PARENTAL EFFORT IN THE NORTHERN FLICKER (*COLAPTES AURATUS*) AND THE

TRADE-OFF BETWEEN QUANTITY AND QUALITY OF OFFSPRING

A Thesis Submitted to the College of

Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Biology

University of Saskatchewan

Saskatoon

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ABSTRACT

The two main goals of my thesis were to further our understanding of how parental effort is related to life-history trade-offs and to see how parental investment is reflected in various potential measures of nestling quality. I looked at how fitness is maximized by examining (1) the trade-off between current and future reproduction, and (2) the trade-off between quantity and quality of offspring. To see how parents responded to energetic demands and whether each sex reacted in a similar way, I experimentally manipulated brood sizes and quantified provisioning rates. Both male and female parents with enlarged broods increased their feeding rates, but provisioning on a per nestling basis declined, so that parents fledged lighter nestlings with shorter wings. Although the incidence of mortality did not differ between control and enlarged broods, nestlings from enlarged broods were lighter than those from control broods with the same brood size, suggesting that clutch size may be individually optimized.

I also looked at how nestlings responded to different levels of nutritional stress in the manipulated broods by quantifying size and body condition, plumage colouration, and the physiological measures of T-cell mediated immune responses, and corticosterone levels in nestling feathers as a long-term integrated measure of stress physiology. The size of melanin ornaments on feathers and the saturation and brightness of carotenoid colouration was associated with nestling mass in such a way that suggested that plumage characteristics reflect nestling quality. The immune function of nestlings was negatively related to brood size and nestlings in better body condition could mount greater immune responses to foreign antigens suggesting that immune responses are energetically costly. Corticosterone levels in the feathers were not related to nestling body condition and were unaffected by the experimental brood manipulation. The

mass of male nestlings, which are the larger sex, was more compromised by brood size than female mass was. I also found sex-specific relationships between plumage characteristics and measures of physiological performance. These findings help to explain optimal clutch size and the classic trade-off between quality and quantity of offspring. They also offer new insights into the reliability of putative measures of quality in nestlings and relationships between physiological and morphological traits.

ACKNOWLEDGMENTS

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

Life-history theory aims to understand how fitness is maximized in a given environment by examining two major trade-offs. The first, between current and future reproduction, determines how much a parent is willing to invest in any one breeding attempt (Williams 1966; Stearns 1976). To maximize fitness, parents must weigh the investment demands of a current clutch against their ability to provide for that clutch, and the impact their investment in the current brood has on future survival and reproductive events. The amount of investment may differ between male and female parents if they have different life spans or breeding strategies (Houston and Davies 1985; Wiebe 2010; Low et al. 2011). In addition to the total amount of resources allocated to the current brood, there is a second trade-off between quantity and quality of offspring (Krebs and Davies 1991; Stearns 1992). Exploring the causes and consequences of reproductive effort in different species and in different ecological contexts has been an ongoing focus of research dedicated to understanding the evolution of life-history strategies (Lack 1947; Martin 2004).

Lack (1947; 1954) pioneered the study of reproductive effort in birds and his field experiments became the foundation for early optimization models for the evolution of clutch size. In general, parents are expected to produce a clutch size that results in the greatest number of surviving offspring, balancing quantity and quality of offspring (Lack 1954; Nur 1984b). The focus of early experiments with birds was to test whether the observed average clutch size in the population corresponded with the most productive clutch size. Subsequently, theory has been

refined to incorporate such factors as unequal distribution of resources among brood members and unpredictability of food supply (Forbes 1993; Mock and Parker 1998; Wright et al. 1998). Another aspect is that male and female parents may react differently to demands from offspring due to potentially differing life histories and prioritizations of the current brood (Wesolowski 1994; Clutton-Brock 1991; Low et al. 2011; Gow et al. 2013a). In these contexts, experimental manipulation of brood size is a useful technique in which to investigate the willingness of the sexes to increase investment in the current brood.

Since Lack (1954), many others have manipulated brood size to study costs to parents and offspring when parents attempt to rear larger broods (Lessells 1986; Dijkstra et al. 1990; Vander Werf 1992). Because the recruitment of offspring usually cannot be tracked directly, most researchers measure traits of nestlings they hope correlate with quality, and ultimately the survival, of the young. Many experiments have assessed quality in fairly simple ways as the weight or size of offspring (Askenmo 1977; Wright et al. 1998; Roulin 1999). However, there is no universally accepted way to measure body condition (Peig and Green 2010) and inferring offspring fitness from morphological traits measured pre-fledging may not provide a complete picture.

Three relatively new tools hypothesized to reflect nestling health and quality are (1) the strength of the immune system, measured in part by a T-cell mediated immune activation response (Saino et al. 1997; Tella et al. 2000b; Smits and Baos 2005), (2) plumage colour, specifically carotenoid- and melanin-based colour within feathers, as an honest indicator of health (Hill et al. 2002; De Ayala et al. 2007; Piault et al. 2012), and (3) the level of the stress hormone, corticosterone (CORT), in feathers (Bortolotti et al. 2008).

Several studies have linked individual variation in colour, hormones or immune function to the more traditional morphological measures of nestling quality and even survival (Saino et al. 1997; Møller et al. 2000; Tella et al. 2000a; Bortolotti et al. 2008). However, relationships between variables were not always straightforward and conflicting results between species may be linked to different life-histories in ways that are not obvious. For example, food-stressed Cory's Shearwater (*Calonectris diomedea*) chicks exhibited lowered feather CORT levels (Fairhurst et al. 2012) whereas food-stressed Red-legged Kittiwakes (*Rissa brevirostris*) had higher levels of CORT (Kitaysky et al. 2001). The patterns of physiological responses by nestlings to food stress are not well understood and needs further investigation in species with different life-history strategies and across different environments.

1.2 Objectives

My thesis examines the costs of increased clutch size in Northern Flickers (*Colaptes auratus;* hereafter "flickers") by measuring parental provisioning rates and potential indicators of quality in nestlings including morphology, plumage colour, and physiology. To understand the impact of nutritional demands on parents and offspring, I experimentally manipulated brood sizes and examined the body condition, immune function, carotenoid- and melanin-based feather colouration, and feather corticosterone levels of flicker nestlings. I also examined parental provisioning levels to see whether male and female parents respond flexibly and equally to the manipulated brood sizes.

In Chapter 2, I investigated the provisioning rates of flicker parents in response to experimentally manipulated brood sizes and looked at the consequences of per-capita provisioning on nestling mass, wing chord, and mortality. I also tested whether control brood

sizes in the population were more productive than manipulated brood size as would be expected if clutch size was individually optimized.

In Chapter 3, I investigated whether nutritional conditions during growth affected melanin and carotenoid-based plumage traits in flicker nestlings and how expression of these plumage ornaments may differ in relation to the brood size manipulation treatment. I predicted that nestlings in enlarged broods would have smaller melanin-based plumage spots on their breast feathers and lower saturation of carotenoid-based pigmentation.

In Chapter 4, I investigated how several indicators of quality (indices of body condition, one aspect of nestling immune function, melanin and carotenoid-based plumage traits, and feather CORT) relate to each other and whether they provide reliable indicators of the health and nutritional status of flicker nestlings. I also tested how brood size affected the physiological measures and may be reflected in the trade-off between quantity and quality of nestlings. I predicted that nestlings in enlarged broods would have lower body condition and immune responses and would have higher feather CORT than nestlings in control broods.

1.3 Anticipated Significance

Most studies of life-history trade-offs to date have been conducted on Passerines. The Northern Flicker, a woodpecker, differs from many other species of birds in a few ways that make it a good study species in which to test hypotheses about life-history trade-offs and differences in investment between the sexes. First, flickers have partially reversed sex roles (the male provides more parental care than the female; Wiebe 2005) and second, there is no extra-pair paternity (Wiebe and Kempenaers 2009). Additionally, flickers are single-brooded, making clutch size potentially the most important factor used by parents to adjust their investment in the

current breeding attempt. In my thesis, I attempted to measure nestling quality in several ways to test hypotheses about relationships between variables and to validate their usefulness as indicators of nutritional stress. In this respect, the use of feather CORT ($CORT_f$) is still very new and the patterns not well documented for nestlings.

Although several studies have examined the relationship between two quality indices such as carotenoid colour and melanin-based plumage characteristics (Griffith et al. 2006; Alonso-Alvarez and Galván 2011), carotenoid or melanin colouration and immune function (Saino et al. 1997, 2013; Soler et al. 2007; Gasparini et al. 2009), carotenoid or melanin colouration and CORT levels (Almasi et al. 2008; Fairhurst et al. 2014), or $CORT_f$ and immune function (Loiseau et al. 2008a; Harms et al. 2010, to my knowledge, no study has addressed the relationship between all four indices, that is, carotenoid colours, melanin-based plumage, immune function, and $CORT_f$, and matched these with indices of body condition and nutritional status. Doing so will broaden our understanding of how the indices relate to each other and will help validate the use of quality indicators in wild birds in general. Although causal relationships between measures could not be addressed in this thesis, future work could potentially use path analysis to show the relative importance of different additive and interactive pathways of indicators explaining variation in nestling quality.

1.4 Thesis Format

This thesis has been organized in manuscript format for publication. As a result, there may be some repetition of information throughout the text.

Chapter 2 has been published in full in *The Auk: Ornithological Advances*, under the joint authorship of Karen L. Wiebe (Musgrove and Wiebe 2014).

Chapter 3 is in preparation for publication in a peer-reviewed journal under the joint authorship of Karen L. Wiebe and Van J. Wishingrad.

Chapter 4 are in preparation for publication in a peer-reviewed journal under the joint authorship of Karen L. Wiebe and L. Michael Romero.

CHAPTER 2: NORTHERN FLICKERS INCREASE PROVISIONING RATES TO RAISE
MORE BUT POORER QUALITY OFFSPRING WHEN GIVEN EXPERIMENTALLY
ENLARGED BROODS

2.1 Introduction

Offspring provisioning is energetically costly for birds raising altricial young (Drent and
Daan 1980; Clutton-Brock 1991). A life history trade-off between provisioning effort and
parental survival means that natural selection should favour an optimal effort to the current brood
such that lifetime reproductive success is maximized (Clutton-Brock 1991; Stearns 1992). For
single-brooded birds, clutch size may be the most important factor used by parents to adjust their
investment in the current breeding attempt (Slagsvold and Lifjeld 1988) and Lack's (1954)
classic hypothesis suggested that clutch size in nidicolous species evolved to correspond with the
maximum number of young that parents could rear. However, because some subsequent field
tests found that parents given enlarged broods sometimes reared more young (Drent and Daan
1980; Lessells 1986; Vander Werf 1992), Lack's hypothesis was refined to acknowledge the
trade-offs between current and future reproductive effort (Nur 1984a; Conrad and Robertson
1993) and between offspring quality and quantity (Nur 1984b). For example, although parents
sometimes reared more young, the per-nestling provisioning rate decreased, resulting in
fledglings with a lower body mass and lower survival prospects (Dijkstra et al. 1990;
Schwagmeyer and Mock 2008). Thus, food may not always be a proximate factor limiting
provisioning levels; rather parents may lay fewer eggs than they could rear to optimize lifetime
reproductive success.

Not all brood enlargement experiments have resulted in increased provisioning effort. Parents with a fixed provisioning strategy do not change their foraging effort when nestling demand is experimentally altered presumably because they are subsequently unwilling to increase effort past a predetermined threshold (Ricklefs 1987; Morris 1987; Johnsen et al. 1994). A fixed provisioning strategy appears to be more common in long-lived birds such as seabirds and raptors (Ricklefs 1987; Sæther et al. 1993; Sasvári and Hegyi 2010) in which increased effort may jeopardize adult survival and severely reduce lifetime reproductive success. However, the extent to which flexible or fixed responses to increased brood demands are adaptive is hard to measure, and the responses of parents across and within species are variable (Drent and Daan 1980; Davies 1991).

Within species, body condition, sex, and age of parents may influence responsiveness to brood demands (Ardia 2007; García-Navas and Sanz 2012). Most brood size manipulations have not measured the distinct contributions of male and female partners despite their potentially different life histories and different prioritization of the current brood. Although results from previous studies are mixed, most female passerines provide more overall care than males (Clutton-Brock 1991; Møller and Cuervo 2000) and are often more responsive to brood size if they have the available energy reserves to respond to increased demands (Markman et al. 2002; Low et al. 2012). Males, on the other hand, may increase provisioning to cover shortfalls when females are already feeding at their maximum levels (Whittingham 1989; Moreno et al. 1995; Low et al. 2012).

In this study, I examine whether the provisioning rate of male and female Northern Flickers (*Colaptes auratus*; order Piciformes; hereafter "flickers") is flexible in relation to brood size. In natural (unmanipulated) broods of woodpeckers, feeding rates were positively correlated

with brood size in flickers (Gow et al. 2013a) and in Lesser Spotted Woodpeckers (*Picoides minor*; Rossmanith et al. 2009). To my knowledge, no studies have previously investigated the responsiveness of biparental birds with partially reversed sex-roles to experimental changes in brood size. Woodpeckers are an interesting group in which to investigate sex-specific parental investment because they are one of only two families of altricial birds with partially reversed sex-roles (Ligon 1999). In particular, the males of most woodpecker species seem to contribute more than females by being the primary excavator at the nest and by being the sole provider of nocturnal incubation and brooding (Winkler et al. 1995; Wiktander et al. 2000; Wiebe 2008). Along with the high investment by males, extra-pair young are very rare among woodpeckers studied to date (Michalek and Winkler 2001; Pechacek et al. 2005; Wiebe and Kempenaers 2009). Thus, I predicted that male flickers would be more responsive to nestling demands compared to many other species and would provision more than females because they may value the current breeding attempt more. Further, if parents increased their provisioning rate to larger broods, I wanted to see at what brood size provisioning rates reached a maximum and how the per-nestling provisioning rates in different sized broods affected the quality of offspring. Finally, I tested whether clutch sizes were individually optimized by comparing fledging mass and success of enlarged broods to unmanipulated (control) broods from a long-term dataset. If clutch sizes are individually optimized I expected the number of fledglings or nestling mass from enlarged broods to be lower than the unmanipulated (control) broods within the population.

2.2 Methods

2.2.1 Study Site and Study Species

I studied breeding flickers near Riske Creek, British Columbia, Canada (51°52'N, 122°21'W) during 2012 and 2013, on an area of approximately 100 km^2 of grassland interspersed with clumps of trembling aspen (*Populus tremuloides*) and surrounded by mixed coniferous forests. The breeding effort of ~100–160 breeding flicker pairs has been monitored here since 1998. Flickers weigh ~150 g, and have a relatively "r-selected" or "fast" life history strategy with large and variable clutch sizes (mean 8 eggs, range of 3–13 in the absence of brood parasitism; Wiebe and Moore 2008) and relatively low annual apparent survival rates of adults of ~42% (Fisher and Wiebe 2006). Incubation lasts ~12 days and the nestling period 25–27 days (Wiebe and Moore 2008). Both sexes provision nestlings at a similar rate, but males make slightly more feeding visits than females during the mid nestling period when nestling energy demands are at their peak (Gow et al. 2013a).

2.2.2 Field Methods

I found nests by using tape-recorded territorial playbacks and visually checking tree cavities. At active nests, small replaceable doors were cut in the tree trunk below the cavity entrance for access to nestlings and parents. Parents were trapped while incubating at the nest by plugging the cavity hole, placing a net over the hole, then removing the plug and flushing the bird into it. I weighed the adults and took six morphometric measurements (length of wing, 9th primary flight feather, and rectrix length were measured to the nearest mm with a ruler; length of bill, bill depth, and tarsus to the nearest 0.01 mm with digital calipers). For each sex, the six measures were entered into a principal components analysis (PCA) and all six measures weighted positively on the first PCA1 axis (Wiebe and Swift 2001), which was used as an overall measure of body size. The residuals of a regression of weight on body size (PCA1) was

used as an index of body condition. At the time of trapping, adults were aged up to 4 years according to moult (Pyle et al. 1997). By visiting nests every 4–6 days (flickers lay one egg per day until the clutch is complete), I determined clutch sizes and hatching dates.

During 2012 and 2013, I experimentally manipulated brood sizes 2–4 days after hatching by transferring ~40% (1-4 nestlings) from reduced broods to enlarged broods. All reduced-enlarged nest pairs were matched with respect to hatching date (± 1 day). I chose the number of nestlings to transfer such that I could maintain brood sizes within the natural range observed for the species (3–12 nestlings at the time of this study). Control broods were not manipulated, but had similar hatch dates as the reduced-enlarged pairs and were visited with the same frequency. The timing of breeding, i.e., hatch date (ANOVA : $F_{2,108} = 0.20$, $P = 0.82$), original clutch size ($F_{2,104} = 2.00$, $P = 0.14$), and original brood size ($F_{2,104} = 1.85$, $P = 0.16$) did not differ between treatments. Sample sizes at the start of the experiment of reduced, control and enlarged nests, respectively in 2012 were 10, 11, and 10, and in 2013 were 12, 8, and 12. However, I used only nests of monogamous pairs and deleted data if one parent subsequently died or disappeared ($n = 58$ at the beginning of the experiment and $n = 41$ at fledging).

Nestlings were weighed using a Pesola spring balance to ± 0.01 g at three stages of development: Stage 1 (ages 5–7 days old), Stage 2 (10–13 days old), and Stage 3 (18–21 days old). Flattened wing chord measurements were taken with a ruler at Stage 3, to the nearest 1 mm and included both the skeletal wing length and the primary feather length. During the three stages, I used video cameras (Sony Handycams) placed 4–6 m from the nest tree to record parental provisioning rates for 3–4 hours on days it was not raining. Preliminary analyses showed that estimates of feeding rates did not change with filming periods longer than 3 hours, so this was a sufficient period for estimation. I used the dimorphic plumage of flickers to identify

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which parent was feeding the young. Flickers regurgitate ants and ant larvae directly into nestlings' mouths and each visit to the nest was assumed to correspond to a feeding event unless a second visit occurred within 5 minutes from the last. Other videos taken from inside flicker cavities show that such visits are associated with nest sanitation and fecal sac removals and not additional feeding (K. Wiebe, unpublished data) and so they were not included in estimates of provisioning rates.

2.2.3 Provisioning Rates

An initial ANCOVA on provisioning rate as the dependent variable and incorporating effects of sex, brood size, nestling stage, and year found no effect of time of day (ANCOVA: $F_{1,275} = 1.29$, $P = 0.26$) or hatch date (ANCOVA: $F_{1,275} = 0.91$, $P = 0.34$) and a model with these two variables included did not fit the data significantly better ($P = 0.46$). Thus, these two variables were excluded from subsequent models. Provisioning rates were normally distributed and the data fit assumptions of homogeneity of variance, so I used linear mixed effect models (LME) to investigate the fixed effects of brood size, nestling stage, parent body condition, parent age, and the interaction between stage and brood size on provisioning rates from nests where both parents were known to be present. Random effects were year and nest in order to account for the repeated measures at the nest. However, because I was interested in sex-specific responses and due to interaction between nest stage and brood size (males: $F = 7.42$, $P < 0.001$; females: $F = 4.68$, $P = 0.009$), I ran separate models for the provisioning of each parent at each stage (Stage 1: $n = 53$, Stage 2: $n = 51$, Stage 3 $n = 40$), with only year included as a random effect (fitted as a random intercept).

I evaluated the support for a full model versus models with fewer parameters (including an intercept only model) by using Akaike's information criterion corrected for sample size (AIC_c) and AIC_c weights (w_i). I show models with Δ_i values of ≤ 6 (following Richards 2005), but consider models with Δ_i value of ≤ 2 AIC_c to be as plausible as the top-ranked model (Burnham and Anderson 2002). AIC_c weights (w_i) sum to 1 across the model set and indicate the relative likelihood of a model being the best at describing the data given the candidate model set (Burnham and Anderson 2002). I further determined the explanatory power of a fixed factor by summing the weights of all models that included the specific factor (Symonds and Moussalli 2011). Because of model uncertainty in the top models, I generated natural model-averaged parameter estimates ± unconditional standard error (SE) and 95% confidence intervals (CI) and examined whether they overlapped with zero. Finally, to test whether provisioning rates increased linearly with brood size or reached a threshold, I pooled provisioning by males and females and compared the fit of a linear versus quadratic regression model at each of the three stages with ANOVA *F*-tests (Zuur et al. 2010).

2.2.4 Nestling Attributes

I used LME models and model selection techniques (AIC_c) to investigate the factors that best predicted nestling mass at each of the three nestling stages. Brood size was the only main effect for Stages 1 ($n = 58$ nests) and 2 ($n = 53$ nests), but in Stage 3 ($n = 40$ nests) when I could sex nestlings by plumage, I also included sex and an interaction between sex and brood size. Nest of rearing was included as a random effect to account for multiple nestlings within each brood (fitted as a random intercept). Wing chord, as a dependent variable, was assessed at Stage 3 using a LME model with the same fixed and random effects used for nestling mass. Because

not all nestlings were measured at exactly the same age and I wanted to pool nestlings in the LME models, I standardized body mass (at Stages 1 and 2) and wing length based on average values for a particular age from the growth curves of control nestlings in Gow et al. (2013a). I did not standardize mass at Stage 3 because mass plateaus during this stage (Gow et al. 2013a). Because of model selection uncertainty, I focused on parameter estimates (± unconditional SE) and unconditional 95% CI for the fixed parameters in the selection of a "best" wing chord LME model.

I used logistic regression to determine the odds ratio of at least one nestling dying according to brood size. To test whether clutch sizes may be individually optimized, I compared data on the chance of nestling mortality and fledging success in manipulated broods to that of control broods of the same size within the natural population collected over 14 years. Similarly, I compared fledging mass between manipulated and control broods of the same size within the natural population (collected over a span of seven years). The mass at fledging of these control nestlings did not differ between years (ANCOVA: $F_{9,1462} = 2.01$, $P = 0.12$) and neither did the fledging success ($F_{9,819} = 1.85$, $P = 0.11$) so the years were similar in environmental conditions.

