correlates of male traits (Lande 1980), in taxa where males contribute substantially to raising offspring, or female fitness is limited by male effort, evidence suggests that males may be also benefit from being selective (Edward and Chapman 2011).

In either sex, exercising mate choice is known to involve costs in the form of search time and energy expenditure (Andersson and Simmons 2006), and should evolve only if the benefits of being selective outweigh the costs. The benefits of selecting mates that express a higher level of ornamentation relative to conspecifics may be direct (e.g., increased access to resources [Wolfenbarger 1999; Keyser and Hill 2000], decreased exposure to parasites [Borgia and Collis 1989; Able 1996], and increased investment in offspring [Hegyi et al. 2015]) or indirect effects that increase the fitness of offspring (e.g., good genes; Andersson 1994; Kokko et al. 2003; Jones and Ratterman 2009). Both direct and indirect benefits have the potential to influence offspring viability, and if the offspring of ornamented individuals perform better than those of less-ornamented individuals there should be strong directional selection on preference for these attributes. This may be of particular importance for the evolution of female ornaments in situations where the trait advertises investment in offspring, as maternal effects have been shown to have a strong influence on the early development of organisms, with long-term consequences for survival and fecundity of individuals (Mousseau and Fox 1998; Lindström 1999).

Blue-green eggshell colour has been proposed as a signal of female condition and quality in birds that advertises maternal investment in components of eggs that are beneficial for embryonic development and post-hatch success (Moreno and Oserno 2003). The blue-green colouration of eggshells is the product of biliverdin (Kennedy and Vevers 1976; Míšík et al. 1996), a metabolically derived antioxidant (Stocker et al. 1990; Barañano et al. 2002), deposited to the outer layer of eggshells during egg formation (Wang et al. 2007). The potential for this trait to function as a quality signal in female birds is suggested by a possible trade-off between endogenous use of biliverdin to prevent oxidative damage and allocating it to eggshells (Moreno and Oserno 2003). Indeed, eggshell colour has been found to reflect female condition (Moreno et al. 2005; Siefferman et al. 2006; Krist and Grim 2007), reveal antioxidant status (Hanley et al. 2008; Morales et al. 2011), and advertise maternal investment (e.g., egg size [López de Hierro and De Neve 2010], egg mass [Moreno et al.

2006a; Siefferman et al. 2006], yolk immunoglobulins [Morales et al. 2006], and yolk carotenoids [Navarro et al. 2011]). Because pre-hatching maternal investment is known to be an important determinant of nestling success (e.g., De Neve et al. 2004), if egg colour advertises maternal investment as the aforementioned studies suggest, it should be positively correlated with nestling performance. Alternately, eggshell colour may also advertise post-hatch conditions in the form of parental provisioning rates, the prevalence of parasites, or other aspects of the nestling environment. Despite their importance to the validity of blue-green eggshell colour as a sexually selected signal, these predictions have not been as thoroughly investigated as other aspects of blue-green eggshell colour, and results have been conflicting. Some studies have shown that blue-green egg colour predicts both immunocompetence and fledging success (Moreno et al. 2005; Morales et al. 2006), but other studies have failed to replicate these results (Krist and Grim 2007), and the relationship between eggshell colour and additional aspects of nestling development such as growth rates have, to my knowledge, not been explored.

To further elucidate the relevance of eggshell colour as an informative signal to male birds, I tested the hypothesis that blue-green eggshell colour is a positive predictor of nestling performance (growth rates and fledging success) in mountain bluebirds (*Sialia currucoides*). Eggshell colour may advertise pre-hatching maternal investment, post-hatching conditions, or both, so I used a cross-fostering experiment to separate pre- and post-hatching influences. In addition, I quantified aspects of the brood-rearing environment such as the intensity of nest ectoparasite infestations, the potential for sibling competition (relative position in the size hierarchy and brood size), and adult body condition during brood rearing. If egg colour signals pre-hatching maternal investment, I anticipated that the performance of cross-fostered young would be positively related to the colour of the eggs in their nest of origin. If egg colour relates to post-hatch conditions such as parental provisioning, territory quality, or level of parasite infestation, then offspring performance should be associated with the colour of the eggs in their foster nest. If both pre- and post-hatch influences affect nestling outcomes, then I expected relationships between nestling success and egg colour to be more apparent when young are not cross-fostered. Finally, if egg colour does not advertise either maternal investment or rearing conditions in this species, I predicted no relationship between eggshell colour and offspring performance.

4.3 Methods

4.3.1 Study site, species, and general field procedures

I studied mountain bluebirds breeding in nest boxes (84 pairs) southwest of Williams Lake in central BC, Canada (51°N, 122°W) from mid-April to early August in 2011 and 2012. The habitat of the study area consisted of arid open grasslands, with scattered patches of Douglas-fir (*Psuedotsuga menziesii*). Mountain bluebirds are medium-sized (~ 30g), sexually dimorphic, socially monogamous passerines with biparental care (Power and Lombardo 1996). At this site, bluebirds returned from wintering grounds in mid-March and began laying eggs in late April. Clutch sizes ranged between two and seven eggs, but clutches of five or six eggs are most common (O'Brien and Dawson 2013). Eggshells of bluebirds range in colour from pale blue to nearly turquoise, although white eggs have been documented (from 2 to 9% of clutches; Peak 2011). Only females construct nests and incubate eggs, but both males and females provision young during brood rearing (18 – 21 days) and provide post-fledging care (Power and Lombardo 1996).

I began monitoring nest boxes early in the breeding season to determine the start of nest construction and date that clutches were initiated. I weighed each egg using a portable balance (nearest 0.01 g) on the day they were laid. After clutches were complete (>24h with no new eggs laid), nests were not disturbed until day 9-10 of incubation (incubation period is ~13 days; Power and Lombardo 1996) when I measured the colour of each egg in a clutch by quantifying their reflective properties using a spectrometer (see section 4.3.3 for details). Following egg colour quantification, I checked nests daily beginning on day 12 of incubation to determine hatching date (day 0 of brood rearing). I weighed nestlings on day 1 post-hatch using a portable balance (nearest 0.01 g) to determine their initial mass, and marked nestlings with non-toxic markers to enable individual identification within the nest. I calculated a standardized size hierarchy score for each nestling based on the initial mass of nestlings relative to their nest mates by subtracting the mean mass of the brood from the mass of each individual and dividing this value by the standard deviation of the brood. On alternating days from day 3 to day 15 post-hatch, I measured mass of nestlings using a spring balance (nearest 0.125 g) and length of tarsus using digital calipers (nearest 0.1 mm). From day 9 to 15, I measured length of the eighth primary flight feather using a ruler (nearest 0.5 mm) every second day. On day 13 post-hatch, I banded nestlings with a single uniquely numbered aluminum band, and determined the sex of nestlings using plumage characteristics (Power and Lombardo 1996).

I captured adult females (n = 34 in 2011, n = 41 in 2012) while they were feeding young and banded them with an individual-specific combination of a numbered aluminum band and three coloured plastic leg bands for identification. I weighed birds using a spring balance (nearest 0.25 g), measured the lengths of the wing and 8^{th} primary flight feather using a ruler (nearest 0.5 mm), and measured tarsus and the combined length of head and bill with digital calipers (nearest 0.1 mm). To obtain an index of body condition of females during brood rearing, I used the residuals from a multiple linear regression ($F_{2,186} = 31.37$, $P < 0.001$) of body mass on the combined length of head and bill ($t = 5.63$, $P < 0.001$) and age of young at the time females were captured (t = -5.85, $P < 0.001$). On day 22 post-hatch I checked nest boxes to determine the number of young that had fledged, and collected the nest in a sealed bag for subsequent quantification of *Protocalliphora* spp.(blow flies), a common blood-sucking ectoparasite of nestling mountain bluebirds (O'Brien and Dawson 2008). Nests were stored for a minimum of seven days following collection, and then baked at 60^{o} C for 48 hours to kill any living ectoparasites. I then sifted through the nest material to quantify the number of pupae and puparia, which provide an estimate of minimum infestation levels by blow flies (Dawson et al. 2005a).

4.3.2 Colour quantification

I measured the shell colour of developed eggs over the range of wavelengths visible to songbirds (300-700 nm) using an Ocean Optics USB2000 spectrometer (Dunedin, FL) with a deuterium tungsten halogen light source (Avantes, Broomfield, CO). I measured reflectance relative to a white standard in three places on the surface of each egg at their widest breadth in a dark environment to eliminate ambient light. I calculated brightness (total reflectance from 300-700 nm), ultraviolet (UV) chroma (relative reflectance from 300-400 nm), blue-green chroma (relative reflectance from 400-605 nm) and hue (wavelength of peak reflectance) from the raw spectra using CLR software (Montgomerie 2008). I averaged these values for each egg and used principal components analysis to reduce colour metrics to a set of uncorrelated variables. Based on the broken stick model (Jackson 1993; see also Fontier 1976), I determined that one component was interpretable. The first component (PC1) explained 66% of the variation in blue-green eggshell colour, and variables loaded as follows: blue-green chroma = -0.94, UV chroma =0.95, brightness = 0.87, hue = -0.31. Eggs with higher PC1 scores therefore had higher UV chroma, were brighter, and had blue-shifted hues but lower blue-green chroma.

4.3.3 Experimental design

To separate pre-hatch effects from the influence of post-hatch rearing conditions, I conducted a cross-fostering experiment. Nests were matched by brood size and hatching date, and nestlings were exchanged on the first day after they hatched (day 1 post-hatch). In 2011, I used a partial cross-fostering design (n = 28) in which one to three nestlings were switched with nestlings from another nest while not altering size hierarchies within both nests. In 2012, I performed full brood cross-fosters (n = 32) in which all young were switched. Concurrent with this study, I performed a supplementation experiment during egg laying designed to determine the effect of food (Fed) and antioxidant (Fed/Carotenoid) availability during pre-breeding and egg laying on eggshell colouration (see Chapter 3 for details), and given that I expected that supplementation would enhance eggshell colouration, I cross-fostered young preferentially between treatment and control nests in an effort to maximize egg colour differences between sets of nests where nestlings were swapped.

4.3.4 Statistical analyses

To determine the relationship between egg colour and nestling performance, I calculated growth rate constants using a logistic model for mass, a Gompertz model for tarsus, and a linear model for eighth primaries, following Dawson et al. (2005b). I then developed and compared linear mixed-effects models using an information theoretic approach to evaluate the factors that influenced nestling growth rates. Brood size and clutch initiation date differed among treatments; additionally, female condition, clutch initiation date, and average brood size were correlated. Using information theory to evaluate models allowed for comparison of the relative importance of these individual variables which, because of issues of collinearity, could not be included in the same models.

I first analyzed data only from cross-fostered young to allow me to determine the relationship between eggshell colour and nestling growth when the eggshell colour that young hatched from was different from the eggshell colour in the nest where they were raised. I then analyzed data only from non-fostered young to assess the natural association between eggshell colour and nestling traits. A series of 30 candidate models were compared for young raised in their native nests, and 41 candidate models for young raised in foster nests. Following the methods of Burnham and Anderson (2002), I evaluated the relative support for each model as an explanation of nestling growth rates. I compared candidate models using Akaike's information criterion adjusted for small sample sizes (AIC_c), and within each candidate set, the model with the smallest AIC_c value was considered the most parsimonious. I considered models with $\Delta AIC_c < 2$ from the best model to be well-supported, and those with $\Delta AIC_c > 2$ and < 4 to be plausible. I report Akaike weights (ω_i; the probability that a candidate model is the best model, given the data and candidate model set [Burnham and Anderson 2002]) and parameter estimates (± 1 standard error: S.E.) for all competitive models. I also evaluated parameter estimates based on confidence intervals, and considered those with 85% confidence intervals that overlapped zero to be uninformative (see Arnold 2010).

In developing candidate models, nestling sex, year, and supplementation treatment were included as fixed factors, and covariates considered were the position of each nestling in the brood-size hierarchy (initial size), female condition, average brood size, clutch initiation date, and eggshell colouration (PC1). In analyses of growth rates of fostered young, I included eggshell colouration of the foster nest as well of the colour of the eggs in their nest of origin as covariates. I considered 31 candidate models, including a null model, and all included nest identity as a random factor to account for non-independence of nestlings raised in the same nest. When competitive models included interactions with year or treatment and I assessed the interactions to be informative based on confidence intervals, further analyses were performed separately by year or treatment respectively.

In addition, I investigated whether average clutch colour was related to brood reduction due to factors other than predation, and to the total number of young fledged (n = 25 in 2011, n = 31 in 2012). Because relatively few nests had brood reduction (n = 14), I examined brood reduction as a binary variable using logistic regression. I assembled a series of 15 candidate models to explain the probability of brood reduction, and included combinations of food supplementation treatment and year as factors, and clutch initiation date, brood size at hatch, and average egg colour (PC1) as covariates. Brood size and treatment were not independent, so no model included both variables. The number of young fledged was not normally distributed (Kolmogorov-Smirnov: $D = 0.25$, $df = 56$, $P < 0.001$), so I used a generalized linear model with a Poisson probability distribution and a log link function. I assembled a series of 12 candidate models that included year and treatment as factors, and clutch initiation date and average egg colour (PC1) as covariates. For both measures I used an information theoretic approach to evaluate models using the criteria outlined above.

I used a series of generalized linear models to test whether eggshell colour was related to the intensity of ectoparasite infestation (blow flies) during brood rearing (n = 47 in 2011, n = 52 in 2012). I calculated the number of pupae/puparia recovered from nests per offspring and used a square-root transformation prior to analysis, as per-capita abundance of blow flies was not normally distributed (Kolmogorov-Smirnov: D = 0.17, *df* = 99, *P* < 0.001). In addition to egg colour, in various models I included year as a fixed factor and clutch initiation date as a covariate to account for between-year and seasonal variation in parasite infestations (Harriman et al. 2013). I also included other variables with the potential to affect susceptibility and total number of hosts available to ectoparasites, including the pre-breeding supplementation treatment experienced by the parents as a fixed factor, and the brood size and sex ratio as covariates (O'Brien and Dawson 2009). I considered 24 candidate models and a null (intercept only) model as potential explanations for per capita parasite numbers. I did not include brood size in models that contained either clutch initiation date or treatment to avoid issues of collinearity as these variables were found to be related.

Analyses were performed using SPSS version 20 (IBM Corp 2011) and I present parameter estimates ± 1 standard error, and 85% confidence intervals.

4.4 Results

4.4.1 Mass growth rate

For non-fostered young, the model that was the most competitive explanation of nestling mass gain, as assessed by AIC_c, included the position of the nestling in the size hierarchy of the brood (initial size), year, and the number of young in the brood (Table 4.1). Young that were initially larger relative to their nest-mates ($\beta = 0.011 \pm 0.0024$, CI: 0.0071 to 0.014), those reared in 2012 ($\beta = -0.028 \pm 0.014$, CI: -0.048 to -0.0082), and those raised in smaller broods ($\beta = -0.026 \pm 0.0068$, CI: -0.034 to -0.015) gained mass faster than those that were smaller, reared in 2011, and raised in larger broods. A second competitive model also included nestling sex ($\beta = 0.0017 \pm 0.0046$, CI: -0.0049 to 0.0084), but this variable was not informative (Table 4.1).

