

**EFFECT OF HABITAT QUALITY ON COMMUNICATION AND SIGNALLING IN  
THE BLACK-CAPPED CHICKADEE**

by

**Thibault Grava**

B.Sc., Université Jean-Monnet, France, 2004

M.Sc., Université Jean-Monnet, France, 2006

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF  
THE REQUIREMENTS FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY  
IN  
NATURAL RESOURCES AND ENVIRONMENTAL STUDIES

UNIVERSITY OF NORTHERN BRITISH COLUMBIA

April 2012

© Thibault Grava, 2012

## ABSTRACT

Vocal performance in songbirds describes the ability to repetitively perform energetically-expensive acts (vocal vigour) and/or to consistently perform difficult motor tasks (vocal skill). This concept is intrinsically correlated with the birds' condition – birds in better condition tend to signal with higher performance than birds in poorer condition (e.g. vocal performance is condition-dependent). Condition of birds, however, is also influenced by the habitat quality in which they settle, and has been well studied in the black-capped chickadee. My Ph.D thesis explores the links between habitat quality and vocal performance in this species.

Previous studies have shown that habitat quality affects song output in this species (reduced vocal vigour in poor-quality habitat) and it was speculated that reduced song output may be linked to poorer condition of birds in these forests. To document the relationship between immediate food availability and vocal performance, I conducted a supplemental feeding experiment on male chickadees during the peak in dawn singing in early spring. My results demonstrate that food availability is an important factor influencing song output, and that song output reflects the condition of the birds at the time of singing. The difference in song output across habitats, thus, appears to relate to difference in food availability at the time of singing.

The ability to maintain internal song structure (vocal skill) has previously been shown to be condition-dependent in chickadees, with dominant birds having better vocal skill than subordinate birds. My supplemental feeding study demonstrated that this vocal skill is not influenced by short-term resource availability to the signaller at the time of singing, but vocal

skill is affected by habitat quality - birds in young forest habitats (poor-quality) are less able to maintain internal frequency ratios in their songs than birds in neighbouring mature forests (high-quality).

The ability to maintain internal song ratios may rather reflect longer-term access to resources that could affect neural development of song centers. The developmental stress hypothesis states that birds suffering from a high stress during song learning are likely to experience lower neural development, which in turn reduces their ability to learn and render their songs. My results suggest that song structure in black-capped chickadees may reflect the birds' resource access or environment during the song learning period. I explored the condition of the juvenile birds settling in either habitat to test whether birds in young forest show indications of higher stress or evidence of lower food access during the time of song learning, which could help explain the difference in song structure observed across habitats. I found that the birds that settle in young forest have lower corticosterone (CORT) levels in tail feathers (associated with poor condition in other studies) prior to settlement than do birds settling in mature forest. This period coincides with the memorization period, which is a critical period for neural development. My results also demonstrate that juvenile males entering their first breeding season, and which settled in young forest, suffer from a lower development of the syrinx and of the RA (robust nucleus of the archistriatum, neural center in the brain associated with song production). This difference in development could arise from resource differences across habitat during the memorization period and could result in the observed differences in song structure.

In the black-capped, structural features of the song are used both by males and females to assess the signaller condition. Because habitat quality affects the ability to

maintain internal song structure, and if this feature is used by males to assess the signaller's condition, habitat quality could have a long-term effect on the relative perception of the bird's status. Using playback experiments, I demonstrated that males show lower responsiveness to song stimuli originating from young forest – this suggests that consistent song structure is used by other males to assess a signaller condition and that songs originating from dominant birds in young forest are not considered as equivalent threat to similarly-ranked stimuli from mature forests.

My results demonstrate the long-term effect of habitat quality on the bird's ability to produce the internal structure of their songs, which may have far reaching influences on the ability to attract mates, extra-pair partners and secure territorial resources in this species.

## CO-AUTHORSHIP

For all chapters in this thesis, I was the primary investigator and led the design of studies, collection of data and conducted all analyses. I wrote the initial drafts of all manuscripts and was responsible for incorporating comments and feedback on previous drafts into the final versions seen in this thesis. However, despite the use of first person singular in writing within the thesis, I would like to acknowledge that this work was not conducted in isolation. Angélique Grava assisted in all aspects of field work for this study, and without her assistance, this work could not have been conducted; to acknowledge her contribution, she is included in authorship on all publications that stem from my work. In addition, the large scope of the analysis associated with chapter 5 of this thesis was reliant on contributions and the expertise of two outside labs, as well as the field assistance of James Bradley at UNBC. Marc Avey, Jillian Avis and Christopher Sturdy (University of Alberta) conducted analysis of brain and syrinx physiology on our collected specimens, and Graham Fairhurst and Garry Bortolotti (University of Saskatchewan) conducted the analysis of feather CORT on our study specimens. All these collaborators are included in publication of Chapter 5. Finally, my supervisor, Ken Otter, contributed to experimental design, data analysis and writing on all studies included in this thesis, and is included in authorship on all resulting manuscripts.

### **Publications stemming from this thesis (published, in press, submitted or in prep)**

Grava T., Grava A. and Otter K.A. 2009. Supplemental feeding and dawn singing in black-capped chickadees. *Condor*. 111:560–564.

Grava T., Grava A. and Otter K.A. 2012. Vocal performance varies with habitat quality in black-capped chickadees (*Poecile atricapillus*). *Behaviour*. 149: 35–50.

Grava T., Grava A. and Otter K.A. Habitat quality affects the perception of dominant males based on their relative song consistency. *In preparation for Animal Behaviour*.

Grava T., Fairhurst G.D., Avey M.T., Grava A., Bradley J., Avis J.L., Bortolotti G.R, Sturdy C.B. and Otter K.A. Effect of habitat quality on early condition and neural development of song center in juvenile black-capped chickadee. *In preparation for Plos (Public Library of Science) Biology*.

## TABLE OF CONTENTS

ABSTRACT.....	i
CO-AUTHORSHIP .....	iv
TABLE OF CONTENTS.....	vi
LIST OF TABLES .....	ix
LIST OF FIGURES .....	x
ACKNOWLEDGEMENTS .....	xiii
1 - GENERAL INTRODUCTION .....	1
1.1 Generalities about performance in birdsong .....	1
1.2 Neurological and mechanical constraints on bird vocal skills .....	3
1.3 Relationships between body condition and vocal vigour in birds:.....	9
1.4 Condition and corticosterone levels in songbirds: .....	11
1.5 Effect of habitat on condition and behaviour .....	14
1.6 Black-capped chickadee – life history and singing behaviour: .....	15
1.7 The study of vocal performance in the black-capped chickadee and the effect of habitat quality: .....	20
1.8 Study site and caterpillar frass assessment.....	22
1.9 Outline of thesis: .....	25
2 - SUPPLEMENTAL FEEDING AND DAWN SINGING IN BLACK-CAPPED CHICKADEES.....	26
2.1 Introduction.....	27
2.2 Methods.....	29
2.2.1. <i>Study species</i> .....	29
2.2.2. <i>Study site</i> .....	29
2.2.3. <i>Winter banding and dominance assessment</i> .....	30
2.2.4. <i>Supplemental feeding and control of other factors affecting dawn singing</i> .....	31
2.2.5. <i>Recording of the dawn chorus</i> .....	32
2.2.6. <i>Statistical analyses</i> .....	34
2.3. Results.....	34
2.4. Discussion .....	39

3 -	VOCAL PERFORMANCE VARIES WITH HABITAT QUALITY IN BLACK-CAPPED CHICKADEES ( <i>POECILE ATRICAPILLUS</i> ) .....	41
3.1.	Introduction .....	42
3.2.	Methods.....	44
3.2.1.	<i>Study species</i> .....	44
3.2.2.	<i>Study sites</i> .....	45
3.2.3.	<i>Winter banding and dominance assessment</i> .....	46
3.2.4.	<i>Experimental design</i> .....	47
3.2.5.	<i>Sound analysis</i> .....	50
3.2.4.	<i>Statistical analysis</i> .....	50
3.3.	Results.....	51
3.3.1.	<i>Principal components analysis</i> .....	51
3.3.2.	<i>Effect of habitat on the ability to maintain internal song structure</i> .....	54
3.4.	Discussion .....	56
4 -	HABITAT QUALITY AFFECTS THE PERCEPTION OF DOMINANT MALES BASED ON THEIR RELATIVE SONG STRUCTURE.....	60
4.1.	Introduction .....	61
4.2.	Methods.....	63
4.2.1.	<i>Study sites</i> .....	63
4.2.2.	<i>Winter banding and dominance assessment</i> .....	65
4.2.3.	<i>Playback design</i> .....	66
4.2.4.	<i>Playback experiment</i> .....	67
4.2.5.	<i>Statistical analyses</i> .....	68
4.3.	Results.....	70
4.3.1.	<i>Principal Component Analysis</i> .....	70
4.3.2.	<i>Effect of habitat on playback response</i> .....	71
4.4.	DISCUSSION .....	74
5 -	EFFECT OF HABITAT QUALITY ON EARLY CONDITION AND NEURAL DEVELOPMENT OF SONG CENTER IN JUVENILE BLACK-CAPPED CHICKADEES.....	78
5.1.	Introduction .....	80
5.2.	Methods.....	84
5.2.1.	<i>Study species</i> .....	84

5.2.2. <i>Song learning in black-capped chickadees</i> .....	85
5.2.3. <i>Study site</i> .....	86
5.2.4. <i>Feather collection</i> .....	87
5.2.5. <i>Ptilochronology</i> .....	88
5.2.6. <i>CORT analysis</i> .....	89
5.2.7. <i>Brain and syrinx analysis</i> .....	90
5.2.8. <i>Statistical analysis</i> .....	92
5.3. Results.....	93
5.3.1. <i>Condition during memorization phase</i> .....	93
5.3.2. <i>Condition during sensorimotor learning period</i> .....	95
5.3.3. <i>Brain and syrinx measurement after song crystallization</i> .....	95
5.4. Discussion .....	100
6- GENERAL DISCUSSION.....	105
6.1. Synopsis .....	105
6.2. The effect of human activities on the production of sexual signal.....	108
6.3. Implications of my results on bird's life history and conservation .....	112
6. 4. Future research .....	116
REFERENCES .....	119

## LIST OF TABLES

Table 2-1 Relative proportion of total variation in dawn singing measures of each of the first three factors in a Principal Component Analysis. ....	36
Table 2-2 The contribution of dawn singing variables to PC1 eigenvalues. ....	37
Table 3-1 Contribution of the coefficient of variation of the seven frequency ratio variables to PC1 eigenvalues. ....	53
Table 4-1 Contribution of the seven variables of song consistency to PC1 eigenvalues and the total percent of variation explained by each of the two first factors. Variables having significant contribution to the PC ( $>0.33$ , Ho et al. 2006) are bolded. ....	69

## LIST OF FIGURES

Figure 1-1 Schematic sagittal drawing of the songbird brain showing projections of major nuclei in the song system. The motor pathway (green arrows) controls the production of song. The green arrows indicate the descending projections from HVC in the neostriatum to RA (Robust nucleus of the archistriatum), and from there to the vocal nucleus (nXIIts), the respiratory nucleus (Ram), and the laryngeal nucleus (Am) in the medulla. The red arrows indicate the anterior forebrain pathway that is essential for song learning. It indirectly links HVC to RA, via Area X in the parolfactory lobe, dorsolateral nucleus of the thalamus (DLM), and lateral magnocellular nucleus of the anterior neostriatum (IMAN). IMAN also projects to Area X. AM-nucleus ambiguus; DLM-medial portion of the dorsolateral nucleus of the thalamus; IMAN-lateral portion of the magnocellular nucleus of the anterior neostriatum; RA-robust nucleus of the archistriatum; RAm-nucleus retroambigualis; X-Area X; nXIIts-tracheosyringeal part of the hypoglossal nucleus. Picture and description modified from Brenowitz et al. (1997). ..... 6

Figure 1-2 Spectrographic representation of a *fee-bee* song of the black-capped chickadee (A) and of one example of a typical pitch shifting during the dawn chorus showing songs from the high-frequency range (the three first songs), of the low-frequency range (the two next songs) and of the mid frequency range (the two last songs). ..... 19

Figure 1-3 (left side) Map of the JPRF young (red lines) and mature (blue lines) forest site. Within each sites, two independent plots of 1600m<sup>2</sup> (yellow squares) were chosen as study sites. (right side) Map of Prince George, BC, showing the mature (blue) and young (red) sites used in chapter 5. .... 24

Figure 2-1 Comparison of the PC1 values (song output and start time) from fed and unfed birds classified by rank. Increasing values of PC1 indicate higher song output and earlier start times during dawn singing. Plots show mean value (dot), and standard error (lines) of the data. .... 38

- Figure 3-1 Extraction of eight frequency variables used to describe the song consistency in the *fee bee* song of the black-capped chickadee (*Poecile atricapillus*). Left: spectrographic representation. Fee: first note of the *fee bee* song. Bee1 and bee2: the two syllables composing the second note of the *fee bee* song. Right: fundamental frequency monitoring. Ffee.start, Ffee.end: frequency variables extracted from the fee note. Fbee1.start, Fbee1.max, Fbee1.end: frequency variables extracted from bee1. Fbee2.start, Fbee2.max, Fbee2.end: frequency variables extracted from bee2. .... 52
- Figure 3-2 Comparison of the PC1 value (song consistency) from birds recorded in young versus mature forest (General Linear Model:  $F(1, 15) = 5.42$ ,  $N = 16$ ,  $p = 0.03$ ). Increasing values of PC1 indicate higher ability to maintain consistent frequency ratios within songs across the chorus. Plots show mean value (square), and standard error (lines) of the data. .... 55
- Figure 4-1 Dominant birds in mature forest respond more aggressively toward a dominant intruder from mature than from an intruder from a young forest (Post hoc Fisher's LSD test,  $df=26.2$ ,  $N=8$ ,  $p=0.005$ ). There was no significant difference in response to either stimuli class among focal males in young forests (Post hoc Fisher's LSD test,  $df=26.2$ ,  $N=10$ ,  $p=0.70$ ). Circles and squares stands for the response to young forest and mature forest stimuli, respectively. Plots show mean and bars show standard errors. .... 73
- Figure 5-1 CORT level in natal feathers are higher in birds that settled in mature forests compared to birds settling in young forests (ANOVA,  $F_{(1,43)}=4.48$ ,  $N=48$ ,  $p=0.03$ ,  $d=0.35$ ). Dots represent mean and whiskers error bars. .... 94
- Figure 5-2 There is no significant difference in average growth bar widths (DGB) in the natal feathers of birds that settled in either mature or young forests (ANOVA,  $F_{(1,45)}=0.35$ ,  $N=48$ ,  $p=0.55$ ,  $d=0.004$ ). Dots represent mean and whiskers error bars. .... 96
- Figure 5-3 Syrinx weight of black-capped chickadee in their first spring is correlated with CORT levels of the natal feathers (regression analysis,  $R^2=0.1$ ,  $N=20$ ,  $p=0.03$ ). 97
- Figure 5-4 (a) Birds from young forests have a lighter syrinx than birds in mature forest ( $d=0.60$ ). (b) HVC volume differ slightly across habitat ( $d=0.13$ ) with birds in

young forests tending to have a smaller HVC than birds in mature forests. (c) An intermediate effect was found between RA volume in young vs. mature forests; birds in young forests tend to have a bigger RA volume than birds in mature forest ( $d=0.38$ ). Dots represent mean and whiskers error bars. .... 98

## ACKNOWLEDGEMENTS

This thesis was made possible thanks to the many people who supervised my work, shared experiences and advice, and supported me scientifically or socially. It has been a real pleasure to work at UNBC in Prince George: this small city has been very welcoming and the University, a real whirlpool of scientific and social experiences.

Foremost amongst those to whom I owe thanks is my supervisor, Dr Ken Otter, who has been extremely efficient, understanding and supportive throughout my PhD. His scientific expertise has been crucial to the design and analysis of experiments presented in this thesis.

This research has also been greatly supported by my committee members – Dezene Huber, Brian Aukema, Glenda Prkachin and Hugues Massicotte. Their participation significantly improved the overall quality of this thesis. I want to thank Hughes in particular for our frequent and refreshing conversations en Français!

All the people who participated to the four field seasons at the JPRF and in Prince George, were also critical in making this thesis possible. I cannot thank enough Angélique Grava and Regis Didier, for their work at the JPRF, and James Bradley, for his immense contribution to chickadee trapping and banding sessions around Prince George. Their commitment and contribution to this work is limitless.

I want to thank Graham Fairhurst and Marc Avey who collaborated with me on this thesis. Their expertise in feathers and brain physiology helped me greatly in the setting up of

the experiment that made chapter 5 possible. It has been a pleasure to work with both of them.

Furthermore, in terms of maintaining sanity, this project would not have been possible had I remained isolated in the birdcave or in the woods and for this I must thank many others in Prince George who made my life more than enjoyable and truly memorable. I had a lot of fun in this town and my range of experiences was made possible by much more than what the thesis alone could have provided. Thanks to all of you, local dudes and NRES students, I can say that my stay in Canada has been an unforgettable slice of life.

Finally I want to say a word about the one and only person who has transcended her role as a partner to become a collaborator, advisor, field assistant and employer collectively! My thesis would not have been possible without the emotional and scientific support of my wife Angélique. Thank you so much for being with me through the thick and thin, good and bad (and for waking up at 3am to record the dawn chorus three springs in a row!). I cherish all our moments thus far and look forward to the good times we will experience together in the future, and now as a small family!

Finally, I dedicate this thesis to loved ones who would have wanted to read these words but are not able to be with me today. I think of my Dad, Jean-Louis Grava, and of Alyssa Shaw – may you rest in peace. This work is for them, and for my son, Sasha, who I hope will one day read this and feel inspired to discover the place he was conceived!

### **1.1 Generalities about performance in birdsong**

Acoustic signals in avian communication are generally termed “vocalizations” and their structures are very diverse in the Order Passeriformes (the songbirds) (Catchpole and Slater 2008). Individual vocalizations are typically defined as syllables (units of continuous sound, the equivalent of notes in music) that have a fundamental frequency, which may or may not vary in pitch over time or contain harmonic frequencies. Syllables may be grouped consistently with other distinct syllables to form discrete vocalization types within species, and these vocalization types may be used consistently in particular circumstances. One of the most complex of these vocalizations in birds is distinguished as “song” and has been described extensively by behavioural ecologist since the invention of sound spectrograms after World War II (Marler 1957; Thorpe 1958; Marler 2004; Marler and Slabbekoorn 2004; Catchpole and Slater 2008). Songs are produced predominately by males, and appear to have evolved through sexual selection and hold two main functions: mate attraction (intersexual interactions) and rivals repelling (intrasexual interactions) (Collins 2004; Marler and Slabbekoorn 2004).

Bird song in Passerines is unlike other vocalizations in that it is partly innate and partly learned (Marler 2004). Unlike the production of many other vocalizations (termed “calls”), a male cannot produce his species-specific song if he does not go through a complex learning process. This involves hearing species-specific songs produced by tutors, and memorizing this structure in the neural centers of the brain devoted to song learning and production (Marler 2004). However, there is also an innate component of song production in

birds, which results from the propensity of birds to preferentially attend to and memorize song types of their own species (with the exceptions of birds learning other species' song through mimicry), suggesting an underlying genetic influence on song learning (Marler 2004). Despite these underlying genetic templates, as well as reliance on learning from tutors, individuals also show variability in the productions of songs that may reflect their underlying physical condition.

There is immense diversity in the structure and complexity of songs across taxa, but variability also occurs between individuals within the same population. Individuals do not necessarily display with the exact same song output (units of vocalizations over a given interval of time) and/or complexity of the syllable structure (e.g. modulation of frequency, number or types of syllables produced during a vocalization). The inter-individual differences in singing has been intensively studied through the concept of vocal performance (Podos 2009; Byers et al. 2010) which states that singing, like any other actions, is limited by factors that are inherent to the individuals.

Motor performance, itself, has been partitioned by Byers et al. (2010) into two independent measures: “vigour”, defined as “the ability to perform energetically expensive acts repeatedly”; and “skill”, which is “the ability to perform difficult motor tasks well”. These definitions apply to the vocal performance in many bird species because vocalizations often represent a challenge in term of production, complexity and/or stereotypy (Podos et al. 2009). In the chorus of many songbird species, song output provides information about the *signaller's* condition (Otter et al. 1997; Welling et al. 1997; Lucas et al. 1999; Poesel et al. 2004; Otter and Ratcliffe 2005; Murphy et al. 2008) and one might describe “vocal vigour” in this context as the birds' ability to perform a chorus with a high song output or song rate.

The internal song structure might also provide information on the signaller's condition in some passerine species (Lampe and Espmark 1994; Forstmeier et al. 2002; Pfaff et al. 2010). For example, the ability to maintain a consistent structure from one song to another correlates with other measures of male quality in black-capped chickadees (Christie et al. 2004; Hoeschele et al. 2010), and may reflect selection on neurological links in song learning and motor pathways (Byers et al. 2010; Podos et al. 2009). Females are known to attend to these structural elements when assessing male quality (Searcy 1984; Forstmeier et al. 2002; Reid et al. 2004; Byers 2006; Botero et al. 2009; Ritschard et al. 2010). Thus, the ability to produce songs with a high level of consistency may be a measure of “vocal skill”.

Song structure and production can, therefore, reveal information on individual condition, and thus be used as a reliable signal in both intra- and intersexual selection. To better understand the inter-individual diversity in vocal performance in bird song, it is important to understand the mechanisms underlying song learning and production, and the constraints associated with them.

## **1.2 Neurological and mechanical constraints on bird vocal skills**

In songbirds, the learning, perception and production of song is controlled by a set of interconnected brain nuclei known as the ‘song system’ (Fig 1.1) (Doupe and Kuhl 1999; Brainard and Doupe 2002; Bolhuis and Gahr 2006; Bolhuis et al. 2010). The song system is present in species that learn their song(s) by the imitation of species-specific sounds (Brainard and Doupe 2002; Doupe and Kuhl 1999). The imitative learning of song occurs during a sensitive period early in development (Bolhuis et al. 2010; Catchpole and Slater 2008), which is divided into two sub-periods – initially with a *memorization learning period*,

which is then followed by a *sensorimotor learning period* (Hultsch and Todt 2004; Bolhuis and Gahr 2006).

During the memorization learning period, the birds hear and memorize the song of a “tutor” – an adult conspecific. During this period, a model of the perceived song is encoded into the bird’s memory and will serve as a pattern to mimic in the subsequent phase. This phase typically starts very early during the bird’s development (often while the bird is still a nestling) and can extend beyond the start of the sensorimotor learning period in some species (Bolhuis and Gahr 2006).