All analyses were conducted in R version 2.15.2 (R Core Development Team 2012). LME models were run using the lme4 package (Bates et al. 2012) and AIC_c values, weights, and natural model-averaging of parameter estimates were run using the AICcmodavg package (Mazerolle 2013). Unless otherwise indicated, data are reported as means ± SE, with statistical significance set at $\alpha \leq 0.05$.

2.3 Results

2.3.1 Parental Provisioning

Averaged over brood sizes, treatments, and years, the mean provisioning rate by males (n = 40) was 1.50 ± 0.08, 1.72 ± 0.06 , and 1.39 ± 0.17 trips per hour and for females ($n = 40$) was 1.41 ± 0.07, 1.69 ± 0.07, and 1.21 ± 0.17 trips per hour for Stages 1, 2, and 3, respectively. The maximum rate of 5–6 trips/h occured in the largest broods of 10–11 nestlings at the oldest nestling stage (Figure 2.1). A *post hoc* test of the total number of provisioning visits to the nest (males and females pooled) varied according to nestling stage (ANOVA: $F_{2,142} = 4.41$, $P = 0.01$), with an increase between Stage 1 and 2 (Tukey HSD: $P = 0.03$), but not between Stage 2 and 3 ($P = 0.98$). Feeding rates between partners did not differ at any stage of the nestling period (Paired *t*-test: Stage 1: $t_{52} = 0.99$, $P = 0.32$; Stage 2: $t_{50} = 0.43$, $P = 0.67$; Stage 3: $t_{39} = 0.32$, $P = 0.75$).

Except for males at Stage 2, brood size always appeared in the top model for provisioning rates and led to a summed w_i of ≥ 0.98 (except for males at Stage 2: $w_i = 0.26$ and females at Stage 1: $w_i = 0.59$; Table 2.1). Parameter estimates ($\pm$ unconditional SE) indicated that males and females increased provisioning with brood size at all stages (Table S2.1A). The body condition index of the parent also sometimes appeared in the top model with brood size, but the unconditional CI overlapped zero indicating non-significance. The best model for provisioning rate in relation to brood size was a linear increase at Stage 1, a decelerating quadratic curve at Stage 2, and an increasing curve at Stage 3 (Figure 2.1). Despite the general increase in provisioning rates with brood size, per-nestling provisioning rates decreased with brood size (ANOVA: Stage 1: $F_{1,51} = 28.27$, $P < 0.001$; Stage 2: $F_{1,49} = 71.21$, $P < 0.001$; Stage 3: $F_{1,39} = 7.09$, $P < 0.01$). The decline in per nestling provisioning rates was fairly linear across the range of brood sizes except at Stage 3 (Figure 2.2). At Stages 1 and 2, nestlings in the smallest broods received about twice as many feedings per hour as those in the largest broods.

2.3.2 Nestling Attributes

Within each stage, nestling mass decreased with brood size (Table 2.2; Figure 2.3; Table S2.1B). The interaction between brood size and nestling sex was the best model at Stage 3 (Table 2; $w_i = 0.75$); mass of male nestlings declined more dramatically with increasing brood size compared to that of females (Figure 2.3). The length of wing chord of male nestlings at Stage 3 (116 ± 0.7 mm, $n = 126$) did not differ significantly from females (116 ± 0.6 mm, $n = 139$; Table 2.2). Standardized to age, length of wing chord decreased with increasing brood size (Table 2.2; Figure 2.4). According to parameter estimates and unconditional CI, only brood size and not sex explained variation in wing length at fledging (Table S2.1B).

Ten of 16 enlarged nests (63%) experienced nestling mortality as did eight of 12 control nests (66%) and two of 13 reduced nests (15%). Of the 41 broods in the experiment that surived through Stage 3, a single nestling died in 10 cases, two nestlings died in six cases and $\geq$ three nestlings died in four broods. The likelihood that at least one nestling died did not differ between control and enlarged nests ($\chi^2_1 = 0.22, P = 0.64$), however the likelihood of mortality tended to be lower in reduced compared to control nests ($\chi^2_1 = 3.6, P = 0.06$), and was significantly lower in reduced compared to enlarged broods ($\chi^2_1 = 5.33, P = 0.02$). The likelihood of mortality increased with brood size (Logistic Regression: Odds Ratio= 1.58, 95% CI: 1.18 – 2.28, $P = 0.005$). To see how mortality changed in relation to the degree of manipulation away from the original brood size, I classified nests according to the number of nestlings added or removed. The likelihood that at least one nestling died declined as nestlings were removed ($\chi^2_4 = 13.5, P = 0.009$), but did not increase in enlarged broods as more nestlings were added.

Although the number of nestlings that died increased with brood size, the number of nestlings that fledged also increased with brood size (ANOVA: brood size effect: $F_{1,39} = 120.6$, $P < 0.001$; treatment effect: $F_{2,38} = 33.23$, $P < 0.001$; Figure 2.5). To determine whether clutch size may be individually optimized, I compared the productivity (number of fledglings) of manipulated broods to control broods of the same size from the natural population. There was no difference in fledging success between enlarged broods and the control broods from the natural population (2-way ANOVA: treatment group effect: $F_{1,491} = 0.33$, $P = 0.56$; brood size effect: $F_{3,491} = 12.89$, $P < 0.001$, interaction effect: $F_{3,491} = 0.75$, $P = 0.52$; Figure 2.5). Similarly, the number of fledglings from experimentally reduced broods did not differ from the control broods in the natural population (2-way ANOVA: treatment group effect: $F_{1,510} = 0.60$, $P = 0.44$; brood size effect: $F_{3,510} = 252.38$, $P < 0.001$, interaction effect: $F_{3,510} = 0.14$, $P = 0.93$; Figure 2.5). In the comparison of nestling mass between enlarged broods and control broods from the natural population, there was a significant interaction between brood size and treatment group (2-way ANOVA: treatment group effect: $F_{1,1138} = 0.53$, $P = 0.47$, brood size effect: $F_{4,1138} = 13.14$, $P < 0.001$, interaction effect: $F_{4,1138} = 4.20$, $P < 0.01$). Inspection of the data showed that for broods $\geq$ 8, nestlings were of lower mass in the enlarged broods compared to those in the control broods from the natural population. Nestlings in reduced broods were significantly heavier than nestlings in the natural population (2-way ANOVA: treatment group effect: $F_{1,768} = 14.41$, $P < 0.001$; brood size effect: $F_{3,768} = 6.91$, $P < 0.001$, interaction effect: $F_{3,768} = 0.29$, $P = 0.84$).

2.4 Discussion

Brood size manipulations in flickers revealed two main findings. First, both male and female parents followed a flexible rather than fixed provisioning strategy in relation to brood

demands. Second, parents could rear more offspring than their original brood size. Provisioning rates and brood size were positively correlated in my control broods and in unmanipulated flicker broods in the population (this study; Gow et al. 2013a) but my current experiment confirms that parents can assess demands from the brood and adjust their effort "in real time" as brood size both decreases and increases. Gow and Wiebe (2014a) reviewed brood size experiments and found that only 50% of six studies on long-lived birds (> 80% adult survival rate) with a "slow" life history (mainly seabirds and raptors) reported an increase in provisioning rates to enlarged broods and none decreased provisioning to reduced broods. This was in contrast to the 90% of 29 studies on short-lived species (mainly passerines) in which parents increased provisioning rates to enlarged broods and 100% of 18 studies where at least one sex decreased provisioning to reduced broods (Gow and Wiebe 2014a). Flickers thus appear to fit the flexible provisioning strategy typical of many other short-lived species.

2.4.1 Responsiveness of the Sexes in Relation to Life History Traits

In my study, both male and female flicker parents responded in a similar way to brood size. One reason that male flickers may be responsive to increased brood demands is that they are as confident of their parentage of a clutch as females. Parasitic eggs in the population may reduce the confidence of both maternity and paternity equally, but there are no extra-pair fertilizations to further reduce confidence of paternity (Wiebe and Kempenaers 2009). In general, species of birds with a high assurance of paternity should be the most willing to increase investment (Trivers 1972; Møller and Cuervo 2000). In another species of woodpecker, however, the provisioning strategy to natural broods was sex-specific; female, but not male, Lesser Spotted Woodpeckers (*Picoides minor*) provisioned in relation to brood size perhaps because females

had lower survival rates and valued the current brood more than males (Rossmanith et al. 2009). In contrast, survival rates do not differ between male and female flickers (Fisher and Wiebe 2006) and indeed, females may be less invested in the current brood than males because they have alternate reproductive opportunities through polyandry and conspecific brood parasitism (Wiebe and Kempenaers 2009).

The similar responses by the sexes also suggests that they face similar energetic constraints during the nestling period and are both equally capable of and/or willing to adjust effort to changes in nestling demand. In my study, the provisioning rates of both parents were nearly equal and it appears that both sexes work equally hard during the nestling period. However, female flickers often terminate care earlier than males in the post-fledging period and so their overall energetic investment in the brood may be lower (Gow and Wiebe 2014b). Among passerines, females often feed at higher rates than males, and thus are often working near their maximum (Low et al. 2012). During poor conditions (e.g., low food abundances, or poor weather) this means that female passerines may be more energetically constrained than males and unable to respond (Whittingham 1989; Moreno et al. 1995; Low et al. 2012), but this was not true for female flickers.

2.4.2 Determinants of Provisioning Rates

Male and female flickers with enlarged broods increased average provisioning rates by ~1.2 and ~1.3 times, respectively, relative to controls. This is lower than the increase of ~1.6 times and ~1.8 times for the sexes seen in a mate removal experiment (Wiebe 2005), but in the current study, I only increased demands by ~40% compared to a 100% increase which would have resulted from a missing partner.

Brood size was by far the strongest predictor of provisioning rate, whereas sex, age, and body condition of the parent had little effect. Feeding rates generally increased with brood size at each stage and reached a maximum at the latest nestling stage in the largest broods of 10–11 nestlings. If this high visitation rate represents a theoretical maximum work rate for parents, they were not working at maximum capacity during the mid-nestling stage (seen in the quadratic concave down model; Figure 2.1A), despite this being the time of peak energy demands of nestlings (Gow et al. 2013a). Woodpeckers in general have unusually long nestling periods relative to other birds their size (Yom-Tov and Ar 1993) and perhaps flicker parents restrain their effort during the mid-nestling period to ensure that they have enough reserves for continued care during the late nestling period (Stage 3) and the post-fledging period.

Although parents increased the rate of feeding to experimentally enlarged broods, the per nestling feeding rate generally declined with brood size in a similar way as in natural, non-manipulated broods of flickers (Gow et al. 2013a). However, the decrease was more dramatic with the manipulated broods in the current study. The decline in per nestling provisioning indicates that parents are not willing or able to fully compensate for additional nestlings in large broods, in agreement with some previous studies on other species (Drent and Daan 1980; Wright and Cuthill 1990). An exception to the declining per capita feeding rate occured in the very largest broods at the oldest nestling stage which had relatively high feeding rates (Figure 2.2C). Possibly, parents under severe brood demands were bringing more frequent, but smaller food loads to nestlings as documented by Lifjeld (1988) for Pied Flycatchers (*Ficedula hypoleuca*). I was not able to measure food loads directly, but the low fledgling mass of nestlings in large broods would be consistent with smaller loads.

2.4.3 Consequences of Provisioning Rates for Nestlings

Brood size influenced the body mass of nestlings at each stage of development, likely as a result of the lower per-capita feeding rates in larger broods. Although parents reduced total feeding rates to smaller broods, these nestlings had higher survival to fledging and higher fledging mass than nestlings in control or enlarged broods. On the other hand, the probabilty of mortality did not differ between enlarged and control broods, but the impact of less food was manifested mainly in terms of reduced nestling body mass. The body mass of male nestlings was more negatively affected by brood size than that of females perhaps because male nestlings (the larger sex) were more energetically costly to produce and thus more sensitive to food shortages. The body mass of the larger sex was also more suseptible to food shortages in several other studies (Eurasian Sparrowhawk, *Accipiter nisus,* Vedder et al. 2005; Lesser Black-backed Gull, *Larus fuscus*, Nager et al. 2000; Blue-footed Booby, *Sula nebouxii,* Torres and Drummond 1997). However, in some species, the larger sex may be at a competitive advantage within the brood if it is dominant and more able to monopolize food from parents (Råberg et al. 2005). My data do not support the idea that male flicker nestlings could outcompete females for access to parental deliveries, because of the greater decline in mass for males.

Flicker nestlings of both sexes had similar wing lengths which decreased with brood size. Thus, despite the slight sexual dimorphism in body mass, the sexes seemed to prioritize wing and feather growth to the same degree. In many nestling birds, structural size (i.e., wing chord) is less influenced by food shortages than body mass, due to the preferential allocation of energy to skeletal elements during growth (Schew and Ricklefs et al. 1998; Sears and Hatch 2008). The fact that wing growth was slower in enlarged flicker broods points to rather severe energy limitations experienced by those nestlings, and not just the males. I was unable to quantify exact

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fledging dates in my study because of the risk of forced-fledging, but in other studies, delays in fledging caused by slow development relative to siblings may lead to starvation (Lemel 1989) or a lengthening of the nestling period which may increase predation risk and decrease flying skills after fledging (Radersma et al. 2011). The general decline of both body mass and wing chord length with brood size suggests a quality versus quantity tradeoff in both manipulated and natural broods (Gow et al. 2013a; this study) of flickers. Unfortunately, local recruitment into the population is low (natal dispersal is high) and so I could not measure the direct effect of mass or size at fledging on recruitment. However, reduced recruitment among fledglings of lower body mass and size is a common pattern across species (Magrath 1991; Lindén et al. 1992).

2.4.4 Implications for Optimal Clutch Size Theory

If clutches (and by extension brood sizes) are individually optimized, unmanipulated brood sizes should be the most productive in the population. Relative to the original brood size, the chance of mortality declined in reduced broods, but parents of reduced broods did not rear significantly more fledglings compared to control broods of the same size in the population. Furthermore, especially heavy fledglings have reduced survival in the post-fledging period (Gow and Wiebe 2014b), suggesting that the number of fledglings can not be increased by having a smaller clutch than the one originally laid. On the other hand, parents of enlarged broods could rear the same number of nestlings as control broods of the same size in the natural population, but for broods larger than seven, the nestlings were lighter than those of unmanipulated broods in the population. This is consistent with individually optimized brood sizes because parents forced to rear unexpectedly large broods could not match the performance of parents with naturally large broods. At the population level, the proportion of the brood that fledged seemed to decline

after a brood size of eight, the mean clutch size in the population. Although I did not quantify food supply, the availabilty of ants (the main prey of flickers; Gow et al. 2013b) or time constraints for foraging may limit the capacity of most parents in the population to fledge more than eight nestlings.

In summary, the flexible feeding response by both flicker parents fits the pattern of "r-selected" species, with both sexes investing heavily in the current brood and discounting future survival prospects. The ability to forage and deliver food did not seem to be the limiting proximate factor constraining the original brood size laid by flickers, but parents had limited ability to provide for unexpectedly large broods. If anything, I may have overestimated the ability of parents to care for enlarged broods because I did not force the parents to lay and incubate extra eggs (e.g., Monaghan and Nager 1997). As flicker eggs are among the smallest and energetically cheapest eggs to produce in relation to body size of any known bird eggs (Wiebe 2006), I do not believe the costs to lay extra eggs are very large. I also did not create unnaturally large broods, but it would be interesting to do so to determine whether the limit to productivity is around 13 nestlings, the maximum recorded clutch size for the species (Wiebe and Moore 2008). It seems likely that the smaller mass and size of fledglings in enlarged broods would be associated with lower recruitment, but further investigation is needed. If true, this would be consistent with individual optimization of brood size in the Northern Flicker.

Table 2.1 A ranking of linear mixed effect models for provisioning rates (trips/hour) by Northern Flickers showing models within 6 ΔAIC_c of the top model. Separate models were evaluated for each sex of parent during three nestling stages (Stage 1: ages 5-7 days old, Stage 2: ages 10-13 days old, and Stage 3: ages 19-21 days old). Year was included as a random effect. k is the number of parameters and sample sizes are listed by each model candidate set.

	Model	k	ΔAIC_c	w_i
Male Stage 1[a]	Brood Size	4	0.00	0.60
(*n* = 44)	Brood size + Body condition	5	1.19	0.33
	Brood size + Age	5	5.25	0.04
Male Stage 2[a]	Null	3	0.00	0.60
(*n* = 43)	Brood size	4	2.05	0.21
	Body condition	4	3.37	0.11
	Brood size + Body condition	5	5.89	0.03
Male Stage 3[a]	Brood size + Body condition	5	0.00	0.62
(*n* = 33)	Brood size + Body condition + Age	6	2.06	0.22
	Brood size	4	3.06	0.13
Female Stage 1[a]	Brood size + Body condition	5	0.00	0.28
(*n* = 47)	Brood size	4	0.72	0.19
	Null	3	0.82	0.18
	Body condition	4	0.86	0.18
	Brood size + Age	5	2.27	0.09
	Age	4	4.09	0.04
	Brood size + Body condition + Age	6	4.73	0.03
Female Stage 2[a]	Brood size	4	0.00	0.91
(*n* = 51)	Brood size + Age	5	5.52	0.06
Female Stage 3[a]	Brood size	4	0.00	0.84
(*n* = 36)	Brood size + Body condition	5	4.61	0.08
	Brood size + Age	5	4.92	0.07

[a] The smallest AIC_c values were 92.2 (male Stage 1), 68.5 (male Stage 2), 84.6 (male Stage 3), 93.0 (female Stage 1), 63.0 (female Stage 2) and 78,7 (female Stage 3).

Table 2.2 A ranking of linear mixed effect models on the body mass and wing chord length of Northern Flicker nestlings, showing models within 6 ΔAIC_c of the top model. The three nestling stages are (Stage1: ages 5-7 days old, Stage 2: ages 10-13 days old, and Stage 3: ages 18-21 days old). Sex of the nestling and the interaction between sex and brood size was only considered at Stage 3. Nest was included as a random effect. k is the number of parameters. Sample sizes are 378 nestlings in 58 nests for Stage 1, 336 nestlings in 53 nests for Stage 2, and 265 nestlings in 40 nests for Stage 3.

	Models[b]	k	ΔAIC_c	w_i
Stage 1 Mass[a,b]	Brood size	4	0.00	0.92
	Null	3	4.85	0.08
Stage 2 Mass[a,b]	Brood size	4	0.00	1
Stage 3 Mass[a]	Brood size * Sex	6	0.00	0.75
	Brood size + Sex	5	2.24	0.25
Stage 3 Wing[a,b]	Brood size + Sex	5	0.00	0.37
	Brood size	4	0.69	0.26
	Sex	4	1.80	0.15
	Brood size * Sex	6	2.38	0.11
	Null	3	2.49	0.11

[a] The smallest AIC_c value was 2818.3 (Stage 1), 2567.2 (Stage 2), 1893.9 (Stage 3) and 1704.2 (wing Stage 3).