Table 4.1. Model selection for factors influencing the rate of mass gain of nestling mountain bluebirds. Only models with ΔAIC_c scores < 4 from the best model, as well as the null (intercept and nest identity) model, are presented. Italicized variables are poorly estimated and considered uninformative based on 85% confidence intervals.

Nestling Category	**Model structure**[a]	**K**[b]	**-2logL**[c]	**AIC_c**[d]	**ΔAIC_c**[e]	ω_i[f]
Non-foster	Size + Year + Brood Size	6	-982.839	-970.548	0	0.48
	Size + Year + *Sex* + Brood Size	8	-982.981	-968.591	1.96	0.18
	Size + Brood Size	4	-978.727	-968.519	2.03	0.17
	Size + Year + *Sex* + Brood Size + *Egg Clr Htch*	9	-983.079	-966.575	3.97	0.07
	Null Model	2	-945.078	-938.996	31.55	<0.001
Cross-fostered	Size + Year + Sex + CID + Egg Clr Fstr + Year*Egg Clr Fstr	12	-385.700	-366.294	0	0.28
	Size + Brood Size	4	-376.402	-365.947	0.34	0.24
	Size + Year + Sex + Brood Size	8	-378.798	-363.937	2.35	0.09
	Size + Year + Brood Size	6	-376.456	-363.815	2.48	0.08
	Size + Year + Sex + Brood Size + Egg Clr Fstr + Year*Egg Clr Fstr	12	-381.821	-362.415	3.88	0.04
	Null Model	2	-356.802	-350.623	15.67	<0.001

[a] Factors included year (Year), nestling sex (Sex), the position of the nestling in the size hierarchy (Size), the average number of young in the nest (Brood Size), the clutch initiation date (CID), the average egg colour (PC1) of the foster nest (Egg Clr Fstr) and the nest of origin (Egg Clr Htch), and the interaction of year and egg colour.

[b] Number of parameters

[c] Model fit

[d] Akaike's Information Criterion corrected for small sample size.

[e] Difference in AIC_c value between each model and the model with the lowest AIC_c score.

[f] The relative model likelihood, given the data and the candidate model set, normalized to sum to 1 (Burnham and Anderson 2002).

For cross-fostered young, the model that best predicted the rate of nestling mass gain included sex and initial size of the nestling, clutch initiation date, and the interaction of year and the colour of the eggs laid in the foster nest; an additional competitive model also included brood size (Table 4.1). As with non-fostered young, cross-fostered young gained mass more quickly when they were higher in the size hierarchy of the nest (β = 0.017 ± 0.0045, CI: 0.010 to 0.023) and raised in smaller broods (β = -0.044 ± 0.015, CI: -0.066 to -0.021). Cross-fostered nestlings also gained mass more quickly when they hatched from clutches initiated earlier in the season (β = -0.012 ± 0.0036, CI: -0.017 to -0.0066), and female nestlings gained mass faster than male nestlings (β = 0.012 ± 0.0085, CI: 0.000005 to 0.025). Analyzing data separately by year revealed a positive relationship between colour of eggshells (PC1) laid in foster nests and growth rates of mass in 2011 (Table 4.2; Fig. 4.1 A), and a negative relationship between eggshell PC1 and growth rates of mass in 2012 (Table 4.2; Fig. 4.1 B). Nestlings gained mass faster in 2012 when they were fostered in nests where the average eggshell colour was less bright and reflected more light in the blue-green portion of the of the spectrum, but less in the UV, and also had longer (green-shifted) hue values (lower PC1), whereas the opposite trend was found in 2011. However, although models that included the colour of eggshells in foster nests were competitive in both years, eggshell colour was estimated as an informative variable only in 2012 (β = -0.031 ± 0.02, CI: -0.06 to -0.0021; 2011: β = 0.021 ± 0.015, CI: -0.0013 to 0.044).

Table 4.2. Model selection for factors influencing the rate of mass gain of nestling mountain bluebird young that were cross-fostered, analyses were conducted separately by year and only models with ΔAIC_c scores < 4 from the best model and null (intercept and nest identity) models are presented. Italicized variables are poorly estimated and considered uninformative based on 85% confidence intervals.

Year	**Model structure**^{a}	**K**^{b}	**-2logL**^{c}	**AIC$_c$**^{d}	**ΔAIC$_c$**^{e}	ω_i^f
2011	Size + Sex + Brood Size	6	-108.069	-93.069	0	0.51
	Size + Sex + Brood Size + *Egg Clr Fstr*	7	-109.94	-91.792	1.28	0.27
	Size + Sex + Brood Size + *Egg Clr Htch*	7	-108.082	-89.934	3.14	0.11
	Null Model	2	-81.154	-74.380	18.69	<0.001
2012	Size + CID + Egg Clr Fstr	5	-295.937	-283.062	0	0.26
	Size + CID	4	-293.524	-282.906	0.16	0.24
	Size + *Sex* + CID + Egg Clr Fstr	7	-296.702	-281.523	1.54	0.12
	Size + *Sex* + CID	6	-294.381	-281.506	1.56	0.12
	Size + CID + *Egg Clr Htch*	5	-294.218	-281.343	1.72	0.11
	Size + *Sex* + CID + *Egg Clr Htch*	7	-295.072	-279.893	3.17	0.05
	Null Model	2	-277.669	-271.427	11.64	<0.001

[a] Factors included nestling sex (Sex), the position of the nestling in the size hierarchy (Size), the average number of young in the nest (Brood Size), the clutch initiation date (CID), and the average egg colour (PC1) of the foster nest (Egg Clr Fstr) and the nest of origin (Egg Clr Htch).

[b] Number of parameters

[c] Model fit

[d] Akaike's Information Criterion corrected for small sample size.

[e] Difference in AIC_c value between each model and the model with the lowest AIC_c score.

[f] The relative model likelihood, given the data and the candidate model set, normalized to sum to 1 (Burnham and Anderson 2002).

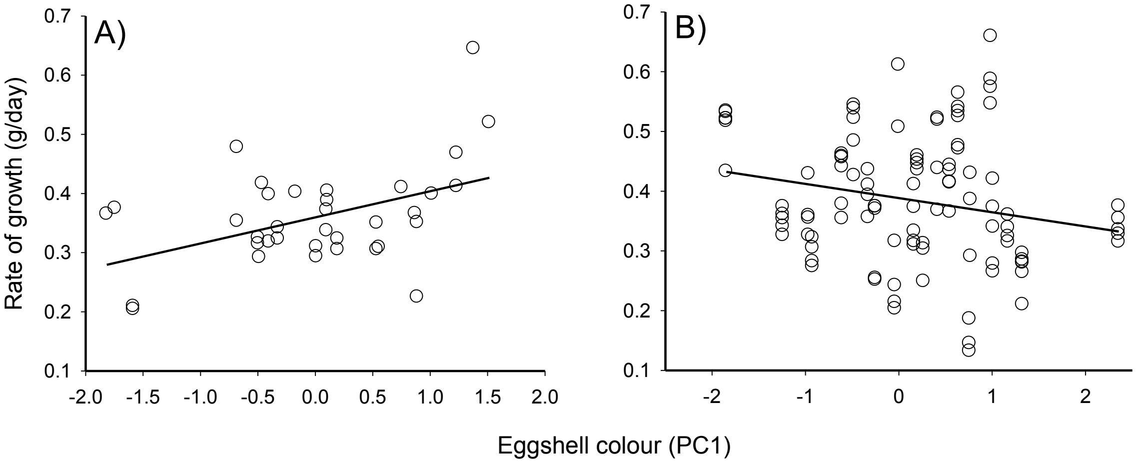

Figure 4.1. Relationship between the colour of eggs laid in the nests that nestling mountain bluebirds were fostered into and the growth rate constants of their mass in A) 2011 and B) 2012. Eggs with higher PC1 scores are brighter, reflect more light in UV and less in the blue-green portion of the spectrum, and have blue-shifted hues. See Methods for details on the calculation of mass growth rates and the quantification of eggshell colouration.

4.4.2 Primary growth rate

For non-fostered nestlings, the top model explaining primary growth rate included year, female body condition, and initial size of nestlings (Table 4.3). Primary growth rates were higher in 2011 than 2012 (*β* = 0.69 ± 0.11, CI: 0.54 to 0.85), and nestlings that were larger relative to their nest mates (*β* = 0.079 ± 0.024, CI: 0.045 to 0.11) and raised by females with lower body condition (*β* = 0.016 ± 0.0017, CI: 0.014 to 0.019) had faster primary feather growth rates than those lower in the size hierarchy and raised by females in better body condition. Other models deemed to be plausible for primary growth rates included egg colour (*β* = -0.063 ± 0.050, CI: -0.14 to 0.0099), nestling sex (*β* = 0.029 ± 0.045, CI: -0.037 to 0.094), and the interaction of year and egg colour (*β* = -0.11 ± 0.10, CI: -0.25 to 0.040), but none of these variables or the interaction were informative.

The growth rate of the primary feathers of cross-fostered nestlings was best explained by a model that included the initial size of nestlings, year, and brood size (Table 4.3). As was the case for non-fostered young, primary feather growth rates were higher in 2011 than 2012 (*β* = 0.75 ± 0.14, CI: 0.55 to 0.95), and for nestlings that were larger relative to their nest mates (*β* = 0.14 ± 0.031, CI: 0.097 to 0.19) and those raised in smaller broods (*β* = 0-0.14 ± 0.08, CI: -0.25 to -0.018). An additional plausible model included the interaction of year and the colour of the eggs in the foster nest (*β* = 0.24 ± 0.14, CI: 0.034 to 0.45). Analyses conducted separately by year revealed that nestlings had higher primary growth rates when they were fostered in nests with higher average eggshell PC1 values in 2011(Fig. 4.2 A); the opposite trend was found in 2012 (Fig. 4.2 B). Although models including foster eggshell colour were competitive in both years (Table 4.4), eggshell colour was estimated as an informative variable only in 2011 (*β* = 0.17 ± 0.11, CI: 0.0024 to 0.33; 2012: *β* = -0.089 ± 0.085, CI: -0.22 to 0.037). However, in 2012 the food supplementation treatment experienced

Table 4.3. Model selection for factors influencing the rate of growth of eighth primary flight feathers of nestling mountain bluebirds. Only models with ΔAIC_c scores < 4 from the best model, as well as the null (intercept and nest identity) model, are presented. Italicized variables are poorly estimated and considered uninformative based on 85% confidence intervals.

Nestling Category	**Model structure**[a]	K^b	$-2\log L^c$	AIC_c^d	ΔAIC_c^e	ω_i^f
Non-foster	Size + Year + Fem Condition	6	342.053	354.345	0	0.19
	Size + Year + Fem Condition + *Egg Clr Htch* + *Year*Egg Clr Htch*	10	339.432	355.935	1.59	0.09
	Size + Year + Fem Condition + *Sex*	8	341.656	356.046	1.70	0.08
	Size + Year + Fem Condition + *Sex* + *Egg Clr Htch*	9	340.098	356.601	2.26	0.06
	Size + Year	5	346.615	356.823	2.48	0.06
	Size + Year + *CID*	6	344.697	356.988	2.64	0.05
	Size + Year + *Egg Clr Htch*	6	345.559	357.85	3.51	0.03
	Size + Year + *Egg Clr Htch* + *Year*Egg Clr Htch*	9	343.843	358.233	3.89	0.03
	Null Model	2	392.318	398.400	44.06	<0.001
Cross-fostered	Size + Year + Brood Size	6	126.951	139.592	0	0.25
	Size + Year	5	129.688	140.153	0.56	0.19
	Size + Year + Egg Clr Fstr + Year*Egg Clr Fstr	9	126.746	141.608	2.02	0.09
	Size + Year + *Sex* + Brood Size	7	126.868	141.729	2.14	0.08

Size + Year + *CID*	6	129.509	142.151	2.56	0.07
Null Model	2	168.763	174.942	35.35	<0.001

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[a] Factors included year (Year), nestling sex (Sex), the position of the nestling in the size hierarchy (Size), the condition of the female parent (Fem condition), the clutch initiation date (CID) the average number of young in the nest (Brood Size), the average egg colour (PC1) of the foster nest (Egg Clr Fstr) and the nest of origin (Egg Clr Htch), and the interaction between year and egg colour.

[b] Number of parameters

[c] Model fit

[d] Akaike's Information Criterion corrected for small sample size.

[e] Difference in AIC_c value of the model between each model and the model with the lowest AIC_c score.

[f] The relative model likelihood, given the data and the candidate model set, normalized to sum to 1 (Burnham and Anderson 2002).

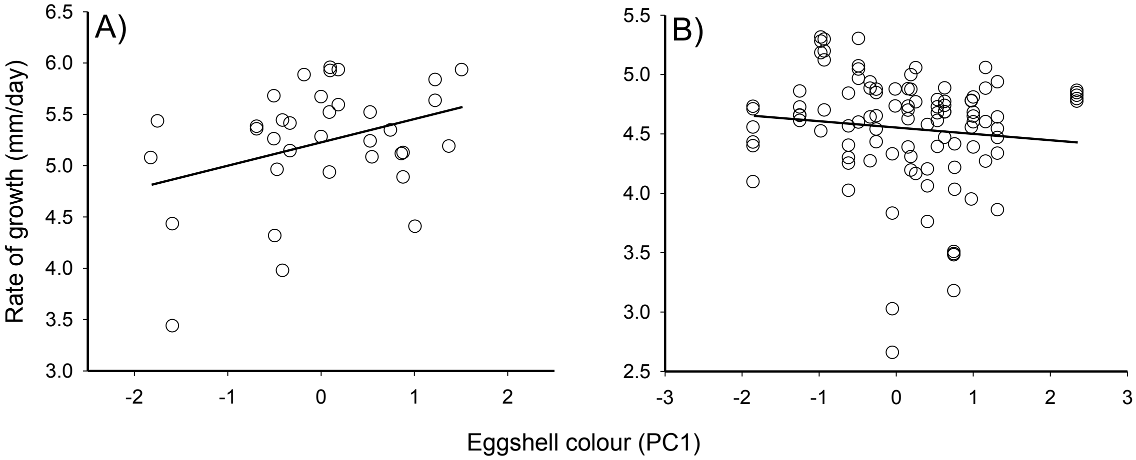

Figure 4.2. Relationship between the colour of eggs laid in the nests that nestling mountain bluebirds were fostered into and the rate of their primary growth in A) 2011 and B) 2012. Eggs with higher PC1 scores are brighter, reflect more light in UV and less in the blue-green portion of the spectrum, and have blue-shifted hues. See Methods for details on the calculation of mass growth rates and the quantification of eggshell colouration.