During the sensorimotor learning period, birds attempt to match their singing performance to the internally-memorized song they learned from tutors. This period involves neural feedback loops with the song system in the brain. The auditory feedback of the produced song appears to be compared to the internal template, and neural development in the brain continues to be refined during this period until the produced song matches the memorized song (Bolhuis and Gahr 2006). This complex neural system makes the link between song learning and song production. This phase takes place until the birds enter their first breeding season, when a peak of testosterone stops the development of the nuclei, a phenomenon called brain crystallization (Hultsch and Todt 2004). In most species, which are termed close-ended learners, this process happens once prior to the first breeding season, and no changes in song development are seen in subsequent years. However, this is not necessarily as static as once thought, as there are a number of species that appear to be open-ended learners. The Island Canary (*Serinus canaria*), for example, is able to develop new songs after the first year, even as sexually mature adults (Nottebohm 2004). The plasticity is made possible through the recruitments of new neurons, particularly in two nuclei of the

song system: the HVC (Higher Vocal Center) and area X (see fig 1.1). The recruitment of new neurons, however, also happens in the song sparrow (*Melospiza melodia*) song system, a close-ended learner, without the development of new song types. Thus, the distinction between close-ended and open-ended learners is not as dichotomous as previously suspected, and that these two groups differ more in the degree of plasticity rather than in the presence or absence of plasticity (Benowitz et al. 2005).

The process of song learning in birds therefore involves the complex neural development linking the auditory perception system to the nuclei of the brain used to store the template, and then the neural linking of these centers to the syringeal muscles used in song production. Thus, the vocal skill of birds (e.g. their ability to perform accurate renditions of their songs) may be limited by neuro-muscular development during song learning. The nutritional stress hypothesis (Nowicki et al., 2002) suggests that a bird's condition during its early development stages can affect brain development, which could result in a diminished ability to accurately acquire and/or produce their song. Nowicki et al. (2000) demonstrated that a significant positive relationship existed between feather growth (a food-limited trait) and song repertoire size in the great reed warbler (*Acrocephalus arundinaceus*). Birds with limited food resources during their early developmental stage also tend to display less stereotyped songs than birds that had unlimited food resources. Food restriction, for example, seems to affect song learning ability of both nestling (swamp sparrow, *Melospiza georgiana*, Nowicki et al. 2002; zebra finch, *Taeniopygia guttata*, Zann and Cash 2008) and juvenile birds (european starlings, *Sturnus vulgaris*, Buchanan et al. 2003; Spencer et al. 2004). The effect of condition on song learning has been linked with an effect on neural development. In zebra finches, birds who undergo developmental stress

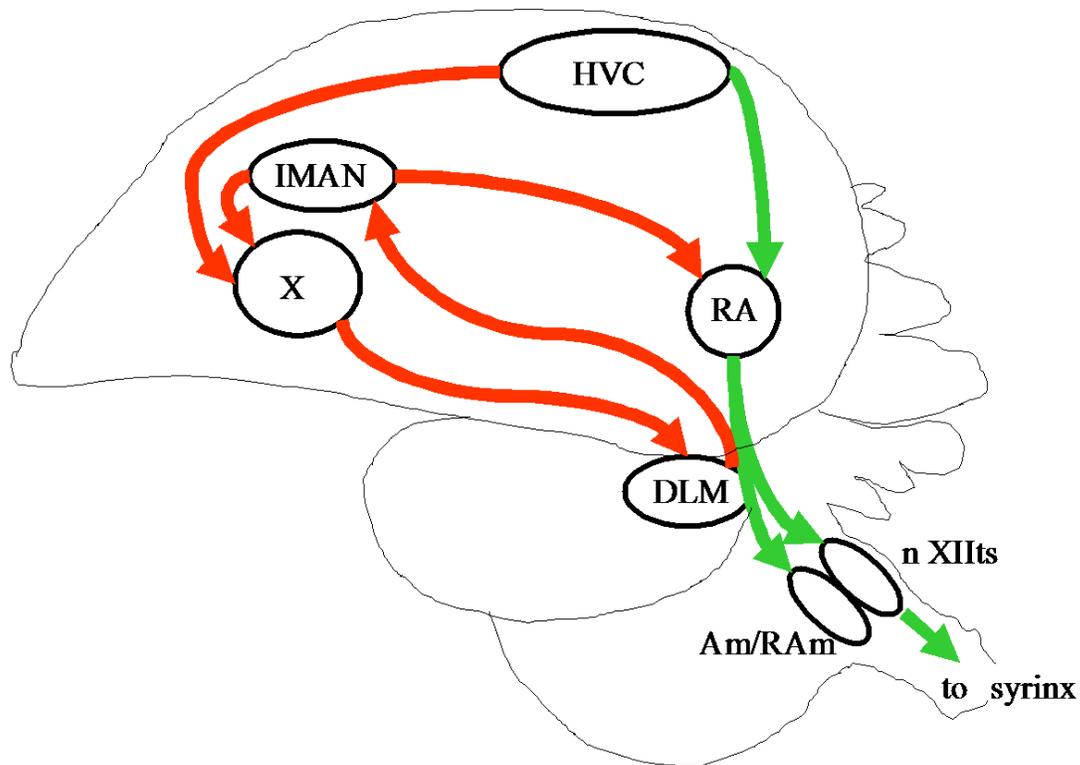


Figure 1.1 Schematic sagittal drawing of the songbird brain showing projections of major nuclei in the song system. The motor pathway (green arrows) controls the production of song. The green arrows indicate the descending projections from HVC (Higher Vocal Center) in the neostriatum to RA (Robust nucleus of the archistriatum), and from there to the vocal nucleus (nXIIIts), the respiratory nucleus (Ram), and the laryngeal nucleus (Am) in the medulla. The red arrows indicate the anterior forebrain pathway that is essential for song learning. It indirectly links HVC to RA, via Area X in the parolfactory lobe, dorsolateral nucleus of the thalamus (DLM), and lateral magnocellular nucleus of the anterior neostriatum (IMAN). IMAN also projects to Area X. AM-nucleus ambiguus; DLM-medial portion of the dorsolateral nucleus of the thalamus; IMAN-lateral portion of the magnocellular nucleus of the anterior neostriatum; RA-robust nucleus of the archistriatum; RAM-nucleus retroambiguus; X-Area X; nXIIIts-tracheosyringeal part of the hypoglossal nucleus. Picture and description modified from Brenowitz et al. (1997).

during the song learning period (through nutritional and hormonal stress, or parasitic infections) have lower development of the HVC region, but RA volume or brain size was not affected (Buchanan et al. 2004; Spencer et al. 2003; Spencer et al. 2005a).

The neuromuscular activity of song production involves a complex interaction between three sub-systems: respiratory, craniomandibular muscle groups and the bird's vocal organ - the syrinx (reviewed by Suthers et al. 1999). When singing, the energy responsible for sound production is provided by the respiratory system, which forces air into the vocal tract. In the vast majority of bird species, sound is produced during the expiration, whereas inspiration happens between syllables. The pattern of expiration/inspiration gives the rhythmic pattern of the song, or its temporal variation. The vocal organ transforming this energy into sound is called the syrinx and consists of two separate sound sources. The muscles on each side of the syrinx are separately innervated by the ipsilateral tracheosyringeal branch of the hypoglossal nerve (NXII<sub>ts</sub>) (Suthers et al. 1999). This configuration potentially allows for independent motor control of both sides of the syrinx and consequently for the extreme complexity and virtuosity that can be heard in certain bird species. Finally, the sound produced in the syrinx may be modified by the craniomandibular muscle group in the suprasyringeal vocal tract, primarily through beak movement (Suthers et al. 1999). Beak gape (i.e. opening) in particular has been demonstrated to correlate with fundamental frequency in most of the notes that were analyzed in the swamp sparrows (*Melospiza georgiana*) and white-throated sparrows (*Zonotrichia albicollis*) (Westneat et al. 1993). This correlation suggests that beak movement may play a role in filtering and enhancing particular frequencies (Suthers et al. 1999).

While this system is highly evolved for allowing the large inter-species diversity seen in bird song, there are mechanical limitations to the birds' vocal skills which are both species and individually-specific (Byers et al. 2010). Studies of vocal mechanics during the last 25 years have illustrated that song production by male birds is intrinsically challenging, involving the simultaneous control and coordination of breathing, syrinx modulations (the left and right sides having separate innervations) and vocal tract modulations including those of the trachea, mandible and oropharyngeal cavity (Nowicki, 1987; Podos and Nowicki, 2004; Suthers, 2004; Riede et al., 2006). A good example of those mechanical constraints involves the production of trills (the rapid repetition of the same note in a short amount of time – corresponding to the tremolo in music) in the swamp sparrow (*Melospiza georgiana*, Podos 1996; 1997). Hand-reared birds that are trained to learn songs with a trill rate higher than normal are not able to perform those songs despite evidence that they have actually memorized and learned them (Podos 1996). These studies show that the constraints on vocal skills are not necessarily based on neural limitations, but may also result from motor constraints (Podos 1997).

The bird's vocal skills are, thus, limited by various constraints at different stages. Brain development can be limited during the song learning period. If the bird's development is perturbed during this learning period, the bird's ability to perform complex or consistent songs (the bird's vocal skills) will be affected. Also, even if no problems occur during song learning, the bird's vocal skills might be affected during the production stage, if the learned song is beyond the bird's mechanical skills. At this stage, nothing can alleviate the mechanical constraints that limit the production of the song.

### 1.3 Relationships between body condition and vocal vigour in birds

An important role of birdsong is for the signaller to give information about its own body condition, to both attract females and/or to discourage intruding males. Thus, the song has to “honestly” reflect the signaller’s condition. This characteristic is known as condition-dependence (e.g. a condition-dependent signal) and can be true only if singing is energetically costly (Zahavi 1975). Therefore, the singing behaviour of the signaller should reflect its body condition. “Vocal vigour” (ability to produce songs at a high rate and sustain this production over time) depends on the bird’s ability to invest energy into singing (Godfrey 2003). As such, the song output is limited by the signaller’s “vigour” which reflects its body condition at the time of singing.

Because song production involves a complexity of different subsystems (see above), the muscular activity involved during song production increases the metabolic requirements for the male (Suthers et al. 1999). An increase in oxygen consumption during song production has been detected in three passerine species displaying songs differing in complexity: zebra finches, Waterslager canaries (*Serinus canaria*) and European starlings (Oberweger and Goller 2001). An increase in the metabolic rate also reflected song production in the Carolina wrens (*Thryothorus ludovicianus*, Eberhardt 1994). While studying the European robin (*Erithacus rubecula*), Godfrey and Bryant (2000) concluded that behaviour, including vocalizations and activity levels, and energy expenditures, were sensitive to manipulations of body condition. Because singing is a non-maintenance activity that requires extra energetic investment, it will be undertaken only if the bird has enough energy to sustain its other primary life-sustaining activities (Godfrey 2003). When a bird

attempts to sing, the amount of song produced will then reflect the amount of excess energy the bird has available to invest into singing (its vocal vigour).

Because birds in better condition supposedly have more energy available than birds in lower condition, their song production will therefore be higher. Studies looking at the relationship between song output and condition showed that birds in higher condition have a higher song output. For example, Lucas et al. (1999) showed that vocalization rate increased with body mass and food availability in Carolina chickadees (*Poecile carolinensis*). Murphy et al. (2008) demonstrated that song rate increases with body size and plumage quality in eastern kingbirds (*Tyrannus tyrannus*). In the european robin (*Erithacus rubecula*), it has been shown that males sing more at dawn when their food resources were constant than when they were variable (Thomas 1999). In the black-capped chickadee (*Poecile atricapillus*), dominant birds have increased song output at dawn (Otter et al. 1997). Therefore, the vocal vigour or song output, which reflects the amount of energy the birds can invest into singing, should be directly correlated to the condition of the birds at the time of singing.

As a result, vocal vigour and vocal skill are two independent condition-dependent signals entrenched in bird song. However, the temporal association between condition and singing ability may not be the same – vocal skill may reflect condition at some time in the past when song acquisition or neural development pathways were occurring, but the immediate energy to invest in present singing may be reflected more in vocal vigour at the time of signalling.

#### 1.4 Condition and corticosterone levels in songbirds

In vertebrates, stress response is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. In response to a stressor, a cascade of hormonal secretion induces the synthesis of glucocorticoids, such as corticosterone, from the adrenal cortex (Breuner et al. 2008). Glucocorticoids mobilize energy reserves, which allow the individuals to respond to stressors. However, long-term increases in both basal and stress-induced glucocorticoids can have detrimental effects on immune function or reproductive success (Sapolsky et al. 2000).

In birds, many studies compared the corticosterone levels (CORT) in individuals and their relationship to the individual condition (for review see Walker et al. 2005). The hypothesis supposed that birds in higher condition would have lower CORT levels than birds in lower condition; as a result, CORT levels were considered a proxy measure of relative individual condition (the *CORT-fitness hypothesis*; e.g. Marra and Holberton 1998; Arlettaz et al. 2007).

However, several problems occur with generalizing this hypothesis. First, many different methods of measuring CORT exist, and cross-comparison of measurements using different techniques are difficult. The initial technique used to measure CORT levels was through blood samples. This technique typically involves catching the birds and collecting a blood sample before the stress associated with handling causes CORT to increase (usually less 3 min after the birds enter the mistnet). This measure serves as baseline CORT level which is then compared to a second blood sample done after a period of induced stress (for example keeping the birds 30 min in a bag in Schmidt et al. (2012)). This technique gives a measure of baseline and induced CORT level at a very specific time, and allows researchers to compare the inter-individuals acute stress-induced response. However, baseline CORT and

acute stress-induced CORT level do not give the same information. While the stress-induced CORT levels give information on the ability of the bird to activate the HPA axis, the baseline CORT levels provide information about the long-term activation of the HPA axis in the background (Sapolsky et al. 2000).

Other techniques measure baseline CORT levels in feces or feathers. These two measures give information about the CORT level over a longer-term than does blood sampling. However, the time period reflected by CORT in feces vary across taxa, and is associated with the rate at which food is processed, which can be relatively rapid in bird species. CORT in feathers, though, provides an indication about the CORT level during the time period in which the feather was grown, which reflects about three weeks for birds as small as chickadees.

All these methods give measures of CORT that differ greatly in their significance and time scale, thus comparisons between studies are best made using a common technique to measure CORT. Even within techniques, inter-individual variation in CORT can change associated with different phases of the organisms life history. Bonnier et al. (2009) compared the baseline CORT levels in breeding females tress swallows (*Tachycineta bicolor*) and found that females investing in reproduction (higher clutch mass) show lower baseline CORT levels than females with lower clutch mass during the incubation stage, but the inverse relationship occurs during the nestling stage. This study demonstrates the variability in CORT level across individuals and the difficulty in using CORT levels as a measure of individual condition unless the context is considered. As a result, to fully understand inter-individual variation in CORT, we have to compare similar metrics of CORT (baseline vs

induced), preferably using the same technique, and compare individuals which are at the same stage of their life history.

Using this approach, studies looking at the relationship between baseline CORT levels and individual condition in nestling birds are beginning to reveal emerging patterns. Using CORT injection *in ovo* in eggs of European starlings (*Sturnus vulgaris*), Chin et al. (2009) demonstrated that embryos with elevated baseline CORT ultimately grow as fledging with more functionally mature pectoral muscles and larger wing areas (the two best predictors of flight performance) than controls. Those results tend to show that elevated baseline CORT level might be advantageous for nestling, allowing them to develop faster and achieve greater size. In a study comparing baseline CORT in feather, Fairhurst et al. (2012) showed a similar trend between Cory's shearwater chicks grown under different nutritional conditions. Chicks from the nests of parents whose flight ability has been impaired were of lower condition (smaller, lighter and reduced immune response) than controls, and showed associated lower CORT level in their feather. Similarly, Kitayski et al. (2005) found the same trend in tufted puffin chicks using baseline CORT level in blood; food deprived chicks show lower CORT levels than control. These studies suggest that birds that are in lower condition or have lower access to resources are less likely to invest in growth. The mobilization of the HPA axis effectively starts mobilizing reserves and increasing the metabolism. Thus, individuals with low food intake may be unable to sustain this metabolic activity and might down-regulate the HPA axis (which would in turn lead to lower baseline CORT levels in measures). As a result, low baseline CORT levels at the earliest developmental stage could be predicted to be associated with lower growth or condition

(Kitayski et al. 2005; Chin et al. 2012; Fairhurst et al. 2012). This may, then, prove a metric for studying the effects of habitat and early condition on song acquisition.

### **1.5 Effect of habitat on condition and behaviour**

It appears that vocal performance in birds reflect the signaller's condition whether it is at the time of singing or at the time of song learning. However, many parameters can influence a bird's body condition, such as the quality of habitat in which the bird has settled. Strong and Sherry (2000) used ptilochronology (measurement of daily growth bars on feathers which gives an indication of food availability during the period of feather regrowth; Grubb 1989) and biomass to show that ovenbirds (*Seiurus aurocapillus*) in habitat with low access to resources were in poorer conditions than those where resource access was higher. Grubb and Yosef (1994) also used ptilochronology to assess nutritional condition, and found that loggerhead shrikes (*Lanius ludovicianus*) were affected by habitat quality. Similarly, Carlson (1998) found that the condition of white-backed woodpeckers (*Dendrocopos leucotos*) was a reflection of their territory quality. In the Paridae family, wintering chickadees in poor-quality habitat carry higher levels of furcular fat than do birds in high-quality habitat (van Oort and Otter, 2005); such high fat levels in other members of the Paridae family occur among birds that experience decreased or inconsistent access to food (Gosler, 1996; Gosler and Carruthers, 1999).

In turn, the effect of habitat quality on the bird's body-condition can have serious consequences on the behaviour of those birds, particularly on condition-dependent behaviour. The effect of habitat on avian behaviour has been extensively studied in the black-capped chickadee in North-Central BC. In this species, birds nesting in poor-quality

habitat (young forest) have a lower reproductive success than birds nesting in high-quality habitat (mature forest) (Fort and Otter 2004a). Territorial behaviour is also reduced in young forest compared to mature forest in this species (Fort and Otter 2004b), which is typically the result of these areas being perceived as lower in resources worth defending. Also, birds in the young forests have higher hematocrit values (indicative of higher metabolic costs) during the breeding season (van Oort et al. 2007). This suggests they might have to work harder, despite still having lower breeding prospects, which in turn can affect their overall condition. Finally, birds nesting in young forest have a lower song output during the dawn chorus than the birds in mature forest (van Oort et al. 2006), which seems to indicate that the bird's vocal vigour is affected by habitat quality.

It appears that habitat quality has a direct effect on the behaviour of the black-capped chickadee in north-central BC through an effect on the body condition. Habitat quality affects vocal vigour encoded in the *fee-bee* song through song output (van Oort et al. 2007). However, whether this effect is due to ecological, mechanical or neural constraints and whether habitat has an effect on song structure is not known. Because the black-capped chickadee behaviour has been extensively studied over the past decade, this species presents itself as a good model for the detailed study of the effect of habitat quality on the bird's vocal performance.

## **1.6 Black-capped chickadee – life history and singing behaviour**

Black-capped chickadee is a small resident generalist, common in deciduous and mixed forests across northern North America. Birds overwinter in stable flocks characterized by linear dominance hierarchies (Smith 1991). Flocks are composed of two to five mated

pairs that forage and travel together during most of the nonbreeding season. As the birds maintain a strict hierarchy during these winter months, the rank of each individual can be determined from interactions involving two birds (e.g. dyadic interaction) at winter feeding stations (Smith 1991; Otter et al. 1997).

When the birds enter the breeding season, flocks break down and mated pairs start to defend individual territories. Black-capped chickadees are primary cavity nesters, excavating a new cavity each year; this nest construction begins soon after the pairs separate out of flocks onto their breeding territories. During the period of excavation and during the early period of egg laying, females are fertile and males begin a period of extensive singing at dawn (the dawn chorus). Males sing from within their territory, typically close to the nest site, at dawn, and choruses can typically last 20-40 min with males singing continuously at rates of 12-20 songs/minute throughout this period. The chorus typically ends when the female emerges from the nest cavity, at which point the pair often copulates. Chorus activity typically stops when a male's mate begins incubation of the clutch (Otter et al. 1997; Mennill and Otter 2007; Foote et al. 2010), resuming only if the clutch fails and the pairs reneest (pers obs). Nestlings fledge at day 16, typically early July in the study area. During the nestling phase, a modified form of the song (*faint fee-bees*) is used by both males and females in coordinating nesting activity (Smith 1991; Foote et al. 2010). While faint *fee-bees* are lower in amplitude, and much more variable in frequency structure than the pure *fee-bees*, they are noticeably similar in tonal structure, and may serve as an initial template for nestling song learning (e.g. Baker et al. 2003). Following a fledgling period of several weeks, juveniles disperse and little is known about the subsequent flock establishment, except that it can occur during the early fall and individual birds can disperse as far as 10km from the nest before

settling into a flock (Smith 1991). During most of the year, chickadees consume a mixed diet of seeds, berries, and invertebrates, but during the breeding season, they switch to a diet predominantly composed of insects (Smith 1989).

Black-capped chickadee song consists of a very stereotyped two-note signal known as the *fee-bee*. Very few examples of variation of this signal have been described in this species; in fact, songs are remarkably invariant across most of their range consisting of the northern half of the North American continent from the Atlantic to Pacific coast (Hailman 1989; Kroodsma et al. 1995; reviewed in Mennill and Otter 2007). In early breeding season, breeding males sing long bouts of continuous “*fee-bee*” songs close to the nest site at dawn (Otter et al. 1997; Gammon 2004; Fig 1.2 A). These dawn song bouts often last over 45 min with average singing rates of 12–15 songs per minute, with some males achieving maximum rates of more than 20 songs per minute during the peak of the chorus. The dawn singing in the black-capped chickadee appears to be directed towards both females and males (Otter and Ratcliffe 1993; Mennill and Otter 2007). During the chorus, the signaller may shift the song to a higher or lower frequency (a process known as “pitch shifting”; Fig 1.2 B), but the frequency ratio between the *fee* and the *bee* note is stringently maintained by birds when they transpose the overall pitch of the song (Weisman *et al.* 1990).

As with all passerines, the black-capped chickadees use their song to claim a territory, and the *fee-bee* song is used also in this context during male diurnal interaction. In this case, the song is typically used to resolve conflicts over territorial occupancy without escalating direct fights. When interacting, males signals their willingness to escalate contests by matching the opponents songs in frequency (frequency matching) and/or in time (song overlapping) (Mennill and Ratcliffe 2004), a behaviour known as countersinging.