[b] Values standardized to age from the equations in Gow et al. (2013a).
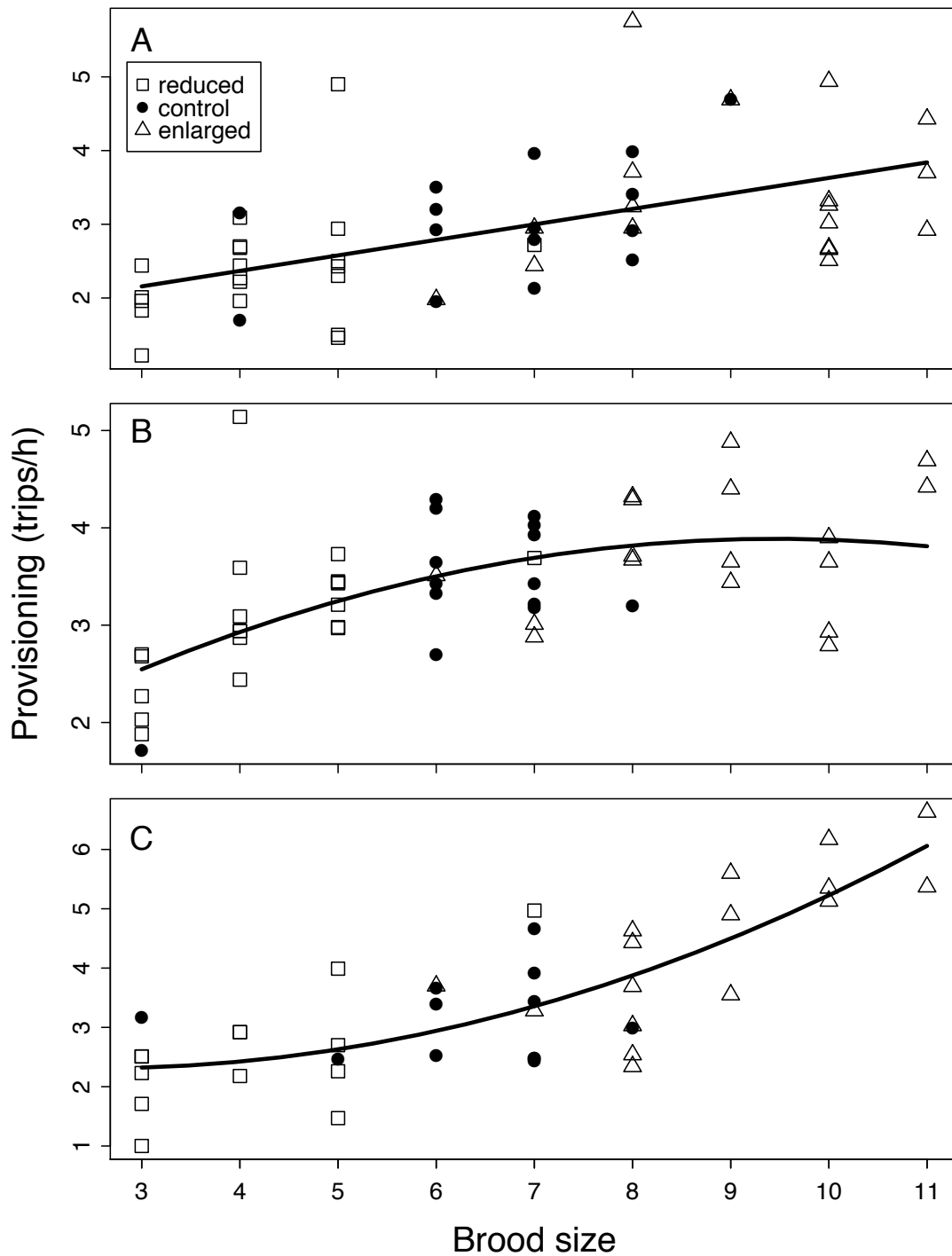
Figure 2.1 Total provisioning by parent Northern Flickers (sexes combined) in relation to brood size for three nestling stages: (**A**) age 5-7 days old, (**B**) age 10-13 days old, and (**C**) age 18-21 days old. The best-fit model was linear at Stage 1 (n = 53 nests), and quadratic at Stages 2 (n = 51 nests) and 3 (n = 41 nests). Symbols represent the experimental treatments.
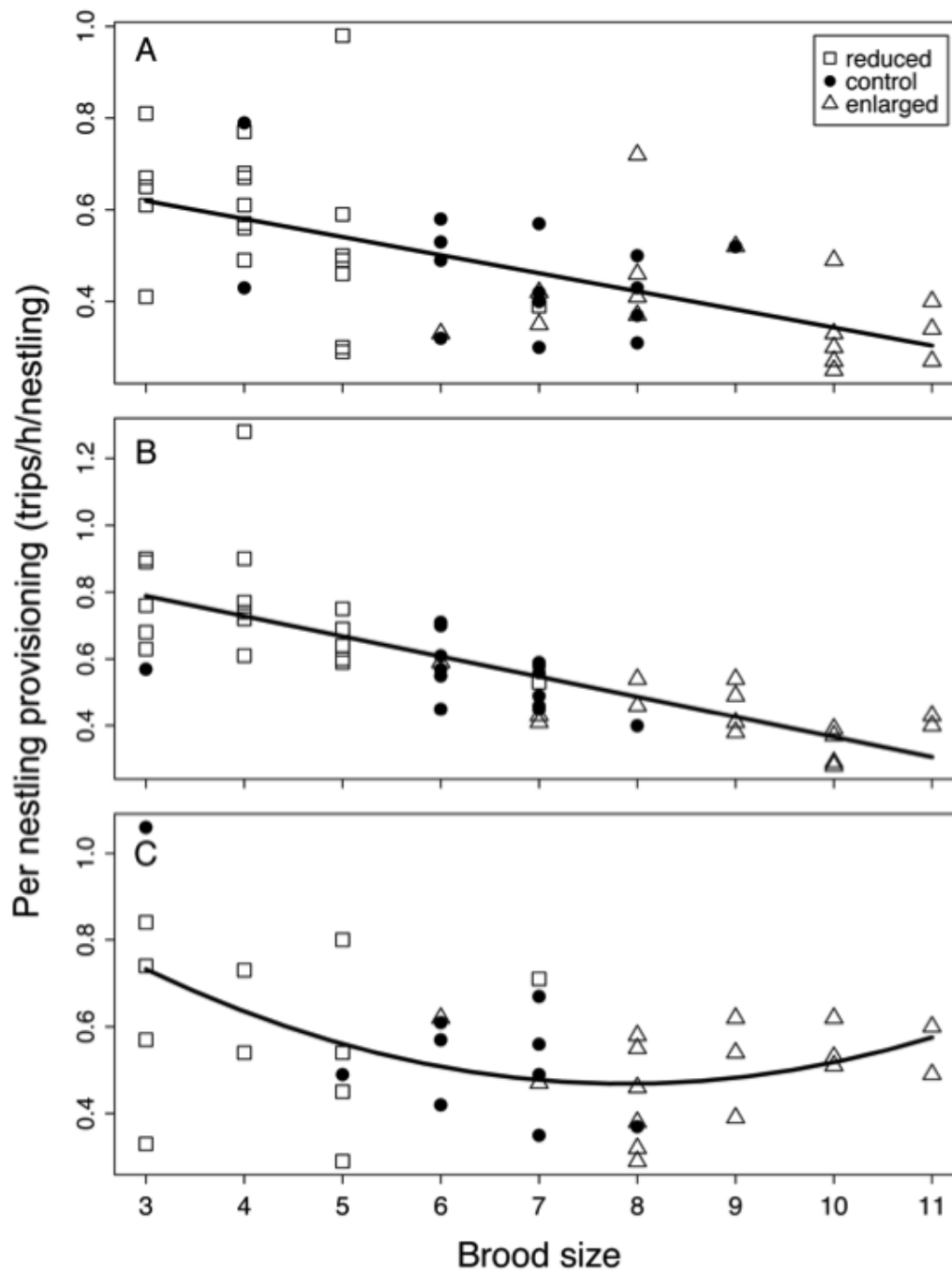
Figure 2.2 Per nestling provisioning (trips/h/nestling) by parent Northern Flickers in relation to brood size for three nestling stages: (**A**) age 5-7 days old, (**B**) age 10-13 days old, and (**C**) age 18-21 days old). The best fit models were linear for Stages 1 ($n = 53$ nests) and 2 ($n = 51$ nests), and quadratic for Stage 3 ($n = 41$ nests). Symbols represent the experimental treatments.
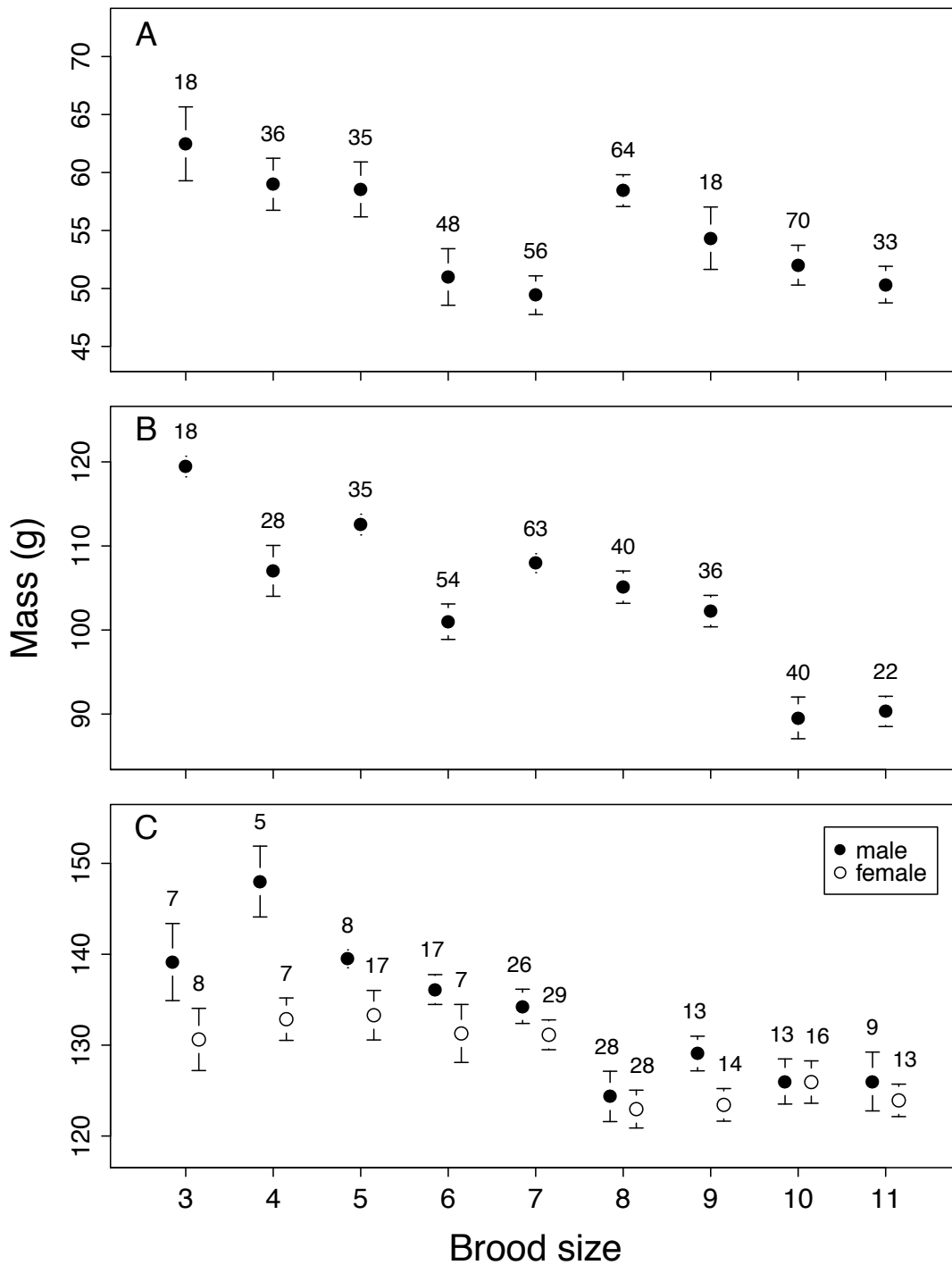
Figure 2.3 Body mass of Northern Flicker nestlings in relation to brood size at (**A**) Stage 1: 5-7 day old nestlings, (**B**) Stage 2: 10-13 days, and (**C**) Stage 3: 18-21 days. Sex-specific nestling mass is shown at Stage 3 with male (filled circles) and females (open circles). Sample sizes of nestlings are listed above the standard error bars.
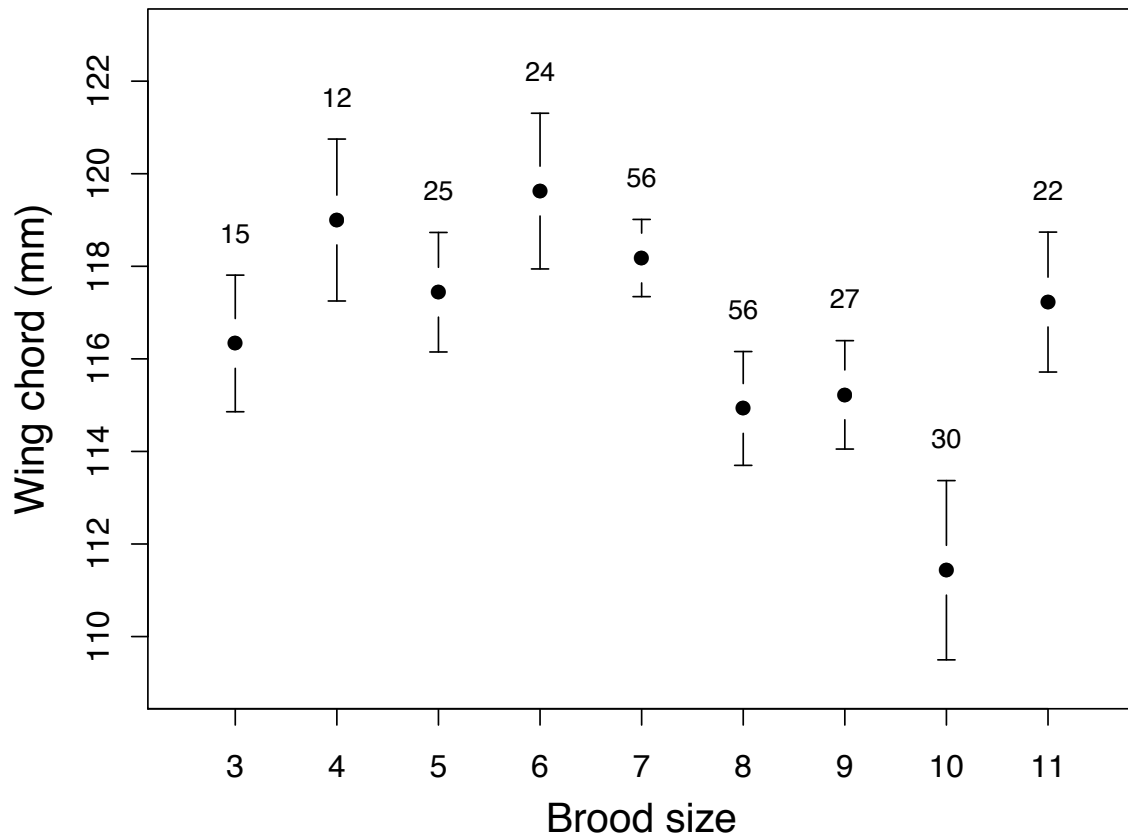
Figure 2.4 Wing chord of Northern Flicker nestlings in relation to brood size at Stage 3 (nestling age 18-21 days old). Sample sizes of nestlings are listed above the standard error bars.
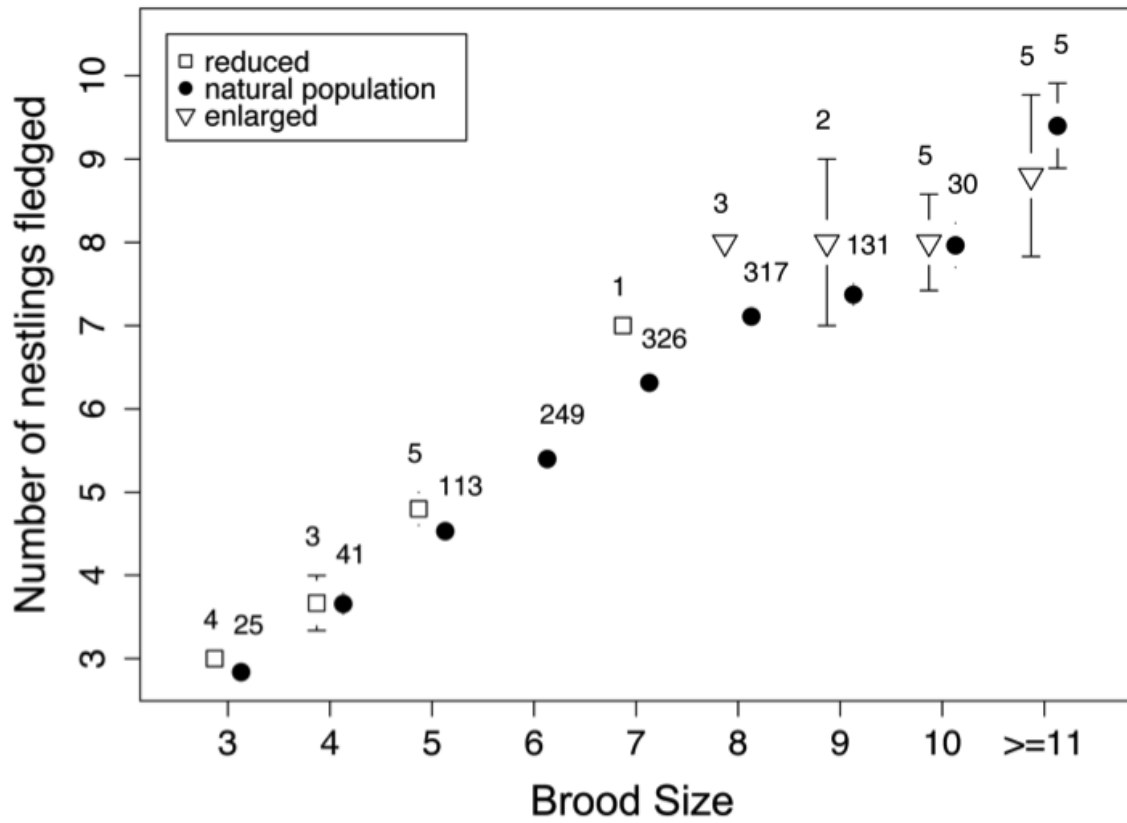
Figure 2.5 Mean number of Northern Flicker fledglings according to brood size in the natural population (filled circles) and in broods with experimentally reduced (open squares) and enlarged (open triangles) brood size. Nests with 11 and 12 nestlings are pooled. Sample sizes are the number of broods listed above standard error bars.

CHAPTER 3: CONDITION DEPENDENCE OF CAROTENOID- AND MELANIN-BASED
PLUMAGE COLOUR OF NORTHERN FLICKER NESTLINGS REVEALED BY
MANIPULATION OF BROOD SIZE

3.1 Introduction

Plumage colour is a prominent aspect of avian visual communication, functioning in

sexual selection (Hill 1990; Andersson 1994; Johnstone 1995), social competition (Senar 2006),

and parent-offspring interactions (Kilner 1997; Tschirren et al. 2005). In birds, the two most

common types of pigments responsible for colour are carotenoids (yellows, oranges, and reds;

Brush 1978) and melanins (blacks, grays, and browns; Fox 1976). These pigments differ

fundamentally in that birds can synthesize melanins within their body from amino acids (Hearing

1993), but they can only obtain carotenoid pigments from their diet (Brush 1978; Goodwin

1984). In addition to this biochemical difference in synthesis mechanisms, the two pigments are

believed to interact in different ways with other physiological processes, which suggests that

there may be a fundamental difference in the way that melanins and carotenoids are used in

animal visual communication and maintained as honest signals of quality (reviewed in Badyaev

and Hill 2000; Griffith et al. 2006).

Carotenoid colour, like other extravagant ornaments, is widely accepted to be a reliable

signal of the health status of an individual, functioning as a condition-dependent indicator of

quality (Andersson 1994; Badyaev and Hill 2000). There are three major hypotheses for why the

display of carotenoids may be energetically costly. First, due to the inability of birds to

synthesise carotenoids, acquisition of these pigments may reflect an individual's nutritional status

or foraging ability (Hill 1992; Hill and Montgomerie 1994). In wild adult House Finches

(*Carpodacus mexicanus*), dietary carotenoids have been directly related to nutritional condition and have been used to predict plumage colouration (Hill 2000). Second, the energy demands of carotenoid allocation, absorption, and metabolism into usable pigments for ornamentation appears to require good health and a strong immune system (Andersson 1994; Hill 2000; Vinkler and Albrecht 2010). Finally, carotenoids may function as an indicator of quality due to the trade-offs associated with their allocation to plumage display functions versus physiological functions like immunity and reproduction (Hõrak and Saks 2003; McGraw et al. 2005; Biard et al. 2009).

In contrast, the condition-dependence of melanin colours in plumage has been highly debated. Some studies have found that melanin colour is under strong genetic control (Berthold et al. 1996; Theron et al. 2001) and not sensitive to nutritional condition and environmental context (Roulin and Dijkstra 2003; Senar et al. 2003), or health status (Hill and Brawner 1998; McGraw and Hill 2000). However, other empirical evidence points to nutritional and energetic limitations to melanin production, suggesting that melanin pigments in plumage may in fact be an honest indicator of quality or reproductive potential (Veiga and Puerta 1996; Griffith et al. 2006). In Eastern Bluebirds (*Sialia sialis*) for example, males with larger melanised breast patches reproduced earlier, provisioned their nestlings more, and fledged larger offspring (Siefferman and Hill 2003). A recent meta-analysis supports the idea that both melanistic and carotenoid-based plumage can be condition-dependent; generally, darker or larger areas of melanin plumage signal better condition (Guindre-Parker and Love 2014). One of the few studies to experimentally manipulate body condition to examine its effects on melanin ornaments found that Eurasian Kestrel (*Falco tinnunculus*) nestlings in good body condition had larger melanin tail bands (Piault et al. 2012).

In birds, carotenoid and melanin colouration have been investigated primarily in adults in the context of sexual selection (Johnstone 1995). Some recent work has focused on parent-offspring communication, primarily on how nestlings may signal hunger (Kilner 1997) or health (Saino et al. 2000) through carotenoid-based mouth colour, but we still know little about what influences the plumage colouration in nestlings and what its adaptive significance may be. One reason that research on nestling plumage is lacking is that nestling plumage is typically duller than adult plumage in most species and usually lacks obvious ornamentation such as carotenoids (Brush 1990). Nevertheless, colour expression determined early in nestling life may have consequences for survival and reproductive success (Fitze et al. 2003; Jacot and Kempenaers 2007; Roulin and Altwegg 2007).

The main purpose of this study was to test whether nutritional condition during rearing could affect the carotenoid and melanin pigments deposited in the plumage of nestlings and hence provide a signal of quality. I studied Northern Flickers (*Colaptes auratus),* which are an ideal species in which to investigate the condition-dependency of plumage colouration because both sexes of nestlings have strong carotenoid-based colouration and distinct melanin patches at fledging. My goal was not to examine fitness consequences of nestling colour, although flickers breed in their first year of life (Wiebe and Moore 2008) and thus colour may play an important role in securing mates or nest sites. As in Piault et al. (2012) and Hõrak et al. (2000), I experimentally manipulated brood size to create rearing conditions with greater and lesser nutritional constraints than unmanipulated control broods. I predicted that the greater nutritional constraints experienced by nestlings in enlarged broods would be reflected in less carotenoid colour and smaller melanin breast spots. Additionally, I examined the effect of the brood

manipulation on nestling body mass and analyzed the relationships between mass and multiple

plumage variables.

3.2 Methods

3.2.1 Study Site and Species

I studied Northern Flickers at Riske Creek, British Columbia, Canada (51°52'N,

122°21'W) during breeding in 2012 and 2013. The study area is ~100 km^2 of grassland

interspersed with clumps of trembling aspen (*Populus tremuloides*) and mixed conifers. A

variety of flicker phenotypes occur at Riske Creek, with many flickers displaying plumage

characteristics of hybrids. Flickers have been studied here since 1998, and the breeding effort of

approximately 85-160 pairs has been monitored each year between late April and late July.