Table 4.4. Model selection for factors influencing the rate of growth of eighth primary flight feathers of nestling mountain bluebirds that were reared in foster nests. Analyses were conducted separately by year and only models with $\Delta\mathrm{AIC}_c$ scores < 4 from the best model, as well as the null (intercept and nest identity) model, are presented. Italicized parameters are poorly estimated and considered uninformative based on 85% confidence intervals.

Year	**Model structure**[a]	**K**[b]	**-2logL**[c]	**AIC_c**[d]	**$\Delta\mathrm{AIC}_c$**[e]	ω_i[f]
2011	Size	3	51.239	60.627	0	0.18
	Size + Egg Clr Fstr	4	49.207	61.276	0.65	0.13
	Null Model	2	55.539	62.313	1.69	0.08
	Size + *Brood Size*	4	50.322	62.391	1.76	0.07
	Size + *CID*	4	50.932	63.001	2.37	0.05
	Size + *Fem Condition*	4	51.140	63.209	2.58	0.05
	Size + *Sex*	5	51.247	63.316	2.69	0.05
	Size + *Egg Clr Htch*	4	51.293	63.362	2.74	0.05
	Size + *Egg Clr Htch* + Egg Clr Fstr	5	48.731	63.731	3.10	0.04
	Size + *Brood Size* + *Egg Clr Fstr*	5	48.915	63.915	3.29	0.03
	Size + *Fem Condition* + Egg Clr Fstr	5	49.000	64.000	3.37	0.03
	Size + *Sex* + Egg Clr Fstr	6	49.183	64.183	3.56	0.03
	Size + *CID* + *Egg Clr Fstr*	5	49.190	64.190	3.56	0.03
	Size + *Treat Htch* + *Egg Clr Fstr*	7	46.125	64.273	3.65	0.03

2012	Size + Treat Htch	6	70.667	83.542	0	0.11
	Size	3	75.297	83.705	0.16	0.10
	Size + *Brood Size*	4	73.255	83.874	0.33	0.09
	Size + Treat Htch + *Egg Clr Fstr*	7	69.583	84.762	1.22	0.06
	Size + *CID*	4	74.383	85.002	1.46	0.05
	Size + *Egg Clr Fstr*	4	74.615	85.234	1.69	0.05
	Size + *Egg Clr Htch*	4	74.737	85.356	1.81	0.04
	Size + *Brood Size* + *Egg Clr Fstr*	5	72.517	85.392	1.85	0.04
	Size + Treat Htch + *Egg Clr Htch*	7	70.283	85.462	1.92	0.04
	Size + *Sex*	5	75.058	85.676	2.13	0.04
	Size + *Sex* + Treat Htch	8	70.553	85.732	2.19	0.04
	Size + *Sex* + *Brood Size*	6	73.020	85.895	2.35	0.03
	Size + *Fem Condition*	4	75.296	85.915	2.37	0.03
	Size + *Brood Size* + *Egg Clr Htch*	5	73.076	85.951	2.41	0.03
	Size + *CID* + *Egg Clr Fstr*	5	73.344	86.219	2.68	0.03
	Size + *CID* + *Egg Clr Htch*	5	73.874	86.749	3.21	0.02
	Size + *Egg Clr Htch* + *Egg Clr Fstr*	5	74.048	86.923	3.38	0.02

Size + *Sex* + Treat Htch + *Egg Clr Fstr*	9	69.430	86.962	3.42	0.02
Size + *Sex* + *CID*	6	74.169	87.044	3.50	0.02
Size + *Sex* + *Egg Clr Fstr*	6	74.335	87.210	3.67	0.02
Size + *Fem Condition* + *Egg Clr Fstr*	5	74.472	87.302	3.76	0.02
Size + *Sex* + *Egg Clr Htch*	6	74.497	87.372	3.83	0.02
Size + *Sex* + *Brood Size* + *Egg Clr Fstr*	7	72.236	87.415	3.87	0.02

[a] Factors included nestling sex (Sex), the position of the nestling in the size hierarchy (Size), the average number of young in the nest (Brood Size), the clutch initiation date (CID), and the average egg colour (PC1) of the foster nest (Egg Clr Fstr) and the nest of origin (Egg Clr Htch).

[b] Number of parameters

[c] Model fit

[d] Akaike's Information Criterion corrected for small sample size.

[e] Difference in AIC_c value between each model and the model with the lowest AIC_c score.

[f] The relative model likelihood, given the data and the candidate model set, normalized to sum to 1 (Burnham and Anderson 2002)

by the adults in the nests where the young hatched had an effect on the primary growth rates of nestlings that were cross-fostered (Table 4.4). Primary growth rates were lower in nests where the foster parents received supplemental food during laying than control pairs (Fig. 4.3; $\beta = -0.43 \pm 0.21$, CI: -0.74 to -0.13), but there was no difference between the primary growth rates of nestlings hatched in nests where parents were food and carotenoid supplemented and those hatched in the nests of control pairs (Fig. 4.3; $\beta = -0.049 \pm 0.19$, CI: -0.33 to 0.23).

4.4.3 Tarsus growth rate

The model that best explained growth rates of the tarsi of non-fostered nestlings included initial size and female body condition (Table 4.5). Nestlings that were larger relative to their nest mates ($\beta = 0.016 \pm 0.0017$, CI: 0.014 to 0.019) and those reared by females with lower body condition scores had faster-growing tarsi. There were two additional plausible models that contained brood size and clutch initiation date, in addition to initial nestling size (Table 4.5). Nestlings raised in smaller broods ($\beta = -0.0095 \pm 0.0058$, CI: -0.018 to -0.011) and those hatched from clutches initiated earlier in the season ($\beta = 0.0013 \pm 0.00084$, CI: 0.00010 to 0.0026) had faster rates of tarsus growth than those raised in larger broods and those hatched later in the season.

The growth rates of the tarsi of cross-fostered nestlings were best predicted by the initial size of nestlings and the food supplementation treatment in the nest of origin (Table 4.5). As with non-foster young, nestlings that were larger relative to their nest mates grew their tarsi faster than those that were smaller ($\beta = 0.015 \pm 0.0023$, CI: 0.012 to 0.018). In addition, young hatched in food supplemented nests showed lower rates of tarsus growth than young hatched in control nests ($\beta = -0.29 \pm 0.016$, CI: -0.052 to -0.0063; Fig. 4.4).

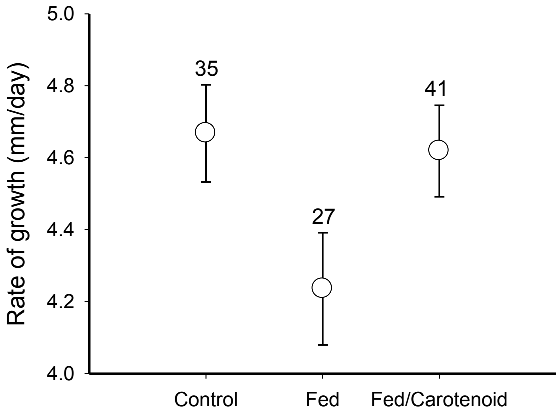

Figure 4.3. Growth constants (mean ± SE) for eighth primary flight feathers of cross-fostered nestling mountain bluebirds in relation to whether their genetic mother and her mate received supplemental food, supplemental food and carotenoids, or acted as controls during pre-breeding and egg laying in 2012. Means were calculated after controlling for other variables in the model (see Results) and sample sizes (number of young) are indicated about error bars. See Methods for details on the calculation of growth rates.

Table 4.5. Model selection for factors influencing the rate of tarsus growth of nestling mountain bluebirds. Only models with ΔAIC$_c$ scores < 4 from the best model and null (intercept and nest identity) models are presented. Italicized variables are poorly estimated and considered uninformative based on 85% confidence intervals.

Nestling Category	**Model structure**^{a}	**K**^{b}	**-2logL**^{c}	**AIC$_c$**^{d}	**ΔAIC$_c$**^{e}	ω_i^f
Non-fostered	Size + Fem Condition	4	-1161.378	-1151.179	0	0.43
	Size + *Year* + *Sex* + Fem Condition	7	-1162.766	-1148.375	2.80	0.11
	Size + Brood Size	4	-1157.936	-1147.728	3.44	0.08
	Size + CID	4	-1157.714	-1147.506	3.66	0.07
	Null Model	2	-1079.330	-1073.247	77.92	<0.001
Cross-fostered	Size + Treat Htch	6	-564.054	-551.413	0	0.23
	Size + *Year* + Treat Htch	8	-565.441	-550.579	0.83	0.15
	Size	3	-557.993	-549.692	1.72	0.10
	Size + *Year* + *Sex* + Treat Htch	10	-565.821	-548.705	2.71	0.06
	Size + *Fem Condition*	4	-559.033	-548.579	2.83	0.05
	Size + *Egg Clr Htch*	4	-558.872	-548.418	3.00	0.05
	Size + *Brood Size*	4	-558.739	-548.284	3.13	0.05
	Size + *CID*	4	-558.708	-548.253	3.16	0.05

Size + *Sex*	5	-558.294	-547.840	3.57	0.04
Size + *Year*	5	-558.220	-547.766	3.65	0.04
Size + *Egg Clr Fstr*	4	-557.999	-547.544	3.87	0.03
Null Model	2	-522.630	-516.451	34.96	<0.001

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[a] Factors included year (Year), nestling sex (Sex), the position of the nestling in the size hierarchy (Size), the condition of the female parent (Fem Condition), the clutch initiation date (CID) the average number of young in the nest (Brood Size), the average egg colour (PC1) of the foster nest (Egg Clr Fstr) and the nest of origin (Egg Clr Htch), and the pre-breeding supplementation treatment of the nest of origin (Treat Htch).

[b] Number of parameters

[c] Model fit

[d] Akaike's Information Criterion corrected for small sample size.

[e] Difference in AIC_c value of the model between each model and the model with the lowest AIC_c score.

[f] The relative model likelihood, given the data and the candidate model set, normalized to sum to 1 (Burnham and Anderson 2002).

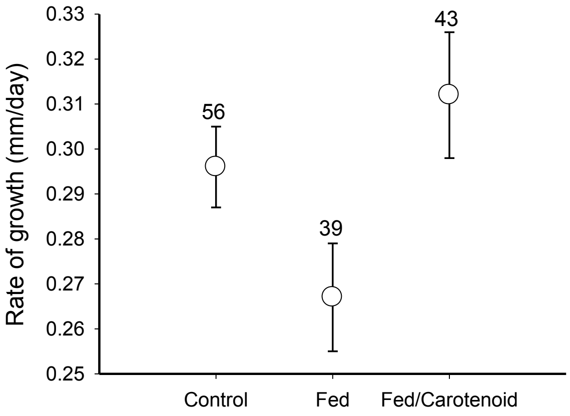

Figure 4.4. Growth constants (mean ± SE) for the tarsi of cross-fostered nestling mountain bluebirds in relation to whether their genetic mother and her mate received supplemental food, supplemental food and carotenoids, or acted as controls during pre-breeding and egg laying. Means were calculated after controlling for other variables in the model (see Results) and sample sizes (number of young) are indicated about error bars. See Methods for details on the calculation of growth rates.

However, tarsus growth rates were not different between Fed/Carotenoid supplemented nests and control nests ($\beta = 0.017 \pm 0.017$, CI: -0.0079 to 0.041; Fig. 4.4). An additional plausible models for tarsus growth rate also included year ($\beta = 0.017 \pm 0.014$, CI: -0.004 to 0.038), but it was not informative.

4.4.4 Fledging success

The probability of brood reduction was best explained by clutch initiation date (Table 4.6). Nests that were initiated later in the season were less likely to have brood reduction due to factors independent of depredation ($\beta = 0.78 \pm 0.41$, CI: 0.19 to 1.36). A second competitive model included food supplementation treatment in addition to clutch initiation date (Table 4.6). The broods of food supplemented pairs were less likely to suffer brood reduction than control pairs ($\beta = 1.99 \pm 1.22$, CI: 0.23 to 3.74). Only 9% (1 out of 11) of nests in the food supplemented treatment had brood reduction while 39% (9 out of 23) of control nests did. However, the rate of brood reduction for pairs in the Fed/Carotenoid supplementation treatment was not different from control broods ($\beta = -0.49 \pm 0.78$, CI: -1.17 to 1.07). Brood size at hatch was also a competitive explanation for brood reduction (Table 4.6); larger broods had slightly higher levels of brood reduction ($\beta = -0.50 \pm 0.35$, CI: -1.00 to -0.001). However, the null model was also competitive based on the ΔAIC_c, and average eggshell colour (PC1) was not included in any competitive model found to predict brood reduction.

The model that best predicted the number of young fledged included the food supplementation treatment experienced by the breeding pair (Table 4.6). Food-supplemented pairs fledged more young than pairs that were not supplemented ($\beta = 0.30 \pm 0.15$, CI: 0.078 to 0.51; Fig. 4.5). However, there was no difference in the number of young fledged between

Table 4.6. Model selection for factors influencing the probability of brood reduction and the number of young fledged from the nests of mountain bluebirds. Only models with ΔAIC_c scores < 4 from the best model and null (intercept only) models are presented. Italicized variables are poorly estimated and considered uninformative based on 85% confidence intervals.

Response variable	**Model structure**[a]	**K**[b]	**-2logL**[c]	**AIC_c**[d]	**ΔAIC_c**[e]	**w_i**[f]
Brood reduction	CID	2	58.645	62.871	0	0.36
	Treat + CID	5	54.627	63.411	0.54	0.27
	Null Model	1	62.982	64.982	2.11	0.12
	Brood Size	2	60.768	64.994	2.12	0.12
	CID + *Year*	4	58.632	65.093	2.22	0.12
	CID + *Year* + *Year*CID*	6	56.929	65.714	2.84	0.09
	Treat	4	60.185	66.646	3.78	0.05
Number of young fledged	Treat	4	222.722	229.183	0	0.18
	Treat + Egg Clr Htch	5	220.432	229.216	0.03	0.18
	Treat + *Year*	6	221.124	229.909	0.73	0.13
	Null Model	1	227.912	229.986	0.80	0.12
	Year	3	226.432	230.658	1.48	0.09
	Treat + *CID*	5	222.046	230.831	1.65	0.08

Egg Clr Htch	2	226.876	231.101	1.92	0.07
CID	2	227.396	231.622	2.44	0.05
Year + Egg Clr Htch	4	225.686	232.148	2.97	0.04
Year + CID	4	225.932	232.393	3.21	0.04

[a] Factors included year (Year), pre-breeding food supplementation treatment (Treat), clutch initiation date (CID), the number of young hatched (Brood Size), the average egg colour (PC1) of the clutch (Egg Clr Htch), and the interaction between year and clutch initiation date.

[b] Number of parameters.

[c] Model fit.

[d] Akaike's Information Criterion corrected for small sample size.

[e] Difference in AIC_c value of the model between each model and the model with the lowest AIC_c score.

[f] The relative model likelihood, given the data and the candidate model set, normalized to sum to 1 (Burnham and Anderson 2002).