Among wild black-capped chickadees, juvenile birds start singing approximately at day 20 (Baker et al. 2003, confirmed by personal observations), which correspond to the date they fledge from their parents. I feel confident in saying that this represents the starting point of the sensorimotor learning in this species, and that memorization therefore occurs prior to this date when the birds are still fed by their parents i.e. from hatching to fledging). During this first phase occurring prior to fledging, the chickadees memorize the template of their species-specific songs that they will try to reproduce in the next phase. Once the chickadees disperse, the males enter the sensorimotor learning period. During this phase, the young males practice singing songs they have previously memorized, attempting to match their subsong attempts with their learned template songs (Shackleton and Ratcliffe 1993). This sensorimotor phase lasts until the birds enter their first breeding season (Shackleton and Ratcliffe 1993; Baker et al. 2003; Hultsch and Todt 2004).

During the entire learning period, the song system develops in parallel with the acquisition of adult song (Bolhuis et al. 2010). As a songbird, the template for the tutor's song in the black-capped chickadee is thought to be encoded in the neural substrate of NCM (caudomedial nidopallium) and CMM (caudomedial mesopallium; Bolhuis et al. 2006). During development, the anterior forebrain pathway is involved in the acquisition of song as well as auditory-vocal feedback processing. The posterior pathway or song motor pathway (SMP) controls the production of song and includes HVC (higher vocal center) and RA (robust nucleus of the archistriatum), which innervate nXIIts (tracheosyringeal portion of the hypoglossal nucleus) that control the syrinx ((for review see Mooney 2009; Fig 1.1). Normal development of the song system is crucial during the sensitive period for normal development of song (Nowicki et al. 2002; Christie et al. 2004; Grava et al. 2012).

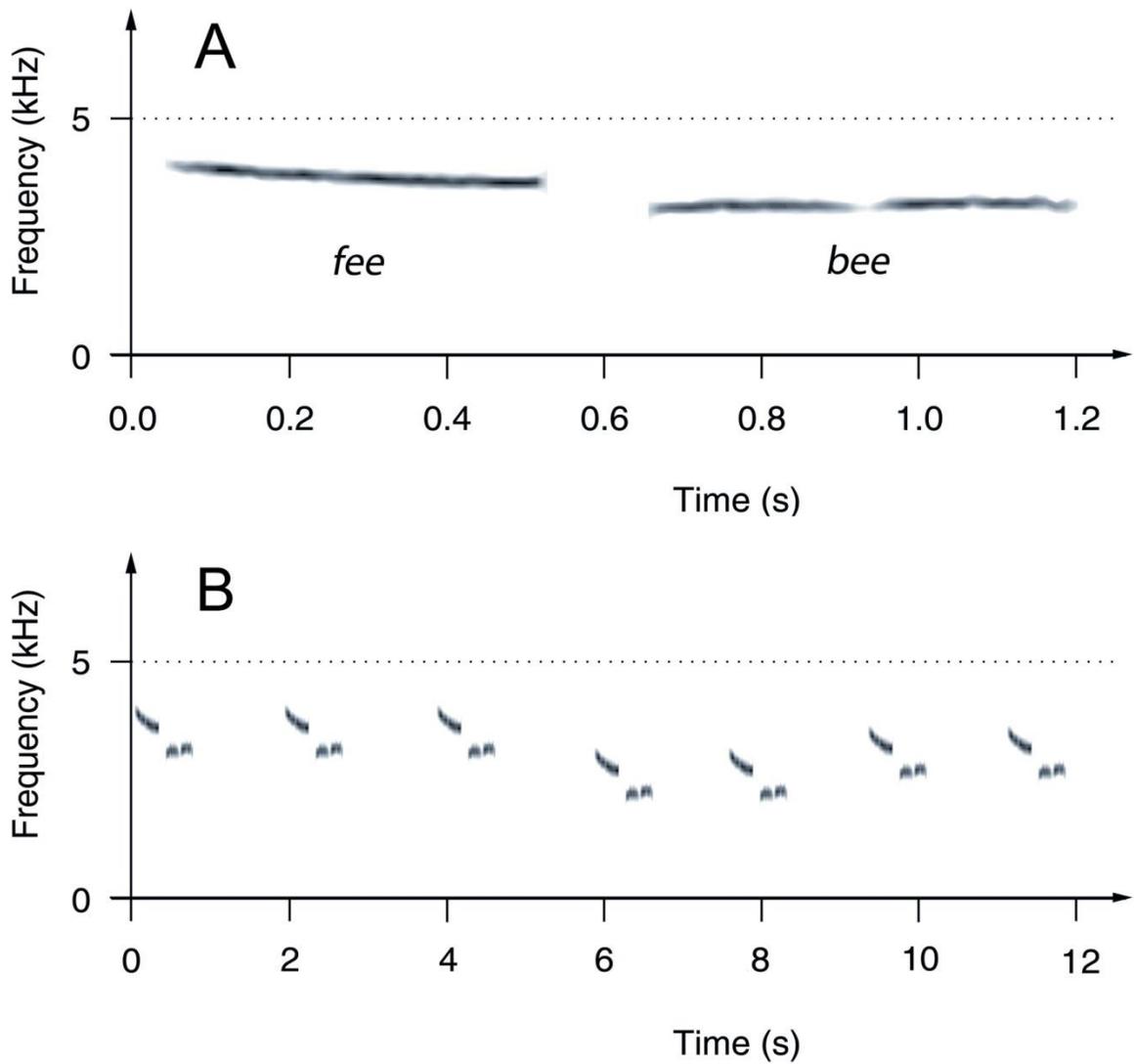


Figure 1.2 Spectrographic representation of a *fee-bee* song of the black-capped chickadee (A) and of one example of a typical pitch shifting during the dawn chorus showing songs from the high-frequency range (the three first songs), of the low-frequency range (the two next songs) and of the mid frequency range (the two last songs).

## **1.7 The study of vocal performance in the black-capped chickadee and the effect of habitat quality**

Vocal communication underlies most aspects of chickadee ecology, from overwintering survival through breeding behaviour. Yet, while the mechanisms of how vocal interactions between birds convey particular kinds of information are becoming better understood in the black-capped chickadee (Mennill and Otter 2007), most of this work suggests that the context and/or reliability of signals can be significantly affected by habitat alteration.

Two different signals within the *fee-bee* song in the black-capped chickadee give reliable information about the signaller's condition. First, song output at dawn (or vocal vigour) is known to reflect the male condition (Otter et al. 1997) and it is assumed that the females can utilize this information to assess male quality (Otter and Ratcliffe 1993, 2005; Mennill and Otter 2007). Female chickadees and other titmice appear to use this information to choose potential partners for extra-pair copulation (Kempnaers et al. 1997; Mennill and Otter 2007). However, the reliability of this signalling depends on high-quality males singing at higher rates than lower-quality males, which may indicate their ability to acquire territories that supply the male with resources needed to sustain such costly singing. Recent work has found that variation in habitat quality may alter the relative signalling of dominant versus subordinate males (van Oort et al. 2006) and may jeopardize the ability of females and males to judge the relative status of different males in the chorus. However, the exact link between habitat quality and song output is still missing. It is suggested that difference in condition at the time of singing across habitat would be responsible for a lower song output in poor-

quality habitat, but no study has directly tested whether song output at dawn was affected by condition.

Second, the internal structure of the songs can also give reliable information about the signaller condition independently from the song output. Christie et al. (2004) found that the ability to maintain a consistent within-song internal acoustic structure (which has been defined as a vocal skill by Byers et al. 2010) during the chorus (“song consistency” hereafter) is lower in subordinate males than in dominant males. The authors suggest that song consistency does not reflect the condition of the birds at the time of singing (Christie et al. 2004) but at the time of song learning. Birds in poorer condition during song learning would face a higher developmental stress, therefore affecting their ability to accurately learn their species-specific song (see “developmental stress hypothesis” described previously). Female black-capped chickadees also appear to attend to signals encoded into the structure of the *fee-bee* song that are related to a signaller’s condition (Hoeschele et al. 2010). Therefore, song consistency seems to both signal the condition of the signaller and is used by other individuals as cues to the signaller’s condition. However, the mechanisms that link song consistency to condition in chickadees and how these are affected by habitat quality remain unexplored. By comparison, vocal skills of a European parid, the great tit (*Parus major*), are negatively affected by the presence of heavy metal pollutants at the time of song learning (Gorissen et al. 2003). Though it has been assumed that song consistency in chickadees reflects the early condition of the signaller, this has not been formally tested. Nor has the influence of habitat quality on song consistency been addressed.

## **1.8 Study site and caterpillar frass assessment**

My studies took place in the John Prince Research Forest (JPRF), north of Fort Saint James, BC as well as in the surroundings of Prince George, BC (Fig 1.3). I studied two populations of black-capped chickadee settling in two different habitats that differ mainly by their age. I compared birds living in mature sub-boreal forest to birds living in very young forest (harvested approximately 20 years ago and left to naturally regenerate).

In order to test whether young forest actually has more limited food resources for the chickadees than the mature forest, I conducted a study to monitor the population of caterpillars during the spring of 2008, 2009, and 2010. During the breeding season, black-capped chickadees rely heavily on caterpillars that feed upon the trees' leaves. I used frass-fall traps to collect caterpillar waste falling from the canopy on my study site. Frass-fall traps consisted of a plastic bucket (diameter = 30cm) elevated at approximately 1m off the ground on a wooden stand. The open end of each bucket was covered with a mesh in a slightly inverted funnel shape, upon which I placed paper napkin filters. These filters were the collection surface for frass falling from the canopy. I positioned four traps in each focal bird's territory. The traps were positioned around the bird nest, as it is typically at the center of the territory. I set up four buckets five meters from each black-capped chickadee cavity, one in each cardinal direction. Every three days, I collected the napkins and installed new ones. Napkins were folded in upon themselves to ensure that no frass was lost during removal, and these were immediately placed into paper bags for transport and storage.

Samples were then desiccated at 40°C in a drying oven for 48 hours. With a magnifying lens, I sorted frass from plant debris, and weighed the frass with an analytical scale to the nearest 0.01mg. The four samples for each nest were then added and I averaged

the data across nests (N=36) for the total amount of frass in the habitat per period in milligram/cm<sup>2</sup>/hour. Seven territories were monitored in 2008, 15 in 2009 and 14 in 2010.

After running an ANCOVA on caterpillar frass quantities with forest age (young and mature) as the categorizing variable and year as a covariate, I found that more frass was collected in mature forest than in young forest during the incubation period ( $F_{(1,26)}=4.35$ ,  $N=36$ ,  $p=0.047$ ) but not later in the breeding season, although a strong trend tends to suggest the difference in frass availability continues into the early nesting period ( $F_{(1,26)}=2.65$ ,  $N=36$ ,  $p=0.115$ ). The incubation period occurs early in the season, and typically overlaps with the dawn chorus period (the birds enter the incubation period towards the end of the dawn chorus period). As a result, based on the quantities of the caterpillar frass collected in three consecutive breeding seasons, my results suggest that the population of caterpillars is effectively less important in young forest than in mature forest during the chorusing period.

t

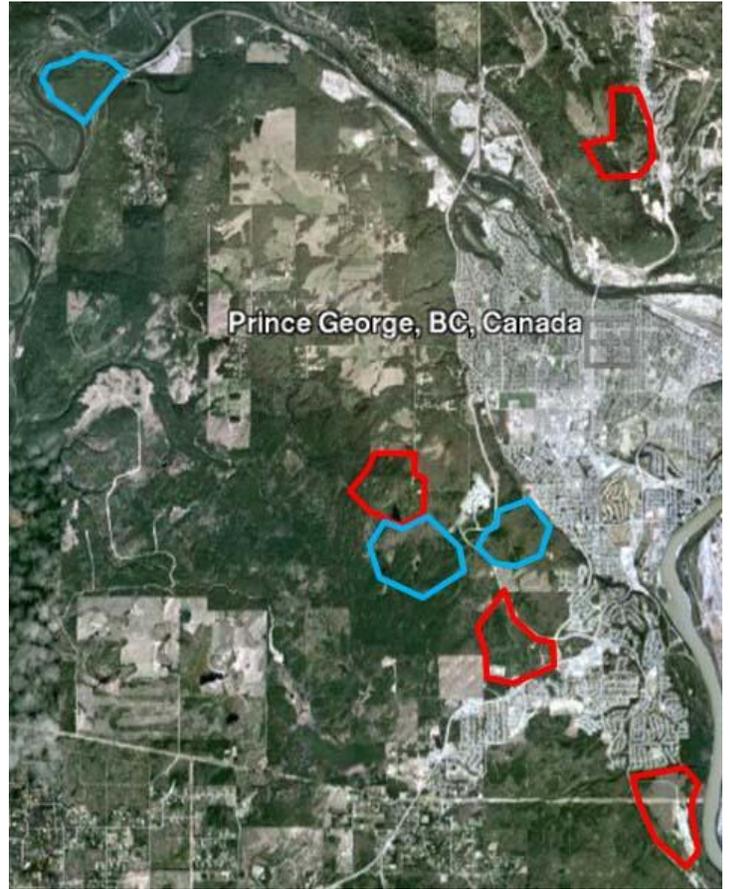
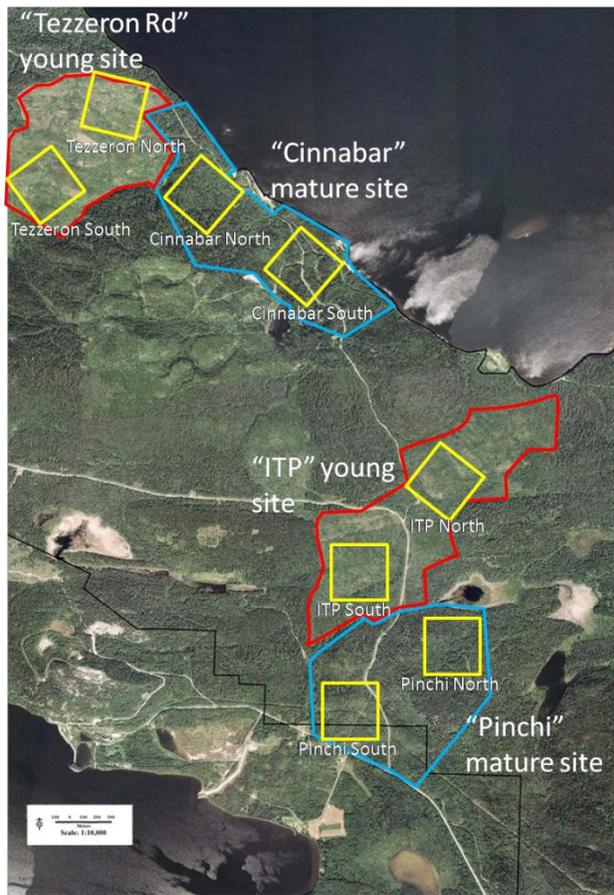


Figure 1-3 (left side) Map of the JPRF young (red lines) and mature (blue lines) forest site. Within each sites, two independent plots of 1600m<sup>2</sup> (yellow squares) were chosen as study sites. (right side) Map of Prince George, BC, showing the mature (blue) and young (red) sites used in chapter 5.

## **1.9 Outline of thesis**

My thesis focuses on the effect of habitat quality on vocal performance in the black-capped chickadee. I was particularly interested in the study of the interactions between the constraints that limit vocal performance and the quality of the environment. My work therefore concentrates on the role of habitat quality and food availability at different stages of the birds' developmental history - during song learning and at the time of chorusing - on condition and consequently on vocal skills and vigour in this species. In chapter 2, I tested whether food availability can affect song output, and thus whether the reduction in output in poor-quality habitat could be equated to low resources. I secondarily tested whether short-term supplemental provisioning of food during the chorus period has a greater relative impact on vocal vigour among birds in young versus mature forests. In chapter 3, I determined whether habitat alters the ability of males to produce more stereotyped songs and whether song structure is affected by short-term feeding. The 4<sup>th</sup> chapter presents the results of an experiment which explored the consequences of lowered-consistency in song structure found in young-forest males, when these songs are broadcast to rivals during diurnal singing interactions. In the 5<sup>th</sup> chapter, I studied the effect of habitat on the development of black-capped chickadees during song learning. Using feathers collected from birds in both habitats, I studied the food availability and the condition of juvenile birds at different stage of song learning. This study was followed by physiological measurements made on euthanized birds to compare the development of brain and syrinx in juvenile males newly settled in both young and mature forest. I then discuss and compare my main findings in chapter 6.

## 2 - SUPPLEMENTAL FEEDING AND DAWN SINGING IN BLACK-CAPPED CHICKADEES

**Abstract-** The dawn chorus is a collective behaviour involving a network of birds signalling at the same time. Two hypotheses can explain the mechanisms of dawn singing. The “condition-dependent hypothesis” states that the dawn chorus consists of a “broadcast network” in which the signal is given in all directions but is not dependent on the singer’s neighbors’ chorus. The song output is condition-dependent and can thus be limited by the amount of food to which a signaller has access to in its territory. On the other hand, the “social-dynamic hypothesis” states that during the chorus the birds are constantly interacting with their neighbors. Under this scenario, the dawn chorus consists of an “interactive network” and the song output is not dependent on the condition of the signaller but on the interaction with the neighbors. To determine which scenario best explains the mechanisms of the dawn chorus in the black-capped chickadee (*Poecile atricapillus*), I set up a supplemental-feeding experiment in a dyadic fashion by providing 3.5 g of mealworms to selected birds and compared their song output to that of unfed counterparts by controlling for rank and habitat. I found that fed birds, both dominant and subordinate males, sang more than unfed birds. I thus conclude that body condition is a critical variable contributing to individual differences in song output in the black-capped chickadee and consequently the functioning of the dawn-chorus network.

## 2.1 Introduction

Dawn singing in birds has often been viewed as a “broadcast network” (Burt and Vehrencamp 2005); signallers broadcast songs omnidirectionally, and the songs can be detected by multiple receivers simultaneously. In such a scenario, singing males need not be interacting with each other directly, as in dyadic countersinging during territorial contests. Rather, during the chorus, song output of males may honestly signal individual condition—males display their quality to listening receivers by outsignalling rivals (Otter and Ratcliffe 2005). In support of this hypothesis, studies have found that song output at dawn often closely approximates attributes of males’ energetic state or access to resources (Welling et al. 1997; Lucas et al. 1999; Poesel et al. 2004; Murphy et al. 2008). In such a scenario, females are able to assess individual differences in males’ quality by assessing song output from a large number of males simultaneously (Otter and Ratcliffe 2005), thus facilitating comparison of her mate against potential extra-pair sires. This scenario also implies that song output at dawn is resource-limited; for example, the more food a signaller has at its disposal, the more energy it can invest in singing (Alatalo et al. 1990; Cuthill and Macdonald 1990; Thomas 1999).

Burt and Vehrencamp (2005) and Foote et al. (2008), however, have used microphone arrays to record multiple males chorusing simultaneously and found that male–male signalling may affect patterns of singing during the chorus. This result strongly supports the social-dynamic hypothesis (Staicer et al. 1996), which proposes that the function of dawn chorus is the interactive communication and adjustment of social relationships among males. This finding implies that the dawn chorus functions as an “interactive network” in which all signallers are interacting with their neighbors. These findings could

suggest that mechanisms other than simply the male's energetic state may control song output during the dawn chorus. Previous findings that song output in chickadees is correlated with social rank of males (Otter et al. 1997) could thus be interpreted in one of two ways: either rank reflects differences in males' energetic state or resource access, which are also signalled via differences in song output, or, alternatively, the correlation between social rank and song output in the chorus could result from dominant males intimidating neighboring subordinate males through interactive signalling during the chorus. The latter may not necessarily imply a limitation of signalling in relation to immediate resource availability on the part of subordinates but rather the suppression of song output by dominants. Under this suppression hypothesis, chorus output is unlikely to increase with provision of moderate amounts of supplemental food, as supplementation is unlikely to change the long-term rank association between two neighboring birds. If the capacity of individuals to maintain song output during the chorus (and thus engage in countersignalling) is resource-limited, however, then food supplementation should increase song output by both dominant and subordinate birds.

I supplementally fed a subset of male black-capped chickadees (*Poecile atricapillus*) and recorded their dawn singing behaviour against that of unfed males of similar rank and occupying similar nesting habitat, two factors known to influence song output at dawn (van Oort et al. 2006; Otter et al. 2007). I therefore attempt to assess the importance of a *signaller's* short-term resource access on song output during the dawn chorus.

## 2.2 Methods

### 2.2.1. *Study species*

The black-capped chickadee is a small resident generalist, common in deciduous and mixed forest across northern North America. The birds overwinter in stable flocks characterized by linear dominance hierarchies (Smith 1991). These flocks are composed of two to five mated pairs that forage and travel together during most of the nonbreeding season. As the birds maintain a strict hierarchy during these winter months, the rank of each individual can be determined from dyadic interactions at winter feeding stations (Smith 1991; Otter et al. 1997). During most of the year, chickadees consume a mixed diet of seeds, berries, and invertebrates, but during the breeding season, they switch to a diet predominantly of insects (Smith 1991). In early spring, flocking is replaced by territoriality as pairs begin excavating nests (Smith 1991). During this period, breeding males sing long bouts of continuous “*fee bee*” songs close to the nest site at dawn (Otter et al. 1997; Gammon 2004). These dawn song bouts often last over 45 min with average singing rates of 12–15 songs  $\text{min}^{-1}$ , with some males achieving maximum rates of more than 20 songs  $\text{min}^{-1}$  during the peak of the chorus.

### 2.2.2. *Study site*

My study took place in the John Prince Research Forest, Fort St. James, British Columbia, Canada (54° 40' N, 124° 24' W). I chose two plots of mature forest and two plots of young forest as study sites. Each plot consisted of ~16 ha of forest of homogenous age. Both of the two mature-forest sites were characteristic of the mixed woods of the sub-boreal spruce subzone in northern British Columbia (see Otter et al. 2007). Furthermore, both sites were similar in age structure, neither having undergone commercial logging for more than 80

years. At both sites, trees were characterized by an average trunk diameter of 25 cm (measured at breast height) and a canopy at approximately 25 m. At the two young-forest sites, the trees had undergone nearly complete clearing in the past 30 years. Both sites had a flora characteristic of a young, regenerating sub-boreal forest. These sites were characterized by an average trunk diameter of 10 cm (measured at breast height) and a canopy at approximately 8 m high.

### *2.2.3. Winter banding and dominance assessment*

I attracted birds to temporary feeders filled with sunflower seeds using playback of the chickadee's mobbing calls during February 2006 and 2008. I captured male and female chickadees on my study sites by using either mist nets or box (Potter) traps mounted on platforms. I then marked birds with a numbered aluminum ring (Canadian Wildlife Service) and three colored leg bands. Each bird was given a unique color combination allowing me to recognize individuals in the field. I determined the bird's sex from body measurements (Desrochers 1990). I confirmed the sex by observing behaviour during the breeding season. I aged the birds by rectrix shape (Meigs et al. 1983), categorizing them as either second year (i.e., entering their second calendar year and therefore approaching their first breeding season) or after second year (i.e., entering their third or later calendar year and second or later breeding season).