Flickers weigh approximately 150 g, and have an "r-selected" or "fast" life-history strategy with

large and variable clutch sizes (mean 8 eggs, range of 3-13; Wiebe and Elchuk 2003). Incubation

lasts ~12 days and the nestling period lasts between 25 and 27 days (Wiebe and Moore 2008).

Up to 5% of females annually are polyandrous and there is no extra-pair paternity (Wiebe and

Kempenaers 2009).

3.2.2 Field Methods

I found nests by visually checking tree cavities. At active nests, a small replaceable door

was cut in the tree trunk below the cavity entrance for access to nestlings. By visiting the nests

every 4-6 days on average, I was able to determine clutch sizes, hatching dates, and nest fates (as

flickers lay one egg per day until the clutch is complete). During summer of 2012 and 2013, I

experimentally manipulated brood sizes 2-4 days after hatching by transferring (on average)

~40% of the nestlings (1-4) from reduced broods to enlarged broods. All reduced-enlarged nest pairs were matched with respect to hatching date (± 1 day). I varied the number of nestlings to transfer so as to maintain brood sizes within the natural range for the species. Control broods were not manipulated but had similar hatch dates as the reduced-enlarged pairs and were visited with the same frequency. Transferred nestlings were marked with coloured thread around the tarsus for individual identification. The brood size manipulation was balanced so that timing of breeding, (i.e., lay date), original clutch size, and original brood size did not differ between treatments (ANOVA: $F_{2,104} = 0.58$, $P = 0.56$; $F_{2,104} = 2.00$, $P = 0.14$; $F_{2,104} = 1.85$, $P = 0.16$, respectively).

When the nestlings were 18-21 days old, (i.e., a few days before fledging), they were weighed using a Pesola spring balance (precision of 0.01 g) and sexed according to dimorphic plumage. Several feathers were also taken for analysis: I clipped a secondary (S2) wing feather distal to the vascularized region of the calamus and plucked three feathers from the central breast which were characteristic of the type of spots on the breast. In flickers, all breast feathers have a single dark melanin spot on the distal end. All feathers were stored in opaque envelopes until they could be analyzed and photographed in the lab.

3.2.3 Plumage Measurements

Of the 205 nestlings measured, 107 were from experimental two-parent nests in 11 control, 16 enlarged, and 13 reduced nests. In the lab, I measured the spectral reflectance of all secondary feathers with a Minolta CM-2600d portable spectrometer (Minolta Co., Ltd., Osaka, Japan) equipped with a UV xenon light source and a visible light standard illuminant (D65). I measured two regions on each nestling's secondary feather: the shaft, by centering the probe on

the shaft 20.5 mm from the distal end of the feather, and the vane, with the probe centered on the vane 8.5 mm from the distal end and about 5.5 mm from the shaft. Before each measurement, I calibrated the spectrometer against a white standard and obtained a reflectance spectrum at 10 nm intervals between 360-740 nm. I took three measures at each part of the feather and averaged them to obtain a separate spectral reflectance curve for the vane and shaft. The spectral curves were double peaked and consistent with those of carotenoid pigments in other studies (e.g., Toral et al. 2008) with the highest values around 600 nm and the lowest between 440 and 470 nm, the area of peak carotenoid absorption.

Colour indices often involve quantifying brightness, chroma, and hue, which correspond to the three major axes of colour variation (Montgomerie 2006; Butler et al. 2011). I ignored hue (the wavelength of maximum reflectance giving rise to the yellow, orange, or red colours) in my study because hybrid flickers may have slightly different colours, ranging from yellow to reddish, which are genetically based and irrelevant to measures of quality (Stradi 1998; Flockhart and Wiebe 2009). All calculated indices of colour were restricted at 700 nm, which is the limit of avian visual sensitivity (Endler 1990).

I measured total brightness (B_{total}), (i.e., how much light is reflected at all wavelengths), as the sum of reflectance over 360-700 nm ($R_{360-700}$) and then calculated average brightness (B_{mean}), which controls for the wavelength increments as $R_{360-700} / N_{360-700}$ (where N = number of wavelengths) and which facilitates comparisons between studies (Montgomerie 2006). Brightness is reduced as greater concentration of carotenoid pigments covers the underlying white keratin of the feather (Andersson and Prager 2006), but brightness may also be influenced by the nanostructure of feather barbs (Shawkey and Hill 2005). I also measured chroma, or spectral saturation, which is positively related to pigment concentration and is likely to convey a

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condition-dependent signal (Saks et al. 2003; Andersson and Prager 2006). I calculated a value that controls for brightness to eliminate potential biases which may occur when pigments are saturated (Andersson and Prager 2006) and which incorporates the reflectance where peak absorbance of carotenoids takes place: $(R_{700}-R_{450})/R_{700}$ (Cuthill et al.1999). See Table S3.1A for repeatabilities of the carotenoid colour variables.

I photographed the breast feathers (and a size scale) using a digital camera (Panasonic Lumix DMC FZ28) mounted on a tripod and calculated the total area of the melanin breast spots using Adobe Photoshop CS5. I calculated the mean spot area based on the three feathers from an individual nestling (See Table S3.1B for repeatabilities). I also classified the shape of the spot as circular, long, heart-shaped or wide, noted whether there were additional dark areas of melanin at the proximal end of the feather and if the spot shape was symmetrical on either side of the shaft (see Figure 3.1).

3.2.4 Statistical Analysis

To interpret the reflectance data, I first compared the calculated shaft and vane chroma and brightness scores of feathers perceived by me as "pale" ($n = 9$) to feathers perceived by me as more intensely coloured ($n = 139$). The comparisons were done only with feathers that had the same hue. Although my focus was not on adult plumage, I also analyzed the size of breast spots of adult flickers that had been measured with calipers in the field to better interpret the size of breast spots observed in nestlings. I used two datasets to analyze the effects of nestling mass (~quality) on plumage variables. A smaller dataset, which included only nestlings in the brood size experiment ($n = 107$) that had both parents alive and present until fledging, was used to establish a causal relationship based on treatment group as a predictor variable. A larger dataset

included non-experimental nestlings from the population that could be sexed ($n = 204$, as one individual could not be sexed) to test for correlations between body mass at fledging and colouration with a larger sample size.

I used linear mixed effect models (LME) to investigate the fixed effects of sex and treatment (or mass). Random effects were year and nest to account for the repeated measures at each nest and variation between years. An additional fixed effect 'nest of origin' was included in the linear mixed effect model for all plumage characteristics to test for genetic vs. environmental effects on the carotenoid colouration and melanin spot sizes in enlarged nests. The models were initially run with interaction terms, but these were dropped if the term was not significant in order to increase power. When interactions between sex and other effects in the LME were significant, I also investigated sex-specific plumage characteristics with correlations and ANOVAs. All analyses were conducted in R (version 3.1.0, R Core Development Team 2014) with the LME models using the lme4 package (Bates et al. 2012). *P*-values were estimated with type III orthogonal sums of squares using the car package in R (Fox and Weisberg 2011), with statistical significance set at $\alpha = 0.05$. Chroma, brightness, and melanin spot size residuals were normally distributed and the data fit assumptions of homogeneity of variance. Values are reported as means ± SE unless otherwise stated.

3.3 Results

3.3.1 Interpretation of Plumage Variables

Chroma of vanes and shafts was lower in the pale compared to the intense feathers ($t = 2.26$, DF $= 8.64$, $P = 0.05$; and $t = 2.81$, DF $= 8.44$, $P = 0.02$, respectively). The shafts of pale feathers were brighter, i.e., reflected more light, than those of intensely coloured feathers ($t = $ -

3.69, DF = 9.68, $P < 0.01$), but there was no difference in the vane brightness between pale and intense feathers ($t = 0.65$, DF = 9.10, $P = 0.53$). Within the entire sample of feathers ($n = 205$), the chroma of the vane and shaft portions of the feather were strongly positively correlated ($r = 0.61$, $P < 0.001$) and so I averaged the two for a single "feather chroma" variable. However, because the brightness of the vane and shafts showed different trends between the pale versus intense feathers, I kept these variables separate despite a correlation ($r = 0.42$, $P < 0.001$).

There was a significant negative correlation between feather chroma and shaft brightness for male nestlings ($n = 100$, $r = -0.23$, $P = 0.02$) and female nestlings ($n = 104$, $r = -0.31$, $P < 0.01$), but no correlation between chroma and vane brightness within either sex (males: $r = -0.01$, $P = 0.90$; females: $r = -0.02$, $P = 0.84$). Carotenoid chroma and melanin spot size was positively correlated for male nestlings ($r = 0.24$, $P = 0.02$), and approached significance for females ($r = 0.17$, $P = 0.09$; Figure 3.2A). On the other hand, female, but not male, nestlings with brighter shafts had smaller breast spots ($r = -0.22$, $P = 0.03$; and $r = -0.14$, $P = 0.16$, respectively, Figure 3.2B). However, there was no correlation between spot size and vane brightness for either females ($r = -0.05$, $P = 0.64$) or for males ($r = 0.15$, $P = 0.15$). Among adult flickers, males had larger spots than females (two-way ANOVA sex effect: $F_{1,87} = 14.47$, $P < 0.001$) and spots increased with age from the first to the second year (age effect: $F_{2,87} = 5.50$, $P < 0.01$).

3.3.2 Effects of Brood Size Manipulation

At fledging, brood sizes (mean ± SD) were 4.5 ± 1.3 nestlings in reduced broods, 6.7 ± 0.8 nestlings in control broods, and 9.0 ± 1.3 nestlings in enlarged broods, with a total of 265 nestlings in 40 broods. The experiment had the intended effect on nestling mass, causing both male and female nestlings to be heaviest in reduced broods and lightest in the enlarged broods

(LME: treatment: $F = 10.43$, $P < 0.001$; Figure 3.3). Males were heavier than females (LME: sex: $F = 18.86$, $P < 0.001$), being on average ~7.8 g heavier in reduced broods, ~5.4 g heavier in control broods, and ~2.9 g heavier in enlarged broods.

Within the smaller dataset with experimental nestlings ($n = 107$ nestlings in 40 nests) there was no effect of treatment on feather brightness (LME: vane: $F = 0.30$, $P = 0.75$; shaft: $F = 0.46$, $P = 0.63$) or chroma ($F = 0.05$, $P = 0.95$), but female nestlings ($n = 56$) had brighter (i.e., more reflective) feathers than males ($n = 51$; LME: vane: $F = 13.85$, $P < 0.001$; shaft: $F = 8.17$, $P < 0.01$; Figure 3.4A,B) and males had slightly higher chroma values than females (LME: $F = 3.56$, $P = 0.059$). For breast spot size, there was no sex effect (LME: $F = 0.75$, $P = 0.36$), but there was a treatment effect (LME: $F = 6.32$, $P = 0.043$); nestlings in reduced broods had larger spots than those from enlarged broods (Figure 3.4C).

3.3.3 Effects of Mass and Sex on Nestling Plumage

Within the larger dataset there was a significant interaction between sex and mass for vane brightness (Table 3.1), so I analyzed the sexes separately. Overall, females had brighter vane and shafts than males (t-test: $t = -2.94$, DF = 196.30, $P < 0.01$; and $t = -2.39$, DF = 197.63, $P = 0.018$, respectively); however, feather vane brightness in male nestlings ($n = 99$), but not females ($n = 104$), increased with mass ($r = 0.26$, $P = 0.011$; and $r = -0.01$, $P = 0.91$, for males and females, respectively; Figure 3.5A). Heavier nestlings of both sexes had slightly higher chroma values (Table 3.1). Females had larger melanin spots than males and spot size increased with mass in both sexes (Table 3.1). The correlation between spot size and mass for male and female nestlings, respectively was $r = 0.38$ ($P < 0.001$) and $r = 0.30$ ($P < 0.01$; Figure 3.5B).

3.3.4 Relatedness and Plumage Variables

I included 'nest of origin' as a factor in LME models along with mass and sex to see whether it explained significant variation in the colour variables within the enlarged broods ($n = 47$ nestlings in 16 broods). There were no effects of nest of origin on any plumage variable (LME nest of origin effect: vane brightness: $F = 0.61$, $P = 0.61$; shaft brightness: $F = 0.72$, $P = 0.40$; chroma: $F = 0.02$, $P = 0.69$; spot size: $F = 1.20$, $P = 0.27$). Furthermore, average spot size of parents was not correlated with the spot size of their biological offspring for either male ($n = 14$, $P = 0.96$) or female ($n = 15$, $P = 0.15$) parents.

3.3.5 Melanin Spot Shape

The most common spot shape was circular, accounting for 99 of 205 feathers (48%) whereas heart-shaped spots were the most rare (9% of feathers). For the three most common spot shapes, the distribution was approximately equal between the sexes (50% of long spots, 49% of circular spots and 45% of wide spots were found in female nestlings). However, 78% of heart-shaped spots ($n = 18$) were in females and this distribution differed between the sexes relative to that of the other spot shapes ($\chi^2 = 4.56$, DF $= 1$, $P = 0.033$). Of 205 nestlings, the majority had an additional melanin region at the base of the breast feather (85%), and this was not related to nestling sex ($\chi^2 = 0.50$, DF $= 1$, $P = 0.48$). Similarly, 48% of nestlings had asymmetrical spots and this was unrelated to sex ($\chi^2 = 0.02$, DF $= 1$, $P = 0.88$). There was no difference in mean body mass for nestlings with different melanin spot shapes for males ($n = 99$) or females ($n = 104$; ANOVA: $F_{3,95} = 1.19$, $P = 0.32$; $F_{3,100} = 0.68$, $P = 0.57$, respectively; Figure 3.6) or in relation to the symmetry of the spots (t-test: $t = -0.18$, $P = 0.86$, $t = 0.22$, $P = 0.82$, respectively; Figure 3.7). Female nestlings with and without additional melanin at the feather base did not

differ in mass ($t = 1.63$, $P = 0.13$), but heavier male nestlings had additional melanin areas ($t = 2.55$, $P = 0.019$; Figure 3.8).

3.4 Discussion

The brood size manipulation experiment provided strong evidence that nutritional conditions during rearing influenced melanin-based plumage patterns in Northern Flicker nestlings. To my knowledge, this is only the second experimental test of the effect of nutritional status during rearing on nestling melanin plumage and the positive relationship between body condition and melanin deposition I found is in accordance with the findings in Eurasian Kestrels (Piault et al. 2012). My results, which point to a positive relationship between nutritional status during growth and the intensity of carotenoid colours in flicker nestlings, also agree with the general findings of several other species (Hõrak et al. 2000; Tschirren et al. 2005; but see Laaksonen et al. 2008). My experiment does not rule out a fixed, genetic contribution to plumage colour, but it does suggest that the environment during rearing has a relatively larger impact on colour expression.

3.4.1 Explaining Carotenoid Colour Variables

Calculated measures of colour based on spectrophotometer readings (i.e., chroma and brightness) can be difficult to interpret. Chroma is perhaps the most straightforward because it has been repeatedly shown to depend primarily on the carotenoid content of the feathers (Andersson and Prager 2006), which in turn, reflects access to carotenoids, both in terms of quantity and quality in the diet (Slagsvold and Lifjeld 1985; Hill 2006). Generally, higher carotenoid content of the feather increases chroma and decreases brightness (Andersson and

Prager 2006). Consistent with this idea, paler feather shafts as perceived by the human eye had lower chroma values and higher brightness scores, presumably because a lack of pigments exposed the white keratin substrate of the rachis. On the other hand, the relationship between vane brightness and carotenoid content was less clear because pale feathers had similar vane brightness measurements as those of the intensely coloured feathers. One explanation is that flicker secondaries have dark (melanin-based) pigments on the dorsal surface and a layer of carotenoids on the ventral surface. Thus, a lack of carotenoid deposition in the vanes possibly exposes a darker vane area, which may result in little change to the overall reflectance or perhaps even a decrease in reflectance (i.e., perceived brightness). Alternatively, the microstructure of the vane itself, which is sensitive to developmental stress and abrasion (Fitzpatrick 1998), may have been altered with nutritional stress in a way that reduced reflectance. Other studies have also noted the different effects of pigmentation versus feather structure on spectral reflectance (e.g., Jacot et al. 2010) and this is reinforced, in this study, by the lack of correlation between feather vane brightness and carotenoid chroma in flicker plumage.

3.4.2 Carotenoid Deposition According to Nestling Mass and Sex

The experimental manipulation of brood size did not affect the carotenoid colouration of the nestlings as strongly as it did the melanin spots, but there was a relationship between nestling mass and some of the carotenoid measures within the larger sample of unmanipulated nestlings. Because brood sizes overlapped between the treatment groups, differences in rearing and nutritional conditions experienced by nestlings may be more accurately reflected in their mass, rather than the more general treatment group. Musgrove and Wiebe (2014) found that per-nestling provisioning rates in flickers decreased with brood size across treatment groups and that

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provisioning rates of adult flickers were positively associated with nestling mass (see Chapter 2 of this thesis). However, the diet composition delivered to different brood sizes likely did not change as flicker parents ($n = 73$) with a range of brood sizes in the natural population consumed and delivered a diet of 99% ants and ant larvae to nestlings (Gow et al. 2013b). Hence, I attribute a difference in colour of the nestlings to a difference in the quantity, rather than the type of prey delivered by parents.

The experiment highlighted a difference in carotenoid deposition patterns between male and female nestlings. Namely, females tended to be brighter than males, which may indicate a lower deposition of carotenoids and males were slightly more saturated in carotenoid pigmentation (higher chroma values) than females. However, this sex difference in chroma was not apparent in the larger dataset and was subtle at least to the human eye. Across bird species, most nestlings do not display obvious sexually dimorphic plumage, but when it occurs, it is usually late in the nestling period and anticipates the sex differences displayed in adulthood (Kilner 2006). Adult male flickers had slightly more intense carotenoid colours than females which was detectable only by camera (Wiebe and Bortolotti 2002), so the sex difference observed in nestlings appears to be subtle and similar to the pattern found in adults.

Among adult birds, there are often sex-based differences in assimilation and/or allocation of dietary and/or circulating carotenoids to integuments (Negro et al. 2001; McGraw et al. 2003), suggestive of fundamental differences in the physiological processes governing carotenoid deposition between the sexes. However, studies on the regulation of carotenoids in nestlings are scant. Benito et al. (2011) found that female Common Tern (*Sterna hirundo*) chicks prioritized the allocation of dietary carotenoids to red foot (integument) colour to a greater extent than male chicks, but at a cost to immunocompentence. In contrast, Sternalski et al. (2012) found that

nestling male Marsh Harriers (*Circus aeruginosus*) prioritized the allocation of circulating

carotenoids to integument colouration more than females. Thus nestlings, like adults, appear to

allocate carotenoids differently in relation to ecological and physiological pressures related to

sex. Presumably male flickers, both nestlings and adults, benefit more by displaying brighter

carotenoid colours in the feathers than do females, but further study is needed to determine if this

involves trade-offs with other physiological functions such as immunocompetence.

3.4.3 Condition-dependence

Consistent with the widely-held hypothesis that better nutritional conditions lead to a

higher deposition of carotenoids in the plumage (Senar et al. 2003; Hill 2006), I found that

chroma of both male and female flicker nestlings was positively associated with body mass.

Somewhat harder to explain was the pattern that males with greater mass had increased vane

brightness. However, as discussed above, the vane brightness variable was somewhat ambiguous

and higher values could indicate higher structural quality of feathers, rather than a lack of

pigment. In any case, the greater sensitivity of the male's plumage to body mass could be

explained by the fact that they are slightly larger than females and probably have higher

energetic demands that make them more sensitive to food shortages. Several other studies have

demonstrated greater susceptibility of the larger sex to food shortages (Vedder et al. 2005), and

this may be a reason for the increase in both chroma and the brightness (via structural

background quality) of males' feathers with resource availability.

Food-stressed adult male House Finches (*Carpodacus mexicanus*) were paler red than

well-fed finches, although all finches had the same access to carotenoids, suggesting that

nutritional stress can affect carotenoid metabolism and deposition (Hill 2000). In one of the few

studies on nestlings, the intensity of colour in Montagu Harriers (*C. pygargus*) was not always

positively associated with the amount of prey consumed, but rather to availability of carotenoid-

rich prey items (Sternalski et al. 2010), a pattern which seems to contrast with that in adult

House Finches. In Great Tit (*Parus major*) nestlings, however, Hõrak et al. (2000) found an

increase in nestling colouration both according to carotenoid-availability and in response to

experimental manipulations of brood size, although body mass and plumage colour were not

correlated. Tschirren et al. (2003) also found that carotenoid-based coloration was enhanced in

Great Tit nestlings that were raised in broods reduced in size and which had higher body mass

than those in control broods. Further experiments that manipulate both diet composition and

energy content are needed to tease apart the mechanisms of carotenoid deposition in nestling

integuments.

3.4.4 Melanin Deposition

The increased nutritional constraints in the enlarged broods led to smaller melanin breast

spots in both male and female flicker nestlings, pointing to a positive relationship between food

supply and melanisation. Additionally, spot size was positively correlated with mass among the

larger sample of control nestlings, and heavier male nestlings had additional melanised regions at

the base of their breast feathers below the main melanin spot. Within the large sample, female

nestlings had larger spots than males, but among adults, males had slightly larger spots. The

increase in spot size between yearling and older flickers is associated with an improvement in

body condition (K.L. Wiebe unpubl. data) and also lends support to the idea that ample nutrient

reserves are correlated with larger breast spots. Perhaps the relatively small spots of male

nestlings compared to female nestlings, again, reflects the relatively high nutritional constraints males face in the nest as a result of their larger size.