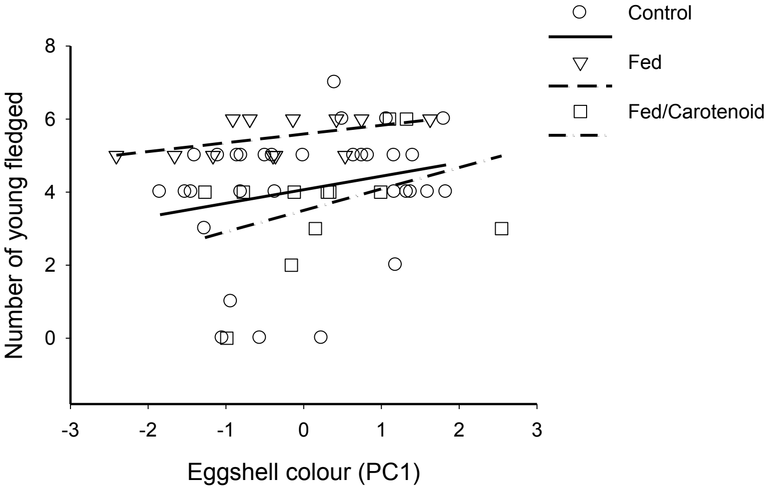

Figure 4.5. The relationship between the average clutch colour (PC1) of the eggs of mountain bluebirds and the number of young fledged from nests when the resident pair received supplemental food, supplemental food and carotenoids, or acted as controls during pre-breeding and egg laying. Eggs with higher PC1 scores are brighter, reflect more light in UV and less in the blue-green portion of the spectrum, and have blue-shifted hues. See Methods for the details of egg colour measurements and principal component analysis.

pairs in the Fed/Carotenoid supplementation treatment and those that were not supplemented (β = -0.11 ± 0.17, CI: -0.36 to 0.14; Fig. 4.5). A second plausible model to explain the number of nestlings fledged included both food supplementation treatment and the average egg colour of the nest (Table 4.6). More young fledged from nests with eggs that had higher PC1 values (brighter, reflected more UV and less blue-green, and had blue-shifted hues) (β = 0.09 ± 0.06, CI: 0.004 to 0.18; Fig. 4.5).

4.4.5 Parasite abundance

The model that best explained the abundance of ectoparasites in nests was year (Table 4.7); parasite numbers were higher in 2011 than 2012 (β = 0.81 ± 0.31, CI: 0.36 to 1.26). A second competitive model included both year and egg colour, but egg colour was poorly estimated (β = 0.14 ± 0.14, CI: -0.06 to 0.34) and not informative. Additional competitive models included average brood size (β = 0.06 ± 0.15, CI: -0.16 to 0.28), clutch initiation date (β = -0.002 ± 0.0099, CI: -0.016 to 0.012), brood sex ratio (β = 0.21 ± 0.57, CI: -0.61 to 1.02), and treatment (Fed/Carotenoid: β = 0.31 ± 0.41, CI: -0.28 to 0.90; Fed: β = -0.20 ± 0.40, CI: -0.78 to 0.38), but these parameters were not informative.

4.5 Discussion

Eggshell colour, as described by PC1, was not a strong or consistent predictor of most aspects of nestling growth or fledging potential in mountain bluebirds. There was no apparent relationship between nestling growth rates for any of the metrics investigated (mass, primary, and tarsus growth rate) and the colour of eggs in the nests where they hatched both for cross-fostered young and those raised in their natal nests. In addition, eggshell colour was not an important predictor either of brood reduction or total brood failure. These findings do not

Table 4.7. Model selection for factors influencing the per-capita infestation intensity of ectoparasites (*Protocalliphora* spp.) in the nests of mountain bluebirds. Only models with ΔAIC_c scores < 4 from the best model and null (intercept only) models are presented. Italicized variables are poorly estimated and considered uninformative based on 85% confidence intervals.

Response variable	**Model structure**[a]	**K**[b]	**-2logL**[c]	**AIC_c**[d]	**ΔAIC_c**[e]	***w_i***[f]
Parasite numbers	Year	2	199.078	205.492	0	0.30
	Year + *Egg Clr Htch*	4	198.008	206.709	1.217	0.16
	Year + *Brood Size*	4	198.924	207.627	2.135	0.10
	Year + *CID*	4	199.050	207.751	2.259	0.10
	Year + *Sex Ratio*	4	198.948	207.947	2.455	0.09
	Year + *Brood Size* + *Egg Clr Htch*	5	197.962	209.033	3.541	0.05
	Year + *Treat*	6	198.006	209.077	3.585	0.05
	Null Model	1	205.666	209.87	4.378	0.03

[a] Factors included year (Year), pre-breeding food supplementation treatment (Treat), clutch initiation date (CID), the number of young hatched (Brood Size), and the average egg colour (PC1) of the clutch (Egg Clr Htch).

[b] Number of parameters.

[c] Model fit.

[d] Akaike's Information Criterion corrected for small sample size.

[e] Difference in AIC_c value of the model between each model and the model with the lowest AIC_c score.

[f] The relative model likelihood, given the data and the candidate model set, normalized to sum to 1 (Burnham and Anderson 2002)

support the hypothesis that eggshell colour represents pre-hatching investment by females that predicts improved nestling performance. Other studies also have failed to support these predictions. Krist and Grim (2007) found no relationship between egg colour metrics and fledging success, nestling weight, or immunity in collared flycatchers (*Ficedula albicolus*). Stoddard et al. (2012) found that although female great tits (*Parus major*) that were heavier laid eggs that were less speckled with protoporphyrin (a pro-oxidant), there was no corresponding positive relationship with nestling growth.

I found some evidence that eggshell colour was related to the brood-rearing environment, but it was weak and not consistent between years. In 2012, young that were cross-fostered into nests that had eggs with more saturated blue-green eggshells (lower PC1) gained mass more quickly and there was also a trend for faster primary growth, supporting the possibility that eggshell colour is related to positive rearing conditions (Fig. 4.1 B & 4.2 B). However, in 2011 the opposite relationships were found, with cross-fostered young growing primaries faster in nests with higher eggshell PC1 and a trend for young fostered in these nests to gain mass more quickly, contradicting what would be expected if more saturated blue-green eggshell colour was a signal of rearing conditions and parental quality (Fig. 4.1 A & 4.2 A). There was no relationship between growth rates and eggshell colour for offspring raised in their natal nests, but more young fledged from nests where the eggs had higher PC1 scores in both years (Fig. 4.5). Neither growth rates nor the higher number of young fledged in relation to eggshell colour appeared to be related to the prevalence of ectoparasites, as the number of larval blow flies per nestling was not related to eggshell colour. High colour saturation in the blue-green portion of the spectrum and low total reflectance (brightness) are thought to be positively related to the concentration of biliverdin in the shell (Moreno et al. 2006a; López-Rull et al. 2008; Cassey et al. 2012); therefore, if biliverdin is similarly related to eggshell colour in this species, these findings indicate that depositing more biliverdin to eggshells does not consistently advertise a positive rearing environment, and in fact the opposite may be true.

Characteristics of the natal environment other than egg colour appeared to have a greater influence on the growth rates of nestlings; both fostered and non-fostered nestlings gained mass faster when they were raised in smaller broods, possibly due to increased resources available to individual offspring (Gebhardt-Henrich and Richner 1998), which is not an uncommon finding (e.g, Dijkstra et al. 1990). Factors influencing growth of tarsus and primary feathers were different between fostered and non-fostered young. Those raised in their original nests grew their tarsi and primary feathers faster in nests where females had lower body condition during brood rearing, which may indicate that these females invested more in offspring and depleted their own resources, but this effect was not found for cross-fostered young. Cross-fostered nestlings that hatched in nests where pairs were food-supplemented during laying grew their tarsi and primary feathers more slowly than those hatched in control nests (Fig. 4.3 & 4.4). This may be because food supplemented females laid larger clutches (see Chapter 3) and may have invested less in individual eggs, which has been shown to influence the performance of nestling birds (Krist 2011). This effect may have been intensified when young were fostered into nests with potentially more competitive siblings (as in 2011) or because parents had not received the benefit of supplemental food during laying (both years), which may have resulted in adults having more reserves during brood rearing. These results suggest that the failure to find a strong and consistent relationship between eggshell colour and nestling growth rate was not due to a lack of connection between either female investment or brood rearing environment and nestling growth, but rather that egg colour may not be an important advertisement.

A complicating factor is that the growth rate of individual offspring was strongly related to their relative position in the brood size hierarchy for all metrics investigated (mass, longest primary, and tarsus) in both cross-fostered and non-foster young. This may complicate interpretations of the relationship between eggshell colour and nestling performance because most studies (including this one) rely on comparisons with average clutch colour rather than the colour of the specific egg each nestling hatched from. Although eggshell colour has been found to be more similar within than among clutches (Chapter 2; Moreno et al. 2004; Seifferman et al. 2006; Krist and Grim 2007; Soler et al. 2008; Hanley and Doucet 2009; Morales et al. 2011; Honza et al. 2012), within-clutch variation in eggshell colour is not distributed randomly. Previous studies have found that eggshell colour generally declines with laying order (Chapter 2; Moreno et al. 2005; Krist and Grim 2007; Morales et al. 2011; López de Hierro and De Neve 2010: but see Siefferman et al. 2006) and nestlings from eggs laid earlier within clutches often hatch first and are generally larger relative to their siblings (Clotfelter et al. 2000; Bitton et al. 2006). Therefore, the position of nestlings in the brood size hierarchy, and the colour of the eggs they hatched from may not be independent, but to my knowledge this has not been tested and further investigation is warranted to separate these effects.

4.6 Conclusion

The lack of relationship between the colour of the eggs that young hatched from and either nestling growth or fledging probability does not support the hypothesis that egg colour advertises maternal investment or that males would benefit from increasing paternal care accordingly. Moreover, the positive relationship between eggshell PC1 and the number of young fledged directly contradicts this supposition. Finally, the contradictory findings for the growth rates of cross-fostered young in each year also indicate that blue-green eggshell colour may advertise a positive rearing environment sometimes, while at other times it may actually relate to poor nestling rearing conditions. Together, these findings suggest that eggshell pigmentation in mountain bluebirds does not consistently predict nestling performance, either through pre-hatching maternal investment, or brood rearing conditions.

5. Blue-green eggshell colour does not have a positive effect on male provisioning in mountain bluebirds (*Sialia currucoides*)

5.1 Abstract

Clarifying the function of elaborate ornamental traits has been a key objective in evolutionary ecology. However, despite the common occurrence of such ornaments in females, the influence of female traits on male behaviour is still poorly understood. Blue-green eggshell colour is a trait produced by female birds in many species which may function as a sexually selected signal of female quality that influences the paternal care decisions of their social mate. Although a positive response to eggshell colour by male birds is a crucial prediction of the theory that eggshell colour is sexually selected, previous tests have been inconclusive and also have not accounted for avian visual capabilities. I examined the relationship between perceptually relevant measures of eggshell colour (achieved r, θ, Φ) and parental provisioning behaviour measured at three stages during brood rearing (early, mid, late). Because eggshell colour and nestling outcomes may be related, I used a cross-fostering design to separate the potential influence of nestling phenotype on provisioning behaviour from the effects of eggshell colour. Contrary to predictions if eggshell colour was sexually selected, achieved r (saturation) of eggs was negatively related to male feeding rates during mid brood rearing. Furthermore, eggshell colour was not related to male feeding rates in early and late brood rearing for any colour metric investigated. These results suggest that eggshell colour may be assessed by male birds, and may influence their provisioning behaviour at some stages of brood rearing, but it does not elicit increased parental care as predicted. Instead, these findings indicate that under some circumstances males provide increased care to young hatched from eggshells with less saturated colour.

5.2 Introduction

The ornamental traits of animals are thought to have evolved through sexual selection (Darwin 1871) by providing mating benefits that outweigh their costs in the context of natural selection. Sexual selection favours features that may be costly for individuals to produce and that are potentially detrimental to survival (Zahavi 1975), but provide a selective advantage through increased access to mates (Anderson 1994). This improved access to mates occurs through both intrasexual competition and mate choice (Kodric-Brown and Brown 1984) by enhancing the perceived quality of an individual to competitors and potential partners (Andersson 1982). Additionally, sexually selected traits may be of value not only during mate selection, but also by influencing parental investment in offspring depending on the apparent quality of the mate with whom offspring are produced. The differential allocation hypothesis predicts that individuals will invest more in the offspring of a mate assessed to be of higher quality based on ornamental traits (Burley 1986; Sheldon 2000; but see Ratikainen and Kokko 2010). Differential allocation has been investigated most often from the perspective of female investment in response to male ornaments (Sheldon 2000; Kiss et al. 2013). However, females in many species also produce ornaments (Amundsen 2000a & b; Amundsen and Pärn 2006), and differential allocation by males may also occur, particularly in species with biparental care.

Differential allocation is thought to occur because parental care can be energetically costly (reviewed in Clutton-Brock 1991) with energetic trade-offs between current and future reproduction for both sexes (Stearns 1989; Bleu et al. 2016). Such trade-offs have been validated experimentally in multiple taxa (Daan et al. 1996; Koivula et al. 2003; Cox et al. 2010; O'Brien and Dawson 2013), and for male birds specifically, there is evidence of trade-offs between reproduction and survival (reviewed in Santos and Nakagawa 2012), and between reproductive effort and the production of cyclically renewed ornaments (Gustafsson et al. 1995; Siefferman and Hill 2005b; Velando et al. 2009). Therefore, in avian species with biparental care, selection should favour males that adjust paternal effort to mediate trade-offs. Although differential allocation by males is theoretically plausible, the few tests of whether levels of paternal care correspond to female ornamentation have produced equivocal results. At least two experimental manipulations of perceived female quality have found an increase in paternal effort relative to female ornamentation (Burley 1988; Mahr et al. 2012), but other studies have found mixed support (Pilastro et al. 2003; Matessi et al. 2009), or attributed their findings as a response to intrinsic nestling quality rather than female traits (Pryke and Griffith 2010; Kiss et al. 2013). Further studies of the flexibility of male investment in response to female ornaments are needed, especially those that manipulate female traits, or cross-foster young, to prevent correlated maternal and nesting phenotypes from confounding results.

Eggshell colour is a trait expressed by females that warrants further investigation to determine if it influences paternal effort through differential allocation. The adaptive value of blue-green eggshell colour has been much debated (Underwood and Sealy 2002), and there is currently little consensus on the subject. Moreno and Oserno (2003) proposed that conspicuous and potentially costly (McGraw 2005) eggshell colours have evolved through sexual selection to elicit increased paternal care in birds with biparental care. There is empirical evidence that eggshell colour advertises the concentration of pigments in eggshells (Moreno et al. 2006a; López-Rull et al. 2008: but see Cassey et al. 2012), and that allocating pigments to eggshells may be costly for female birds (Morales et al. 2008). In addition, evidence suggests that the colour of eggshells depends on the condition of laying females (Moreno et al. 2005; Siefferman et al. 2006; Krist and Grim 2007; Chapter 3; but see Cassey et al. 2008; Hargitai et al. 2008; López-Rull et al. 2008; Johnsen et al. 2011). However, the prediction central to testing the differential allocation hypothesis in this context, that more saturated blue-green eggshell colour elicits higher levels of male investment, remains controversial. Several studies have provided support (Moreno et al. 2004; Moreno et al. 2006b; Soler et al. 2008), but others have found weak (Morales et al. 2010b; English and Montgomerie 2011) or no evidence (Krist and Grim 2007; Hanley and Doucet 2009; Johnsen et al. 2011) for differential allocation by male birds in relation to blue-green eggshell colouration.