I determined the composition of flocks and their home ranges by following the birds attracted to the feeder. I assessed dominance rank by observing dyadic interactions around the feeders. I used three behaviours to identify the flock hierarchy: if (1) a focal bird supplanted or chased away its opponent, (2) the focal bird gave a display that elicited a

submissive posture in an opponent, or (3) the opponent waited for the bird to leave before approaching a feeder (Ficken et al. 1990, Otter et al. 1998), the focal bird was considered to be dominant to its opponent. Thus, I determined the linear dominance matrix for each flock, classifying birds as low, mid, or high ranking, according to their position within the flock. Because a female chickadee's rank is correlated with the rank of her social mate (Smith 1991; Otter et al. 1999), I concentrated on determining relative rank of males within flocks. In flocks consisting of three or more pairs, the male submissive to the alpha male but dominant over the low-ranking male was considered mid ranking. I observed too few interactions to determine the rank of six of the 26 birds; in these cases, I used age as a proxy of rank, as rank is strongly associated with relative age (Smith 1991; Otter et al. 1999; Ratcliffe et al. 2007).

#### *2.2.4. Supplemental feeding and control of other factors affecting dawn singing*

Both rank and habitat strongly influence dawn singing in the black-capped chickadee (Otter et al. 1997; van Oort et al. 2006). Thus, to determine whether song output at dawn accurately reflects variation in access to food, I controlled for the effect of rank and habitat by selecting two males from similar habitat age and similar rank class for dyadic comparisons. The two males in a dyad were selected, as much as possible, so as not to include neighbors; typically, the males I paired resided in different forest patches. Only one dyad was composed of neighbors, the situation resulting from a shift in territory boundaries after the feeding had begun but prior to the day of recording.

I randomly selected one of the two males in each dyad to receive feeding; the other served as the unfed control. Each fed bird received approximately 30 mealworms (~3.5 g)

every second day for six to ten days before I recorded its dawn singing (three to five feeding sessions). As mealworms contain approx.  $2.5 \text{ kcal g}^{-1}$  of gross energy content (Martin et al. 1976), my supplementation provided up to 8.75 kcal of available food to the birds, close to the average daily energy requirement ( $\sim 10 \text{ kcal}$ ) predicted for the black-capped chickadee (Smith 1991). I used a quiet playback of the chickadee's mobbing calls to attract only the fed male to the feeder, which was placed close to the nest excavation on the individual's territory. To be sure that only the correct male came to the feeder, I checked for 15 min after the first feeding to ensure that no other chickadee (other than the male's mate) or Red-breasted Nuthatch (*Sitta canadensis*) ate the mealworms. Most of the time, the mealworms were gone at the end of the 15 min; if not, I checked the feeder regularly during the day to be sure the birds ate all the mealworms. Only males that I visually confirmed to be using the feeders and taking the majority of mealworms provided were included in the study. To control the effects of the playback on the song output of the fed birds, I used the same duration of playback to attract the unfed dyadic control males to an empty feeder in their territory for the same number of days as their fed counterparts.

#### 2.2.5. *Recording of the dawn chorus*

I recorded both males in a dyad on the same day, following the final feeding session. In this manner, I attempted to control for the effects of seasonality or abiotic conditions (weather) on singing behaviour. All the dyads were recorded between 28 April and 12 May 2006 and between 5 May and 13 May 2008. Daily survey of the birds' movements permitted me to find their nests and/or territory boundaries. As this species begins to sing close to the nest, the survey provided good information on the potential locations of singing for each

male. Sometimes, however, the birds shifted territorial boundaries, particularly in young forest where black-capped chickadees tend to be less territorial (Fort and Otter 2004; see above note regarding one dyad being neighboring males). As it was important to ensure that males recorded were the selected manipulated males, I eliminated from the study several recordings in which the identity of the singers could not be conclusively deduced. This left me with a sample comparison of 13 dyads (26 males) to use in analysis among the 26 dyads that I attempted (eight of 12 dyads attempted in 2006; five of 14 dyads attempted in 2008). The lower inclusion rate in 2008 reflects unusually late snow melt that year making both recording and identification of chorusing males much more difficult.

I recorded dawn choruses with Marantz PMD430 audiocassette recorders or Marantz PMD671 digital recorders in conjunction with one of the following directional microphones: a Sennheiser ME 67 with a K6 power supply, a Sennheiser MKH 70 with an MZA14 power supply, or an Audio Technica ATB-815a. I began recording with the first song of the day and recorded continuously until the bird stopped singing for five consecutive minutes (Otter et al. 1993, 1997), taking that pause as the “end” of the chorus. All recordings were made after nests had been constructed but before incubation by the resident female, so my data reflect song output during the mates’ fertile period. The fertile period also reflects a time when resources available to males in the population I studied are probably limited, as it occurs several weeks prior to leaf-burst, when lepidopteran prey become abundant (Blondel et al. 1993; Hunter and Elkinton 2000).

### 2.2.6. *Statistical analyses*

I use five variables from recordings and observations made during the chorus to quantify the dawn song's output and start time: onset time, chorus length, average song rate, maximum song rate, and average shift rate. I entered the five dawn-song variables into a principal component analysis, using STATISTICA (version 6.0, StatSoft, Inc.) to create a composite variable representing chorus-song output. I then compared principal components that contributed significantly to variation in fed and unfed males' chorusing behaviour by using a general linear model with paired design (fed vs. unfed members of the dyad) with rank of the dyad (dominant or subordinate) as a random classifying variable.

### 2.3. **Results**

There was no overall difference in song output of males between 2008 and 2006 (ANOVA,  $n = 26$ ,  $P = 0.29$ ), so I combined data from both years for analysis. Principal component one (PC1) accounted for more than 56% of the total variation among individuals (Table 2.1), and principal component two (PC2) accounted for an additional 20%. When I included five variables in the analysis, however, only PC1 accounted for more variation than expected by chance according to the broken-stick method of factor significance, so I used only PC1 in subsequent tests (Jackson 1993, Legendre and Legendre 1998).

Length of the chorus, maximum song rate, average song rate, and average shift rate all had a strong positive contribution to PC1. The chorus's start time had a smaller and negative contribution (Table 2.2). As a result, increasing PC1 values for an individual

indicate a high song output and earlier start of the chorus than for individuals with a lower PC1 score.

Across treatments, overall song output tended to be higher among dominant dyads than among subordinate dyads ( $F_{\text{rank}} = 4.39, P = 0.06$ ). Supplemental feeding, though, had a greater effect; within dyads, fed birds had a significantly higher PC1 score than unfed birds ( $F_{\text{fed vs. unfed}} = 5.47, P = 0.032$ ) (Fig. 2.1). Thus fed birds began the chorus earlier in the morning and sang more frequently and for a longer period at dawn than their unfed counterparts. However, there was no effect of rank on the difference in song output between fed and unfed birds within a dyad ( $F_{\text{rank} \times (\text{fed vs. unfed})} = 1.67, P = 0.23$ ) (Fig. 2.1).

Table 2.1 Relative proportion of total variation in dawn singing measures of each of the first three factors in a Principal Component Analysis.

	Percentage variation explained	
	Total	Cumulative
PC1	56.9	56.9
PC2	20.2	77.2
PC3	12.9	90.1

Table 2.2 The contribution of dawn singing variables to PC1 eigenvalues.

Variable	PC1
Length of the chorus	0.78
Maximum song rate ( $\text{min}^{-1}$ )	0.92
Average song rate ( $\text{min}^{-1}$ )	0.89
Start time	-0.60
Frequency shift rate	0.46

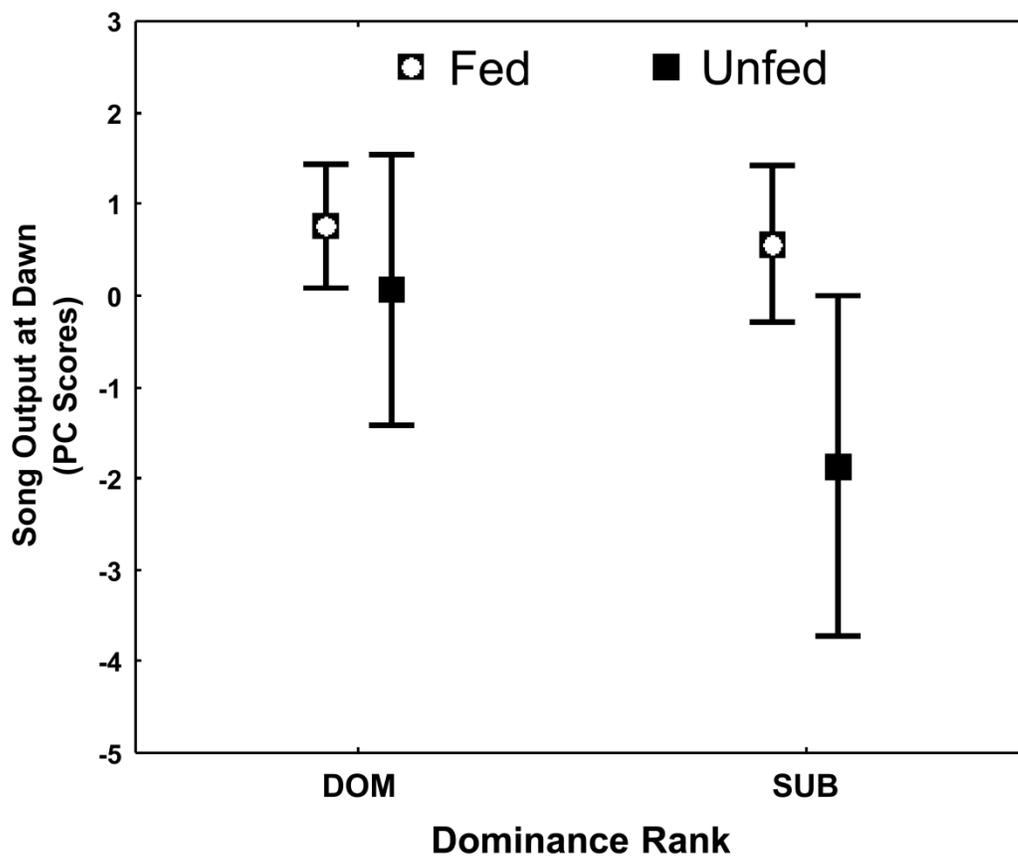


Figure 2.1 Comparison of the PC1 values (song output and start time) from fed and unfed birds classified by rank. Increasing values of PC1 indicate higher song output and earlier start times during dawn singing. Plots show mean value (dot), and standard error (lines) of the data.

## 2.4. Discussion

My results demonstrate the influence of food availability on song output at dawn—birds provided supplemental food have a higher song output at dawn than their unfed counterparts of similar rank. Furthermore, the increase in song output among fed birds was similar whether they were dominant or subordinate. These results allow me to better interpret the disparity in song output between dominant and subordinate birds during the dawn chorus.

If dominant males suppress the song output of their subordinate neighbors, for example, by interactively matching and thus intimidating them during the chorus, one would expect a pattern of higher song output among dominant males, as previously shown (Otter et al. 1997). Under this scenario, the higher song output is akin to a badge of status; differences in song output are maintained by social reinforcement. If the disparity in song output was due simply to suppression of subordinates by dominant males, then I might expect only dominants to increase song output with supplementation. The supplementation I provided constitutes nearly half the birds' daily energy requirement every other day (providing the male ate all the mealworms itself), but such short-term supplementation is unlikely to be sufficient to reverse the very stable, long-term hierarchies of chickadee flocks. However, if the ability of birds both to maintain song output and to interact dynamically at dawn is resource limited, rather than socially regulated, then I would expect the fed birds in both rank classes to increase song output. My study suggests that the difference between dominant and subordinate males in dawn song output comes from, at least proximately, a difference in food availability, which could in turn translate into differences in body condition of dominant and subordinate males during the spring. Dominant males might have access to territories in which food is naturally more available than in territories left to subordinates, which would

allow them to invest more energy in dawn singing. This scenario was supported by van Oort et al. (2007), who found that dominant males' body mass and hematocrit level during the breeding season are higher than those of subordinate males, suggesting that dominant male chickadees are in better condition than subordinates. My findings do not exclude, however, the dawn chorus from being an "interactive" rather than a "broadcast" network. On the contrary, the more energy a bird can acquire, the more it might be able to invest in both high song output and increased countersignalling during the chorus. Whether supplementation increases an individual male's ability to engage in dynamic countersignalling during the chorus, however, remains to be tested directly.

My results also suggest a proximate mechanism for the differences in song output seen between birds settling in different habitat types (van Oort et al. 2006). Decreased song output during the dawn chorus of birds in young forests may be, in part, a result of food limitation in these habitats. Several other lines of evidence also suggest that young forests provide fewer resources than mature forests (Fort and Otter 2004; van Oort et al. 2006; Otter et al. 2007; van Oort et al. 2007). The resulting effect of this food limitation on signalling could reduce the ability of females in poor-quality habitat to accurately assess males for alternative mating strategies (Ramsay et al. 2000; van Oort et al. 2006). Continued studies in supplementation will allow me to determine whether the effect of feeding differentially affects the ability of birds to engage in dawn-chorus signalling in different habitat types.

3 - VOCAL PERFORMANCE VARIES WITH HABITAT QUALITY IN  
BLACK-CAPPED CHICKADEES (*POECILE ATRICAPILLUS*)

**Abstract-** In vocal learners, such as songbirds, the ability to maintain an internal acoustic structure between songs during a chorus seems to be positively correlated with the singer's condition and may, therefore, represent a reliable signal. For instance, some internal ratios in the black-capped chickadee (*Poecile atricapillus*) *fee-bee* song seem to be more stable in the song of dominant males than in the song of subordinate males. Habitat quality is also known to affect the behaviour of this species. Birds settling in young forest have a lower song output and lower reproductive success than birds occupying mature forests, and it is suggested that those differences stem in differential food availability across habitat. Because evidence is accumulating that song performance can be altered by food limitation during the song learning period, I explored whether habitat quality affects song structure in the same manner that has been shown for social rank. The ability to maintain the internal structure of song among birds occupying young forests was consistently lower than birds occupying mature forests. My results demonstrate that the same difference in song structure exists between male differing in social rank and between males that settle in different habitat types. My results suggest that inter-individual differences in song structure originate from differences in food availability during the song learning period.

### **3.1. Introduction**

Motor performance in bird song has been intensively studied in the past few years, and has been recently partitioned by Byers et al. (2010) into two components: “vigour”, defined as the “the ability to perform energetically expensive acts repeatedly”; and “skill”, which is “the ability to perform difficult motor tasks well”. These definitions apply to the vocal performance in many bird species because they often represent a challenge in term of production, complexity and/or stereotypy (Podos et al., 2009). In the chorus of many songbird species, song output provides information about the signallers’ condition (Welling et al. 1997; Lucas et al. 1999; Poesel et al. 2004; Otter and Ratcliffe 2005; Murphy et al. 2008) and I might describe “vocal vigour” in this context as the birds’ ability to perform a chorus with a high song output or song rate. The internal song structure, however, might also provide information on the signaller’s condition in some passerine species (Lampe and Espmark 1994; Forstmeier et al. 2002; Pfaff et al. 2007). For example, the ability to maintain a consistent structure from one song to another correlates with other measures of male quality in chickadees (Christie et al. 2004; Hoeschele et al. 2010), and may reflect selection on neurological links in song learning and motor pathways (Podos et al. 2009; Byers et al. 2010). Females are known to attend to these structural elements when assessing male quality (Hoeschele et al. 2010), and thus, the ability to produce songs with a high level of consistency may be a measure of “vocal skill”.

Most studies that have addressed “vocal skill” in birds have focused on either song complexity or repertoire size (Podos et al. 2009). In some species, however, it may be the ability to maintain highly-stereotyped internal structure of the songs within and between bouts of singing that constitutes vocal skill. Such may be the case with black-capped

chickadees (*Poecile atricapillus*), whose relatively simple *fee bee* whistled song maintains very high stereotypy in internal frequency ratios and amplitudes (Mennill and Otter 2007). During the chorus, individual males can alter the frequency at which song is presented, yet they simultaneously maintain the relative internal frequency structure within and between notes (a process known as *pitch shifting*, which is equivalent to transposing the song up and down in pitch so as to maintain the same ‘tune’). Songs at each frequency are presented with eventual variety – a male will sing a number of songs at a particular pitch, shift to a new frequency and sing a number of songs at this pitch prior to shifting again.

The ability to maintain these internal frequency ratios correlates with the male’s social rank within social winter flocks, which is a measure of relative resource holding potential and lifetime reproductive success in chickadees (Ratcliffe et al. 2007). Dominant males are better at maintaining both the internal song structure and the relative amplitude between the *fee* and the *bee* note than subordinate males (Christie et al. 2004; Hoeschele et al. 2010). Thus, the ability to maintain frequency ratios between songs (vocal skill) in the black-capped chickadee also appears to offer some information on the signaller’s condition, similar to what shown previously for song output during the chorus (Otter et al. 1997; Grava et al. 2009 – chapter 2). It is suggested that the inter-individual differences observed in vocal skill in the black-capped chickadee can be explained through the “developmental stress hypothesis” (Nowicki et al. 2002) – subordinate males may sing with a lower consistency because they suffer from higher stress during song learning, which affects their neural development and consequently their song learning ability.

Song output and condition, however, are also affected by habitat in chickadees. Birds that settle in young forests in northern BC, Canada, experience lower reproductive success

and a general reduction in condition-dependent behaviour than birds that settle in neighboring mature forests (Otter et al. 2007b). In particular, birds in young forest had lower song output during the dawn chorus than birds in mature forest (van Oort et al. 2006). This habitat effect on the birds' behaviour were suggested to be related to food limitation in young forests during the non-breeding season (August to March; van Oort and Otter 2005) as well as during the breeding season from April to July (Otter et al. 2007a; van Oort et al. 2007; Grava et al. 2009). It is currently unknown, though, whether these habitat differences also affect the ability of males to maintain the consistency of frequency ratios across songs during extended bouts of singing. If poor-quality habitat reduces male condition, in particular during song learning, they may be less able to accurately learn their songs. This could result in a diminished ability to maintain this internal consistency of songs. To test this, I compared the songs produced by males of similar social ranks across habitats of differing ages, which have previously been shown to affect other metrics of individual condition (e.g. reproductive success – Otter et al. 2007b).

## **3.2. Methods**

### *3.2.1. Study species*

The black-capped chickadee is a common, resident songbird of northern North America, occupying mixed deciduous/coniferous forests. The birds over-winter in stable flocks composed of two to five mated pairs which forage and travel together. Flocks have a linear dominance hierarchy – males are dominant over females, and within sexes adults are typically dominant over juveniles (Smith 1991). The rank of each bird can be determined by observing dyadic interactions at winter feeding stations (Smith 1991; Otter et al. 1997). In

early spring, flocks begin to disband and pairs establish and defend individual breeding territories (Smith 1991). During this period, breeding males sing long bouts of continuous ‘*fee bee*’ songs at dawn (Otter et al. 1997; Foote et al. 2008). These dawn song-bouts often last over 45 min with average singing rates of 12-15 songs per minute, with some males achieving maximum rates of >20 songs/min during the peak of the chorus (personal data from 2008, 2009 and 2010).

### 3.2.2. Study sites

The study took place in the John Prince Research Forest, Fort St James, British Columbia, Canada (54°40’ N, 124°24’ W). Four plots of mature forest and four plots of young forest were chosen as study sites. Each plot consisted of approximately 1600m<sup>2</sup> (16ha) of forest of homogenous age separated by at least 500m and held between three and seven pairs of chickadees.

Details of the sites are provided in chapters 1 and 2. The four mature sites were characteristic of the mixed woodlands of the sub-boreal spruce sub-zone in Northern British Columbia. Further, all the sites were similar in age structure, with no commercial logging for more than 80 years. Dominant deciduous species in these sites are trembling aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), and black cottonwood (*Populus balsamifera* ssp. *trichocarpa*). Subalpine fir (*Abies lasiocarpa*), lodgepole pine (*Pinus contorta*) and hybrid spruce (*Picea glauca* x *P. engelmannii*) form the predominant conifer species in these mixed forests, but tend to be in lower relative abundance than deciduous species. All these sites were characterized by an average trunk diameter of 25cm and a canopy at approximately 25m.

The four young sites were forests that have undergone near-complete clearing in the past thirty years. All those sites had characteristic flora of a young, regenerating sub-boreal forest. Dominant deciduous tree species and conifer species were similar to those described above for the mature sites, but early successional species, such as lodgepole pine, dominated the coniferous component. These sites were characterized by an average trunk diameter of approximately 10cm and a canopy at approximately 8m.

These forest classes match those previously reported to affect reproductive success and song output levels in this species (see Otter et al. 2007), but are located approximately 200km from those previous sites. Further, my study sites are at approximately 100m higher elevation (850m above sea level) than study sites in Prince George, BC, and are subject to slightly longer winter conditions and greater snow coverage. Monitoring of breeding of birds in the two habitat classes at the John Prince Research Forest since 2006 suggest that the disparity in reproductive success between birds breeding in mature vs young forests is even greater than reported in Otter et al. (2007).

### *3.2.3. Winter banding and dominance assessment*

Birds were attracted to temporary feeders filled with sunflower seeds using playback of chickadee mobbing calls during February 2006, 2008 and 2009. The feeders were used for short periods (several days for banding, and only several hours for dominance assessments) throughout the fall and winter, to alleviate any potential effects of the feeding on the song learning. The feeders were used equally, and were typically placed simultaneously, in both habitats. I captured male and female chickadees on my study sites using mist nets and marked birds with numbered aluminum bands (Canadian Wildlife Service) and three colored

leg bands. Each bird was given a unique color combination allowing me to recognize individuals in the field. Sex was determined by body measurements (Desrochers 1990) and behaviour during the breeding season. Ages were determined by rectrix shape (Meigs et al. 1983), which several years of banding with known-aged birds in this area have confirmed to be consistent for aging birds in either habitat type. Birds were classified as either second-year (SY; i.e. entering their second calendar year and therefore approaching their first breeding season) or after-second-year (ASY; i.e. entering their third or later calendar year and second or later breeding season).

The composition of flocks and relative dominance ranks of birds were determined as outlined in chapter 2. Briefly, three behaviours were used to identify the flock hierarchy - the focal bird was considered dominant to its opponent if: (1) the focal bird supplanted or chased its opponent, (2) the focal bird gave a display that elicited a submissive posture by the opponent, or (3) the opponent waited for the bird to leave before approaching a feeder (Otter et al., 1997). A linear dominance matrix was determined for each flock; birds were classified as low-, mid-, or high-ranking, depending on their position within the flock. In flocks consisting of three pairs, the male submissive to the alpha male but dominant over the low-ranking male was considered mid-ranking. I observed too few dyadic interactions to determine the rank of six of the 26 birds; in these cases, I used age as a proxy of rank, as rank is strongly correlated with relative age (Smith 1991; Otter et al. 1999; Ratcliffe et al. 2007).