Symmetry in plumage ornaments has been hypothesized to reflect developmental stability linked to good nutrition (Møller and Swaddle 1997), and is positively associated with quality and reproductive success (Møller 1990; Hill et al. 1999). However, there was no evidence that the shape or symmetry of breast spots in flickers were associated with body condition and, with the exception of a slight female bias for heart-shaped spots, there were no sex differences in spot shape. Therefore, it is the amount of melanin deposited in the spot (total surface area), and not its arrangement, which is associated with better nutritional condition during growth. Because developmental instability can also be caused by genetic factors (e.g., inbreeding, mutations, etc.; Møller and Swaddle 1997) with offspring resembling their parents, it would be interesting to quantify spot symmetry in parents and their offspring.

In conclusion, I provide some of the first experimental evidence that melanin ornament size in growing nestlings is condition dependent. In fact, melanin pigmentation in flicker nestlings was more strongly affected by nutrition than the carotenoid deposition. Sex differences were apparent in both types of pigmentation and future studies should investigate whether there are any trade-offs for nestlings between investing in carotenoid colouration and melanisation and whether trade-offs differ between the sexes. Reciprocal cross-fostering experiments could also be carried out to quantify the genetic versus environmental components of feather colour.

Table 3.1 Effects of sex and mass on four plumage traits of nestling Northern Flickers sampled between ages 18-21 days old. Results are from four linear mixed effect models with nest and year included as random effects. Interaction terms were removed when non significant. Sample sizes are 203 nestlings in 86 nests.

	Variable	Estimate	*F*	*P*
Chroma	Sex	0.004	1.06	0.30
	Mass	<0.001	5.15	0.043
Vane Brightness	Sex	-3.83	16.73	<0.01
	Mass	-0.01	2.60	0.54
	Sex * Mass	0.03	6.19	0.013
Shaft Brightness	Sex	-0.41	6.07	0.014
	Mass	0.005	0.02	0.57
Melanin spot size	Sex	-1.11	3.61	<0.01
	Mass	0.10	23.33	<0.001

Figure 3.1 Melanin spots on the breast plumage of nestling Northern Flickers differ in size and shape. Shown above, from left to right, is the most common circular spot shape, a heart shaped spot, and an asymmetrical spot with an additional melanin spot at the proximal end of the breast feather.
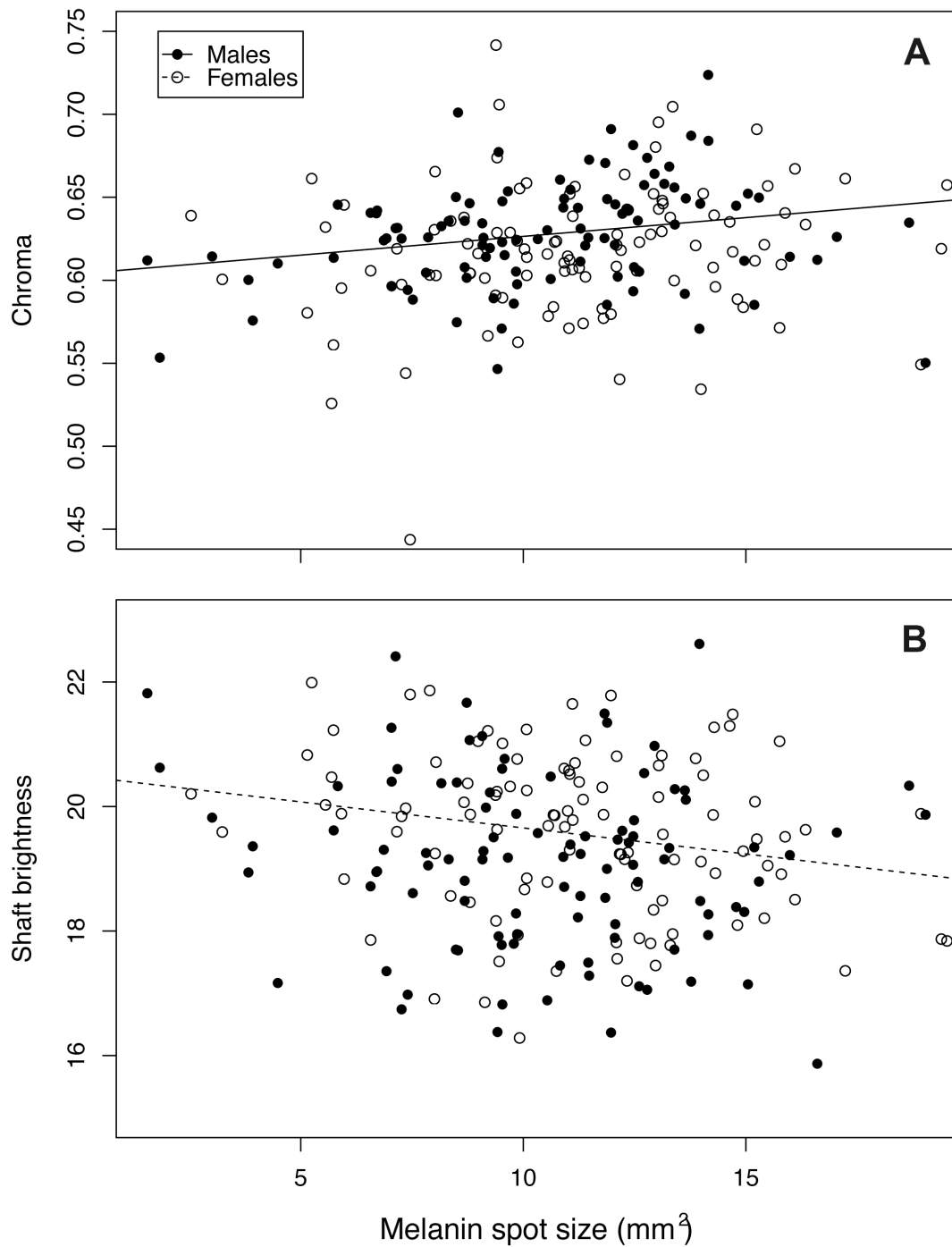
Figure 3.2 Correlations between melanin spot size and (**A**) feather chroma and (**B**) shaft brightness of male (*n* = 100) and female (*n* = 104) Northern Flicker nestlings at the time of fledging.
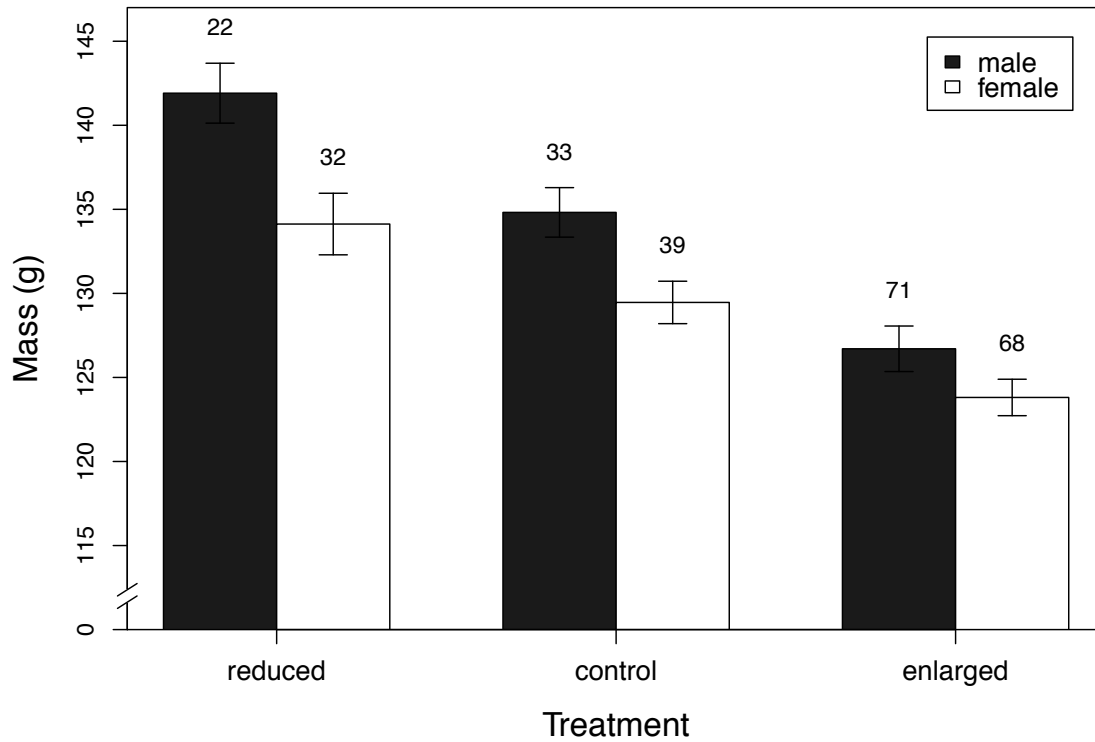
Figure 3.3 Mean mass (± SE) of male and female Northern Flickers at fledging in response to brood size manipulations. The number of nestlings measured in each group is listed above the SE bars.
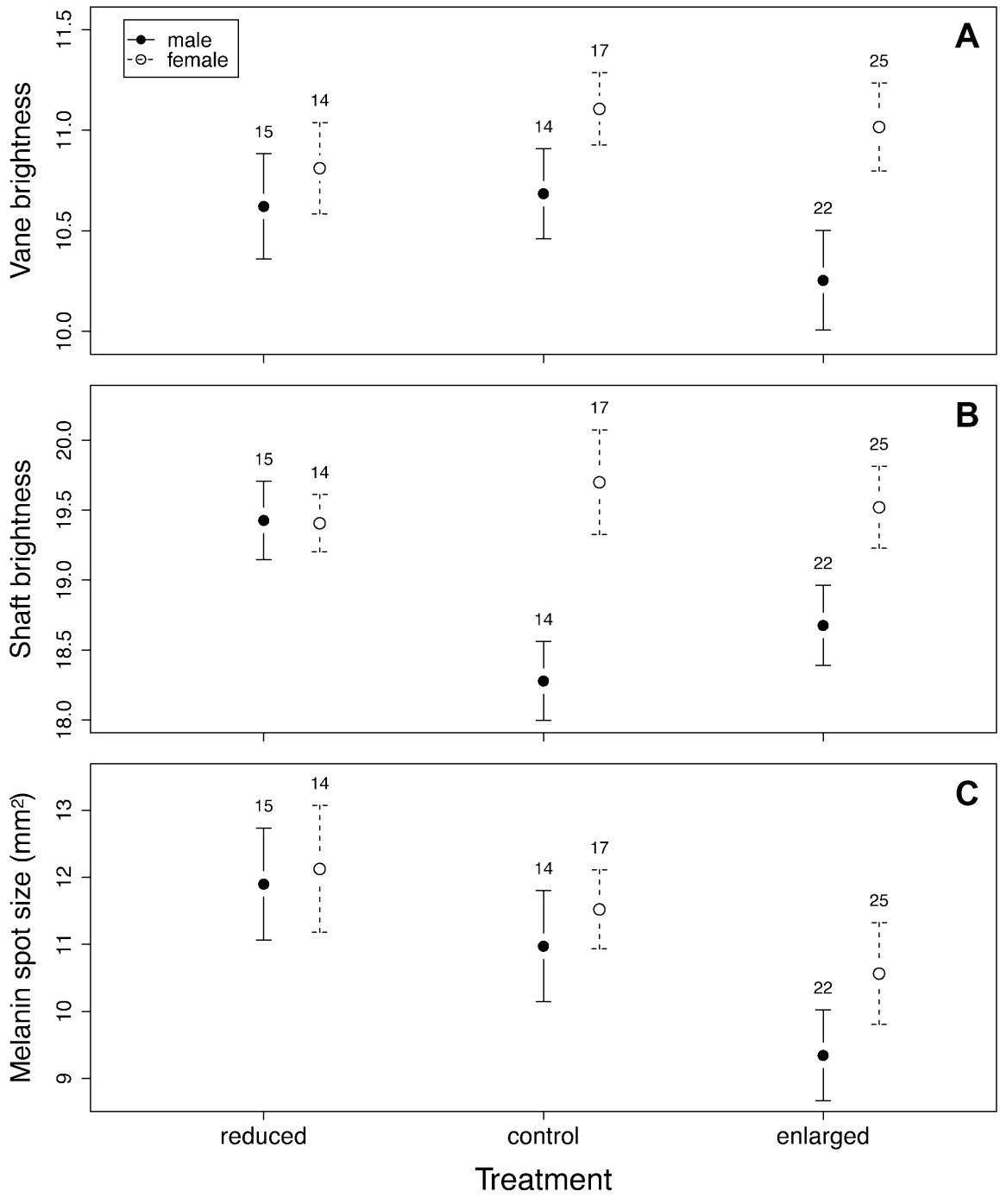
Figure 3.4 The effect of treatment on (**A**) vane brightness, (**B**) shaft brightness, and (**C**) melanin spot size measured on male and female Northern Flicker nestlings at the time of fledging. The number of nestlings measured in each treatment group is listed above the SE bars.
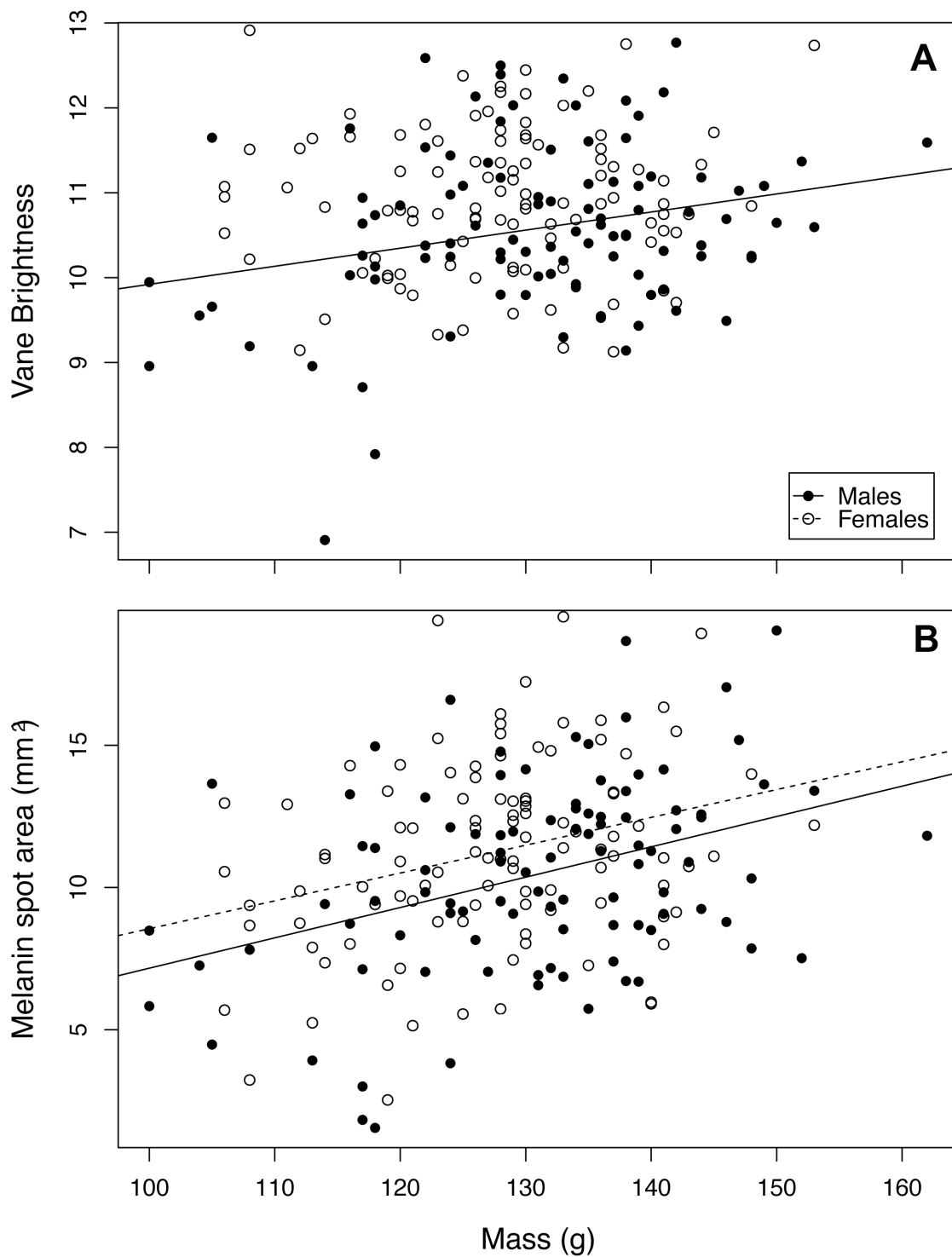
Figure 3.5 Correlations between mass and (**A**) vane brightness and (**B**) melanin spot size for male and female Northern Flicker nestlings at fledging.
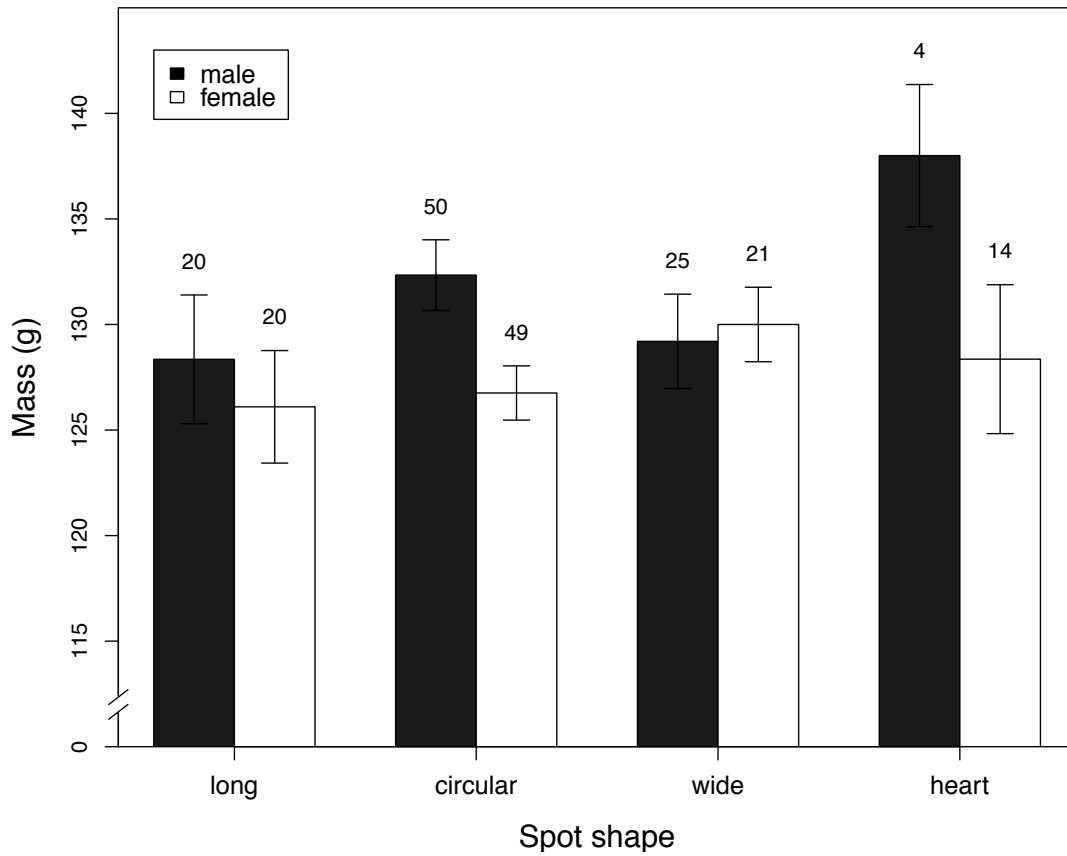
Figure 3.6 Mean mass (± SE) of male and female Northern Flicker nestlings with different shapes of melanin breast spots. The number of nestlings measured in each group is listed above the SE bars.
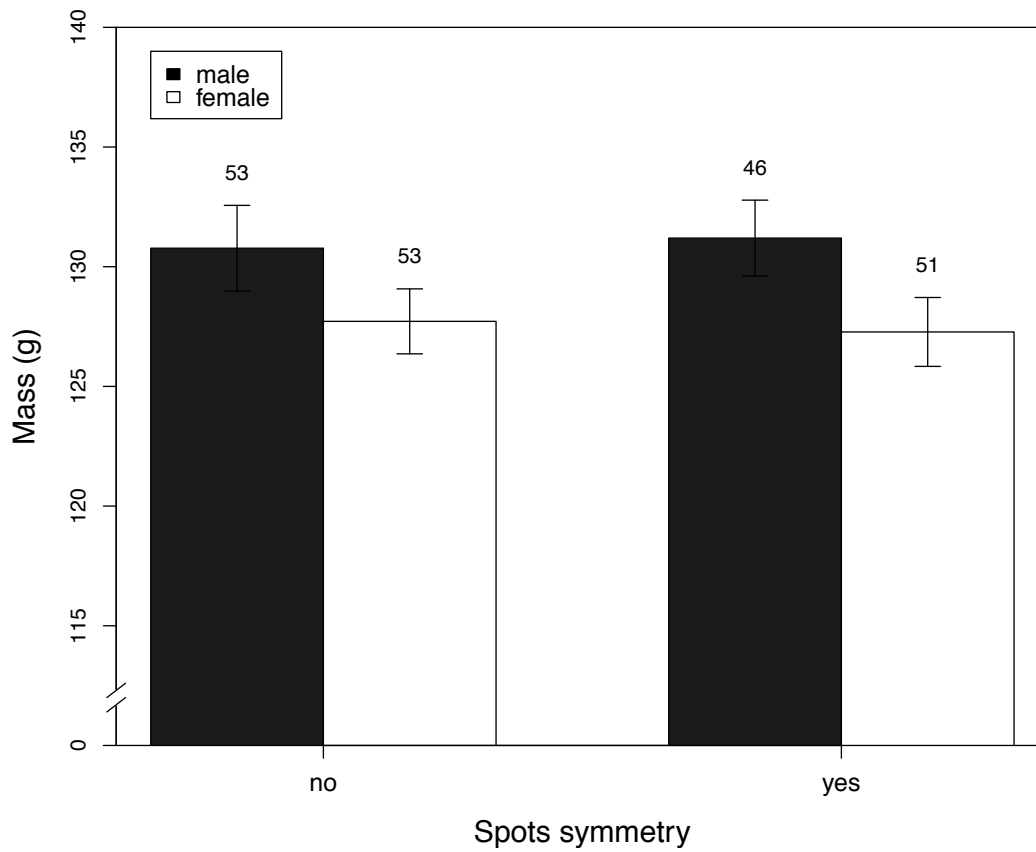
Figure 3.7 Mass (mean ± SE) of Northern Flicker nestlings according to the symmetry of their melanin breast spots. Sample sizes are listed above SE bars.
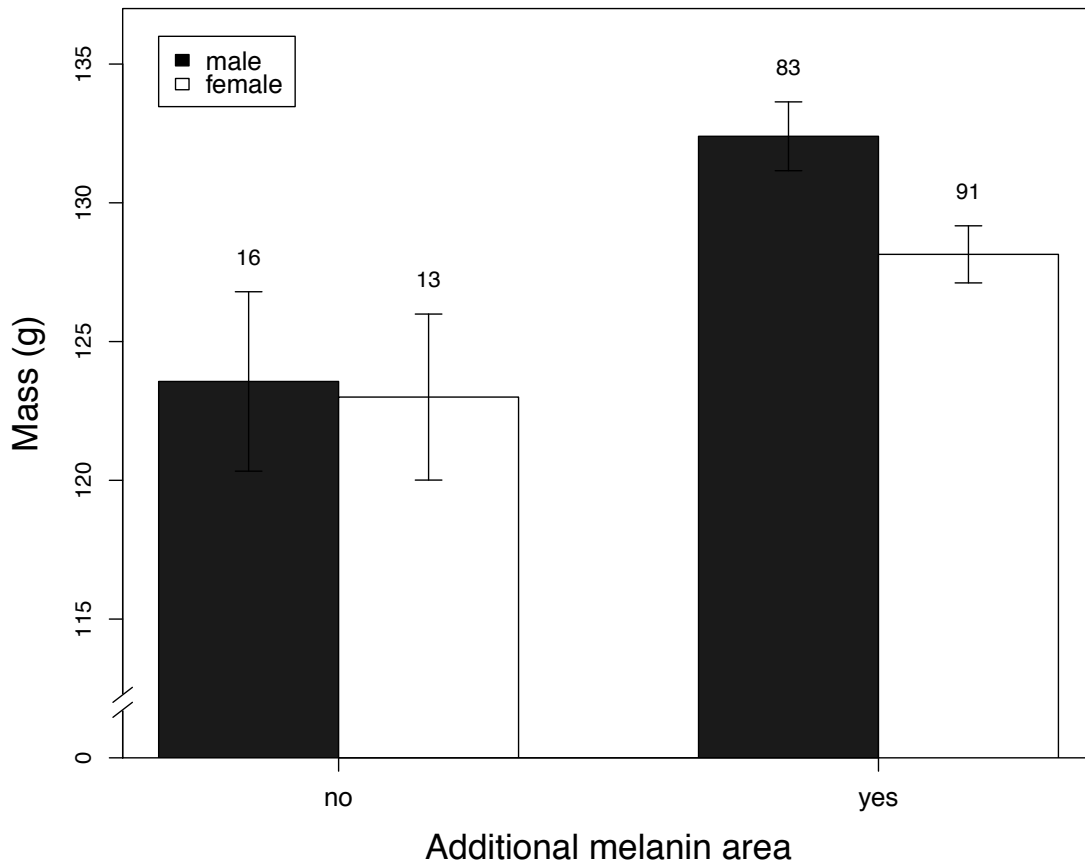
Figure 3.8 Mass (mean ± SE) of male and female Northern Flicker nestlings with and without additional melanin regions at the base of breast feathers. The number of nestlings measured in each group is listed above the SE bars.