One issue in testing the prediction that variation in egg colour influences investment decisions of male birds is that measures of colour used in previous studies may not reflect the actual perception of colour by birds. Colour is produced by light interacting with the pigments and structure of an object's surface, and the perception of this colour depends on the visual capabilities of the receiver (Cuthill 2006). Avian colour vision is based on four types of cones (tetrachromatic; Chen and Goldsmith 1986), allowing birds to perceive colour very differently than in the three cone (trichromatic) visual system that most metrics of colour are based on. Tetrahedral colour space models are one method of examining colour as perceived by birds. These models use quantum catch values for each of the four types of cones in a bird's eye to estimate a colour location in three dimensional space, and model what birds are likely capable of perceiving (see Goldsmith 1990; Endler and Mielke 2005; Stoddard and Prum 2008). Such models do not account for light environment or higher level neural processing of colour and so represent only what a bird is potentially able to perceive (Dakin and Montgomerie 2013), but they do provide a substantial advantage over trichromatic-based colour metrics when investigating behavioural responses to avian colour signals.

I tested whether there is support for differential allocation by male mountain bluebirds (*Sialia currucoides*) in response to perceptually relevant values of blue-green eggshell colour. I used tetrahedral colour space models to quantify eggshell colour, because although bluebirds nest in cavities and light limitation may affect the perception of colour, models attempting to account for reduced visual sensitivity due to light environment, such as photoreceptor noise-limited models (Vorobyev and Osorio 1998), have not been found to match behavioural discrimination experiments (Avilés et al. 2011). This suggests that birds can distinguish colour differences below detection thresholds presently predicted by noise-limited models under low-light conditions. I estimated paternal investment in offspring using male provisioning rates during brood rearing. In addition, because provisioning rates are often adjusted by individuals depending on the effort of their mate (Hinde 2006), I also investigated the relationship between eggshell colour and provisioning rates of females, as well as the proportional provisioning effort of males relative to their mates' provisioning. To separate the potentially confounding influences of nestling phenotype and eggshell colour on male provisioning rate, I conducted a full-brood cross fostering experiment in which approximately half of all broods were swapped on the day that they hatched, so that the eggshell colour observed by provisioning males was not the colour of the eggs the nestlings being provisioned had hatched from. I expected that if the eggshell colour observed by males is the factor that determines their provisioning effort, male provisioning rates should show a positive relationship with the eggshell colour they observed during laying and incubation regardless of cross-fostering treatment. However, if males are responding to nestling phenotype, and nestling phenotype is related to the colour of the eggs they hatched from, then provisioning rates should correlate with eggshell colour only when nestlings were not swapped between nests. If eggshell colour does not signal female quality in a way that affects male investment, then I would expect male provisioning rates to be unrelated to eggshell colour, regardless of whether the nestlings they provisioned were cross-fostered or not.

5.3 Methods

5.3.1 Study site, species, and general field procedures

I conducted this research on mountain bluebirds breeding in nest boxes southwest of Williams Lake, BC, Canada (51°N, 122°W) from mid-April to early August in 2012. The habitat of the study site consisted of xeric open grassland with scattered stands of Douglas-fir (*Psuedotsuga menziesii*). Mountain bluebirds are medium sized (~ 30 g), sexually dimorphic, socially monogamous passerines with altricial young, necessitating biparental care (Power and Lombardo 1996). The study population returned from migration as early as mid-March, and begins breeding in mid- to late-April. Females construct nests and incubate eggs with no assistance from males. Clutch sizes ranged from two to seven eggs, but five and six egg clutches are most common (O'Brien and Dawson 2013). The colour of bluebird eggshells range from pale blue to nearly turquoise, although white eggs can also occur (2-9% of clutches; Peak 2011). Both males and females actively defend their territories, participate in rearing nestlings (18-21 days to fledging), and contribute to post-fledging care (Power and Lombardo 1996).

I monitored nest boxes beginning in mid-April to determine the start of nest construction and clutch initiation dates. After clutches were compete (>24 hours with no new eggs laid), nests were not disturbed until 9-10 days after the last egg was laid (incubation period is ~13 days; Power and Lombardo 1996), when I measured the colour of all eggs in each clutch, quantifying their reflective properties using a spectrometer (see section 5.3.2 for details). I checked nests daily beginning 12 days after the start of incubation to determine hatch date (day 0 of brood rearing) and recorded the number of young that hatched. I quantified the provisioning rates of both parents using video cameras (Kodak PlaySport Zx3) placed near nest boxes at three different stages of brood rearing: early (nestlings aged days 4-6 post-hatch), mid (day 9-11 nestlings), and late (day 14-16 nestlings). Video observations were conducted between 0715 and 1730 PST, and averaged 1.8 ± 0.05 (standard error) hours long. To allow parents to return to their normal feeding behaviour after cameras were set up, I excluded data from the first 15 minutes of each observation period. Provisioning rates were measured separately for each parent as the number of feeds per hour. Additionally, I calculated the proportion of male feeds relative to female feeding rate by dividing male feeding rate by the total feeding rate.

5.3.2 Eggshell colour quantification

I measured the shell colour of developed eggs (see Appendix 1) over the range of wavelengths visible to songbirds (300-700 nm) using an Ocean Optics USB2000 spectrometer (Dunedin, FL) with a deuterium tungsten halogen light source (Avantes, Broomfield, CO). For each egg, I measured reflectance relative to a white standard in three places on the surface of the shell at its widest breadth and calculated colour metrics relevant to avian vision based on tetrahedral models of colour vision (Appendix 2; Goldsmith 1990; Endler and Mielke 2005; Stoddard and Prum 2008) using the package pavo (Maia et al. 2013) in R 3.1.2 (R Core Team, 2014): the relative saturation of colour for a given hue (achieved r: length of the vector relative to the total possible length); hue longitude (θ: hue in the non-UV range); and hue latitude (Φ: hue in the UV range). I took the average value of each of the three metrics for individual eggs and used these values in further analyses.

5.3.3 Experimental design

To separate the influence of the eggshell colour viewed by males from the potential relationship between nestling phenotype and the shell colour of the eggs they hatched from, I performed full brood cross-fosters (n = 27) in which all young were switched on day one of brood rearing. Broods were matched by hatching date, brood size, and, when possible, by pre-hatch clutch size. Additional broods that were not cross-fostered (n = 26) were also monitored and included in analyses as control nests. As part of another study, I carried out a pre-breeding supplementation experiment during egg laying, designed to determine whether food and/or antioxidant availability during pre-breeding and egg laying affected eggshell colouration (see Chapter 3 for details). Although not statistically significant, there was some suggestion that food supplementation enhanced eggshell colouration (see Chapter 3); therefore, I cross-fostered young preferentially between treatment and control nests in an effort to maximize egg colour differences between sets of nests where nestlings were cross-fostered.

5.3.4 Statistical analyses

I used linear mixed-effects models to assess the relationship between eggshell colour and male and female provisioning rates. Because the two measures of hue (θ and Φ) were correlated ($r = 0.74$, $P < 0.001$) and I wished to be able to interpret the effects of hue and saturation (achieved r) independently, the relationships between each metric of colour and parental provisioning rates were analyzed separately. To account for potential relationships between male and female feeding rate, I also evaluated the relationship between eggshell colour and proportion of male feeds relative to female feeds. Mountain bluebirds are facultatively double brooded (Power and Lombardo 1996) and will re-nest after clutch failure, but the pigmentation of second clutches is reduced compared to first clutches (Chapter 2); therefore, similar to O'Brien and Dawson (2009) only first breeding attempts (clutches initiated before 15 May) were used in analyses. I first performed preliminary analyses to investigate the effect of the pre-breeding supplementation treatment on provisioning rates. Supplementation did not influence either male ($F_{2,50} = 0.27$, $P = 0.77$) or female ($F_{2,50} = 0.05$, $P = 0.95$) provisioning rates, or the proportion of feeding visits made by male parents ($F_{2,50} = 0.10$, $P = 0.91$), and so was not considered further in analyses. Initial models had nest identity as a random factor, age category of the young (early, mid, late) when provisioning rates were recorded, and whether the brood was cross-fostered (yes, no) as fixed factors, and clutch initiation date, brood size, start time of the observation period, and eggshell colour as covariates. Initially, I included all two-way interactions between fixed factors and covariates and I removed terms that did not approach significance ($P \geq 0.10$) in a backwards stepwise fashion. However, as they were the predictors of interest, measures of eggshell colour were always retained in final models. In cases where final models included a significant interaction of covariates with age category, further analyses of provisioning rates were conducted separately for each age group using general linear models.

All analyses were performed using SAS software version 9.3 (SAS Institute Inc.) and for mixed-effects models degrees of freedom were estimated using the containment method (Schaalje et al. 2001). I present means ± 1 standard error, and considered results significant at the $P \leq 0.05$ level. Post-hoc tests were conducted when appropriate using a Bonferroni correction for multiple comparisons.

5.4 Results

5.4.1 Male provisioning rate

Male provisioning rates were not influenced by brood size or cross-fostering treatment (both P-values ≥ 0.33), nor were these variables included in any significant interactions (all P-values ≥ 0.26), so these terms were removed from all final models. The relationship between achieved r and male provisioning rate showed a significant interaction between the age of the young when provisioning was quantified and the average achieved r of the eggs in the clutch (Table 5.1), so I conducted further analyses for this colour metric separately by age category. When data were analyzed by age category, I found that male provisioning rates during early brood rearing (day 4-6 nestlings) increased with later clutch initiation dates and tended to decrease later in the day, but were not related to achieved r of eggshells (Table 5.2; Fig. 5.1). Similar to early stages, males fed more during mid-stage observations at nests with later clutch initiation dates, but they also increased provisioning rate when the average achieved r of the eggs in the clutch they observed was lower (Table 5.2; Fig. 5.1). Late in brood rearing (day 14-16), provisioning rates of males were not related to the achieved r of eggshells in the clutch (Table 5.2; Fig. 5.1); clutch initiation date and start time also were not significant (all P-values ≥ 0.58) and these variables were removed from final models.

I found no relationships between male provisioning rate and either of the measures of the hue of eggshells (θ and Φ; Table 5.1). These models did show that males fed young at higher rates later in brood rearing and earlier in the day, and there was a trend for males to feed broods with later clutch initiation dates at higher rates (Table 5.1).

Table 5.1. Results from linear mixed models that test the effect of the eggshell colour (achieved *r*, θ, Φ)[1] of mountain bluebirds on male provisioning rates measured during early (day 4-6), mid (day 9-11), and late (day 14-16) stages of brood rearing. See Methods for details of the quantification of provisioning rates and eggshell colour metrics.

Predictor	***F***	***df***	***P***
Achieved *r*	8.84	1,41	0.005
Age category	3.13	2,41	0.05
Start time	4.60	1,41	0.04
CID	9.85	1,41	0.003
Age category*Achieved *r*	4.54	2,41	0.02
Age category*CID	4.72	2,41	0.01
θ	2.28	1,46	0.14
Age category	6.74	2,46	0.003
Start time	7.86	1,46	0.007
Clutch initiation date	3.82	1,46	0.06
Φ	0.04	1,45	0.85
Age category	6.91	2,45	0.002
Start time	6.35	1,45	0.02
Clutch initiation date	2.87	1,45	0.10

[1] Achieved r is the relative saturation of colour for a specific hue, θ is a lateral measure of hue (hue in the non-UV range), and Φ is a vertical measure of hue (hue in the UV range).

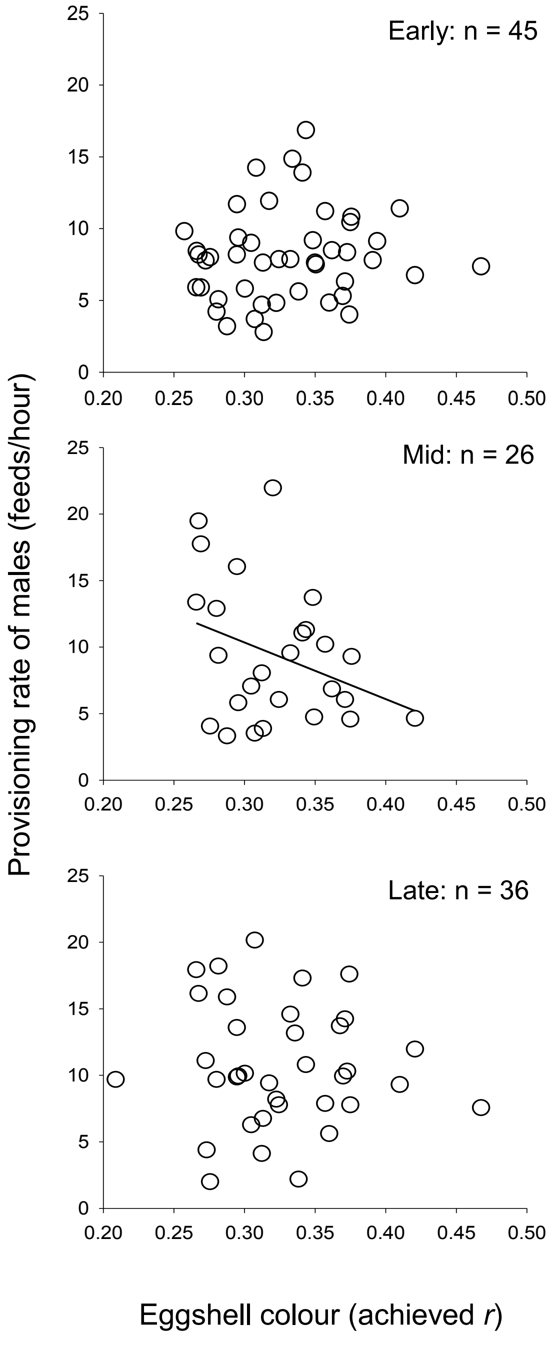

Figure 5.1. Relationship between the average eggshell achieved *r* of clutches and provisioning rates by male mountain bluebirds during the early (age 4-6 days), mid (age 9-11 days), and late (age 14-16 days) stages of the brood-rearing period. See Methods for details of the quantification of achieved *r* and provisioning rates.

Table 5.2. Results of general linear models that test the effect of the achieved *r*[1] of the eggs of mountain bluebirds on male provisioning rates in early (day 4-6), mid (day 9-11), and late (day 14-16) stages of the brood-rearing period. See Methods for details of the quantification of provisioning rates and eggshell colour metrics.