#### *3.2.4. Experimental design*

Some of the birds in my studied population have been associated with a supplemental feeding experiment taking place in my study sites during 2006, 2008 and 2009. This study

compared the song output of fed birds versus unfed birds in a dyadic comparison, while controlling for the factor influencing song output at dawn (see chapter 2). To determine whether short-term food supplementation at the time of singing affected the ability of individual males to maintain consistent frequency ratios in their songs, I compared the ability to maintain internal song structure of fed birds versus unfed birds following the pairing-protocol used in chapter 2. I found no effect of supplemental feeding on the ability of fed versus non-fed males to maintain the internal song structure (Repeated-measures ANOVA:  $F_{(1,18)} = 0.1, N = 19, p = 0.76$ ). I therefore included some of the fed birds in the present experiment. Out of the 22 birds that received short-term food supplementation, 15 entered the analysis of song structure across habitats – nine were recorded as birds in young forest, and six as birds in mature forest. Wherever possible, I paired fed birds together in cross-habitat comparisons, or used the fed birds from the young forest sites in comparisons. In this manner, any residual influence of short-term feeding would be controlled, or would tend to make findings of habitat affects more conservative.

I recorded the dawn choruses of 32 males during 2006 (eight birds recorded), 2008 (16 birds recorded) and 2009 (eight birds recorded). Four birds in young forest and one in mature forest were recorded in more than one year of the study, and so appeared in more than one dyad. However, in all these cases, their dyadic counterpart differed between the two years.

I paired one bird recorded in young forest with one recorded in mature forest. I paired the birds by rank (dominant:dominant dyads or subordinate:subordinate dyads), which also controls for age in chickadees, due to the close relationship between relative rank and relative age (adult/juvenile) of males chickadee (Otter et al. 1999). I also controlled for

seasonality by minimizing the time separating recording of two paired males (difference between two recordings in the same dyads was 4.3 days  $\pm$  2.7). In fact, when more than two birds could potentially be paired up, the date of recording was critical as a final choice – the two birds that were recorded the closest in time were chosen to create a dyad. Those criteria allowed me to reduce the possibilities to only one dyad that were chosen for the analysis. As it was important to ensure that males recorded were the selected males (i.e. dom/sub), several choruses where the identity of the singers could not be conclusively deduced were eliminated from the studies. I also eliminated the recordings that were not of sufficient recording quality to allow acoustical analysis. A total of 16 dyads were recorded (four of six dyads attempted in 2006; eight of 14 dyads attempted in 2008; four of eight dyads in 2009). Of the four dyads recorded in 2006, one was composed with subordinate males and three were of dominant males. Of the eight dyads recorded in 2008, two were composed of subordinate males and six of dominant. Of the four dyads recorded in 2009, two were composed of subordinate and two of dominant males.

Dyads were recorded between the 28<sup>th</sup> of April and the 12<sup>th</sup> of May in 2006, between the sixth and the 16<sup>th</sup> of May in 2008 and between the third and the 15<sup>th</sup> of May in 2009. Chorus singing for each male was recorded from the first song of the morning until the terminal song of the chorus (see Grava et al. (2009) for details on recording equipment and methods). The chorus was deemed to have ended when the male ceased all singing for greater than five consecutive minutes – as chorus song rates are typically 15 songs per minute in my study site, this represented a marked cessation of signalling.

### 3.2.5. *Sound analysis*

I randomly chose nine songs distributed across the chorus for each bird (Christie et al., 2004). To avoid the effect of pitch shift on the ability to maintain an internal song structure, I avoided measuring songs within five songs before or after a pitch shift event. I also selected songs distributed evenly throughout the recordings to have songs representative of the chickadee's frequency range. I used the sound analysis software Seewave (Sueur et al., 2008) to monitor the fundamental frequency of every songs. This technique allows for a more precise data extraction than is possible on raw spectrograms. I then extracted eight frequency variables from the fundamental frequency – the frequency at the start of the fee note (Ffee.start) and at the end of the fee note (Ffee.end); the frequency at the start, end, and maximum, of both syllables composing the bee note (respectively, Fbee1.start and Fbee2.start, Fbee1.end and Fbee2.end, Fbee1.max and Fbee2.max) (see Fig. 3.1 for more details).

I used those frequencies to calculate seven frequency ratios for each song (see Table 2.1). I calculated these ratios for each of the nine songs of every bird entering the analysis. The coefficient of variation for each of those variables was then calculated to represent the intra-individual variation within notes. This analysis created seven coefficients of variation for each bird corresponding to the seven ratios calculated in each of the nine songs.

### 3.2.4. *Statistical analysis*

The seven coefficients of variation used to quantify the ability to maintain an internal song structure were entered into a principal component analysis (statistica version 6.0, StatSoft, Inc.), to create a multivariate measure of vocal skill. I then used a general linear

model to compare principal components that contributed significantly to variation in choruses between young and mature forest.

For every bird entering the analysis, the average absolute pitch at the end of the fee note was calculated and compared across habitats using a repeated measures ANOVA.

### **3.3. Results**

#### *3.3.1. Principal components analysis*

A Kaiser-Meyer-Olkin Measures of Sampling Adequacy (KMO) applied to my data gave a value of 0.52, indicating that a Principal Component Analysis was sound. A Sphericity test applied on my data gave a  $p < 10^{-6}$ , therefore the strength of the relationship among variables is strong enough to perform a Principal Components Analysis.

Principal component one (PC1) accounted for more than 44% of the total variation among individuals, and principal component two (PC2) accounted for an additional 19%. Principal components three, four and five accounted for an additional 12%, 10% and 5% respectively. As I included seven variables in the analysis, however, only PC1 exceeded the value of explained variation expected by chance, using the broken-stick method of factor significance (Jackson 1993; Legendre and Legendre 1998), so only PC1 was analyzed in subsequent tests.

The coefficient of variation of all seven variables had a strong negative contribution to PC1 (Table 3.1). As a result, an increase in PC1 is interpreted as a decrease in variability in all of the seven frequency ratios measured, so an increase in the ability to maintain an internal song structure

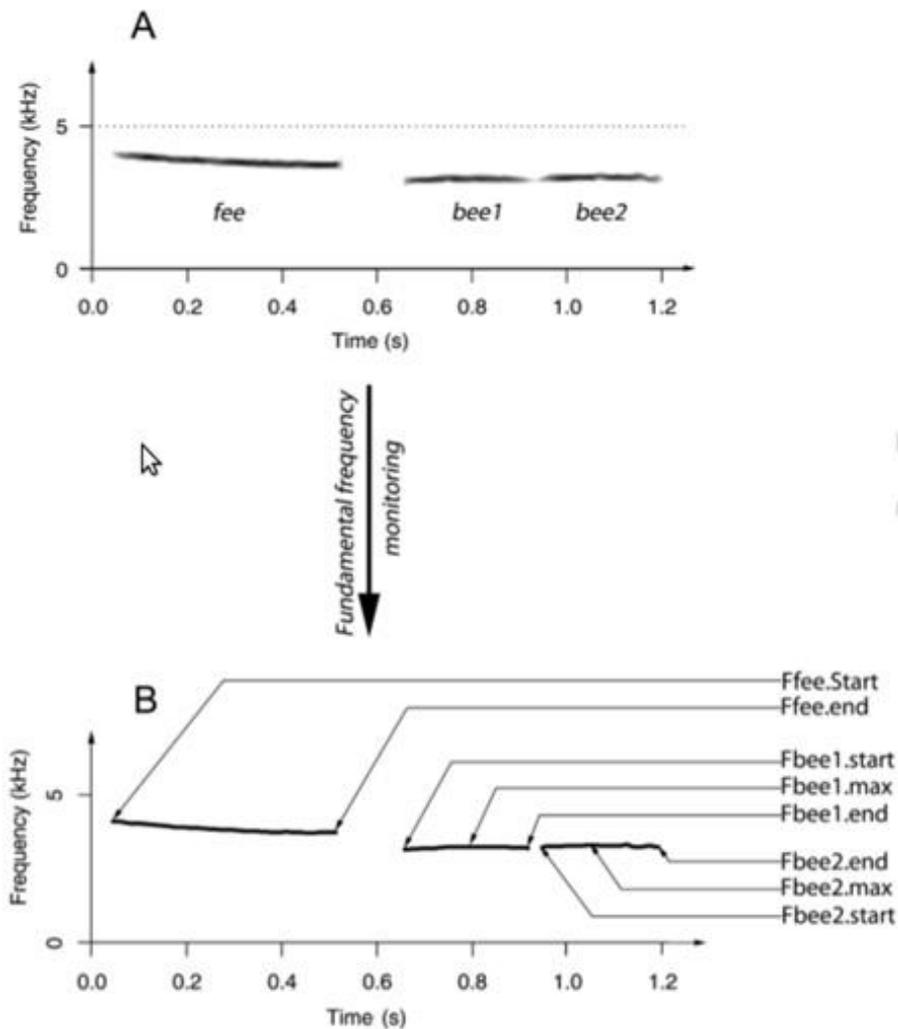


Figure 3.1 Extraction of eight frequency variables used to describe the song consistency in the *fee bee* song of the black-capped chickadee (*Poecile atricapillus*). Left: spectrographic representation. Fee: first note of the *fee bee* song. Bee1 and bee2: the two syllables composing the second note of the *fee bee* song. Right: fundamental frequency monitoring. Ffee.start, Ffee.end: frequency variables extracted from the fee note. Fbee1.start, Fbee1.max, Fbee1end: frequency variables extracted from bee1. Fbee2.start, Fbee2.max, Fbee2.end: frequency variables extracted from bee2.

Table 3.1 Contribution of the coefficient of variation of the seven frequency ratio variables to PC1 eigenvalues.

Variables	Description	PC1
Glissando ratio	Ffee.end / Ffee.start	-0.60
Internote ratio	Ffee.end / Fbee1.start	-0.73
Bee start 1 ratio	Fbee1.start / Fbee1.max	-0.60
Bee end 1 ratio	Fbee1.max / Fbee1.end	-0.61
Bee start 2 ratio	Fbee2.start / Fbee2.max	-0.61
Bee end 2 ratio	Fbee2.max / Fbee2.end	-0.79
Intersyllable ratio	Fbee1.max / Fbee2.max	-0.66

### 3.3.2. *Effect of habitat on the ability to maintain internal song structure*

I found no difference in the absolute pitch of songs between males in either habitat class (repeated measures ANOVA:  $F_{(1, 15)} = 0.04$ ,  $N = 16$ ,  $p = 0.84$ ). I did find, however, that the ability of males to maintain internal song structure was higher in mature than in young forest (repeated measures ANOVA:  $F_{(1, 15)} = 5.42$ ,  $N = 16$ ,  $p = 0.03$ , Fig 3.2). The effect size of my data ( $d=0.72$ ) results in a power of 60% with an alpha level of 0.05.

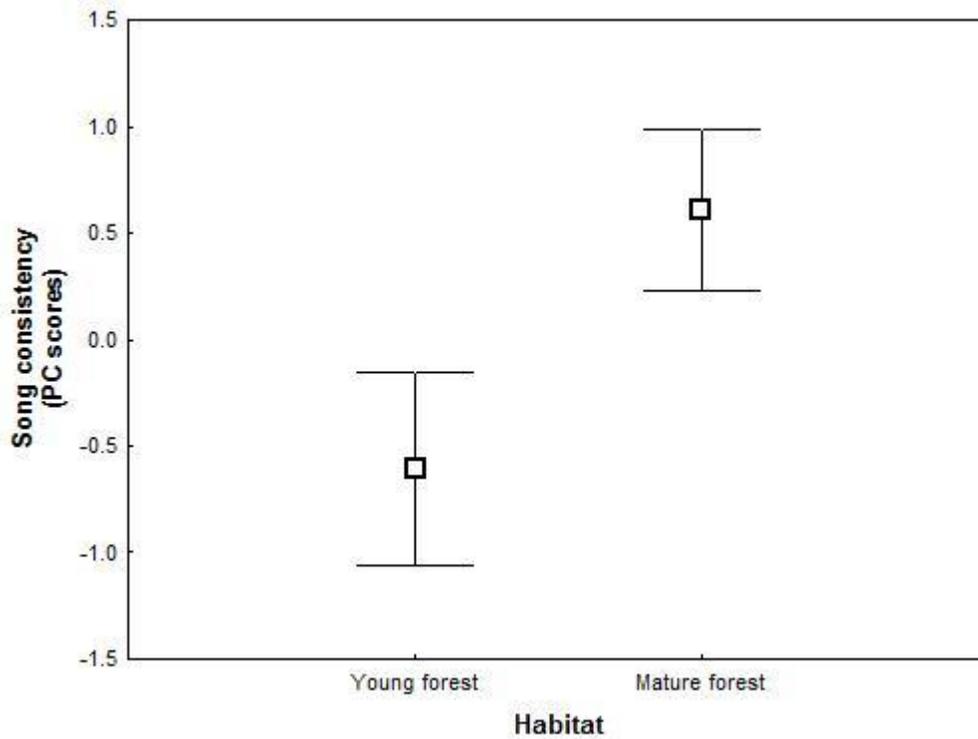


Figure 3.2 Comparison of the PC1 value (song consistency) from birds recorded in young versus mature forest (General Linear Model:  $F(1, 15) = 5.42$ ,  $N = 16$ ,  $p = 0.03$ ). Increasing values of PC1 indicate higher ability to maintain consistent frequency ratios within songs across the chorus. Plots show mean value (square), and standard error (lines) of the data.

### **3.4. Discussion**

I found that males in young forests produce dawn songs that have more variable internal frequency structure than do males occupying neighbouring mature forests. Because there is no difference in pitch across habitat, the differences in song structure cannot be explained by the fact that higher pitch songs may be more challenging to produce than lower pitch songs. Also, no differences in quality of recording can be detected across habitat. This suggests that the distance between the observer and the birds being recorded was not affected by the structure of the habitat (small vs. big trees) and therefore did not affect the results. As these two habitat types have been shown in past studies to differ in quality to chickadees, this suggests that the ability to maintain controlled frequency intervals in the song may be influenced by ecological parameters. The fact that the ability to maintain an internal song structure does not seem to be affected by short-term food supplementation suggests that the difference observed across habitat is entrenched into the individual and cannot be alleviated by an increase in energy available at the time of singing.

Previous work has shown that the ability to maintain frequency ratios across songs in chickadees is correlated with the singer's quality; dominant males having a higher ability to maintain an internal song structure than subordinate males (Christie et al. 2004). Further, birds in young forests matching those used in my study are associated with lower reproductive success (Fort and Otter 2004b), reduced territorial behaviour (Fort and Otter 2004a), lower song output (van Oort et al. 2006) and lower body condition of males (van Oort et al. 2007). I now add that males in these habitats have song structure that is associated with subordinate males in other studies, despite my design of matching males in similar rank categories across habitats.

Interestingly, difference in the ability to maintain an internal frequency ratios did not appear to be influenced by short-term food supplementation, which suggests that the effect of habitat may reflect longer-term differences in singer condition across habitats. One possible mechanism that could explain the differences in the ability of males to maintain internal song ratios is differences in food availability during the song-learning periods (Developmental Stress Hypothesis – Nowicki et al. 2002). Such an effect was demonstrated in swamp sparrows (*Melospiza georgiana*) that were nutritionally stressed during their development; such males produce less accurate copies of the model songs with which they were tutored (Nowicki et al. 2002).

In chickadees, early development of the song center nuclei in the brain likely occurs between the post-fledging period and their first breeding season. The fall and winter following dispersal of fledglings are correlated with settlement into their first winter flocks (Smith 1991), and subsongs are commonly heard during the period between early fall and mid winter (personal observation). Even though the production of subsongs starts before fledglings are fully independent (day 20; Baker et al. 2003), the crystallization does not seem to occur before the end of winter. Therefore, the first fall and winter may be a critical period in chickadee neural development, as the juvenile birds have to forage by themselves for the first time. The developmental stress hypothesis suggests that brain development required for song learning is linked with the amount of food birds can find during this period (Nowicki et al. 2002). The quantity or quality of food available may differ substantially depending on the habitat the birds settle in. Wintering chickadees in the young forest sites carry higher levels of furcular fat than do birds in mature forest (van Oort and Otter 2005); such high fat levels in other members of the Paridae family occur among birds that experience decreased or

inconsistent access to food (Gosler 1996; Gosler and Carruthers 1999). If these differences in fat loading reflect differences in food availability, this may coincide with the period of song learning for the juvenile birds. Birds in young forest may be less able to invest resources in overall brain development, and this may compromise development of song center nuclei, which might explain the differences in song production I found in the current study.

My results suggest habitat may indirectly affect male reproductive success through altering their song structure. Maintenance of internal structure of notes within and between songs is known to accurately reflect male social rank in chickadees, and is also known to be attended to by female within this species (Hoeschele et al. 2010). If the ability to maintain consistency in frequency ratios represents a vocal skill used by the female to assess male quality, males with high consistency should be preferred over males with low consistency. Because males occupying low-quality habitats produce songs with lower consistency in song structure, they may not be able to adequately compete in territorial song contests against males able to occupy high-quality habitats; as a result, they may have a more difficult time attracting mates or extra-pair partners or have to expend extra effort defending their territories from intruding males.

An alternate possibility to explain witnessed differences in vocal skill across habitats is that individual chickadees that settle in either habitat differed before they settled in both habitats. For example, dispersing juveniles in better condition may be more likely, or better able, to integrate themselves into flocks in mature forest than in young forests, creating a settlement bias that is later revealed by differences in ability of males to maintain song structure. van Oort and Otter (2005), however, detected no difference in the condition of feather growth rates (ptilochronology) of males settling in either habitat types used in the

current study. Studies are currently underway to look at other metrics of condition among juvenile birds settling in either habitat class, but even if differences among settling birds exist, differences in habitat quality between the two forest classes (Otter et al. 2007b) are likely to amplify this disparity.

My results imply a strong effect of habitat quality on individual behaviour and reproduction. Habitat quality does not only appear to affect a bird's prospects of successfully nesting during the breeding season, but may also play a role in how they signal their quality throughout their lifetime. A similar long-term effect of habitat on song learning has been described by Gorissen et al. (2005), who showed that male great tits learning their song in areas with elevated heavy-metal pollution display a less stereotyped song than birds in less polluted habitat. In this case, the stress comes from a high exposure to contaminants known to interfere with brain development. Human disturbances often alter habitats - such as the mosaics of abutting young and mature forest patches within northern Canada that result from commercial logging. My results suggest that these may not only impose short-term effects on immediate condition, but also impose potentially permanent effects on communication and development of sexually-selected signals. Conservation measures designed to compensate for decreased habitat quality, such as supplemental feeding during the breeding season, may not alleviate long-term effects of habitat quality on avian communication.

#### 4 - HABITAT QUALITY AFFECTS THE PERCEPTION OF DOMINANT MALES BASED ON THEIR RELATIVE SONG STRUCTURE

**Abstract-** The ability to maintain internal song structure in black-capped chickadees is known to be affected by both rank and habitat quality. Further, lab studies show that females discriminate between songs of dominant and subordinate males that vary in acoustic structure. This study was set up to investigate whether males also rely on acoustic structure to assess males quality during diurnal interactions and whether habitat quality affects the perception of male social rank, based on their relative song structure. I conducted a playback experiment to simulate an intrusion by dominant males recorded either in low-quality (young forest) and high-quality (mature forest) environment into the territory of dominant males breeding in both habitats. The playbacks reflected the difference in song structure already known to exist across habitat. My results show that stimuli from young forest elicited less territorial response from dominant males in either habitat. I also found that males in mature forest responded less to young-forest stimuli compared to mature-forest stimuli, despite the stimuli in both cases being recorded from dominant males within their own winter flocks. I demonstrate in this study that the ability to maintain internal song structure in the black-capped chickadee constitutes a signal that appears to be used by males to assess the level of threat of perceived intruders, and that this perception is affected by habitat quality in which the stimulus was recorded.

#### 4.1. Introduction

Among species with hierarchical social organization, dominance rank can have a major impact on resource access and fitness. This is evident in the higher reproductive success experienced by dominant males among many non-human animals (see Ellis 1995 for review). In birds, most studies on dominance effects on reproductive success and condition focus on the Paridae family (titmice and chickadees) in which many of the species have a stable and hierarchical social organization within flocks. Dominant males have a preferential access to resources (Ekman 1987 (willow tits, *Poecile montanus*); Desrochers 1989 (black-capped chickadee, *Poecile atricapillus*)), enhanced survival (Ekman and Askenmo 1984 (willow tits); Koivula 1994 (willow tits)) and enhanced reproductive success (Ellis 1995; Otter et al. 1998 (black-capped chickadee)). However, ranks relations are a relative measure; hierarchies will be established within flocks regardless of the absolute condition of individuals in the population.

The condition of individuals within a population, however, can be altered by habitat quality. For example, several studies have shown that birds in poor-quality habitat may have lower overall condition than their counterparts in good-quality habitat. Strong and Sherry (2000) used feather regrowth (ptilochronology) and biomass to show that ovenbirds (*Seiurus aurocapillus*) in habitat with low access to resources were in poorer conditions than those where resource access was higher. Grubb and Yosef (1994) also used ptilochronology to assess nutritional condition, and found that loggerhead shrikes (*Lanius ludovicianus*) were affected by habitat quality. Similarly, Carlson (1998) found that the condition of White-backed woodpeckers' (*Dendrocopos leucotos*) was a reflection of their territory quality. Wintering chickadees in poor-quality habitat carry higher levels of furcular fat than do birds

in high-quality habitat (van Oort and Otter 2005); such high fat levels in other members of the Paridae family occur among birds that experience decreased or inconsistent access to food (Gosler 1996; Gosler and Carruthers 1999).

These differences in condition across habitats can alter different condition-dependent behaviours. Within European parids, habitat quality affects the breeding performances of Great tits (*Parus major*) (Sanz et al. 2010) and blue tits (*Cyanistes caeruleus*) (Tremblay et al. 2003; Lambrecht et al. 2004; Tremblay et al. 2005). Among the new-world parids, black-capped chickadees in poor-quality habitat have a decreased territorial behaviour (Fort and Otter 2004a) and a lower reproductive success (Fort and Otter 2004b) than birds in good-quality habitat. Song output in the black-capped chickadee - which reflects resource access of the signaller (chapter 2) - is also lower in poor-quality habitat compared with high-quality habitat (van Oort et al. 2006).