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CHAPTER 4: RELATIONSHIPS BETWEEN MEASURES OF PHYSIOLOGICAL
PERFORMANCE, BODY CONDITION AND PLUMAGE COLOUR: WHAT INDICATES
"QUALITY" IN NESTLINGS?

4.1 Introduction

Nutritional conditions related to social and ecological factors experienced during early
life can have profound effects on an organism's growth, morphology, physiology, and behaviour
(Naguib et al. 2011). Quality- or health-related physical traits in juveniles may be used as cues
by parents to target investment to offspring with the greatest nutritional need and/or reproductive
value (Godfray 1991; Kilner and Johnstone 1997). For example, parents may choose to feed the
most brightly coloured (Kilner 2006; Saino et al. 2000a) or actively begging offspring (Saino et
al. 2000b). In turn, theory suggests that such signals of quality should be costly to produce so as
to maintain honest advertisement of need and/or condition despite competing interests between
parents and offspring (Kilner and Johnstone 1997). Often, nutritional stress, physiological
function, and expression of ornaments such as feather colour underlie the reliable signalling of
nutritional status between parents and offspring (Godfray 1991; Kilner and Johnstone 1997).

In many cases, links between nutritional status and physiological function are well
established, but in other cases such links are complicated by potential trade-offs, multiple
physiological pathways, and/or different short- and long-term effects. For example, it has been
experimentally confirmed in both Eurasian Magpies (*Pica pica*; Soler et al. 2003) and Blue Tits
(*Cyanistes caeruleus*; Brommer 2004) that allocation of resources to immune defence impairs
nestling growth, presumably because immunity is costly and competes for limited nutrients and
energy (Lochmiller and Deerenberg 2000). Corticosterone (CORT), the main glucocorticoid in

birds, plays an important role mediating trade-offs between maintenance and developmental

processes (Sapolsky et al. 2000). Food stress in nestlings can trigger increased secretion of

CORT in a beneficial way to mobilize stored energy (Sapolsky et al. 2000) and increase begging

(Kitaysky et al. 2001a). However, long-term and chronic CORT elevation can be detrimental,

suppressing memory and immune function, causing muscle atrophy, and triggering cell death

(reviewed in Sapolsky et al. 2000). Long-term effects of chronic stress in developing individuals,

however, remain poorly understood. In one of the few studies examining long-term stress in

birds, Saino et al. (2003) found that extended food deprivation increased CORT secretion in

nestling Barn Swallows (*Hirundo rustica*) and depressed their T-cell-mediated immunity. In

another study, Kitaysky et al. (2003) found that Black-legged Kittiwake chicks (*Rissa tridactyla*)

exposed to chronic elevation of corticosterone during development suffered from impaired

cognition.

Links between physiological measures and the development of plumage ornaments in

nestling birds are complicated and not well understood. Birds must obtain carotenoid pigments

from their diet and so food supply may directly limit the intensity of carotenoid colouration in

their plumage (Brush 1978; Goodwin 1984). Even if carotenoids are not limiting in the diet,

carotenoid molecules may be modified along biochemical pathways that may be energetically

costly (Hill 2000). In addition, carotenoids potentially act as antioxidants, scavengers of free

radicals, and immunostimulants (Møller et al. 2000; Pérez-Rodríguez 2009; Simons et al. 2012),

so there may be a trade-off between the allocation of plasma carotenoids to the immune system

and feather deposition (Møller et al. 2000). Recently, it has also been suggested that CORT plays

a role in the augmentation and quality of feathers containing carotenoid pigments because the

hormone is involved in regulating and mobilizing carotenoid-rich fat stores (Loiseau et al. 2007;

McGraw et al. 2011) and can alter feather micro-structure, potentially affecting brightness and feather strength (DesRochers et al. 2009).

On the other hand, melanin pigments are not limited by a bird's diet and have been largely assumed to have few energetic or physiological costs (Hill and Brawner 1998; Badyaev and Hill 2000). Ducrest et al. (2008) suggested that pleiotropic genes both increase the synthesis of melanin and increase anti-inflammatory activity and resistance to stressors. In support of this hypothesis, antibody production was positively correlated with the number of melanin plumage spots in two owl species (Roulin et al. 2000, Gasparini et al. 2009). This suggests that the immune response and melanin-based plumage coloration do not compete for resources; rather, they may co-vary according to the genotype of the individual. The picture is made more complicated, though, by recent evidence that the size of melanin ornaments in some species is related to nutritional conditions during nestling growth and hence can be influenced by the environment (Piault et al. 2012; Chapter 3 of this thesis). Additionally, melanogenesis has been hypothesized to augment resistance to stressors (Ducrest et al. 2008) because darker individuals in several species are less sensitive to stress and/or to CORT administration (Senar et al. 2000; Roulin et al. 2008a; Almasi et al. 2012; Minias et al. 2013). CORT may mediate the condition-dependent component of melanin production through a negative feedback link (Almasi et al. 2008) as demonstrated by a reduction in melanin-based phaeomelanic pigments of nestling Barn Owls (*Tyto alba*) with experimentally increased circulating CORT levels (Roulin et al. 2008b).

Researchers have inferred health or quality of nestlings from multiple measures including measures of body mass or body mass corrected for structural size (body condition; Nur 1984b; Schwagmeyer and Mock 2008), immune function (Saino et al. 1997; Brzek and Konarzewski 2007), CORT levels (Blas et al. 2007), and plumage colour (Pérez-Rodríguez 2008), but few

have examined relationships between more than two quality indices. Such exploratory studies are needed because existing hypotheses may predict both positive and negative correlations between the same set of variables. Experimental manipulation of nutritional stress is a powerful way to examine specific relationships between nutrition, physiology, and expression of ornaments, and brood size manipulations in birds is one way to achieve this. For example, nestlings from enlarged broods with poor food resources have been shown to have lowered T-cell mediated immunocompetence (Saino et al. 1997; Hõrak et al. 1999; Ilmonen et al. 2003), decreased deposition of carotenoids (Hõrak et al. 2000) and smaller melanin ornaments in the plumage (Piault et al. 2012; Chapter 3 of this thesis), presumably because these traits are resource demanding. Additionally, brood enlargements can increase baseline and stress-induced levels of corticosterone in nestlings (Kitaysky et al. 2001b; Saino et al. 2003).

The goal of this study was to examine how various physiological and plumage-related variables reflect food stress in Northern Flicker (*Colaptes auratus*) nestlings, using an experimental manipulation of brood size to alter levels of nutritional stress during development. I was interested in testing correlations between potential measures of quality such as T-cell mediated immunocompetence (one aspect of the immune function), long-term integrated measures of corticosterone in feathers ($CORT_f$), and plumage characteristics to test for hypothesized trade-offs, such as between immune function and carotenoid colouration, or hypothesized positive correlations such as between immune function and melanin. I also tested for some hypothesized links between variables based on the literature. Specifically, that (1) $CORT_f$ inhibits an individual's immune system and thus leads to a lower T-cell mediated immune response to an experimental challenge with phytohaemagglutinin (PHA), (2) high $CORT_f$ increases the transport of carotenoids and so leads to more saturated colours within

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feathers, and (3) individuals with larger melanin spots (i.e., those having undergone more melanogenesis) are less sensitive to stressful factors and thus have lower feather CORT levels. I predicted that nutritionally stressed nestlings from enlarged broods would be of poorer body condition, have lower PHA immune responses, and higher $CORT_f$ levels. Finally, I also tested for sex-specific relationships because physiological strategies related to immunity, plumage ornamentation, and $CORT_f$ may differ according to sex (Fargallo et al. 2002; Kelly et al. 2012).

4.2 Methods

4.2.1 Study Site and Study Species

I studied Northern Flickers at Riske Creek, British Columbia, Canada (51°52'N, 122°21'W) on an area covering ~100 km^2 of grassland interspersed with clumps of trembling aspen (*Populus tremuloides*) and surrounded by mixed coniferous forests. Flickers have been studied here since 1998, and the breeding effort of approximately 85-160 pairs in tree cavities has been monitored each year between late April and late July. Flickers weigh approximately 150 g when fully grown, and have an "r-selected" or "fast" life-history strategy with large and variable clutch sizes (mean 8 eggs, range of 3-13; Wiebe and Elchuk 2003). Incubation lasts ~12 days, and the altricial nestlings are fed at the nest for ~25-27 days (Wiebe and Moore 2008). Both parents contribute to nestling provisioning but males provision slightly more (Gow et al. 2013a) and for a longer period of time during the post-fledging period (Gow and Wiebe 2014a). Up to 5% of females annually are polyandrous and there is no extra-pair paternity (Wiebe and Kempenaers 2009).

4.2.2 Field Methods

I found nests by visually checking tree cavities. At active nests, small replaceable doors were cut in the tree trunk below the cavity entrance to allow access to nestlings. By visiting the nests every 4-6 days on average, I was able to determine clutch sizes, hatching dates, and nest fates. During 2012 and 2013, I experimentally manipulated brood sizes 2-4 days after hatching by transferring (on average) 40% of the nestlings (1-4) from reduced broods to enlarged broods. All reduced-enlarged nest pairs were matched with respect to hatching date (± 1 day). I chose the number of nestlings to transfer such that I could maintain brood sizes within the natural range observed for the species (3-12 nestlings). Control broods were not manipulated, but had similar hatch dates as the reduced-enlarged pairs and were visited with the same frequency. Transferred nestlings were marked with coloured thread around the tarsus for individual identification. The brood size manipulation was balanced so that timing of breeding (i.e., laying date), original clutch size, and original brood size did not differ between treatments (ANOVA: $F_{2,104} = 0.58$, $P = 0.56$; $F_{2,104} = 2.00$, $P = 0.14$; $F_{2,104} = 1.85$, $P = 0.16$, respectively).

Nestlings were weighed using a Pesola spring balance (precision of 0.01 g) at three stages of development: Stage 1 (5-7 days old), Stage 2 (10-13 days), and Stage 3 (18-21 days). Flattened wing chord measurements were taken with a ruler at Stages 2 and 3 (precision of 1 mm). The residuals from stage- and sex-specific regressions of nestling mass against flattened wing cord gave an index of nestling body condition. Nestlings at Stage 3 were sexed by plumage, a secondary (S2) wing feather was clipped distal to the vascularized region of the calamus, and three representative breast feathers with melanin spots were plucked from the central breast. All feathers were stored in dark envelopes until they could be analyzed and photographed in the lab.

4.2.3. Nestling Immune Response

T cell-mediated immune responses were measured on two or three nestlings per brood using a common phytohaemagglutinin (PHA) skin-testing technique (Smits et al. 1999; Tella et al. 2008) which quantifies the proliferation of T cells and other leukocytes to an injection of PHA, a plant lectin. During Stage 2 (10-13 days old nestlings), I interdermally injected the nestling's patagium with a 0.1 mL dose of 1 mg PHA-P (Sigma Chemicals) disolved in 1 mL phosphate-buffered saline solution using a 31 gauge needle. Prior to injection, the site was marked, the web wing thickness measured to the nearest 0.01 mm with a Mitutoyo micrometer, and swabbed with alcohol. The marked injection site was measured again 24 hr later. For each of the pre- and post- measures, the mean of three values was used. The pre-injection web wing thickness serves as a temporal control (Smits et al. 1999) and the increase in patagial thickness serves as a quantitative index of the cutaneous cellular aspect of immunocompentence in nestlings.

4.2.4 Carotenoid Analysis

In the lab, I quantified the colour of vane and shaft portions of the secondary feathers with a Minolta CM-2600d portable spectrometer (Minolta Co., Ltd., Japan) which has an UV xenon flashlight source and a visible light standard illuminant (D65). I measured each vane and shaft portion three times and the resulting measurements were expressed as the percent reflectance relative to a white standard, which reflects 93% of incident light. The reflectance spectrum was obtained at 10 nm intervals between 360-740 nm and the shape of the resulting reflectance curves was consistent with those of carotenoid pigments in other studies (e.g., Toral et al. 2008). All calculated variables of colour indices were restricted at 700 nm, which is the limit of avian visual sensitivity (Endler 1990).

Colour indices often involve quantifying brightness, chroma, and hue, which correspond to the three major axes of colour variation (Montgomerie 2006; Butler et al. 2011). I ignored hue (the wavelength of maximum reflectance) because the hybrid flickers could have slightly different colours, ranging from yellow to red, which are genetically based and irrelevant to measures of quality (Stradi 1998; Flockhart and Wiebe 2009). Brightness, the average reflection of light across the entire interval 360-700, is negatively related to pigment concentration (Andersson and Prager 2006), and may also be affected by the underlying feather structure (Shawkey and Hill 2005). I also measured chroma, or spectral saturation, which is positively related to pigment concentration and is likely to convey a condition-dependent signal related to carotenoid concentration (Saks et al. 2003; Andersson and Prager 2006). The chroma value I used controls for brightness to eliminate potential biases that may occur when pigments are saturated (Andersson and Prager 2006) and incorporates the reflectance where peak absorbance of carotenoids takes place: $(R_{700}-R_{450})/R_{700}$ (Cuthill et al.1999). For more details on the colour variables and measurement repeatability, see Chapter 3 of this thesis.

4.2.5 Melanin Analysis

In the lab, I photographed the breast feathers using a digital camera (Panasonic Lumix DMC FZ28) mounted on a tripod, and calculated the total area of the melanin breast spots using Adobe Photoshop CS5. I used the mean spot area based on the three feathers from an individual in the analysis. For more details on measurement repeatability, see Chapter 3 of this thesis.

4.2.6 Feather CORT Analysis

After measuring the length of the S2 feather, a methanol-based extraction technique was used to extract CORT from feathers following the methods of Bortolotti et al. (2008), with minor modifications. Feathers were minced into ~3-4 mm pieces, mixed with 7 ml of methanol (HPLC grade, Fisher Scientific), and placed in a sonicating water bath at room temperature for 30 minutes followed by an overnight incubation in a sonicating 50°C water bath. Particulate matter was filtrated from methanol and washed twice with ~2.5 mL of additional methanol which was then placed in a 50°C water bath and dried under a fume hood. After evaporation, the extract residues were reconstituted using 500 μL of a Tris-buffered saline and run through a standard radioimmunoassay. Values are expressed as pg of CORT per mm of feather. CORT assays were performed at Tufts University, Massachusetts, USA.

4.2.7 Statistical Analysis

I used linear mixed effect models (LME) with restricted maximum likelihood (REML) to explore the effects of the brood size manipulation treatment and nestling sex on nestling PHA response and CORT$_f$ levels. The experiment resulted in reduced per capita feeding rates and hence reduced mass of nestlings in enlarged versus reduced broods (Chapter 2 of this thesis; Musgrove and Wiebe 2014) and is a useful method to reveal any causal relationship between provisioning rates and the various plumage and physiological measures of quality (Chapter 3 of this thesis). I also analyzed the relationship between body condition and various quality indices using a larger sample of nestlings which were not all part of the experimental treatments. Statistical models were initially run with the fixed factors of sex and treatment (or body conditon in the case of the large dataset), as well as the interaction between treatment/body condition and sex, but the latter term was dropped if not significant. Additionally, I was unable to sex many

nestlings at Stage 2 because their plumage was not developed, so when sex was not a significant predictor in the LME models, I conducted analyses which excluded sex as a fixed factor and which included nestlings of unknown sex to increase sample size. $CORT_f$ values were $\log_{10}$ transformed to normalize model residuals and homogenize the variances. In all cases, nest identity was fitted as a random factor to account for multiple nestlings measured per brood. Similar LME models, with nest as a random factor, were also used to test hypotheses of causal links between measures of physiology and plumage. Sample sizes vary among analyses due to missing data for some variables. I also ran correlations between multiple quality measures. Results for quality measures should be interpreted with caution, however, since they are based on simple correlations between all individuals in the study, and do not imply causation. All analyses were conducted in R (version 3.1.0; R Core Development Team 2014) using the lme4 package (Bates et al. 2012). P-values were estimated with type III orthogonal sums of squares using the lmerTest package in R (Kuznetsova et al. 2014), with statistical significance set at $\alpha = 0.05$.

4.3 Results

As intended, the experiment caused body condition of nestlings to be highest in reduced broods and lowest in enlarged broods ($n = 265$ in 40 nests; LME: brood manipulation treatment: $F = 9.63$, $P < 0.001$; Figure 4.1). The body condition of nestlings at fledging (Stage 3) was correlated with their condition earlier in brood rearing (Stage 2; males: $r = 0.70$, DF = 64, $P < 0.001$; females: $r = 0.56$, DF = 70, $P < 0.001$), suggesting that individuals experienced a consistent relative nutritional status during most of the growth period.

4.3.1 Physiological Traits in Relation to Treatment, Body Condition, and Sex

Considering only experimental nestlings of known sex ($n = 112$ nestlings in 47 nests), there was no effect of sex on the PHA response (LME: $F = 1.97$, $P = 0.16$), but there was a treatment effect ($F = 3.96$, $P = 0.027$); nestlings from reduced treatments had a larger PHA response (i.e., stronger immune responses) than nestlings from control and enlarged broods. The treatment effect remained when including nestlings of unknown sex ($n = 131$ in 52 nests; treatment effect: $F = 4.19$, $P = 0.021$; Figure 4.2). Within the larger dataset, which included nestlings of unknown sex from the population ($n = 170$ nestlings in 69 nests), body condition was postively associated with a higher PHA response ($F = 11.33$, $P < 0.001$; Figure 4.3). This positive effect of body condition on nestling PHA response was also present using body condition measured just prior to fledging ($n = 130$ nestlings in 55 nests; $F = 4.35$, $P = 0.039$).