Age	**Predictor**	***F***	***df***	***P***
Early	Achieved *r*	0.69	1,39	0.41
	Start Time	3.65	1,39	0.06
	CID	7.26	1,39	0.01
Mid	Achieved *r*	8.85	1,20	0.008
	Start Time	3.03	1,20	0.10
	CID	10.69	1,20	0.004
Late	Achieved *r*	0.9	1,31	0.76

[1] Achieved r is the relative saturation of colour for a specific hue.

5.4.2 Female provisioning rate

There was no significant relationship between any of the metrics of perceived eggshell colour (achieved r, θ, Φ) and female provisioning rates (Table 5.3). There were also no significant interactions between eggshell colour and the age of the young at the time of observation or with cross-fostering treatment (all P-values ≥ 0.19). However, there were near-significant interactions between the age of the young when provisioning was quantified and the observation start time (Table 5.3), and between the age of the young and the cross-fostering treatment (Table 5.3); therefore, I conducted further analyses of female provisioning rates by age category. While female provisioning rates varied with clutch initiation date, brood size, and time of day the observation began in several of these analyses, I found that eggshell colour (achieved r, θ, Φ) was again not related to provisioning rate for any age category (Tables 5.4, 5.5, 5.6).

5.4.3 Proportional male provisioning rate

The proportion of total feeds made by males relative to female feeds was not related to any of the eggshell colour metrics investigated (Table 5.7). Relative male feeding rate was significantly related to the age of the young when provisioning was quantified in models testing for the effect of achieved r or θ, and a near-significant relationship emerged in the model for Φ (Table 5.7), with males feeding at a higher proportional rate earlier in brood rearing. However, males fed proportionally less to larger broods, significantly in the model with θ and nearly significant for achieved r and Φ (Table 5.7).

Table 5.3. Results from linear mixed models that test the effect of the eggshell colour (achieved r, θ, Φ)[1] of mountain bluebirds on female provisioning rates measured during the early (day 4-6), mid (day 9-11), and late (day 14-16) stages of brood rearing. See Methods for details of the quantification of provisioning rates and eggshell colour metrics.

Predictor	***F***	***df***	***P***
Achieved *r*	0.43	1,40	0.51
Age category	4.24	2,40	0.02
Cross-fostering treatment	6.90	1,40	0.01
Brood size	6.37	1,40	0.02
Start time	6.45	1,40	0.02
CID	8.53	1,40	0.006
Age category*Start time	3.25	2,40	0.05
Age category*Cross-foster	2.86	2,40	0.07
θ	1.01	1,41	0.32
Age category	3.89	2,41	0.03
Cross-fostering treatment	6.52	1,41	0.01
Brood size	6.07	1,41	0.02
Start time	7.08	1,41	0.01
CID	12.35	1,41	0.001
Age category*Start time	3.06	2,41	0.06
Age category*Cross-foster	2.97	2,41	0.06
Φ	0.01	1,40	0.75
Age category	4.01	2,40	0.03
Cross-fostering treatment	6.79	1,40	0.01

Brood size	6.12	1,40	0.02
Start time	6.45	1,40	0.02
Clutch initiation date	11.16	1,40	0.002
Age category*Start time	3.07	2,40	0.06
Age category*Cross-foster	2.84	2,40	0.07

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[1] Achieved r is the relative saturation of colour for a specific hue, θ is a lateral measure of hue (hue in the non-UV range), and Φ is a vertical measure of hue (hue in the UV range).

Table 5.4. Results of general linear models that test the effect of the eggshell colour (achieved r, θ, Φ)[1] of mountain bluebirds on female provisioning rates during early brood rearing (day 4-6). See Methods for details of the quantification of provisioning rates and eggshell colour metrics.

Predictor	***F***	***df***	***P***
Achieved *r*	1.36	1,39	0.25
CID	2.82	1,39	0.10
Brood size	4.39	1,39	0.04
θ	0.48	1,39	0.49
CID	3.95	1,39	0.05
Brood size	4.02	1,39	0.05
Φ	1.02	1,39	0.32
CID	3.25	1,39	0.08
Brood size	3.72	1,39	0.06

[1] Achieved r is the relative saturation of colour for a specific hue, θ is a lateral measure of hue (hue in the non-UV range), and Φ is a vertical measure of hue (hue in the UV range).

Table 5.5. Results of general linear models that test the effect of the eggshell colour (achieved *r*, θ, Φ)[1] of mountain bluebirds on female provisioning rates during mid brood rearing (day 9-11). See Methods for details of the quantification of provisioning rates and eggshell colour metrics.

Predictor	***F***	***df***	***P***
Achieved *r*	0.49	1,20	0.49
Start Time	8.56	1,20	0.008
CID	10.18	1,20	0.005
θ	0.83	1,20	0.37
Start Time	7.99	1,20	0.01
CID	11.74	1,20	0.003
Φ	0.11	1,20	0.75
Start Time	8.66	1,20	0.008
CID	10.98	1,20	0.004

[1] Achieved r is the relative saturation of colour for a specific hue, θ is a lateral measure of hue (hue in the non-UV range), and Φ is a vertical measure of hue (hue in the UV range).

Table 5.6. Results of general linear models that test the effect of the eggshell colour (achieved *r*, θ, Φ)[1] of mountain bluebirds on female provisioning rates during late brood rearing (day 14-16). See Methods for details of the quantification of provisioning rates and eggshell colour metrics.

Predictor	***F***	***df***	***P***
Achieved *r*	0.71	1,29	0.41
Cross-foster	6.72	1,29	0.01
Brood size	5.35	1,29	0.03
θ	0.71	1,29	0.41
Cross-foster	6.71	1,29	0.01
Brood size	5.57	1,29	0.03
Φ	0.71	1,29	0.41
Cross-foster	9.44	1,28	0.005
CID	3.08	1,28	0.09
Brood size	3.83	1,28	0.06

[1] Achieved r is the relative saturation of colour for a specific hue, θ is a lateral measure of hue (hue in the non-UV range), and Φ is a vertical measure of hue (hue in the UV range).

Table 5.7. Results from linear mixed models that test the effect of the eggshell colour (achieved *r*, θ, Φ)[1] of mountain bluebirds on proportional male provisioning rates relative to the total provisioning rates measured during the early (day 4-6), mid (day 9-11), and late (day 14-16) stages of brood rearing. See Methods for details of the quantification of provisioning rates and eggshell colour metrics.

Predictor	***F***	***df***	***P***
Achieved *r*	0.94	1,46	0.34
Age category	3.95	2,46	0.03
Cross-fostering treatment	2.77	1,46	0.10
Brood size	3.81	1,46	0.06
θ	0.13	1,46	0.72
Age category	3.67	2,46	0.03
Cross-fostering treatment	2.52	1,46	0.12
Brood size	3.81	1,46	0.05
Φ	0.63	1,45	0.43
Age category	3.68	2,45	0.06
Cross-fostering treatment	2.15	1,45	0.15
Brood size	3.63	1,45	0.06

[1] Achieved r is the relative saturation of colour for a specific hue, θ is a lateral measure of hue (hue in the non-UV range), and Φ is a vertical measure of hue (hue in the UV range).

5.5 Discussion

Provisioning rates of males were not related to perceived eggshell hue metrics (θ and Φ) during the early, mid and late brood rearing stage, and colour saturation (achieved r) was not linked to male provisioning behaviour in early and late brood rearing (Fig. 5.1 A & C). However, the provisioning rates of males during the mid brood rearing period were related to achieved r, but in the opposite direction than would be predicted if positive differential allocation was occurring, and more highly saturated blue-green eggshell colour was a signal of female quality (Fig. 5.1 B). Some studies have found a positive relationship between eggshell colour and male provisioning behaviour (Moreno et al. 2006b; Soler et al. 2008), others provide mixed support (Morales et al. 2011; English and Montgomerie 2011), and some have found no association between measures of male parental care (Krist and Grim 2007) and eggshell colour, but to my knowledge, no other study has found a negative relationship between eggshell colour and provisioning rate.

The energetic demands of nestlings are thought to be high during mid brood rearing as it is the period of peak growth (e.g., in closely related western bluebirds (*Sialia mexicana*); Mock 1991); therefore, increased male care may be particularly important at this time. Increased male provisioning at nests where egg colour was paler could represent a compensatory investment (Gowaty et al. 2007). If nestlings hatched from eggs with less saturated eggshell colour are at a disadvantage, for example as a function of compromised immunity (Moreno et al. 2005; Morales et al. 2006) or lower levels of pre-hatching maternal investment (Morales et al. 2006; Moreno et al. 2006a; Siefferman et al. 2006; López de Hierro and De Neve 2010; Navarro et al. 2011), males may attempt to compensate by increasing provisioning to nestlings hatched from pale eggs during this key stage of development. Increased investment in offspring produced with less-preferred mates has been found (Bluhm and Gowaty 2004), but tests of compensatory investment by males are exceedingly rare and this is potentially the first example of this phenomenon in relation to eggshell colour.

Female provisioning rate was not related to any metric of eggshell colour. Instead, female provisioning was generally related to brood size, clutch initiation date, and the time of day when provisioning was observed. An increase in female care post-hatch is not a central prediction of the sexually selected eggshell colour hypothesis, but if eggshell colour is a signal of female quality, a positive relationship between it and maternal provisioning might be expected. English and Montgomerie (2011) found that the provisioning rate of female American robins (*Turdus migratorius*) was higher early in brood rearing when vividly coloured fake eggs were placed in the nest, and Soler et al. (2008) found that spotless starlings (*Sturnus unicolor*) with experimentally enhanced eggshell colour provisioned at higher rates than those with reduced colour. However, natural eggshell colour was not related to female provisioning rates for either spotless starlings (Soler et al. 2008) or pied flycatchers (*Ficedula hypoleuca*) (Johnsen et al. 2011).

Proportional male effort was most strongly related to the age of the young when provisioning was quantified, with males bearing a greater share of the provisioning workload when nestlings were younger. Males may have fed more often relative to females earlier in brood rearing due to the time that females spent brooding the young before they are able to independently thermoregulate (~ day 6; Power 1966) rather than provisioning. In addition, male feeding rates were proportionally lower when brood sizes were larger. Male feeding rates were unrelated to brood size, but females increased their feeding rate with the number of young in the nest; therefore, the reduction in relative feeding rate for males with increasing brood size is likely a function of females provisioning more to larger broods. Moreover, while there was a relationship between male feeding rate and eggshell colour for mid-aged broods, the effect was not detected when proportional feeding rates were analyzed, indicating that adjustments in male feeding behaviour as a consequence of eggshell colour did not result in males taking on a larger relative share of the burden of nestling care during this period. I also did not find evidence during early or late brood rearing that the proportion of male effort relative to female effort was influenced by eggshell colour.

Finally, there was no evidence that the relationship between provisioning rates of males and eggshell colour was different when brood swaps were performed to isolate effects of the phenotype of the nestlings from the colour of the eggshells males observed. Had different relationships been found between male provisioning behaviour and eggshell colour for broods where nestlings were cross-fostered compared to those that were not, it would indicate that males may have been responding to something other than the colour of the eggs they observed during laying and incubation, such as nestling phenotype. Although my study design did not account for any potentially confounding effects of territory quality, or the influence of other aspects of female phenotype on male investment, these findings do suggest that when male provisioning behaviour is related to eggshell colour (i.e., during mid brood rearing), it is not the appearance or behaviour of the nestlings males are responding to when deciding on how much parental effort to expend. Similarly, when Moreno et al. (2006) performed a cross-fostering experiment with pied flycatchers, they found that male provisioning behaviour was related to the colour of the eggs they observed, rather than the colour of the eggs the young hatched from.

5.6 Conclusion

Although aspects of eggshell colour (saturation) do influence male provisioning behaviour under some circumstances, the results of my field manipulation do not support the hypothesis that blue-green eggshell colour functions as a signal of female quality that results in differential allocation by male mountain bluebirds. The increase in male provisioning when eggshell colour was less saturated could represent some form of compensatory investment, but this effect was only detected at one stage in brood rearing, and was not substantial enough to change the frequency of male feeds relative to female feeds, which may indicate that the biological significance of this effect was marginal. In addition female provisioning behaviour was not related to eggshell colour. Together, these findings suggest that eggshell colour is likely not an important determinant of parental effort in this species.

6. Synthesis

Although colourful ornamental traits are widespread among the females of many taxonomic groups (Amundsen 2000a), their evolution and function is still not well understood. Traits such as the blue-green eggshell colour produced by the females of many species of birds have previously been considered in the context of natural selection (Underwood and Sealy 2002), but are now being scrutinized as sexually selected advertisements of female quality that function to elicit differential allocation from males (Moreno and Oserno 2003). I used observations of naturally occurring patterns of eggshell colour variation and parental provisioning rates along with experimental manipulations of food and nutrient availability and cross-fostering treatments to determine the causes and consequences of the blue-green eggshell colour of mountain bluebirds (*Sialia currucoides*). My overall goal was to clarify whether eggshell colouration functions as a sexually selected signal in this species.

To interpret how a trait functions as a signal, it is important to understand how it varies within and among individuals and what drives these differences (Maynard-Smith and Harper 2003). In Chapter 2, I measured natural patterns of within- and among-clutch colour variation and found that eggshell colour was more consistent within clutches than among them, and was repeatable between years for first clutches laid by the same female, indicating that eggshell colour has an intrinsic component. These findings support previous research which suggests that eggshell colour is heritable (Morales et al. 2010a) and specific to individual females (e.g., Moreno et al. 2004). If eggshell colour is a signal of female quality, I expected that it would be related to other potential indicators of quality such as plumage colour (Siefferman and Hill 2005a) and clutch initiation date (O'Brien and Dawson 2013). I found some support for this, as females that initiated clutches earlier laid eggs that were less bright (potentially more pigmented) and those with bluer rump feathers laid eggs with more saturated blue-green colour, but these findings were not consistent between study years or among colour metrics, providing only weak evidence that eggshell colour is a signal of female quality.

Sexually selected signals are supposedly costly to produce and maintain (Andersson 1994), and the expression of some colourful ornaments is expected to be limited by the availability of pigments (McGraw 2005). The results I presented in Chapter 2 indicate that eggshell pigment, biliverdin, is limited. I found that pigment-related colouration declined naturally within clutches, and eggs laid later in the laying sequence had less saturated colour than early-laid eggs. Additionally, the eggs in second clutches had less-saturated colour than those in first clutches laid by the same female earlier in the same breeding season. Pigment limitation has also been demonstrated in a number of other species that lay blue-green eggs including pied flycatchers (*Ficedula hypoleuca*; Moreno et al. 2005; Krist and Grim 2007), house sparrows (*Passer domesticus*; López de Hierro and De Neve 2010), and blue-footed boobies (*Sula nebouxi*; Morales et al. 2011).