Males of the same relative stature within their population may still differ in overall condition when comparing between populations. Population health cannot necessarily be measured simply by the presence/absence of dominant/subordinate relationships and stable hierarchies within a population. Should emigration of individuals from one population to another occur, the ability of residents to determine the status of intruders may rely on assessment of traits that vary on a continuous scale, as they may not have information on the relative rank status of the intruder. In most songbirds, evidence suggests that song features can be used by both males and females to assess a signaller's condition. For example, among black-capped chickadees (a common forest songbird in northern North America with a linear dominance hierarchy within flocks), dominant birds are known to have a higher song output than subordinate males (Otter et al. 1997). The study of the fine acoustic structure of the

songs in the black-capped chickadee showed that dominant birds have a higher ability to maintain consistent, within-songs internal frequency ratios (aka song consistency) than subordinate males (Christie et al. 2004). Both of these signals are therefore condition-dependent and may be used by males and females to assess a signaller's condition. However, habitat is also known to affect both of these features. Males in poor-quality habitat (young forest) have both lower song output and lower song consistency than males of the same rank in good quality habitat (mature forest). When controlling for both rank and habitat quality, only song output is affected by immediate access to food resources (chapter 2). Song consistency may reflect a longer-term conditional signal (chapter 3) and therefore might be a useful metric for males to assess the relative condition of unknown rivals. It has been shown that features inherent in the song of male black-capped chickadees are indicative of their condition (Hoeschele et al. 2010), and that those signals are attended by females during playbacks. However, whether inherent features in the song of the black-capped chickadee are used by males to assess a signaller's condition, and whether differences in such traits across habitat affect the relative perception of males within rank classes, are not known. In this study, I tested whether song consistency can be used by dominant males to assess an intruder's condition and whether the differences in song consistency within rank class across habitat affect the perceived status of the simulated intruder.

## **4.2. Methods**

### *4.2.1. Study sites*

The study took place in the John Prince Research Forest, Fort St James, British Columbia, Canada (54°40' N, 124°24' W). Four plots of mature forest and four plots of

young forest were chosen as study sites. Each plot consisted of approximately 1600m<sup>2</sup> (16ha) of forest of homogenous age separated by at least 500m and held between three and seven pairs of chickadees.

Details of the sites are provided in chapters 1 and 2. The four mature sites were characteristic of the mixed woodlands of the sub-boreal spruce sub-zone in Northern British Columbia. Further, all the sites were similar in age structure, with no commercial logging for more than 80 years. Dominant deciduous species in these sites are trembling aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), and black cottonwood (*Populus balsamifera* ssp *trichocarpa*). Subalpine fir (*Abies lasiocarpa*), lodgepole pine (*Pinus contorta*) and hybrid spruce (*Picea glauca* x *P. engelmannii*) form the predominant conifer species in these mixed forests, but tend to be in lower relative abundance than deciduous species. All these sites were characterized by an average trunk diameter of 25cm and a canopy at approximately 25m.

The four young sites were forests that have undergone near-complete clearing in the past thirty years. All those sites had characteristic flora of a young, regenerating sub-boreal forest. Dominant deciduous tree species and conifer species were similar to those described above for the mature sites, but early successional species, such as lodgepole pine, dominated the coniferous component. These sites were characterized by an average trunk diameter of approximately 10cm and a canopy at approximately 8m.

These forest classes match those previously reported to affect reproductive success and song output levels in this species (see Otter et al. 2007b), but are located approximately 200km from those previous sites.

#### 4.2.2. Winter banding and dominance assessment

Birds were attracted to temporary feeders filled with sunflower seeds using playback of chickadee mobbing calls during February 2010. I captured male and female chickadees on my study sites using mist nets and marked birds with numbered aluminum bands (Canadian Wildlife Service) and three colored leg bands. Each bird was given a unique color combination, allowing me to recognize individuals in the field. Sex was determined by body measurements (Desrochers 1990) and *behaviour* during the breeding season. Ages were determined by rectrix shape (Meigs et al. 1983). Birds were classified as either second-year (SY; i.e. entering their second calendar year and therefore approaching their first breeding season) or after-second-year (ASY; i.e. entering their third or later calendar year and second or later breeding season).

The composition of flocks and relative dominance ranks of birds were determined as outlined in chapter 2. Briefly, three *behaviours* were used to identify the flock hierarchy - the focal bird was considered dominant to its opponent if: (1) the focal bird supplanted or chased its opponent, (2) the focal bird gave a display that elicited a submissive posture by the opponent, or (3) the opponent waited for the bird to leave before approaching a feeder (Otter et al. 1997). A linear dominance matrix was determined for each flock; birds were classified as low-, mid-, or high-ranking, depending on their position within the flock. In flocks consisting of three pairs, the male submissive to the alpha male but dominant over the low-ranking male was considered mid-ranking.

#### 4.2.3. Playback design

I compared the reaction of focal dominant birds to both a *young-forest* stimulus (songs recorded from a dominant male from a territory in a young forest) and to a *mature-forest* stimulus (songs recorded from a dominant male from a territory in a mature forest). To produce my stimuli sets, I used songs recorded from dominant males recorded from 2000 to 2003 in young and mature forest around UNBC in Prince George, BC (54° 53' N, 122° 48' W), and dominant birds recorded in 2006, 2008 and 2009 in the young and mature forest in the JPRF (see study site). I did an initial screening of stimuli for recordings that contained both good recording-quality of songs within individual males as well as of comparable recording-quality between males. For each male, I then analysed song consistency as described in chapter 3. This entailed measuring seven frequency ratios from nine *fee bee* songs extracted from the dawn chorus of the stimulus males and calculating a coefficient of variation of those ratios for each male. I then conducted a Principal Components Analysis (PCA) on those coefficients of variation to obtain a single value (PC score) describing song consistency. I ranked stimuli in each habitat based on their relative song consistency (highest to lowest within an individual male). I then paired stimuli between habitats for these rankings; e.g. males in either habitat with most consistent songs against each other, males with 2<sup>nd</sup> most consistent songs against each other etc. Finally, I standardized playbacks for amplitude so that these matched both between dyadic pairs and across all playbacks. Using these criteria, ten dyadic stimuli sets (*young-forest* vs *mature-forest* stimuli) were constructed from recordings involving 20 stimuli males.

Playbacks were standardized for length, average song rate and rate of frequency shifting to reflect average singing patterns witnessed in my study site. Each playback was

composed of 12 different songs extracted from a male's chorus— six songs were extracted from a frequency in the lower range of the bird's chorus, and six were extracted from the higher frequency range of the bird's chorus. I then randomized whether the playback would start with the low or high frequency. Six songs at the first frequency were broadcast every five seconds, then the playback switched to the other frequency for the next six songs. This pattern was then repeated to create a 2 min playback with 12 songs per minute and three frequency shifts. The volume of all playbacks was normalized at 80db 1.5m from the speaker.

#### 4.2.4. *Playback experiment*

For each subject male, I randomly chose one of the dyadic stimuli sets for the playback trials, and then randomized whether I started with the *young-forest* or *mature-forest* stimuli. The focal birds were tested in the middle of their territories. I set up the playback speaker (Honeytone, Danelectric) on a tripod at about 1.2m from the ground. Before I started the playback I made sure the bird was within hearing distance from the speaker. When the birds could not be spotted, I used a mobbing call to attract the bird toward the playback speaker. When the birds either approached or vocally responded to the mobbing call, I ceased broadcasting, waited 30 seconds, and then began the playbacks. I used a 40m rope marked off at five meter intervals centred on the playback speaker to help estimate the distances of the bird relative to the speaker during the playback. Using a directional microphone connected to a digital recorder, I recorded all of the bird's vocalization during the trial. Following presentation of the first stimulus, I waited at least one hour and then tested the same bird with the other playback of the dyad. All birds were tested with both playbacks on

the same day. I extracted ten variables potentially describing the strength of the response of the tested males to the playbacks – seven variables on the spatial position and movement of the birds during the trial and three variables of the vocal behaviour of the tested birds (See table 4.1)

I tested a total of 18 subject males – all 18 were dominant males within their own flocks, but ten subject males were occupying territory in young forest and eight in mature forest. To select the dyadic stimuli to be used on a focal male, I tried to pick a dyad that had not previously been used, while also making sure that none of the birds used to create stimulus are known by the subject male. This resulted in two different dyadic stimuli that had to be used twice in both habitats, but otherwise the stimulus sets were used only once per habitat.

#### 4.2.5. Statistical analyses

The ten variables used to quantify the territorial response were entered into a Principal Component Analysis (statistica version 6.0, StatSoft, Inc.), to create a multivariate measure of response to playback. I then used a mixed repeated-measures ANOVA model to compare the principal components for both within-subject effects (response to *young-forest* vs *mature-forest* stimuli) and between-subject effects (whether response to stimuli differed depending on whether the subject males themselves were occupying either mature or young forest). I did mean comparison, using a post hoc Fisher's LSD test, where significant differences exist.

Table 4.1 Contribution of the seven variables of song consistency to PC eigenvalues and the total percent of variation explained by each of the two first factors. Variables having significant contribution to the PC ( $>0.33$ , Ho et al 2006) are bolded.

Variables	PC1	PC2
Latency to first response	<b>-0.60</b>	-0.13
Closest approach	<b>-0.77</b>	<b>0.54</b>
Time spent between 0 and 5m	<b>0.60</b>	<b>-0.63</b>
Time spent between 5 and 10m	<b>0.43</b>	0.12
Time spent between 10 and 15m	0.05	<b>-0.65</b>
Time spent between 15 and 20m	<b>0.62</b>	0.26
Time spent more 20m	<b>-0.84</b>	<b>0.48</b>
Number of <i>fee bee</i> songs	<b>0.60</b>	<b>0.62</b>
Number of frequency matching	<b>0.75</b>	<b>0.43</b>
Number of overlapping	<b>0.54</b>	<b>0.46</b>
% Total Variation explained by each Principal Component	38.10	22.34

### **4.3. Results**

#### *4.3.1. Principal Component Analysis*

Both a Kaiser-Meyer-Olkin Measures of Sampling Adequacy (KMO >0.05) and Bartlett's test of sphericity ( $P < 0.05$ ) applied to my factors indicated that the Principal Component Analysis was sound.

Principal component one (PC1) accounted for more than 38% of the total variation among individuals (Table 4.1), and principal component two (PC2) accounted for an additional 22%. As I included ten variables in the analysis, both PC1 and PC2 exceeded the value of explained variation expected by chance using the broken-stick method of factor significance (Jackson 1993; Legendre and Legendre 1998), so both PC1 and PC2 were interpreted and analyzed in subsequent tests.

As a rule of thumb, Ho et al. (2006) suggest that variables with individual contributions of 0.33 or higher should be considered as contributing significantly to the individual principal component measure. Nine of the ten variables exceeded this contribution to PC1, and seven to PC2 (Table 4.1). Out of the nine variables, three variables had a strong negative contribution to PC1 (Latency to first response, closest approach and time spent further than 20m from the speaker), and six had a strong positive contribution to PC1 (time spent less than 5m, between 5 and 10m, and between 15 and 20m from the playback, the total number of songs, the number of songs matching the playback in frequency and the number of songs overlapping the playback in time). Thus, higher values of PC1 indicate rapid responses, closer approaches and higher aggressive vocal responses to the playback, and thus would be interpreted as strong aggressive response.

Out of the seven variables contributing to PC2, two had a strong negative contribution (time spent less than 5m and between 10 and 15m from the speaker) and five had a strong positive contribution (closest approach, time spent further than 20m from the speaker, total number of songs given during the playback and total number of songs overlapping the playback in time as well as in frequency). As a result, an increase in PC2 indicates birds maintaining a larger distance from the playback speaker and increasing their songs rates. This would likely be interpreted as a more tentative or weaker response to playback than PC1, but still indicative of a territorial response to the stimuli.

#### *4.3.2. Effect of habitat on playback response*

Overall, mature-forest stimuli elicited a more aggressive response than young-forest stimuli among subject males (repeated measure ANOVA,  $F_{(1,16)}=7.01$ ,  $N=18$ ,  $p=0.02$ ), but there was also a between-subject effect of habitat on how subject males responded to stimuli (repeated measure ANOVA,  $F_{(1,16)}=4.55$ ,  $N=18$ ,  $p=0.049$ ). Subject males occupying mature forest had a significantly greater response to mature-forest stimuli than young-forest stimuli (Post hoc Fisher's LSD test,  $df=26.2$ ,  $N=8$ ,  $p=0.005$ ; Fig 4.1). Conversely, there was no significant difference in response to either stimuli class among subject males that occupied young forests (Post hoc Fisher's LSD test,  $df=26.2$ ,  $N=10$ ,  $p=0.70$ ; Fig 4.1). No differences were detected to the response of the young-forest stimuli across habitat (Post hoc Fisher's LSD test,  $df=26.2$ ,  $N=18$ ,  $p=0.25$ ; Fig 4.1) nor in the response to the mature-forest stimuli across habitat (Post hoc Fisher's LSD test,  $df=26.2$ ,  $N=18$ ,  $p=0.32$ ; Fig 4.1). The response of subject males in young forest to both stimuli seems to be intermediate compared to the response of subject males in mature forest to both stimuli (see Fig 4.1).

I found no significant difference in PC2 scores for the within-subject (comparison of response to two stimuli types-General linear model on PC2,  $F_{(1,16)}=0.7$ ,  $N=18$ ,  $p=0.42$ ), or between-subject (habitat) effects (General linear model on PC2,  $F_{(1,16)}=0.41$ ,  $N=18$ ,  $p=0.53$ ).

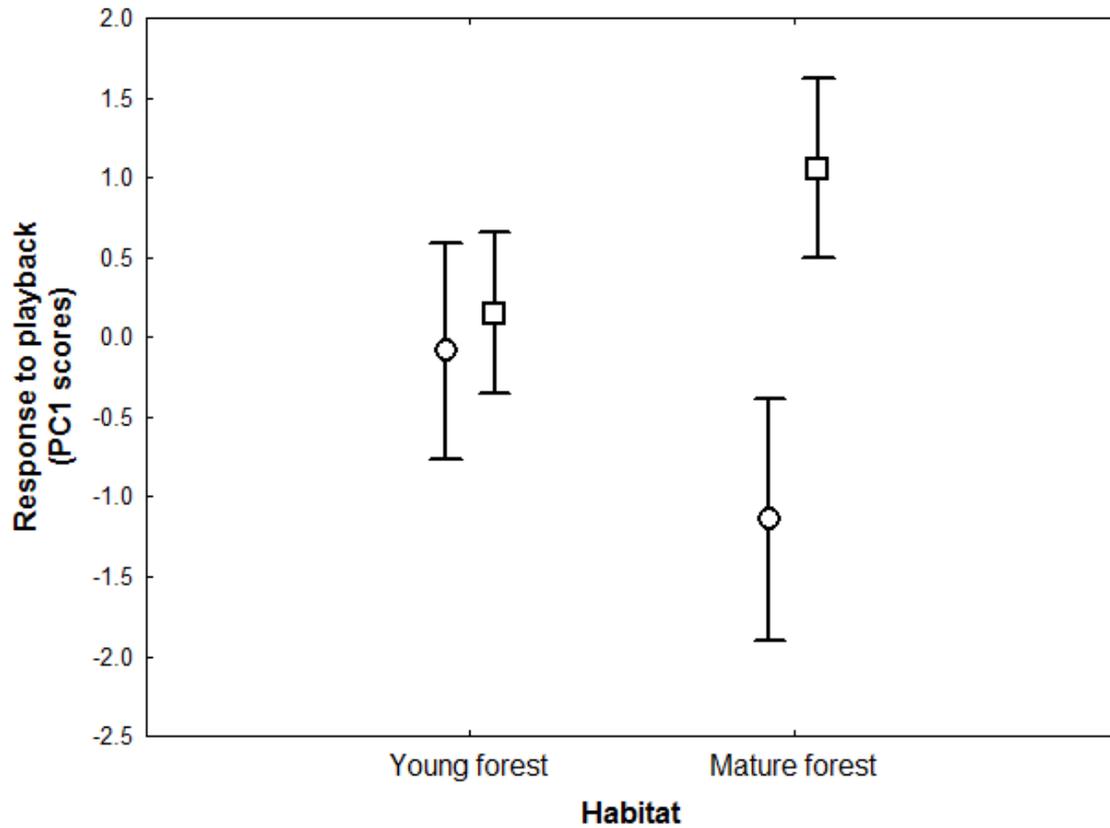


Figure 4.1 Dominant birds in mature forest respond more aggressively toward a dominant intruder from mature forest than from an intruder from a young forest (Post hoc Fisher's LSD test,  $df=26.2$ ,  $N=8$ ,  $p=0.005$ ). There was no significant difference in response to either stimuli class among focal males in young forests (Post hoc Fisher's LSD test,  $df=26.2$ ,  $N=10$ ,  $p=0.70$ ). Circles and squares stands for the response to young forest and mature forest stimuli, respectively. Plots show mean and bars show standard errors.

#### 4.4. DISCUSSION

I observed differences in the strength of response among subject males depending on song consistency of playback stimuli and whether the stimuli originated from either young or mature forest. Subject males respond more strongly towards stimuli with a high song consistency (young-forest stimuli) than toward stimuli with a low song consistency (mature-forest stimuli). This differential response occurred despite controlling for relative rank of the subjects and stimuli – all tested subject males were dominant males within flocks in their populations, and the stimuli were taken only from dominant males as well. Since song consistency is known to vary with male rank (Christie et al. 2004), it is very likely that song consistency is correlated with a signaller's underlying condition. Although song consistency has been shown to differ across rank classes (Christie et al. 2004) and females show differential response to songs of dominant and subordinate males that differ only in internal structure (Hoechele et al. 2010), my study provides the first evidence that this condition-dependent signal appears to be used by males to assess the quality of other males.

Song consistency in the black-capped chickadee is suggested to reflect the bird condition at the time of song learning (Christie et al. 2004; chapter 3). Birds who face higher developmental stress, because they are not able to forage effectively by themselves after fledging for example, may experience lower development of the brain nuclei involved in song learning, and therefore would not be able to accurately learn songs from tutors (Nowicki et al. 2002). This in turn may lead to either inability to accurately produce species-specific songs or high variability in renditions of songs. This has already been demonstrated in other species in the Paridae such as in the great tits (Doutrelant et al. 2000; Gorissen et al. 2005), and also in other species outside of the Paridae (swamp sparrow *Melospiza georgiana*

(Nowicki et al. 2002), zebra finch (Zann and Cash 2008) and European starling *Sturnus vulgaris* (Buchanan et al. 2003; Spencer et al. 2004)). Thus, in the black-capped chickadee, song consistency could provide reliable information about the ability of the signaller to effectively find and secure resources during the post-fledging period. However, this signal is entrenched into the signaller and cannot be altered subsequently, even if its body condition improves.

I further found that the response of subject males was context-specific: subject males in mature forest respond more strongly toward the *mature-forest* stimuli than toward the *young-forest* stimuli, despite the fact that these stimuli both arose from dominant males within their individual populations. By comparison, subject males that were tested in young forests showed no differential response to either stimulus class, and the magnitude of their overall response was intermediate to those of subject males tested in mature forests. The intensity of response of territorial males to intruders is expected to be correlated with the threat represented by the intruders. If the intruder signalling is in poorer condition, subject males may perceive the threat to its territory as minimal, and the predicted response would be lower. Subject males in mature forest may not have responded as strongly to *young-forest* stimuli because they perceived the intruder as being a lesser threat than the *mature-forest* stimuli. Subject males tested in young forest, however, responded with similar levels to both stimuli, suggesting that they consider both “intruders” as of being of a similar condition. The overall response level of these males fell between that given to *mature-forest* stimuli versus *young-forest* stimuli by subject males tested in mature forest. This does not mean that subject birds tested in young forest do not use song consistency to assess an intruder’s quality, but it means that the two stimuli presented to these subject males may be perceived

as of equivalent threat. This may stem from subject males, themselves, being in poorer condition due to occupying poorer habitat quality. Such males may be limited (either by energy or motivation) to mount as strong a response on a perceived threat as did subject males in mature forest to *mature-forest* stimuli, but simultaneously perceived young-forest stimuli to be more equivalent to themselves in condition, and thus requiring a higher level of response.

This interpretation of the results is predicated on the metrics used as constituting a high-aggression response. To describe the response of subject males to the playback, I recorded the movement as well as the vocal behaviour of the birds. It is well accepted that a closer approach toward an intruder is considered as an aggressive response. However, whether some vocal signals (such as frequency matching and song overlapping) can be considered as aggressive signalling is the subject of current debate (Searcy and Beecher 2009; Naguib and Mennill 2010; Searcy and Beecher 2011). Among the variables used in my study, closer approaches toward and sustained activity close to the playback speaker was correlated with both high frequency matching and song overlapping (all contributing strongly to PC1). These latter two signals (frequency matching and signal overlapping) were performed by the subject male towards a static playback, indicating that the male may be altering his own signalling behaviour to intentionally match and overlap that of the perceived intruder. Past criticism of these metrics has pointed to a lack of such matching and overlapping in natural encounters as evidence of these not constituting escalating signals in birds (Searcy and Beecher 2009). However, natural encounters allow signallers to dynamically adjust their signals if rivals attempt to match or overlap them, and thus these patterns in natural encounters may not be clearly evident. My study suggests that males do

attempt to match and overlap rivals, and that such behaviours are correlated with other traditional metrics of aggressive response, and may be used to accentuate aggressive behaviour toward an intruder.

The ability to produce songs with consistent internal structure may be linked to early neural and motor development. This in turn may be dependent upon ability to secure resources early in life, and thus song consistency in chickadees may constitute a condition-dependent trait that reflects information on early development. Even though males may achieve the status of high-ranking males in young forest, thanks to their relatively higher condition, their song consistency may continue to reflect limited resource availability. Chickadees who settle in mature forest might face less developmental stress, and achieve higher levels of condition, and song consistency. As my results show that males appear to differentiate between rivals on variation in song consistency, and this is context-dependent, it suggests that males are able to use song consistency as a measure of absolute condition of rivals regardless of the rival's social rank. While social rank remains a strong metric for comparing males within populations (Ratcliffe et al. 2007), the ability to use traits such as song consistency that vary on a more continuous scale may allow comparisons of the relative condition of birds across habitats that differ in resource quality. This would allow males to assess relative condition of unknown rivals where habitat patches intersect - such as the mosaics of abutting young and mature forest patches within northern Canada that result from commercial logging. As suggested by Godfrey (2003), condition-dependent signals such as song consistency may also provide researchers with metrics that vary on a continuous scale to compare the relative condition of birds breeding in different habitats, and thus be used as a mean of assessing habitat health.