There was no significant effect of treatment (LME: $F = 1.72$, $P = 0.19$) or sex ($F = 0.05$, $P = 0.83$) on $CORT_f$ values for experimental nestlings ($n = 100$ nestlings in 40 nests), although the average concentration of $CORT_f$ in the feathers increased from reduced to enlarged broods (Figure 4.4). Within the sample of all individuals measured ($n = 133$ nestlings in 54 nests), there was also no effect of body condition ($F = 0.01$, $P = 0.92$) or sex (LME: $F = 0.002$, $P = 0.97$) on the level of $CORT_f$.

4.3.2 Relationships Between Quality Measures

The PHA response increased with melanin spot size for males and approached significance for females (Table 4.1; Figure 5); however, the PHA response was not correlated with carotenoid chroma for either sex (Table 4.1). Among male nestlings, but not females, individuals with larger immune responses also had higher vane brightness (Table 4.1).

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Conversely, $CORT_f$ was correlated with feather brightness of female nestlings, but only weakly so for males (Table 4.1).

Controlling for the multiple nestlings per brood ($n = 121$ nestlings in 54 nests), $CORT_f$ levels did not affect the PHA response (LME: $F = 0.05$, $P = 0.83$), but the PHA response was affected by nestling sex ($F = 4.31$, $P = 0.041$), with males having slightly larger immune responses than females. $CORT_f$ was not related to some feather attributes such as melanin spot size ($n = 133$ in 54 nests; $F = 0.01$, $P = 0.94$) or feather chroma ($F = 0.50$, $P = 0.48$), but it did vary weakly with brightness (vane: $F = 3.06$, $P = 0.082$, shaft: $F = 4.24$, $P = 0.042$). The brightness of feathers decreased with increasing $CORT_f$ levels for both sexes (Figure 4.6), with females having brighter feathers overall compared to males (sex effect vane: $F = 27.81$, $P < 0.001$, shaft: $F = 14.35$, $P < 0.001$). The size of melanin spots did not vary with nestling $CORT_f$ levels ($F = 0.02$, $P = 0.88$), and there was no difference between the sexes in the level of $CORT_f$ ($F < 0.001$, $P = 0.98$).

4.4 Discussion

The nutritional state experienced by Northern Flicker nestlings in experimentally enlarged broods was manifested most strongly in the body condition index and in T-cell mediated immune responses, but not in $CORT_f$ levels. This study is one of few to examine the relationship between multiple physiological measures and multiple plumage characteristics, and it was surprising that melanin spot size appeared to be more strongly associated with body condition and immune responses than carotenoid pigmentation because the latter has received the most attention as an indicator of quality (Hill and Brawner 1998; Hill 2000, but see Fitze and Richner 2002).

4.4.1 Physiological Measures of Nutritional Stress and Nestling Quality

In agreement with other studies (Saino et al. 1997; Hõrak et al. 1999; Alonso-Alvarez and Tella 2001), I found support for the hypothesis that the strength of the T-cell mediated immune response is energetically costly because nestlings in good body condition (i.e., those with more nutrient reserves relative to their body size) were better able to mount immune responses to a novel antigen. Interestingly, the immune response of nestlings from reduced broods was markedly better than nestlings from either control or enlarged broods. This suggests that even control nestlings are mounting sub-maximal immune responses and that parents may be choosing an optimal brood size that favours quantity over maximum disease resistance of their offspring (i.e., nestling quality).

The level of immune response did not depend on the sex of the nestling, suggesting that both sexes had the same strategy for allocating resources to immunity versus to other competing functions. Despite the obvious importance of immunity in many aspects of sexual selection and parental care (Møller and Saino 1994), sex-specific differences are just starting to be examined in wild adult birds, with similar responses being found in both sexes for the Zebra Finch (*Taeniopygia guttata*), Tree Swallows (*Tachycineta bicolor*) and Leach's Storm-petrels (*Oceanodroma leucorhoa*; Haussmann et al. 2005). However, the larger sex in species with significant dimorphism has been found to be able to mount a stronger PHA response than the smaller sex (e.g.: Ruffs, *Philomachus pugnax*, Lozano and Lank 2003; Magellanic Penguins, (*Sphenisucs magellanicus*, Moreno et al. 2001). Sex-differences in the immune function of nestlings however, are still largely unknown. In one study, male Eurasian Kestrel nestlings (the smaller sex by ~ 20% at adulthood) had lower cell-mediated immunity than females, but only in

treatments with food restrictions (Fargallo et al. 2002). Dubiec et al. (2006) found that the enlargement of brood sizes had different effects on the cellular immune responses of male and female Blue Tit (*Parus caeruleus*) nestlings, with males (which at fledging are about 3.3% heavier than females) being more negatively affected than females. Likewise, Tschirren et al. (2003) found sexual dimorphism in body size, susceptibility to parasites, and T-cell-mediated immunity in nestling Great Tits (*Parus major*), with males showing reduced immunocompetence. Male and female nestling flickers were the same size at the time of PHA injection in my study, but males are about 6% heavier than females at fledging, so it would be interesting to assess sex-specific immune responses at older ages.

In contrast to the immune function, $CORT_f$ values did not differ significantly between treatments, although there was a clear trend of increased $CORT_f$ with brood size. Ilmonen et al. (2003) also documented a positive relationship between manipulated brood size and plasma CORT in nestling Pied Flycatchers (*Ficedula hypoleuca*). This pattern is consistent with the idea that nutritionally stressed, and hence the poorest quality nestlings display higher chronic CORT levels (e.g., plasma CORT: Red-legged Kittiwake chicks, *Rissa brevirostris*, Kitaysky et al. 2001b; Barn Swallows, *Hirundo rustica*, Saino et al. 2003). However, the magnitude of the CORT effect measured in flicker feathers was small despite a considerable difference in nutritional stress between reduced and enlarged broods as shown by the difference in mass and chance of mortality between treatments (Chapter 3 of this thesis). Additionally, the lack of a relationship between flicker body condition and $CORT_f$ suggests that the trend of increased $CORT_f$ in enlarged broods may not be related to nutritional stress *per se*, but to other factors related to the brood manipulation experiment, such as sibling competition. $CORT_f$ values in relationship to nestling sex have rarely been examined, and plasma CORT has been found to be

independent of sex in nestlings of other species (e.g.: American Kestrel, *Falco sparverius*, Sockman and Schwabl 2001). $CORT_f$ is hypothesized to affect survival and recruitment (Blas et al. 2007), but $CORT_f$ levels in flickers were not strongly associated with other conventional measures of nestling quality - mass, immunity, or feather colour - so the long-term significance of $CORT_f$ for fitness is uncertain.

As corticosterone may suppress immune function (Padgett and Glaser 2003), I expected a negative relationship between $CORT_f$ and nestling immune response, but this was not found. However, the inverse relationship between PHA response and $CORT_f$ across the treatment groups lends support to the hypothesis that a trade-off between quantity and quality of offspring could be mediated by stress-induced immunosuppression (i.e., Pied Flycatcher, *Ficedula hypoleuca,* Ilmonen et al. 2003). More work is needed, however, to test this.

4.4.2 Relationships Between Physiological and Colour-based Measures of Quality

I found no evidence for a trade-off between the uses of carotenoids for the immune system versus for colouration. However, among male nestlings, those with brighter feathers had greater PHA responses. If feather brightness (i.e., greater reflectance) is caused by less pigment deposition, then it is consistent with the idea of a trade-off in the use of pigments. However, brightness may also result from structural changes in the feather related to nutritional status during growth or enhanced abrasion due to sibling competition (Neuenschwander et al. 2003; Jacot et al. 2010). Because there was no relationship between PHA response and feather chroma for either male or female nestlings, the data suggest that the relationship between carotenoid deposition and immune function is weak or absent in flicker nestlings.

With respect to melanin deposition, males and to a lesser extent females, showed a positive correlation between melanin breast spot size and immune response. The fact that individuals with greater melanin deposition have stronger immune responses is in agreement with adult Tawny Owls (*Strix aluco*; Gasparini et al. 2009). Furthermore, melanin-based plumage ornaments are smaller when exposed to reduce ectoparasites in Great Tits (*Parus major*; Fitze and Richner 2002). My results suggest that the immune response and melanin-based plumage coloration do not compete for resources; rather, nestlings in good condition could both mount a strong immune response and deposit melanin according to their underlying nutritional status. In contrast to some others, I found no evidence that more melanised individuals were better able to withstand food stress and thus had lower CORT levels (e.g.: Ducrest et al. 2008; Roulin et al. 2008a; Almasi et al. 2008, 2010). My results also do not support the idea that CORT reduces melanogenesis by inhibiting the secretion of melanocortins and tyrosinase (Roulin et al. 2008b), or that CORT relates to melanin production in any way.

There was also no clear relationship between $CORT_f$ and carotenoid deposition as measured by chroma in the feathers for either sex. However, $CORT_f$ negatively affected brightness for both sexes. Unlike some other studies (Kitaysky et al. 2001a, but see Loiseau et al. 2008a,b), higher $CORT_f$ in flicker nestlings did not increase food intake and therefore access to pigments in the diet. Rather, if feather brightness in flickers is more a result of its microstructure, poor nutritional status could both trigger high $CORT_f$ levels and impair the microstructure of feather vanes during growth. In fact high plasma CORT causes feathers to be lighter and weaker (DesRochers et al. 2009). Similar to my results, the brightness of the epaulets of Red-winged Blackbirds (*Agelaius phoeniceus*) was negatively associated with $CORT_f$ (Kennedy et al. (2013)

and in both studies, $CORT_f$ did not affect feather chroma. This suggests that $CORT_f$ affects the background keratin matrix of the feather rather than its carotenoid content.

In summary, the T-cell-mediated immune response in nestling flickers was dependent on nutritional stress during rearing and was more closely associated with traditional measures of nestling quality (body mass or body condition) than was the long-term integrated measure of CORT level in the feathers. By studying multiple quality indicators in nestlings simultaneously, I revealed for the first time how physiological measures can affect various types of pigment deposition in the feathers quite differently. Whereas carotenoid deposition is widely believed to be an honest indicator of quality, the results of this study show that melaninisation in flicker nestlings is more strongly related to both body condition and immune function. I found that the body condition index and PHA immune response (to a lesser degree) were most reflective of nutritional stress, whereas $CORT_f$ did not appear to strongly reflect the nutritional status of individuals. Relationships between quality indices are obviously complex and may vary among species and ecological contexts. Researchers therefore must consider potential trade-offs and co-variation between potential indices of quality when deciding which variables are most valid to use as bioindicators of nutritional stress in a particular context.

Table 4.1 Correlations between multiple quality indices for male and female Northern Flicker nestlings. PHA was measured at Stage 2 (10-13 days old) and all feather measurements (colour and feather CORT) were measured at Stage 3 prior to fledging (18-21 days old). Body condition values are the residuals of a stage- and sex-specific regression of mass on wing chord length.

FEMALES ↓	MALES → Body Condition Index	Immune Response (PHA)	Melanin spot size	Log CORT$_f$	Feather Chroma	Feather Vane Brightness	Feather Shaft Brightness
Body Condition Index		$r = 0.29$ DF = 59 **P = 0.024**	$r = 0.26$ DF = 97 **P = 0.011**	$r = -0.08$ DF = 61 P = 0.54	$r = -0.07$ DF = 97 P = 0.50	$r = 0.21$ DF = 97 **P = 0.036**	$r = 0.13$ DF = 97 P = 0.20
Immune response (PHA)	$r = 0.16$ DF = 64 P = 0.20		$r = 0.30$ DF = 58 **P = 0.020**	$r = -0.10$ DF = 54 P = 0.47	$r = -0.11$ DF = 58 P = 0.42	$r = 0.27$ DF = 58 **P = 0.038**	$r = 0.22$ DF = 58 P = 0.086
Melanin spot size	$r = 0.24$ DF = 102 **P = 0.014**	$r = 0.21$ DF = 67 P = 0.084		$r = 0.095$ DF = 61 P = 0.48	$r = 0.24$ DF = 98 **P = 0.019**	$r = 0.15$ DF = 98 P = 0.15	$r = -0.14$ DF = 98 P = 0.16
Log CORT$_f$	$r = -0.05$ DF = 68 P = 0.71	$r = 0.19$ DF = 63 P = 0.14	$r = -0.10$ DF = 68 P = 0.44		$r = 0.15$ DF = 61 P = 0.24	$r = -0.22$ DF = 61 P = 0.085	$r = -0.22$ DF = 61 P = 0.081
Feather Chroma	$r = -0.06$ DF = 102 P = 0.57	$r = -0.05$ DF = 67 P = 0.68	$r = 0.17$ DF = 102 P = 0.092	$r = 0.16$ df DF = 68 P = 0.19		$r = -0.01$ DF = 98 P = 0.90	$r = -0.23$ DF = 98 **P = 0.024**
Feather Vane Brightness	$r = -0.02$ DF = 102 P = 0.86	$r = 0.05$ DF = 67 P = 0.72	$r = -0.05$ DF = 102 P = 0.64	$r = -0.22$ DF = 68 P = 0.062	$r = -0.02$ DF = 102 P = 0.84		
Feather Shaft Brightness	$r = -0.14$ DF = 102 P = 0.17	$r = -0.08$ DF = 67 P = 0.54	$r = -0.22$ DF = 102 **P = 0.029**	$r = -0.26$ DF = 68 **P = 0.027**	$r = -0.31$ DF = 102 **P = 0.001**		