In Chapter 3, I used an experimental approach, providing supplemental food and micronutrients (carotenoids) to pairs during pre-breeding and egg laying, to further explore the effect of conditions during this period on blue-green eggshell colouration and pigment limitation. Females that received supplemental food prior to, and during egg laying, laid clutches of eggs with more saturated blue-green colour and lower brightness, but the effect was only significant in one of two study years. Moreover, birds that received carotenoids in addition to the supplemental food laid eggs that were not different from those laid by control females. In addition, neither food nor nutrient supplementation mitigated the decrease in eggshell colour later in the laying sequence. My results show that the production of blue-green eggshell colour among clutches is sensitive to food availability during pre-breeding and laying, but that the effect of supplementation may depend on factors that vary annually, and that supplemental food does not affect within-clutch colour changes. Studies conducted in a single breeding season (e.g., Moreno et al. 2006a; Morales et al. 2011) may not sufficiently capture the effect of environmental heterogeneity and fluctuating conditions which have been shown to be important for the expression and selection of ornamental traits (Cornwallis and Uller 2010).

One of the points of conflict regarding the potential for female traits to function as sexually selected signals is that the relationship between female ornaments and fecundity has not been well validated, and predictions are contradictory regarding the expected direction of relationships (reviewed in Nordeide et al. 2013). In Chapter 2, I found a positive relationship between eggshell colour and both egg mass and relative yolk volume, indicating that eggshell colour advertises investment in eggs. However, in Chapter 4 I found that there was no association between nestling growth rates and the colour of the eggs in the nest where they hatched, and a negative relationship between eggshell colour and the number of young that fledged from a nest. The results of the cross-fostering experiment reported in Chapter 4 are also conflicting; when full broods of young were cross-fostered the rate of the mass gain and growth of primary flight feathers of nestlings were positively related to the eggshell colour of the eggs in their foster nest, but when partial broods were fostered I found the opposite relationship. The link between eggshell colour and investment in eggs is intriguing, but it may be a by-product of both features being sensitive to food availability (see Chapter 2) and the lack of a positive relationship with nestling outcomes calls into question the value of eggshell colour as a signal.

One of the critical predictions of the sexually selected eggshell colour hypothesis is that males allocate more paternal care to offspring in nests where females laid eggs with higher levels of pigmentation. I investigated this prediction in Chapter 5 and my results did not provide support. I did not find a relationship between male feeding rates early or late during brood rearing, but I found that during mid brood rearing when the energetic needs of nestlings are likely to be high (Mock 1991), males fed at higher rates in nests where the eggs they observed during incubation had less-saturated blue-green colour. This could represent a compensatory investment by males to ensure that lower quality young survive this crucial period (Harris and Uller 2009). However, the effect was not strong enough to change the frequency of male feeds relative to female feeds, and there was no relationship between eggshell colour and female provisioning rate, so it seems unlikely this would provide a strong source of selection on the trait.

Together, my findings do not provide strong support for the hypothesis that blue-green eggshell colour is maintained by sexual selection. My results suggest that eggshell colour is affected by food availability, which may be the underlying cause of the relationship between eggshell colour and egg mass and yolk volume, and fluctuating patterns between years indicate that it is sensitive to annual variation in environmental conditions. However, the lack of a clear relationship between eggshell colour and nestling growth, and the lack of a positive response to eggshell colour by male birds, indicate that sexual selection is unlikely to be the explanation for the persistence of eggshell pigmentation in this species and other hypotheses should be pursued. In cavity-nesting birds, including mountain bluebirds, blue-green eggshell colour also reflects ultraviolet wavelengths, which may serve to increase the detectability of eggs to parents in dimly lit cavities, making activities such as egg turning and settling on eggs to incubate more efficient (Avilés et al. 2006; Węgrzyn et al. 2011). An alternative hypothesis is that eggshell pigmentation functions primarily to protect the developing embryo from harmful solar radiation (Lahiti and Ardia 2016). While it is unlikely that this would be an important current function for cavity-nesting mountain bluebirds, blue-green eggshell colour may have evolved in an open-cup nesting ancestral thrush for this purpose. If blue-green eggshell colour is a phylogenetic artefact in bluebirds, this would suggest that the costs of allocating biliverdin to eggshells is low, although few studies have directly measured the cost of deposition of biliverdin (but see Morales et al. 2008). Future research should consider the nutritional/metabolic component of blue-green eggshell pigmentation, but alternative functional explanations for the evolution and persistence of blue-green egg colour should be pursued.

Appendix 1. Incubation and embryonic development affect eggshell colouration

A1.1 Introduction

In studies of avian eggshell colouration, it is important to consider both the timing (e.g., laying or incubation) and the development of the embryo when colour is measured. Unfortunately, the timing of eggshell colour measurements has not been consistent among published studies. Some studies measured the shell colour of freshly laid eggs (Moreno et al. 2005, 2006a; Morales et al. 2006, 2008, 2010a, b, 2011; Krist and Grim 2007; Hargitai et al. 2008), while others have taken measurements later during laying (Cassey et al. 2008, 2012), in early incubation (Soler et al. 2008; Johnsen et al. 2011; Węgrzyn et al. 2011), or late in incubation (Hanley et al. 2008). In addition, several studies do not explicitly state what stage eggshell colour was quantified during (Moreno et al. 2006b, 2008, Siefferman et al. 2006, Hanley and Doucet 2009). The appearance of eggs changes noticeably throughout incubation; as the embryo and extra-embryonic membranes develop, less light is able to pass through and the egg becomes progressively more opaque (Enemar and Arheimer 1980; Deeming 2002). Furthermore, changes in the composition and microstructure of eggshells occur during embryonic development (Deeming 2002; Blom and Lilja 2004; Pisklak et al. 2012). The shell is the principle source of calcium for the growing embryo (Simkiss 1961), particularly in the later stages of development (Karlsson and Lilja 2008). Calcium removal causes erosion of the mammillary (inner shell) layer (Karlsson and Lilja 2008), thinning the shell and potentially affecting the light reflecting properties. With these changes in the content and structural components, it is plausible that the shell colour may change as well, though this has not been directly examined in the literature. If the colour of individual eggs is found to vary between laying and hatching, then comparisons among studies where colour was measured at different stages must be made with caution, or may not be appropriate at all. Additionally, colour measurements taken late in the incubation period are likely to include inviable eggs resulting from clutch abandonment, unfertilized eggs, or embryos that died early in development. It is therefore important to understand not only how colour of individual eggs change between the laying and incubation phases, but also how colour differs between viable and inviable eggs. Such knowledge is essential for making meaningful comparisons among studies, and to determine whether or not inviable eggs can be included in analyses of egg colour when measurements are taken late in the incubation period. My objective was to determine whether the timing of colour measurements, or the inclusion of infertile eggs in analyses, has an effect on the quantification of eggshell colour. This is important to understand as not all studies use the same methods and protocols, and differences among studies may result in inconsistent conclusions and render comparisons among studies problematic.

A1.2 Methods

A1.2.1 Study species and study site

This study was conducted using mountain bluebirds (*Sialia currucoides*) breeding in nest boxes at a study site located southwest of Williams Lake, B.C. (51°N, 122°W). The habitat is arid open grassland with scattered patches of Douglas-fir (*Pseudotsuga menziesii*). There were 84 pairs of nest boxes installed on fence posts, with boxes within pairs spaced approximately 5 m apart to avoid competition with other cavity-nesting species, particularly tree swallows (*Tachycineta bicolor*). Pairs of boxes were approximately 200 m apart to avoid intraspecific competition. Mountain bluebirds are migratory thrushes that favour open grasslands for breeding (Power and Lombardo 1996). They are secondary-cavity nesters and readily use nest boxes. Females lay clutches of primarily blue-green eggs (though between 2-9% lay white eggs; Peak 2011), containing from two to eight eggs, although five to six egg clutches are most common (O'Brien and Dawson 2013). The incubation period is approximately 13 days, and eggs are incubated solely by the female (Power and Lombardo 1996).

A1.2.2 Eggshell colour quantification

Colour measurements were taken over two breeding seasons (2011 and 2012) late in incubation (9-10 days after clutch completion); however, in 2012, I also measured a subsample of 44 eggs from 15 clutches during laying or very early incubation, and again on day 9-10 of incubation. The timing of the early measurements ranged from 0 to 3 days after eggs were laid. All colour measurements were taken using an Ocean Optics USB2000 spectrometer (Dunedin, FL) with a deuterium tungsten halogen light source (Avantes, Broomfield, CO). I extracted four colour variables from spectrometric data: brightness (total reflectance from 300-700 nm), hue (peak reflectance), blue-green chroma (relative reflectance from 400-605 nm), and ultraviolet (UV) chroma (relative reflectance from 300-400 nm) using the program CLR (Montgomerie 2008).

A1.2.3 Statistical analyses

I compared the brightness, hue, blue-green and UV chroma of viable eggs ($n = 44$ eggs from 15 clutches) measured during laying/early incubation and again in late incubation using a paired *t*-test to determine if eggshell colour was different between the two stages. I used a Pearson's correlation to determine if there was a predictable relationship between measurements of these variables for pre-incubation and late-incubation eggs. I found that hue values were not normally distributed (Shapiro-Wilk, $P < 0.001$ for early measures and $P=$

0.04 for late measures) and also failed to meet the assumption of homogeneity of variance (Levene's test *P*=0.01); therefore, I evaluated the difference between shell hue measured pre-incubation and in late incubation using a Sign test. I assessed the degree of association between the two measures with a Spearman's rank correlation.

For comparisons of late incubation measurements of viable and inviable eggs, I selected all clutches that contained at least one viable and one inviable egg from both years (n = 19 clutches for 2011, n = 17 clutches for 2012). One clutch containing white eggs was excluded from analyses. I used repeated measures analyses of variance to compare values within clutches. I averaged brightness, blue-green chroma, UV chroma, and hue separately for all viable and inviable eggs in each clutch, and used mean values in analyses. Year was included as a between-subject factor to test for the possibility of annual differences. Initial models were fully factorial, and I used a backwards stepwise approach to remove non-significant terms and interactions. Neither year nor the year-by-viability interactions were found to be significant so they were removed from all final models. I used Pearson's correlations to test for significant relationships for colour variables between viable and inviable eggs.

All analyses were performed using SPSS version 20 (SPSS IBM, New York U.S.A.), I present means ± 1 standard error, and considered results significant at the $P \leq 0.05$ level.

A1.3 Results

For viable eggs, all four of the colour variables (brightness, hue, blue-green and UV chroma) were significantly different between pre-incubation and late incubation measures (Table A1.1). Brightness, blue-green chroma, and hue all increased later in incubation, but UV chroma decreased (Fig. Al.1). However, although colour values changed between early

Table A1.1. Results of a paired *t*-tests comparing brightness, blue-green chroma, and ultraviolet (UV) chroma, and a Sign test comparing hue of the eggs of mountain bluebirds measured prior to, and in late incubation, and correlations between each variable in 2012 (n = 44 eggs from 15 clutches). See Methods for calculation of colour variables.

	Difference between egg colour measured early and late			**Correlation between egg colour measured early and late**	
Variable	***t***	***df***	***P***	***r***	***P***
Brightness	19.72	43	<0.001	0.74	<0.001
Blue-green Chroma	8.72	43	<0.001	0.76	<0.001
UV Chroma	-11.14	43	<0.001	0.70	<0.001
Hue	.	.	<0.001	0.34*	0.024

* r_s correlation

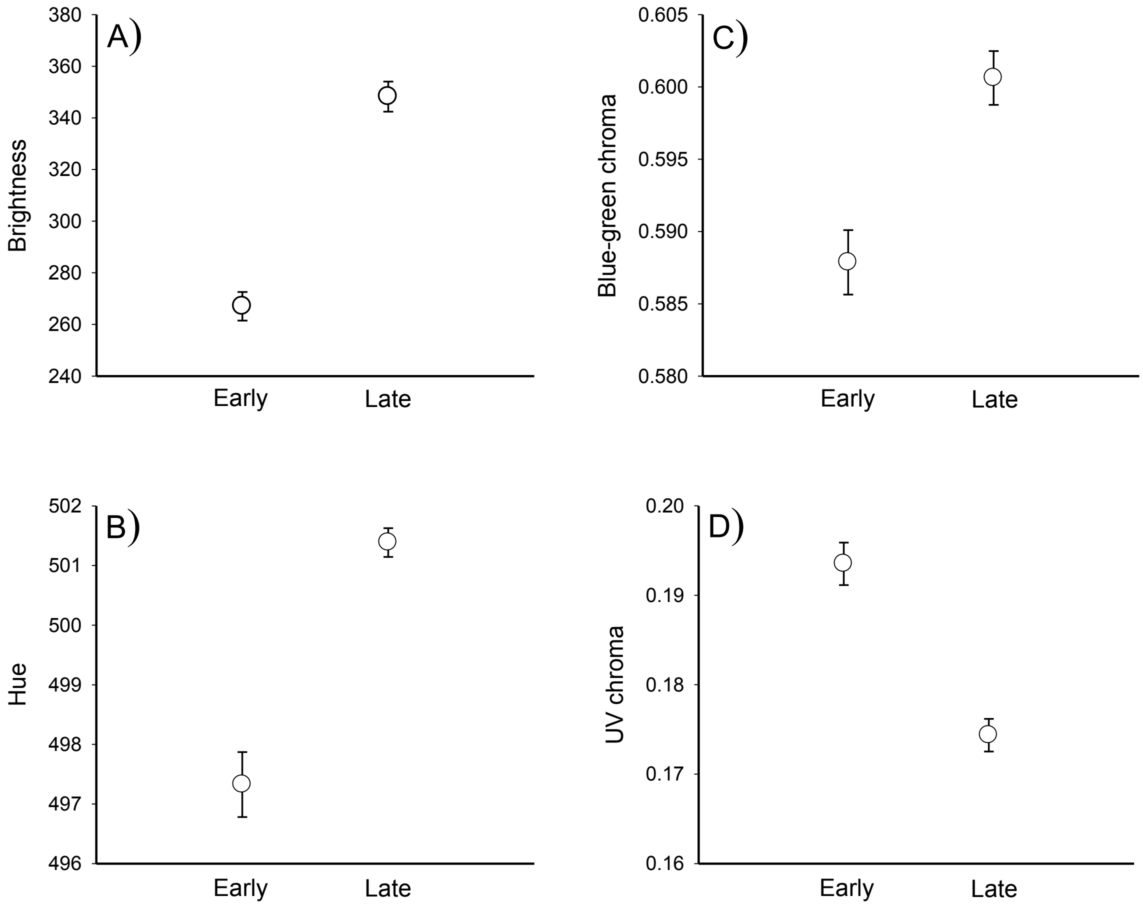

Figure A1.1. Mean (± SE) A) brightness, B) hue, C) blue-green chroma, and D) ultraviolet (UV) chroma of the eggs of mountain bluebirds measured prior to the start of incubation (0 to 3 days after clutch completion) and near the end of incubation (9 to 10 days after the clutch was complete) (n = 44 eggs from 15 clutches). See Methods for calculation of colour variables.

and late measurements, they were positively correlated (Table A1.1). The strength of correlations between pre-incubation and late incubation measures was similar for brightness, blue-green and UV chroma, but considerably lower for hue (Table A1.1; Fig. Al.2).