## 5 - EFFECT OF HABITAT QUALITY ON EARLY CONDITION AND NEURAL DEVELOPMENT OF SONG CENTER IN JUVENILE BLACK-CAPPED CHICKADEES

**Abstract-** In songbirds, the ability to learn and render the species-specific song is influenced by the development of both the song nuclei in the brain and of the syrinx (bird's vocal apparatus) early in the bird's life. In black-capped chickadees, habitat quality is known to affect song structure, with birds in high-quality habitat (mature forest) having a greater ability to render songs with consistent internal frequency ratios than birds in low-quality habitat (young forest). Although this difference is suspected to stem from differences in development, the developmental status of juvenile birds in either habitat remains unexplored. In this study, I used ptilochronology and feather corticosterone (CORT) to compare the condition of juvenile chickadees in young and mature forest during two distinct periods of song learning – the *memorization period*, which occurs prior to settlement, and the *sensorimotor learning period*, which occurs post-settlement. A sample of juvenile males was captured and euthanized several weeks prior to their first breeding season to compare the development of song center nuclei and syrinx in both habitats. The natal - feather CORT levels were greater among birds that settled in mature than young forests – as these feathers were grown pre-settlement, they might reflect differences in condition during the memorization period. This difference in condition is reflected by differences in syrinx and song center nuclei development later during the sensorimotor learning period – birds in young forest have smaller syrinx and HVC, and bigger RA, than birds in mature forest.

Those differences could possibly be responsible for the difference in consistency in song structure observed across habitats. Birds settling in young forest seem to differ from birds in mature forest prior to settlement – as this difference is equated with lower condition in other species, and young forests are known to be lower in quality to chickadees, this finding suggests a despotic distribution of black-capped chickadees across habitats. This difference in condition across habitats, combined with potential compounding effect of differences in winter resources between habitats, could influence the difference in syrinx and neural development seen in juveniles males during the early spring, and influence the male's ability to learn and render their species-specific song.

## 5.1. Introduction

In songbirds, the learning, perception and production of song are controlled by a set of interconnected brain nuclei known as the ‘song system’ (Doupe and Kuhl 1999; Brainard and Doupe 2002; Bolhuis and Gahr 2006; Bolhuis et al. 2010). The song system is present in species that learn their song(s) by the imitation of species-specific communication sounds (Doupe and Kuhl 1999; Brainard and Doupe 2002). The imitative learning of song occurs during a sensitive period early in development (Catchpole and Slater 2008; Bolhuis et al. 2010) that is divided into two sub-periods – initially, there is a *memorization learning period*, which is then followed by the *sensorimotor learning period* (Hultsch and Todt 2004; Bolhuis and Gahr 2006). In this second sensorimotor phase, the bird repeats the song initially learned during the memorization phase, and tries to match this produced song to its internalized template (Bolhuis and Gahr 2006). In this phase, the brain nuclei of the song system develop intensively until the first breeding season, where the whole brain development is set and the song crystallized (Hultsch and Todt 2004). This phase is critical for normal song development; if there are perturbations to their development, birds do not properly produce their songs (Nowicki et al. 2002).

The developmental stress hypothesis proposes that the condition of the birds during the period of song learning may affect the birds’ neural development and consequently the song production quality (Nowicki et al. 2002). Nowicki et al. (2002) found brain nuclei of the song system, the robust nucleus of the arcopallium (RA) and HVC (Higher Vocal Center; Reiner et al. 2004; Jarvis et al. 2005), were both significantly smaller in nutritionally-stressed birds. Such condition-dependence may explain the acquisition of song types in birds with a vocal repertoire. For example, Doutrelant et al. (2000) showed that tarsus length correlated

positively with song repertoire in blue tits (*Cyanistes caeruleus*) and Nowicki et al. (2000) demonstrated that a significant relationship existed between feather growth and song repertoire in the great reed warbler (*Acrocephalus arundinaceus*). Food availability is an important factor dictating a bird's condition and birds suffering from low food availability at the time of song learning have lower song learning abilities, whether this happens when the birds are nestlings (swamp sparrow *Melospiza georgiana*, Nowicki et al. 2002), during the early post natal period (zebra finch, Zann and Cash 2008) or when the birds are juveniles (european starlings *Sturnus vulgaris*, Buchanan et al. 2003; Spencer et al. 2004).

One environmental factor that may limit food availability, and thus impact the condition of the birds, is habitat quality. Strong and Sherry (2000) used rates of feather regrowth (ptilochronology) and biomass to show that ovenbirds (*Seiurus aurocapillus*) in habitat with low access to resources were in poorer condition than those where resource access was higher. Grubb and Yosef (1994) also used ptilochronology to assess nutritional condition, and found that loggerhead shrikes (*Lanius ludovicianus*) were affected by habitat quality. Similarly, Carlson (1998) found that the condition of white-backed woodpeckers' (*Dendrocopos leucotos*) was a reflection of their territory quality. In the Paridae family (chickadees and titmice), wintering black-capped chickadees (*Poecile atricapillus*) in poor-quality habitat carry higher levels of furcular fat than do birds in high-quality habitat (van Oort and Otter 2005); such high fat levels in other members of the Paridae occur among birds that experience decreased or inconsistent access to food (Gosler 1996; Gosler and Carruthers 1999).

Habitat quality is also known to impact condition-dependent behaviours in chickadees. Birds in young forest have a lower reproductive success (Fort and Otter 2004a),

lower territorial behaviour (Fort and Otter 2004b) and lower song output (van Oort et al. 2006). These condition-dependent behaviours appear to reflect low food access in poor-quality habitat, but have been measured primarily during the breeding season. In support of this hypothesis, Otter et al. (2007a) found subordinate female chickadees in young forest had a higher food-solicitation calling rates than subordinate females in mature forests. Because these calling rates were correlated with immediate hunger levels (Otter et al. 2007a), the results suggest that birds in young forest tend to have reduced access to food relative to birds in mature forest. In a comparison of somatic condition in chickadees during the breeding season, van Oort et al. (2007) found that there was greater disparity in provisioning rate among dominant and subordinate males among birds occupying poor-quality vs. good-quality habitat, suggesting that resources may be limited and males in low condition may reduce parental behaviour to compensate. Further, reduced song output at dawn that is seen across habitat (van Oort et al. 2006) is alleviated by supplemental feeding in the early spring (Grava et al. 2009; Chapter 2), suggesting a link between song output and the condition of the birds during the breeding season.

Although song output is increased with supplementation of resources, other condition-dependent signals in the chorus are not affected by supplemental feeding in the early spring, even though this likely increases the immediate condition of the birds. This is the case with the fidelity with which birds render their songs: subordinate birds are less able than dominant birds to maintain internal frequency parameters between successive renditions of their songs (Christie et al. 2004), and birds in high-quality habitat also show a higher ability to maintain consistent note structure between songs than do birds in poor-quality habitat (Grava et al. 2012; Chapter 2). I have also shown that males assess rivals on this

ability to accurately maintain internal note structure; males show lower response to perceived intruders with low consistency in song structure than they do to playbacks that maintain higher consistency in song structure (Grava et al. in prep; Chapter 3). These combined results suggest that the ability to maintain consistent song structure throughout the chorus is a condition-dependent signal in the black-capped chickadee. However, food supplementation at the time of singing has no effect on this ability, suggesting that it does not reflect the short-term condition of the birds at the time of singing (Grava et al. 2012; chapter 2). Rather, internal song structure consistency may reflect the condition of the birds at the time of song learning, but no study has tried to estimate the early condition of the birds and its implication on song structure in the black-capped chickadee. Further, it is unknown whether habitat affects the birds during the sensorimotor learning period (post dispersal period) or whether the birds that settle in young and mature forest already differ prior to settlement.

To address these questions, I followed a cohort of chickadees settling in either young forests (poor-quality habitat) or mature forests (high-quality habitat) during their first winter. First in the fall and then subsequently during mid-winter and again in early spring, birds were captured and feathers removed for analysis of growth rates and levels of corticosterone (CORT) because both can reflect nutritional status and environmental variation, including habitat (Suorsa et al. 2003; Grubb 2006; Fairhurst et al. 2012; for reviews see Breuner et al. 2008, Bonier et al. 2009a). A subset of males caught in early spring was then euthanized to assess neural and muscular development of the song nuclei and syrinx. I used feather growth rates (ptilochronology) and feather CORT levels as proxies of conditional state during two potentially important periods of song acquisition and development within the first year of

male chickadees: the natal period, when it is thought that the birds might be memorizing song, and the post-dispersal/first winter period, when the neural song system is developing.

This study investigated whether a difference in condition between juvenile black-capped chickadees exist across habitats during these two song learning periods and whether this difference affects the development of the organs involved in song learning and song production. This work contributes importantly to my understanding of the interplay of environment, physiology and signalling behaviour in passerine species.

## **5.2. Methods**

### *5.2.1. Study species*

Black-capped chickadees are resident songbirds of northern North America. They over-winter in stable flocks composed of two to five mated pairs during the non-breeding season, typically from August to early May in my study site. The breeding season typically starts in May when the flocks break up and the mated pairs defend their own territory. The pairs raise on average five to seven chicks, which fledge at ca. 18 days posthatch (late June to early July). After fledging, the chicks are fed by their parents in the territory surrounding the nest for an additional two weeks. By mid July to late August, the juveniles disperse randomly and can travel as far as 10km before settling into a flock (Smith 1991; Foote et al. 2010). Once settled, these birds will typically remain and attempt to breed in this settled habitat for the remainder of their lives. The song of the black-capped chickadee is a simple two-note *fee bee* song, which is commonly heard during the breeding season. As with other songbirds, black-capped chickadees undergo a complex learning process in order to produce

their species-specific song (Baker et al. 2003; Hultsch and Todt 2004; Bolhuis and Gahr 2006).

### *5.2.2. Song learning in black-capped chickadees*

In wild populations of black-capped chickadees, juvenile birds starts subsong at approximately day 20 postfledging (Baker et al. 2003; personal observation), which corresponds to the date they become independent from their parents. This likely represents the starting point of the sensorimotor learning in this species, and that memorization therefore occurs prior to this date when the birds are still fed by their parents (i.e. from hatching to independence). During this first phase, the chickadees memorize the template of their species-specific songs that they will try to reproduce in the next phase. Once the chickadees disperse, the males enter the sensorimotor learning period. During this phase, the young males practice singing songs they have previously memorized, attempting to match their subsong attempts with their learned template songs (Shackleton and Ratcliffe 1993). This sensorimotor phase lasts until the birds enter their first breeding season (Shackleton and Ratcliffe 1993; Baker et al. 2003; Hultsch and Todt 2004).

During the entire sensitive period, the song system develops in parallel to the acquisition of adult song (for review see Bolhuis et al. 2010). As a songbird, the template for the tutor's song in the black-capped chickadee is thought to be encoded in the neural substrate of NCM (caudomedial nidopallium) and CMM (caudomedial mesopallium; Bolhuis et al. 2006). During development, the anterior forebrain pathway (AFP) is involved in the acquisition of song as well as auditory-vocal feedback processing (for review see Mooney 2009). The AFP connects the brain nuclei for learning of song including Area X (Area X of

the striatum), HVC, LMAN (lateral magnocellular nucleus of the anterior nidopallium), and RA (Bolhuis et al. 2010). The posterior pathway or song motor pathway (SMP) controls the production of song and includes HVC and RA, which innervate nXIIts (tracheosyringeal portion of the hypoglossal nucleus) that control the syrinx (Mooney, 2009). Normal development of the song system is crucial during the sensitive period for normal development of song (Nowicki et al. 2002; Christie et al. 2004; Grava et al. 2012; Chapter 3).

### 5.2.3. Study site

The study took place in Prince George, British Columbia, Canada (53°55'01'N 122°44'58'W) on three plots of mature forest, at least 850m apart, and four plots of young forest at least 3.7km apart. Plots of both types were intermixed throughout the study region (which covered 16.35km from N to S and 13.9km from E to W, centered on the city), and each study plot consisted of forest of homogenous age.

The three mature sites were characteristic of the mixed woodlands of the sub-boreal spruce sub-zone in Northern British Columbia. Further, all sites were similar in age structure, with no commercial logging for more than 80 years. Dominant deciduous species in these sites are trembling aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), and black cottonwood (*Populus balsamifera* ssp. *trichocarpa*). Subalpine fir (*Abies lasiocarpa*), lodgepole pine (*Pinus contorta*) and hybrid spruce (*Picea glauca* x *P. engelmannii*) form the predominant conifer species in these mixed forests, but tend to be in lower relative abundance than deciduous species. All these sites were characterized by an average trunk diameter of 25cm and a canopy at approximately 25m.

The four young sites were forests that have undergone near-complete clearing in the past thirty years. All these sites had characteristic flora of a young, regenerating sub-boreal forest. Dominant deciduous tree species and conifer species were similar to those described above for the mature sites, but early successional species, such as lodgepole pine, dominated the coniferous component. These sites were characterized by an average trunks diameter of approximately 10cm and a canopy at approximately 8m.

#### 5.2.4. Feather collection

I caught birds during three distinct catching sessions during the period of Aug 2009 to April 2010. The first catching session lasted from August 28th to September 6<sup>th</sup> 2009 (Fall), the second catching session was conducted between November 11<sup>th</sup> 2009 and January 21<sup>st</sup> 2010 (Winter), the third between March 23<sup>rd</sup> and April 12<sup>th</sup> 2010 (Spring). I used a variety of techniques to attract the birds into mistnets, including: playback of *chick-a-dee* calls recorded in the context of either food-finding or mobbing; presentation of feeders filled with sunflower seeds; presentation of stuffed chickadee (intruder) or saw-whet owl (predator). In most circumstances, combinations of these stimuli were used to catch birds.

Only birds that hatched during the preceding spring (identified by shape and wear of outer rectrices (Meigs et al. 1983)) were sampled for this study. I plucked the two outer tail feathers at the beginning of the first fall (August) of 120 birds settling in both young and mature forests; these “natal” feathers reflect those grown in the nest and during early post-fledging in juvenile chickadees. This plucking induced feather regrowth during the fall song learning period. In the winter period, I re-caught 30 of the birds from the fall sample, as well as an additional 30 new birds. For recaptured birds, I plucked the induced feathers to

measure growth rates during the first fall period. For new birds, I plucked feathers so as to induce regrowth in the winter across habitats; plucked feathers from these birds also added to my sample of natal-grown feathers. Finally, in spring sampling, I returned to the same catch locations and recaptured as many of the previously banded birds as possible. This constituted six birds captured in all three periods, 14 birds captured only in fall and winter periods, six birds caught in winter and spring and six caught in fall and spring (but not in winter). In addition, a sample of 16 birds was sampled for the first time in the spring collection period, but I did not attempt to recapture these birds a second time. Out of the 32 birds for which I managed to induce feather re-growth, 26 re-grew their feathers in fall and six re-grew their feathers in winter. For each bird, I measured growth bands on one tail feather, using ptilochronology, and the other feather was sent to collaborators at the University of Saskatchewan for CORT analysis. All feathers were mature (i.e., fully-grown) when collected.

Finally, out of the 48 birds for which I collected feathers grown in the natal territory, 26 had settled in their post-dispersal habitat as early as September, and 22 were definitely settled by the onset of winter. Because CORT is known to affect the juvenile birds dispersal and settlement behaviour in at least one other species of old-world Parids (willow tits *Poecile montanus*, Silverin 1997), I addressed time of settlement in the subsequent analysis.

#### 5.2.5. Ptilochronology

Ptilochronology is the study of the bird daily growth through the study of the tail feathers (Grubb 1989). The tail feathers record evidence of their daily growth in visible “bands” that can be measured, similar to growth rings on tree. The size of these daily growth

bands correlates with food availability, and thus ptilochronology can be used as an index of relative condition of individuals under different ecological conditions (Grubb 2006).

The methods for measuring daily growth bars (DGB) on the feathers were taken from Grubb (1989). Briefly, the feathers were placed on a card and pinned at their extremities. I measured the total length of the feathers and calculated and marked the location of the 2/3rds position along the feather's length from the proximal end. I then pushed a pin perpendicularly through the feather at the distal edge of each growth bars. I then determined the average growth bar width by calculating the mean length of ten growth bars centered on the 2/3<sup>rd</sup> point with four additional growth bars proximal to and five distal to the central growth bar. This measure gave me the average daily growth bar of the natal feathers that grew during the first few weeks after the birds fledge, and of the induced feathers of the birds I re-caught at least once.

#### 5.2.6. *CORT analysis*

Feather CORT assays followed Bortolotti et al. (2008). Briefly, I extracted CORT from feathers using a methanol-based technique. The length of the feather was measured, the calamus was removed and discarded, and then the sample was cut into pieces  $<5 \text{ mm}^2$  with scissors. I then added 10 mL of methanol (HPLC grade; Fisher Scientific, Fairlawn, New Jersey, USA) and placed the samples in a sonicating water bath at room temperature for 30 min, followed by incubation at 50° C overnight in a shaking water bath. To separate the methanol from the feather material, I used a vacuum filtration system that included a plug of synthetic polyester fibre in a filtration funnel. The methanol extract was placed in a 50° C water bath and subsequently evaporated in a fume hood. Extract residues were reconstituted

in a small volume of phosphate buffer system (PBS; 0.05M, pH 7.6) and frozen at  $-20^{\circ}\text{C}$  until analyzed by radioimmunoassay (RIA). I assessed the recovery of the methanol extraction by including feather samples spiked with a small amount (approximately 5000 CPM) of  $^3\text{H}$ -corticosterone in the extraction. Samples were extracted in two batches, and 92% and 96% of the radioactivity was recoverable in the reconstituted samples, respectively. Final values were adjusted by recoveries. For more information about validation, see Supplementary Appendix S1 available in online version of Bortolotti et al. (2008).

Feather CORT levels were determined by RIA as in previous studies (Wayland et al. 2002; Bortolotti et al. 2008; Fairhurst et al. 2011; Fairhurst et al. 2012). Measurements were performed on reconstituted methanol extracts and were duplicated. Samples were measured in two assays with an intra-assay coefficient of variation of 8.2%, an inter-assay coefficient of variation of 9.6%, and mean ( $\pm$  SD) limit of detection (ED80) of  $10.63 \pm 0.26$  pg CORT/assay tube. Data values are expressed as pg CORT per mm of feather, which gives a valid estimate of CORT per unit time of feather growth (for validation see Bortolotti et al. 2008; Bortolotti et al. 2009a; Bortolotti 2010). CORT assays were performed at the University of Saskatchewan, Canada.

#### *5.2.7. Brain and syrinx analysis*

##### *Histology*

During the spring sampling, and after the flocks broke up (after song crystallized), 20 second-year males hatched the previous summer (the 15 additional birds from the previous analysis, plus five birds which were part of the re-grown feather analysis) were euthanized (Animal Care approval - A2009 0119 013) for brain and syrinx histology. Ten males from

either of the two habitat classes were sampled. Sex was confirmed via inspection of gonads following euthanization. Birds were euthanized with 0.03 ml of 100 mg/ml ketamine and 20 mg/ml xylazine given intramuscularly (1:1) and then transcardially perfused with heparinized 0.1M phosphate buffered saline (PBS) and then 4% paraformaldehyde. Following perfusion, the brain, gonads and syrinx were removed and placed in 4% paraformaldehyde and transported to the University of Alberta for further analysis. After one week, the brain, gonads and syrinx were weighed three times each and the average value was recorded. The average syrinx weight was then corrected for body size for statistical analysis. The length and width of the gonads was also measured and all measurements were carried out blind to the habitat condition of the specimens. The brain was then placed in 30% sucrose in PBS for approximately 24 h until saturated. The brains were then snap-frozen in isopentane at -80°C and stored at -80°C. Brains were then warmed to -20°C and the entire brain was sectioned coronally at 40µm using a cryostat and mounted on gelatin-embedded slides in four series. The first series of mounted sections, every fourth section, were then stained with Thionin (0.25%) and coverslipped.

### *Brain morphometry*

To calculate total brain volume, I used methods similar to Phillmore et al. (2006) and Smulders et al. (2006). First, each slide was scanned into ImageJ, using an Hp 4470c ScanJet. The total area of the brain as well as the total areas of the brain nuclei for HVC and RA in every section in which they appeared were traced using a WACOM tablet. The total volume of the brain, HVC and RA were calculated by multiplying the given surface area by

the distance between measurements (Smulders et al. 2006). All imaging and tracing was done by a research assistant unaware of the habitat condition of the specimens.

#### *5.2.8. Statistical analysis*

##### *Condition during memorization phase*

To study the condition of the birds during the memorization period, I analyzed the width of daily growth bars (DGB) and CORT levels in the natal feathers collected.

I used general linear models (STATISTICA version 6.0, StatSoft, Inc) to compare the levels of CORT in the natal feathers (dependent variables) between habitats in which the birds were sampled (categorical variables), controlling for period of known settlement (fall or winter - categorical variables) as well as for the DGB in the same natal feathers (continuous variables).

To detect an effect of habitat on DGB in the natal feathers, I used general linear models to compare the natal DGB (dependent variables) between habitats in which the birds were sampled (categorical variables), controlling for period of settlement (categorical variables) as well as for the CORT in the same natal feathers (continuous variables).

##### *Condition during sensorimotor learning phase*

To study the condition of the birds during the post-settlement period, I performed analysis on DGB and CORT in the induced feathers.

I used general linear models to compare the levels of CORT in the induced feathers (dependent variable) between habitats in which the birds were sampled (categorical variable), controlling for the period in which the feather grew (winter or fall) (categorical variables) as

well as for the DGB in the same feathers and for the DGB and CORT in the natal feathers previously sampled from the same birds entering this analysis (continuous variables).

I used general linear models to compare the levels of DGB in the induced feathers (dependent variable) between habitats in which the birds were sampled (categorical variable), controlling for the period in which the feather grew (winter or fall) (categorical variable) as well as for the CORT in the same feathers and for the DGB and CORT in the natal feathers previously sampled from the same birds entering this analysis.

### *Brain and syrinx measurement after song crystallization*

Because my sample size of ten birds euthanized per habitat was limited by ethical considerations, my analyses lacked sufficient power to detect statistically meaningful patterns. Hence, I used Cohen's  $d$  to compare the effect size of differences in brain and syrinx measurements between birds that had settled in either young or mature forests. I used the guidelines of Cohen (1988) who suggests that a  $d=0.1$  represents a small effect,  $d=0.3$  an intermediate effect and  $d=0.5$  a large effect. When the power of the analysis was above 60% I performed an ANOVA to compare the measurement across habitats.

## **5.3. Results**

### *5.3.1. Condition during memorization phase*

When controlling for time of establishment as well as for the DGB in the same natal feathers, I found that the CORT level in natal feathers were higher in birds that settled in mature forests compared to birds settling in young forests (ANOVA,  $F_{(1,43)}=4.48$ ,  $N=48$ ,  $p=0.03$ ,  $d=0.35$ ; Fig 5.1).