Significant correlations are in bold.
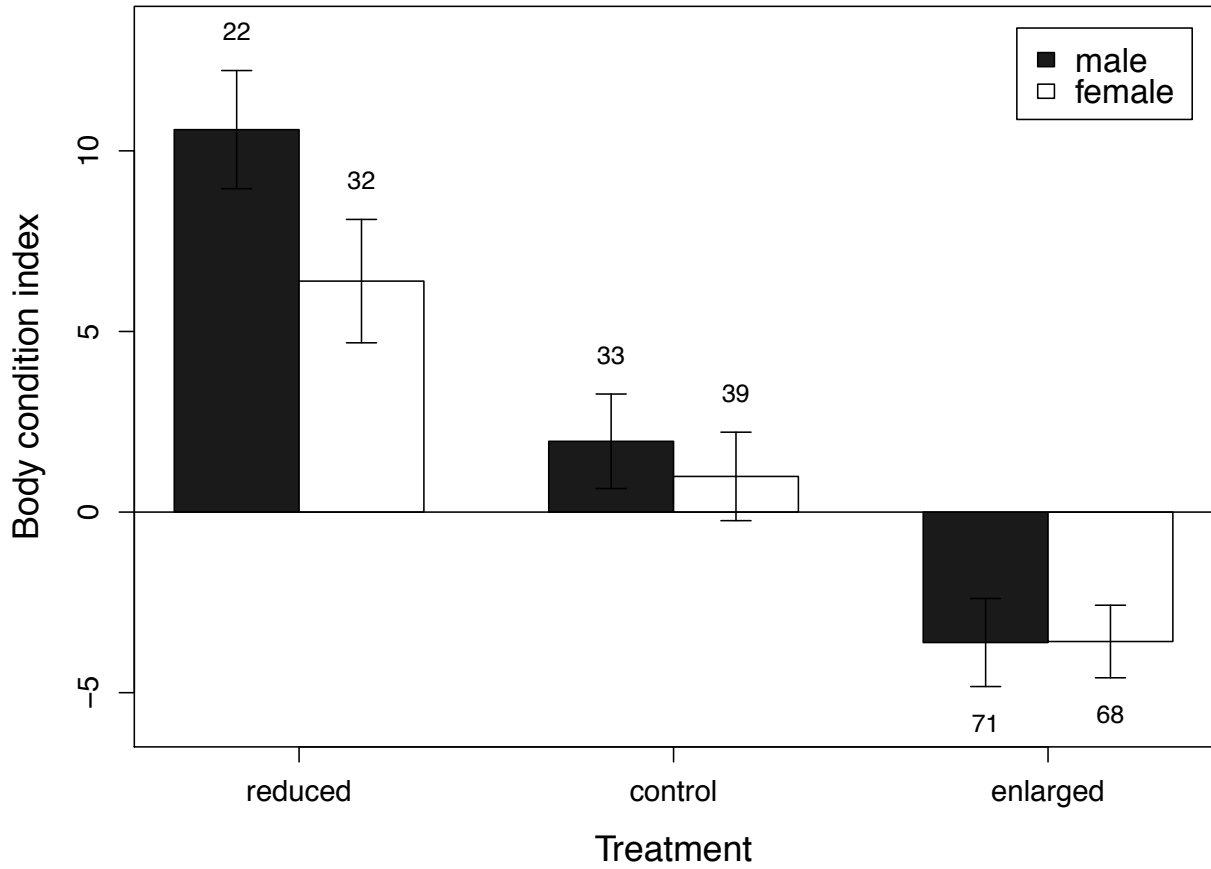
Figure 4.1 Body condition of male and female Northern Flicker nestlings at fledging (18-21 days old) in response to brood size manipulations. Sample sizes of nestlings in each group are listed above or below SE bars.
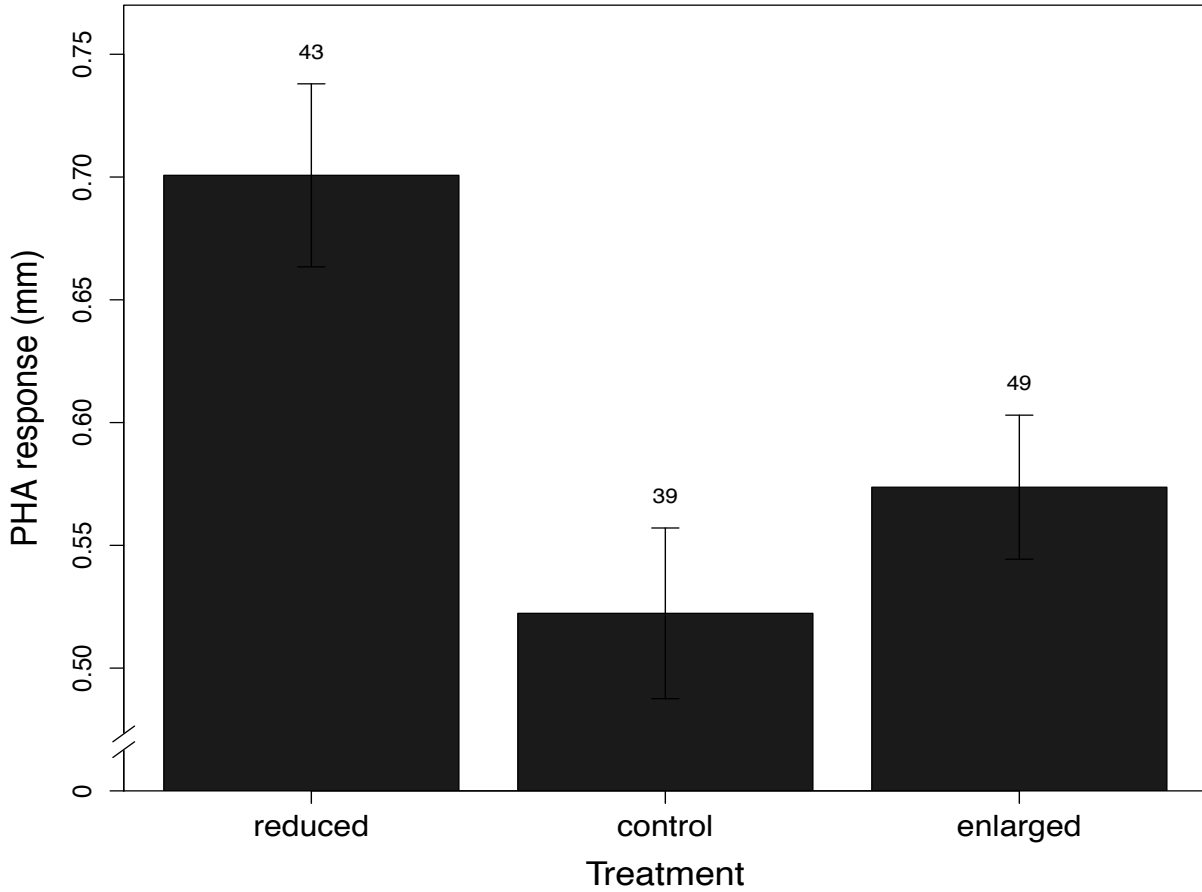
Figure 4.2 The T-cell-mediated immune response of Northern Flicker nestlings, as measured by the Phytohaemagglutinin (PHA) skin testing technique, at Stage 2 (10-13 days old) in response to brood size manipulations. Sample sizes of nestlings in each group are listed above SE bars.
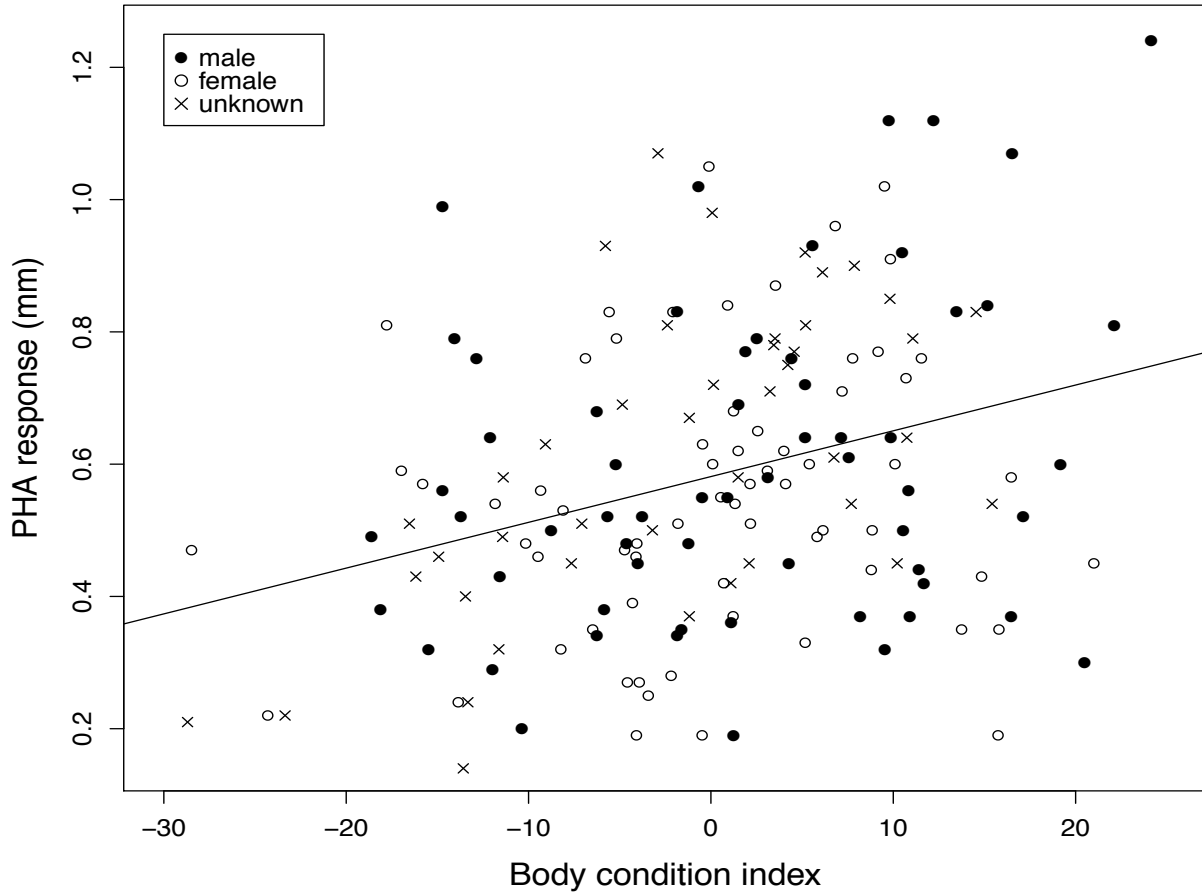
Figure 4.3 Relationship between the T-cell mediated Phytohaemagglutinin (PHA) immune response and a body condition index of Northern Flicker nestlings at Stage 2 (10-13 days old).
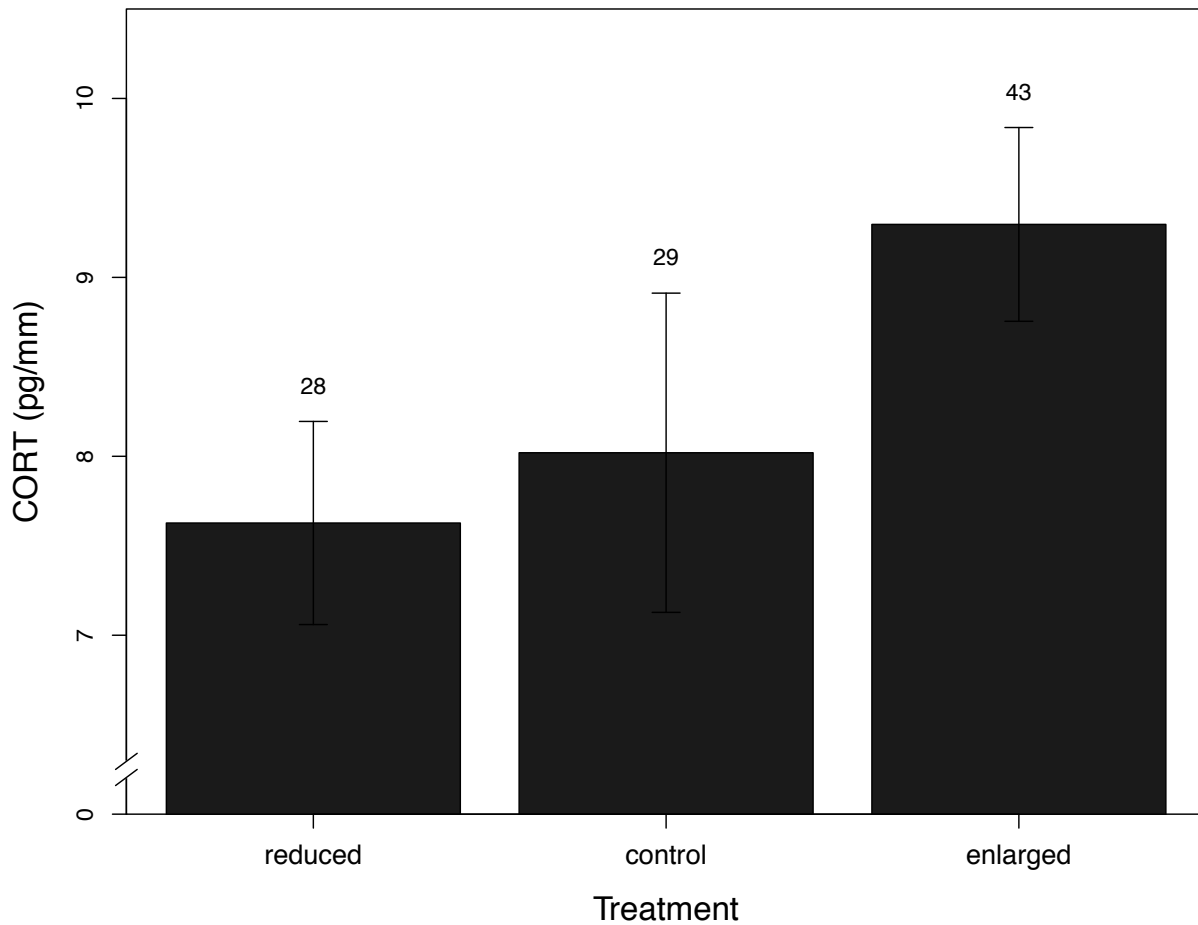
Figure 4.4 Stress hormone corticosterone (CORT) levels in feathers of Northern Flickers in response to brood size manipulations. CORT was sampled from feathers collected prior to fledging (Stage 3: 18-21 days old). Sample sizes of nestlings in each group are listed above SE bars.
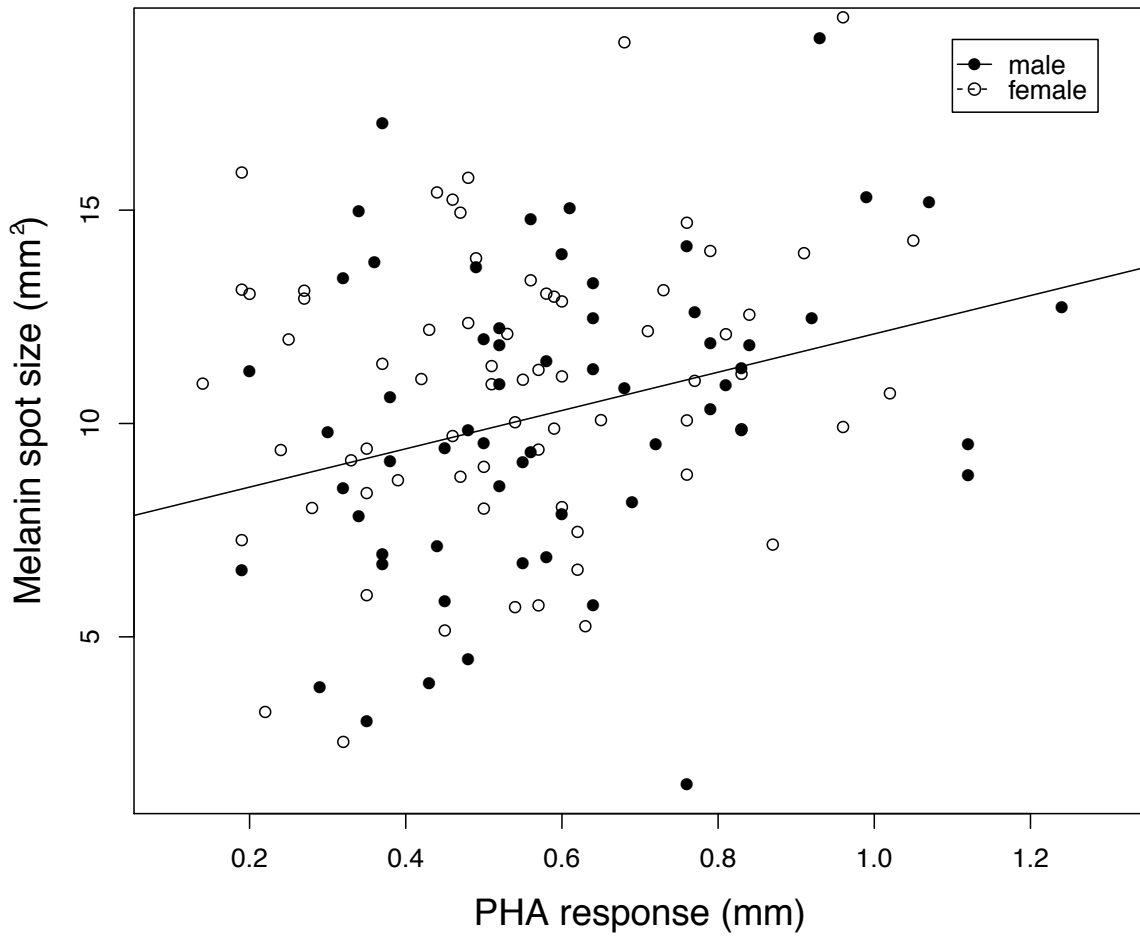
Figure 4.5 Correlation between the T-cell-mediated Phytohaemagglutinin (PHA) immune response and melanin spot size for male and female Northern Flicker nestlings at the time of fledging (Stage 3: 18-21 days old).
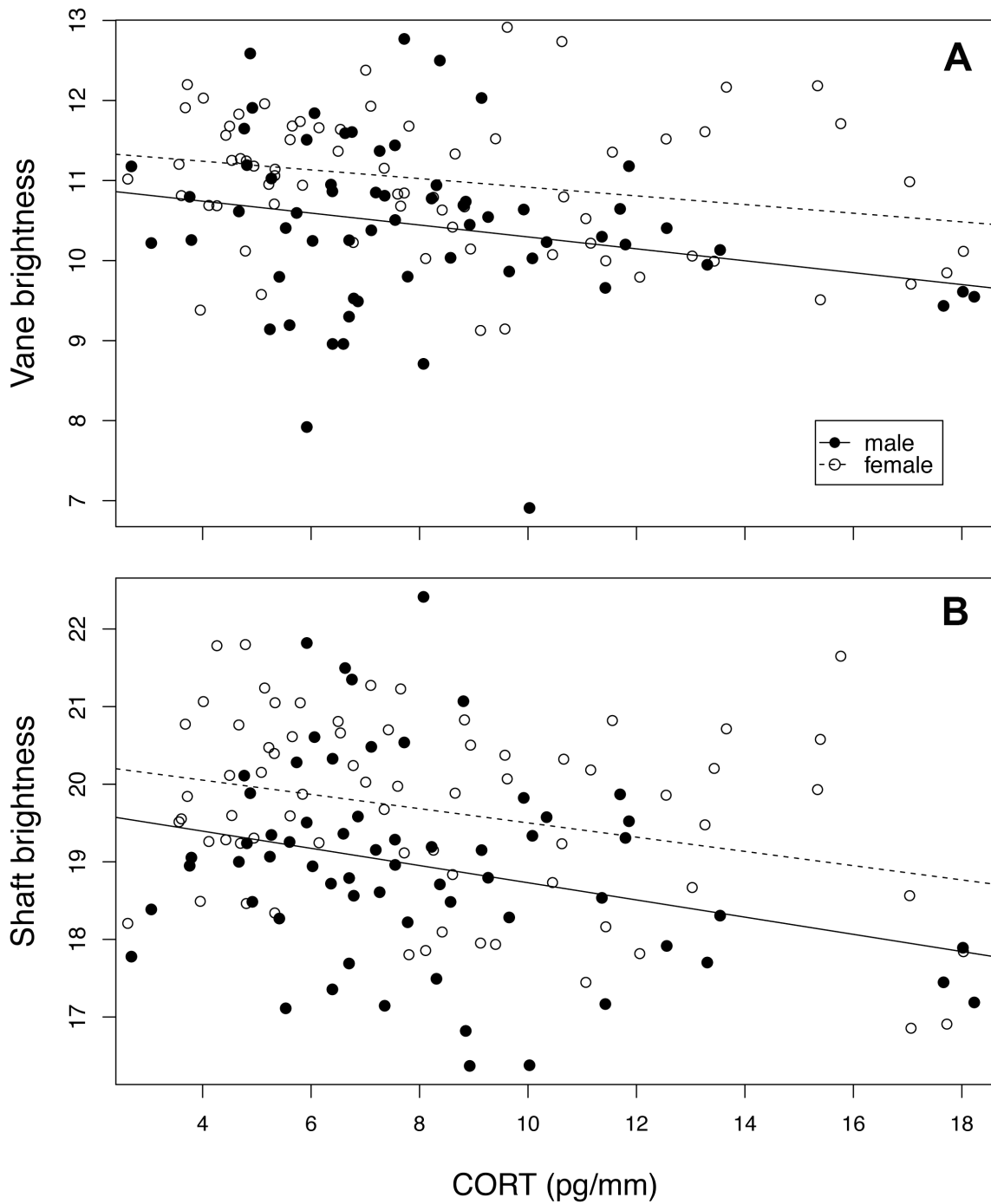
Figure 4.6 Relationship between feather corticosterone (CORT) levels and (**A**) vane brightness and (**B**) shaft brightness for Northern Flicker nestlings at the time of fledging (Stage 3: 18-21 days old).

CHAPTER 5: GENERAL DISCUSSION

5.1 Overview

The brood size experiment showed that parent flickers can increase provisioning effort when forced to do so, but that they lay a clutch size that is below their maximal capacity to provision (Chapter 2). In this respect, they are similar to many other species with "fast" or r-selected life histories (reviewed in Gow and Wiebe 2014a). Laying a smaller than maximal clutch size implies life-history trade-offs and I was able to demonstrate a trade-off in quality and quantity of offspring as measured by nestling mass and wing length (chapter 2), carotenoid chroma and melanin colouration (Chapter 3), and nestling body condition and immune function (Chapter 4).

A second goal of my thesis was to assess potential measures of nestling quality by examining the effect of food supply on morphology and physiology. To date, the focus has been on carotenoids as reliable signals of quality and nutrition, but the condition-dependence of melanin-based plumage has been much more debated. I was able to experimentally confirm that nutritional conditions during rearing influenced both melanin and carotenoid deposition in flicker nestlings (chapter 3), although there may also be genetic contributions to plumage colour. My findings are among the first to provide experimental evidence that the size of melanin ornaments in growing nestlings is condition dependent and, in fact, melanin was more strongly affected by nutrition than carotenoid colouration.

The body mass (Chapter 2) or condition index and the strength of the immune response most strongly reflected nutritional conditions during rearing, whereas $CORT_f$ levels were not

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influenced by food supply (Chapter 4). The study of $CORT_f$ is still in its infancy, but at least for flickers, the large degree of variation in $CORT_f$ was unrelated to food stress within broods, and suggests that it may not be a reliable biomarker of nutritional conditions during growth (although a larger sample size may reveal this link in flickers).

In contrast to with previous studies on size dimorphic birds, nutritional "stress" in nestling flickers did not induce sex-specific cell-mediated immunity or differences in $CORT_f$ levels (Chapter 4), suggesting that physiological strategies in coping with nutritional stress are similar between the sexes. However, brood size did affect nestling mass in a slightly sex-specific manner, with the larger sex being more negatively affected by large brood sizes (Chapter 2), and sex-specific correlations among quality measures were found (Chapter 4).

The final goal of this research was to understand sex differences in parental provisioning. The brood manipulation experiment I conducted confirms that both parents are assessing the demands from the brood (similar to Gow et al. 2013a) and adjusting their effort in "real time" (Chapter 2). In a short-term (24h) brood manipulation experiment with flickers, Gow and Wiebe (2014a) found that parents were incapable or unwilling to increase effort in enlarged broods. Results from Chapter 2 of this thesis, however, suggest that both parents are able to increase effort in enlarged broods when the demands are increased in the long-term throughout the nestling period. Despite male flickers potentially valuing the brood more than females (because of their high investment when the entire breeding season is considered), I found that both sexes in manipulated broods responded similarly to brood size (Chapter 2). Other cues (besides brood size) that flicker parents use to decide how much to provision in experimentally manipulated broods should be examined in future studies.

My work on flickers adds to our understanding of life-history trade-off between quality and quantity of offspring. It identifies consequences to flicker nestlings raised in experimentally enlarged broods and shows that some of these consequences have sex-biased relationships with other measures of quality. Additionally, as sex differences in parental care in birds with facultative polyandrous mating strategies have rarely been investigated (but see Gow 2014), my findings may add to our knowledge of reproductive decisions in sex-role reversed species.

5.2 Future Research Directions and Conservation Notes

I was able to measure the life-history trade-off of offspring quality versus quantity, but it would be interesting to measure the impact of parental effort on survival and future reproduction of parents. Such a trade-off may be more easily examined in a longer-term study and in species where larger sample sizes are possible or in which adults and juveniles are more easily tracked throughout the season.

Due to time constraints, I did not focus on many aspects of parental quality, but the effect of relatedness and inheritance on indices of quality could be examined with reciprocal transfers of nestlings between breeding pairs. The effect of gene-environment interactions on phenotype and especially epigenetic effects is an area of growing interest in biology. Specifically, as the condition-dependence of melanin-based plumage is highly debated, more work is needed to investigate the environment versus genetic effects on this ornament in flicker nestlings and in birds in general.

As many signals of health or quality in nestlings can indicate environmental conditions as well as predict the chances of reproduction and survival for individuals, future work should focus on identifying the most accurate bioindicators, elucidating the relationships between quality

measures, and especially determining their applicability to a range of species with different life histories and ecological traits. Understanding individual and population level variation in quality measures and determining which measure(s) best indicates "quality" in species may contribute to the success of conservation and wildlife management programs with limited time and money.

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APPENDIX

CHAPTER 2 SUPPLEMENTARY MATERIAL

Table S2.1 Natural model-averaged parameter estimates ± unconditional standard error (SE) and 95% unconditional confidence intervals (CI) for each of the fixed factors in the linear mixed effect models describing (**A**) parental provisioning for males and females and (**B**) nestling mass and wing chord length at three stages of development (Stage 1: ages 5-7, $n = 378$ nestlings in 58 nests; Stage 2: ages 10-13, $n = 336$ nestlings in 53 nests; and Stage 3: ages 19-21, $n = 265$ nestlings in 40 nests) in Northern Flickers. Factor weight is the relative importance of the specific factor.

		Factor	Estimate ± SE	CI	Factor weight
(**A**)	Male Provisioning				
	Stage 1	Brood size	0.13 ± 0.03	0.07, 0.19	0.98
	($n = 44$)	Body condition	0.01 ± 0.01	-0.1, 0.03	0.35
		Age	-0.01 ± 0.06	-0.12, 0.10	0.05
	Stage 2	Brood size	0.06 ± 0.03	0.01, 0.11	0.25
	($n = 43$)	Body condition	0 ± 0.01	-0.01, 0.20	0.14
		Age	0 ± 0.05	-0.09, 0.10	0.05
	Stage 3	Brood size	0.23 ± 0.05	0.13, 0.32	1
	($n = 33$)	Body condition	0.01 ± 0.01	-0.01, 0.03	0.84
		Age	-0.15 ± 0.08	-0.31, 0	0.24
	Female Provisioning				
	Stage 1	Brood size	0.09 ± 0.03	0.03, 0.15	0.57
	($n = 47$)	Body condition	0 ± 0.01	-0.02, 0.02	0.50
		Age	0.07 ± 0.07	-0.06, 0.21	0.17
	Stage 2	Brood size	0.12 ± 0.02	0.07, 0.17	1
	($n = 51$)	Body condition	0 ± 0.01	-0.01, 0.01	0.03
		Age	-0.13 ± 0.05	-0.13, 0.07	0.06
	Stage 3	Brood size	0.22 ± 0.04	0.14, 0.29	1

($n = 36$)	Body condition	-0.01 ± 0.01	$-0.03, 0.01$	0.09
	Age	-0.08 ± 0.08	$-0.24, 0.08$	0.08
(B) Nestling mass				
Stage 1[a]	Brood size	-1.09 ± 0.41	$-1.89, -0.29$	0.92
Stage 2[a]	Brood size	-2.86 ± 0.56	$-3.95, -1.76$	1
Stage 3	Brood size	-1.9 ± 0.53	$-2.94, -0.86$	0.75
	Sex	4.32 ± 0.99	$2.38, 6.25$	0.25
Nestling wing chord				
Stage 3[a]	Brood size	-0.68 ± 0.03	$-1.30, -0.05$	0.74
	Sex	0.89 ± 0.01	$-0.47, 2.26$	0.63

[a] Values standardized to age from the equations in Gow et al. (2013a).

Table S3.1 Repeatabilities (Lessells and Boag 1987) for Northern Flicker nestling plumage measurements of (**A**) carotenoid colour variables of vane and shaft portions of the secondary feather and (**B**) melanin spot size of the breast feather, with associated F and P-values from intraclass regressions. All repeatabilities were calculated on three measurements for each individual nestling. As vane and shaft chroma measurements were highly correlated, subsequent analysis was based on average chroma values.

	Measure		r	F	P
(**A**)	Vane[a]	Brightness	0.97	91.07	<0.001
		Chroma	0.90	28.62	<0.001
	Shaft[a]	Brightness	0.90	28.68	<0.001
		Chroma	0.98	132.5	<0.001
(**B**)	Melanin[b]	Spot Size	0.99	9.95	<0.001

[a]$n = 203$ nestlings

[b]$n = 204$ nestlings