Egg colour measured late in incubation also was found to be significantly different between viable and inviable eggs within a clutch (Table Al.2; Fig. A1.3). As with comparisons of egg colour measured early and late, all of the colour metrics calculated were positively correlated between the average values of viable and inviable eggs within a clutch. The strength of the correlations was comparable among the four colour values, although brightness and blue-green chroma showed slightly stronger relationships between viable and inviable eggs, than did UV chroma and hue.

A1.4 Discussion

My results indicate that egg colour changes significantly between laying and late incubation (Fig. A1.1) and that the colour of viable and inviable eggs measured late in incubation differs within clutches (Fig. A1.3). Given that eggs undergo substantial physical and physiological changes during incubation (Romanoff and Romanoff 1949; Deeming 2002), this is not a surprising result. Not only does the growing embryo block light from passing through the egg, but biominerals are also eroded from the inner surface of the shell to support the growth of the developing embryo (Romanoff and Romanoff 1949; Simkiss 1961; Deeming 2002; Karlsson and Lilja 2008). It is unlikely that pigment molecules are directly removed from the shell, as they are most concentrated in the outer calcite layer (Romanoff and Romanoff 1949) and calcium is mobilized from the inner surface of the shell (Karlsson and Lilja 2008). However, changes in the shell thickness and composition could still influence the reflective properties. To my knowledge the potential for either direct leaching

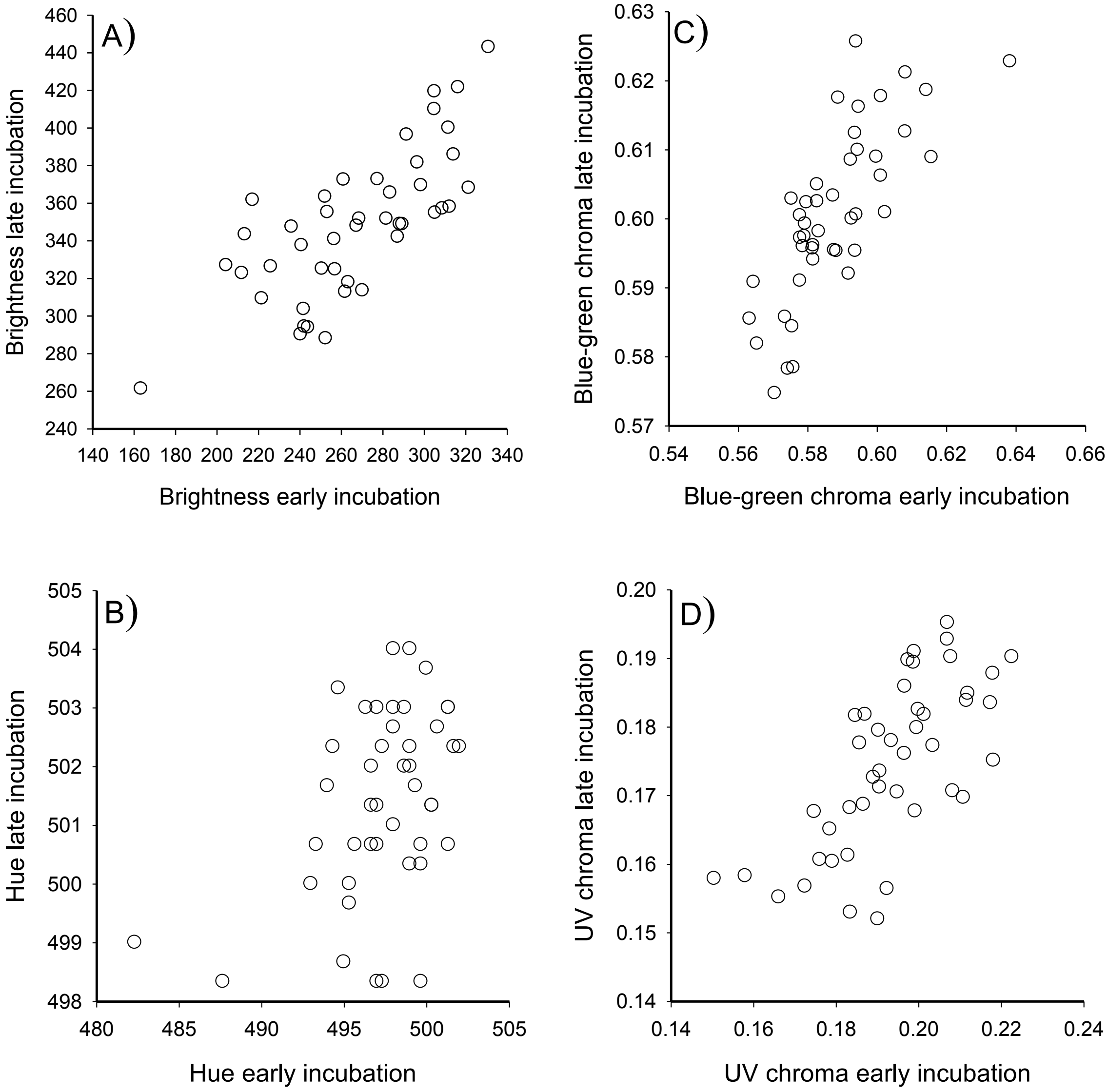

Figure A1.2. Relationships between eggshell A) brightness, B) hue, C) blue-green chroma, and D) ultraviolet (UV) chroma of the eggs of mountain bluebirds measured prior to the start of incubation (0 to 3 days after clutch completion) and near the end of incubation (9 to 10 days after the clutch was complete) in 2012 (n = 44 eggs from 15 clutches). See Methods for calculation of colour variables.

Table A1.2. Results of repeated measures analysis of variance testing the difference between measures of the shell colour (brightness, blue-green chroma, ultraviolet (UV) chroma, and hue of viable and inviable eggs of mountain bluebirds within clutches and correlations between each variable (n = 36 clutches). See Methods for calculation of colour variables.

	Difference between egg colour measured early and late			**Correlation between egg colour measured early and late**	
Variable	***F***	***df***	***P***	***r***	***P***
Brightness	37.3	1,35	<0.001	0.80	< 0.001
Blue-green Chroma	59.3	1,35	<0.001	0.81	< 0.001
UV Chroma	76.7	1,35	<0.001	0.74	< 0.001
Hue	38.6	1,35	<0.001	0.72	< 0.001

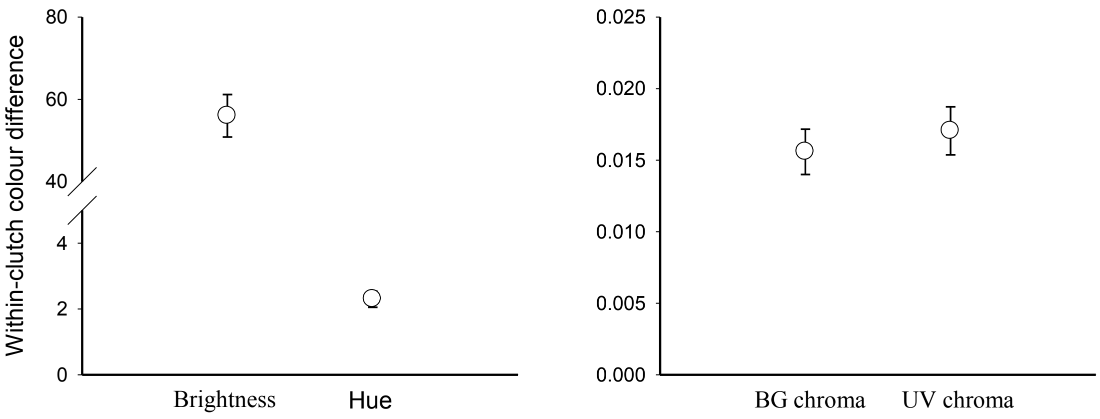

Figure A1.3. Mean within-clutch differences (± SE) in eggshell brightness, hue, blue-green chroma, and ultraviolet (UV) chroma between viable and inviable eggs of mountain bluebirds (n = 36 clutches). See Methods for calculation of colour variables.

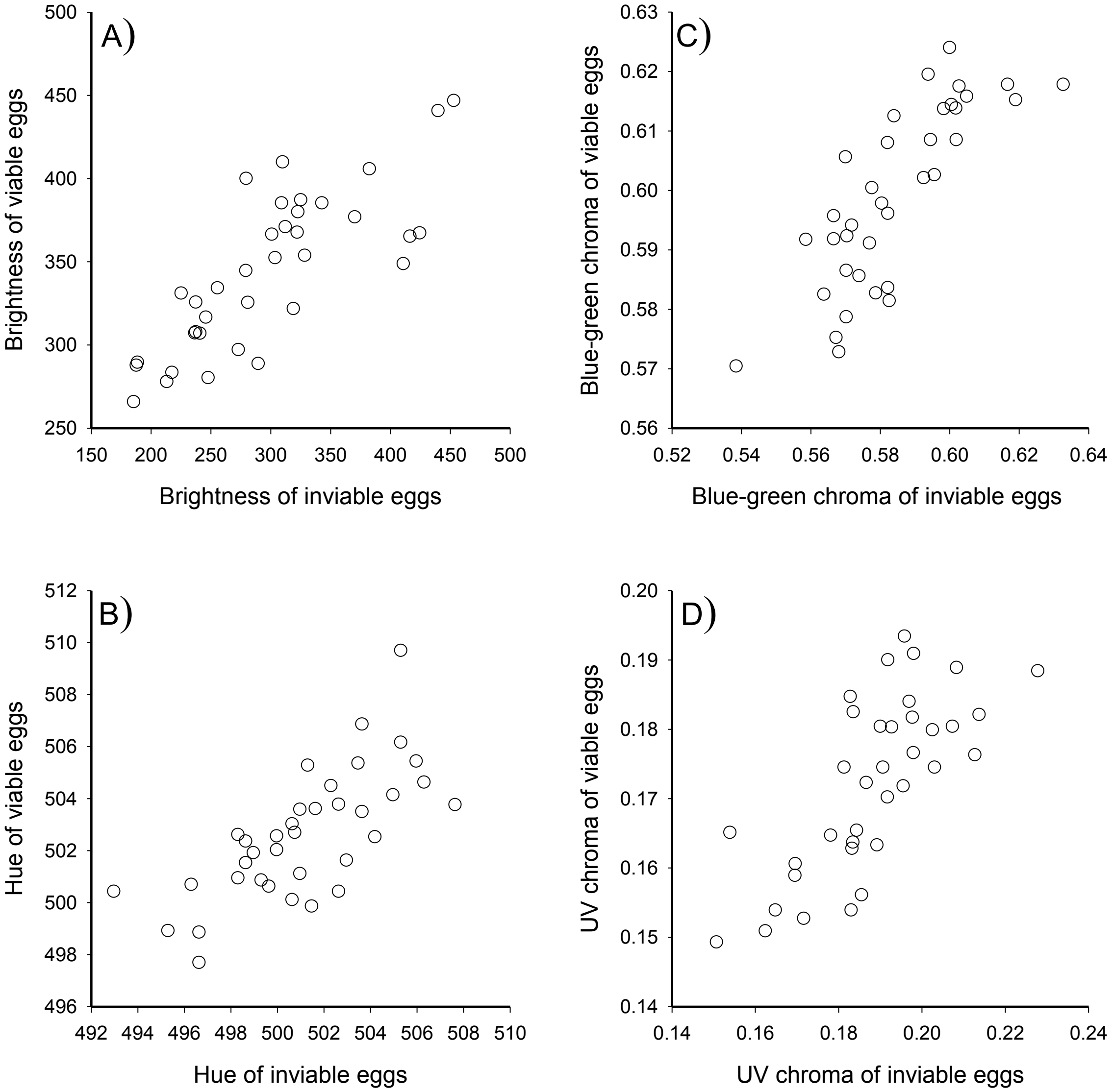

Figure A1.4. Relationships between eggshell A) brightness, B) hue, C) blue-green chroma, and D) ultraviolet (UV) chroma of the viable and inviable eggs of mountain bluebirds (n = 36 clutches). See Methods for the calculation of colour variables.

of pigment molecules or the influence of calcium removal on egg colour has yet to be investigated.

All metrics of egg colour measured in this study were significantly correlated both between early and late measurements (Fig. A1.2), and between viable and inviable eggs within clutches (Fig. A1.4), but these relationships were generally not strong. Given this observation, including inviable or abandoned eggs in analyses is inadvisable if colour is measured late in incubation. The correlations between colour metrics in fertile developed eggs measured during laying and late in incubation show that early colour does not entirely predict late colour, but that it may be possible to compare general patterns among studies that measured colour at different points in egg laying and incubation.

Appendix 2. Tetrahedral colour space

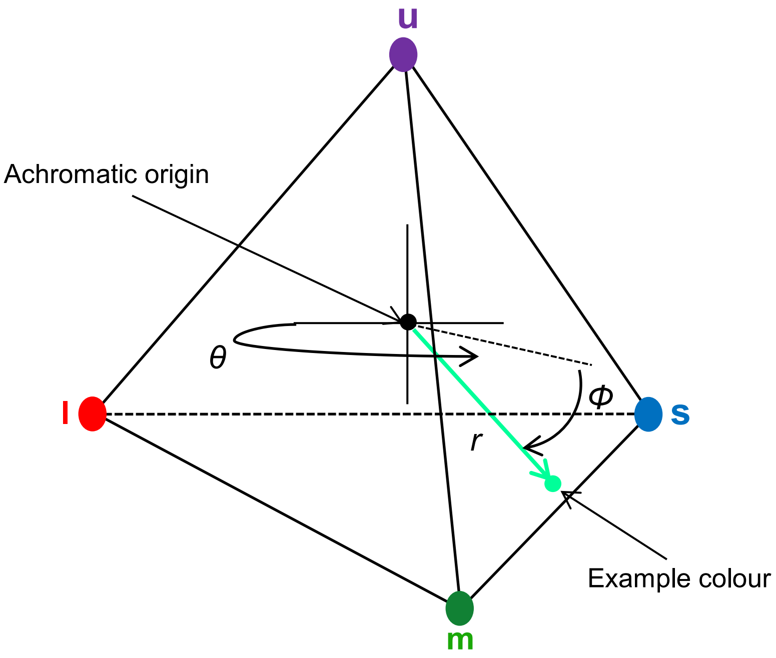

Figure A2.1. A schematic of tetrahedral colour space representing avian perceptual capabilities. Each of the four nodes represent one of the four photoreceptor types in avian colour vision, u (uvs – violet and ultraviolet sensitive), s (sws – short wavelength blue sensitive), m (msw – medium wavelength green sensitive), and l (lws – long wavelength red sensitive). Hue is characterised by the angle of the colour vector from the achromatic origin represented by the horizontal angle (*Θ*) and the vertical angle (*Φ*). The length of this vector is the saturation of the colour (*r*), which illustrates how different the colour is from achromatic (chroma). Figure modified from Stoddard and Prum (2008) and Dakin and Montgomery supplementary material (2013).

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