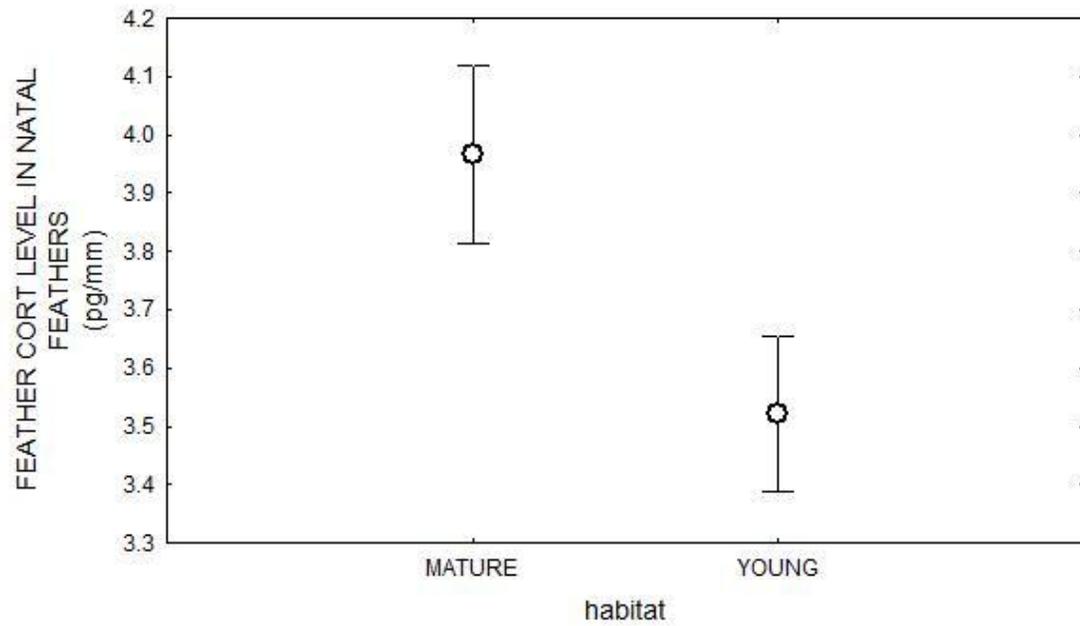


Figure 5.1 CORT level in natal feathers are higher in birds that settled in mature forests compared to birds settling in young forests (ANOVA,  $F_{(1,43)}=4.48$ ,  $N=48$ ,  $p=0.03$ ,  $d=0.35$ ). Dots represent mean and whiskers error bars.

However, when controlling for CORT in natal feathers, there was no significant difference in average growth bar widths (DGB) in the original feathers of birds that settled in either mature or young forests (ANOVA,  $F_{(1,45)}=0.35$ ,  $N=48$ ,  $p=0.55$ ,  $d=0.004$ ; Fig 5.2).

### 5.3.2. Condition during sensorimotor learning period

I found no differences in CORT in induced feathers among birds settling in young versus mature forest (ANOVA,  $F_{(1,25)}=0.34$ ,  $N=32$ ,  $p=0.57$ ,  $d=0.05$ ) and no difference in DGB across habitats (ANOVA,  $F_{(1,25)}=0.43$ ,  $N=32$ ,  $p=0.51$ ,  $d=0.05$ ).

### 5.3.3. Brain and syrinx measurement after song crystallization

Syrinx weight in the first spring correlated significantly with CORT levels of the natal feathers (regression analysis,  $R^2=0.2$ ,  $F_{(1,18)}=4.9$ ,  $N=20$ ,  $p=0.03$ ; Fig 5.3) but not with DGB in these feathers (regression analysis,  $R^2=0.003$ ,  $F_{(1,18)}=0.7$ ,  $N=20$ ,  $p=0.79$ ). Neither HVC nor RA volumes were correlated with CORT levels (regression analysis,  $R^2=0.08$ ,  $F_{(1,18)}=1.5$ ,  $N=20$ ,  $p=0.23$ ; regression analysis,  $R^2=0.04$ ,  $F_{(1,18)}=0.75$ ,  $p=0.38$ ; respectively) or DGB in natal feathers (regression analysis,  $R^2=0.003$ ,  $F_{(1,18)}=0.7$ ,  $N=20$ ,  $p=0.79$ ; regression analysis,  $R^2<0.0001$ ,  $F_{(1,18)}<0.001$ ,  $p=0.98$ ; respectively). As several of these males were only caught in the spring sampling period, there was insufficient data to compare syrinx or brain measures to CORT or DGB of re-grown feathers.

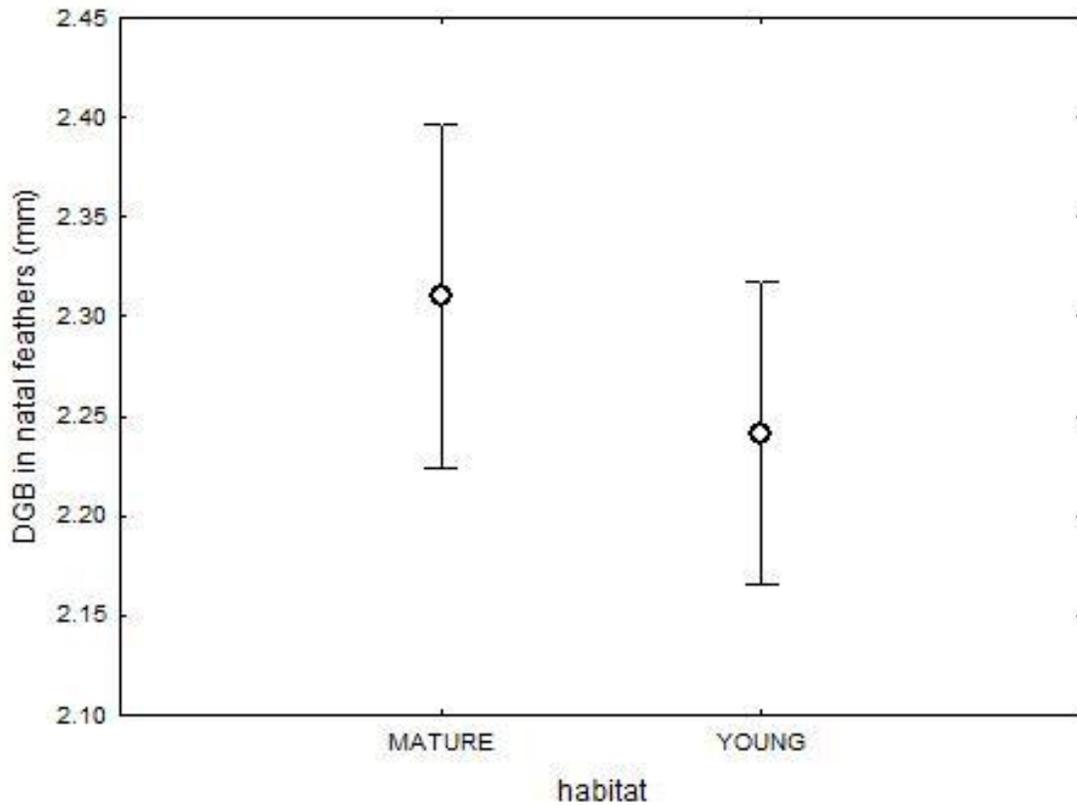


Figure 5.2 There is no significant difference in average growth bar widths (DGB) in the natal feathers of birds that settled in either mature or young forests (ANOVA,  $F_{(1,45)}=0.35$ ,  $N=48$ ,  $p=0.55$ ,  $d=0.004$ ). Dots represent mean and whiskers error bars.

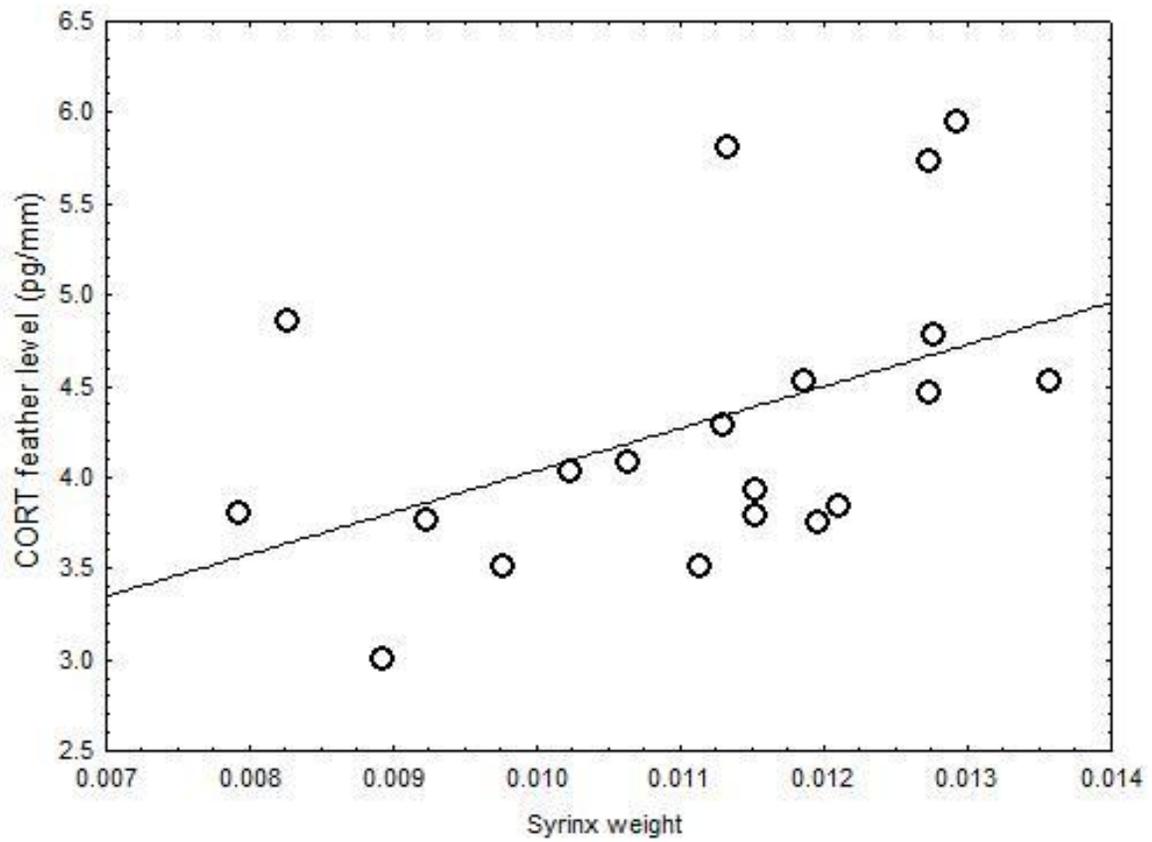


Figure 5.3 Syrix weight of black-capped chickadee in their first spring is correlated with CORT levels of the natal feathers (regression analysis,  $R^2=0.1$ ,  $N=20$ ,  $p=0.03$ ).

The power of the analysis comparing syrinx weight across habitats gave a value of 63% allowing me to perform statistical comparisons. However, all comparisons between song nuclei across habitat have a power below 14%. I found that birds from young forests have a smaller syrinx mass than birds in mature forest (ANOVA,  $F_{(1,17)}=5.86$ ,  $N=20$ ,  $p=0.02$ ,  $d=0.60$ ; Fig 5.4a). Using a Cohen's  $d$  index of effect size, an intermediate effect was found between RA volume in young vs. mature forests; birds in young forests tend to have a bigger RA volume than birds in mature forest ( $d=0.38$ ; Fig 5.4c). HVC volume differs very slightly across habitats ( $d=0.13$ ; Fig 5.4b) with birds in young forests tending to have a smaller HVC than birds in mature forests.

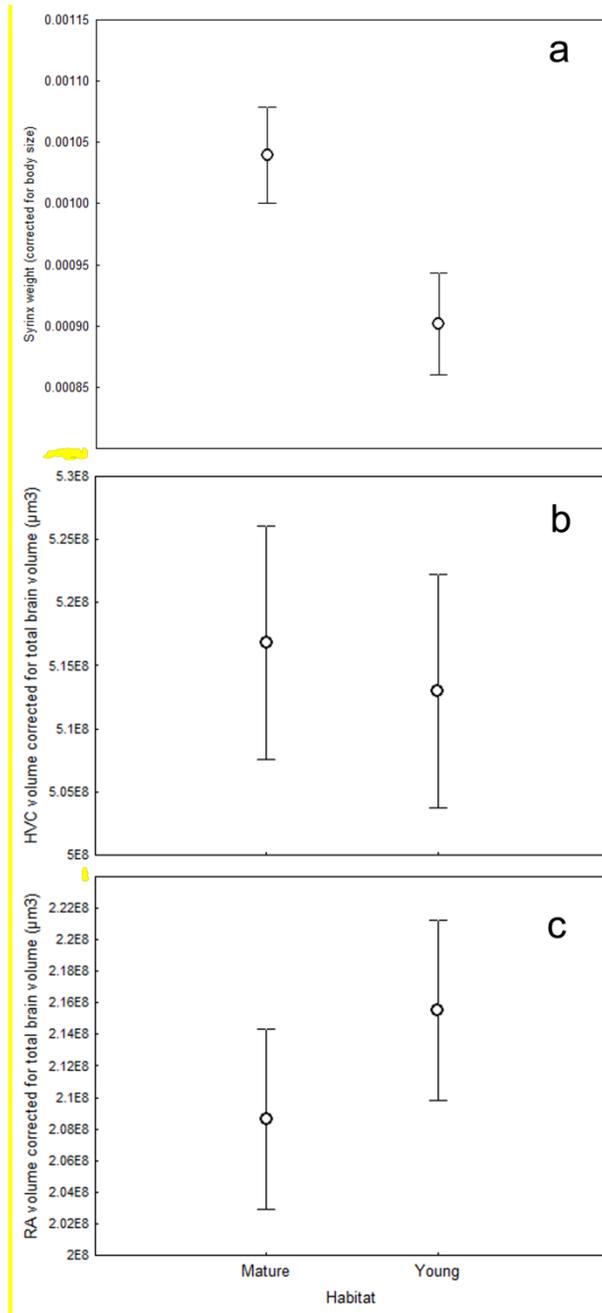


Figure 5.4 (a) Birds from young forests have a lighter syrinx than birds in mature forest (ANOVA,  $F_{(1,17)}=5.86$ ,  $N=20$ ,  $p=0.02$ ,  $d=0.60$ ). (b) HVC volume differ slightly across habitat ( $d=0.13$ ) with birds in young forests tending to have a smaller HVC than birds in mature forests. (c) An intermediate effect was found between RA volume in young vs. mature forests; birds in young forests tend to have a bigger RA volume than birds in mature forest ( $d=0.38$ ). Dots represent mean and whiskers error bars.

#### 5.4. Discussion

I found that the birds that ultimately settled in young forests had lower CORT in their natal feathers than did birds that established in mature forests. This result seems at odds with the idea that birds in poorer condition are predicted to be under higher levels of stress, reflected by higher CORT level, than birds in better condition (Bonier et al. 2009). All previous studies of birds settling in similar habitat types in the region would indicate that birds in young forest would be likely to encounter higher levels of resource limitation (van Oort et al. 2006; Fort and Otter 2004a, 2004b; Otter et al. 2007; Grava et al. 2009). This discrepancy, however, might be associated with my metric for measuring CORT levels. I assessed CORT using feathers, which provides a longer-term perspective compared to instantaneous blood sampling typical of other studies. CORT from feather samples provides an integrated measure (Bortolotti et al. 2008), and current studies have found a positive relationship between feather CORT and nutritional status (Fairhurst et al. 2012), rather than the inverse relationship proposed by previous work (Bonier et al. 2009). Moreover, the lack of consistency in the relationship between CORT and condition across taxa questions the generality of assuming high CORT is associated with poor condition. In particular, in the Paridae family, the relationship between CORT and condition is not consistent across two closely related species - the great tit (non-significant relationship - Eeva et al. 2003) and the blue tit (positive relationship - Muller et al. 2007). My results differ from Lucas et al. (2006) who documented an elevated CORT levels in disturbed habitat in the Carolina chickadees (*Poecile carolinensis*) based on fecal CORT on both adult and juvenile chickadees. The fact that my results differ from theirs is likely based on the difference in the experimental

designs, and is representative of the problem of generalization when studying condition and CORT levels.

My results, however, show similar patterns to other studies using feather CORT to assess relative nutritional condition among individuals. Fairhurst et al. (2012) for example, found that Cory's shearwater (*Calonectris diomedea*) nestlings, facing conditions of diminished provisioning rates, had lower feather CORT, which shows a link between lower energetic condition and lower CORT in nestling birds, a result also found using blood CORT levels (Kitaysky et al. 2005). My findings that birds settling in young forest have lower CORT in their natally-grown feathers may indicate that these birds were in poorer energetic state than those that ultimately settled in mature forests, but the factors responsible for CORT differences need to be addressed with additional research. As previous research has demonstrated a strong positive influence of mature forests on reproductive success in these birds (Otter et al. 2007b), any settlement bias based on competitive advantage of juvenile birds would tend to favour settlement in the mature forests. Once the birds establish in their habitat, however, my results suggest they do not differ in CORT deposited into induced feathers.

My results also suggest that the condition of the black-capped chickadee nestlings may predict whether the birds will establish in good or poor-quality habitat, and suggest that a despotic distribution occurs when black-capped chickadee juveniles disperse – the birds in better condition tend to establish in higher-quality habitat, while birds in lower condition are pushed towards sub-optimal habitat (Godfrey 2003). A similar study in the black-capped chickadee showed that birds across habitat did not differ in DGB before they settled in their habitat (van Oort and Otter 2005). The authors concluded that there was no evidence for

differential settlement (at least among males) based on individual condition. By adding the CORT level as another measure of condition in this study, I was able to detect a difference in condition between juvenile birds settling in young versus mature forest through a study of the feather CORT, even though I confirmed the previous results of van Oort and Otter (2005), that there were no differences across habitats in DGB of settling birds.

During sensorimotor learning period, I found no effect of habitat on either CORT or DGB in induced feathers. However, my study of the condition of the birds during the sensorimotor learning period suffers from a smaller sample size compared to the study of the song memorization period, which could explain the lack of consistency between those results.

The song memorization period occurs when the birds are still nestlings. This period is extremely important for song development. It has been shown that birds in lower condition during the song memorization have a lower ability to learn their species-specific songs because of a lower development of the organs involved in song learning (Nowicki et al. 2002). Because I found a physiological difference between birds that eventually settle in young forest compared to those in mature forest, these differences in feather CORT may reflect general differences among the birds during the song memorization period. If these differences in CORT are correlated with other physiological measures associated with later song production, it may indicate a connection between natal condition and song learning.

This connection appears in the correlation between CORT levels in natal feathers and the syringeal mass in juvenile males entering their first breeding season. I also found differences in song center nuclei across habitats. Birds in young forest tend to have a smaller HVC volume and a bigger RA volume. Other studies suggest that HVC is affected more by

early condition than RA. In zebra finch, birds who have undergone developmental stress during song learning (through nutritional and hormonal stress, or parasitic infections) have a lower development of the HVC region while RA volume or brain size was not affected (Buchanan et al. 2004, Spencer et al. 2003, Spencer et al. 2005). My results show that even though birds in young forest tend to have a bigger RA volume, they still have a lower ability to maintain internal song structure (Grava et al. 2012; chapter 3).

My results also suggest that birds occupying habitats associated with lower consistency in song rendition also tend to have a lower HVC volume. The relationship between HVC volume and song learning ability has been well demonstrated (DeVoogd et al. 1993; Szekely et al. 1996; Airey and DeVoogd 2000; Airey et al. 2000; Garamszegi and Eens 2004, although see Gahr et al. 2008 for a recent study that found no difference in learning ability among males that differ in HVC volume).

Syrinx mass was smaller among my young-forest birds than in the mature-forest birds. Because a smaller syrinx is associated with less accurate imitation of a tutor song (Solis and Doupe 2000), my results may explain why motor production of songs among young-forest birds tends to be less consistent than those of mature-forest birds (Grava et al. 2012; chapter 3). The fact that syrinx weight is correlated with natal CORT level may suggest a link between the condition of the birds during the song memorization period and later development of the neuro-motor pathways responsible for song learning in the black-capped chickadee. However, it is possible that there is no causality between development and condition and that birds in both habitats differ genetically - the birds having a better genotype (higher CORT, higher DGB, faster neural and syringeal development) preferentially settle into optimal habitat, while birds with a lower-quality phenotypes are pushed towards sub-

optimal habitat. In any case, it seems that the establishment of the birds into good and poor-quality habitat may be linked to the natal condition of the birds, and that settlement within these habitats leads to reduced development of neural centers and motor pathways associated with song development. This current study may provide a mechanistic connection as to why adults birds in these same habitats differ in the ability to produce songs with consistent frequency ratio (Grava et al. 2012; chapter 3), and the ability of maintaining these components of song structure appear to positively influence male perception of intruder condition (Grava et al. in prep.; chapter 4). Song consistency may therefore be a condition-dependent signal. That I did not find differences in condition in birds in both habitats during the sensorimotor period might reflect a trade-off where birds with limited energy reserves invest first in somatic maintenance (body condition) and less in the development of sexually selected traits (Godfrey 2003). This may explain why I see little difference in condition of birds between good and poor habitats, but major differences in the expression of sexually-selected signals.

## 6- GENERAL DISCUSSION

### 6.1. Synopsis

My thesis explored how habitat quality affects individual birds at different stages in their life-history and consequently their vocal performance. In northern BC, intensive forestry activity in the last half century has created a mosaic of habitat types across a landscape that was traditionally continuous sub-boreal forest. Although forest practices advocate mimicking natural disturbance events (forest fires and insect epidemics), the extent of disturbance over much of northern BC is larger and more regular than natural events, and creates a diversity of small, adjacent patches of forest differing in ages. Patches of old-growth forest now abut newly clear-cut patches of forest, and/or patches of forest regenerating from a ten, 20 or 30 year-old clear-cut. Black-capped chickadees are a generalist forest species that can occupy forests in a range of ages (Smith 1991; Otter et al. 2007). The juvenile birds dispersing in this habitat mosaic can settle in territory that can range in age from 20-year old regeneration to old-growth forest. However, previous assertions that such generalist species were not sensitive to habitat disturbance are too simplistic. Chickadees occupying low-quality habitat have lower breeding success than those in high-quality habitat (Fort and Otter 2004a; Otter et al. 2007), and their behaviour is significantly altered by habitat quality (van Oort et al. 2007; Fort and Otter 2004a; Fort and Otter 2004b; Otter et al. 2007). However, previous studies had found no difference in condition between juvenile males that settled in either habitat type (van Oort et al. 2007). Thus, the influence of habitat on condition was considered to begin post-settlement (Otter et al. 2007). By adding data about the energetic condition using feather CORT, I showed that

juvenile black-capped chickadee settling in young forest had lower CORT levels prior to settlement compare to juvenile birds settling in mature forest (Chapter 5). As low CORT levels are associated with lower energetic condition in some other species (Fairhurst et al. 2012), my results may suggest that young forests are occupied by birds in greater energetic stress during a period that also corresponds to the song memorization period. At the onset of the breeding season, I also found that these birds had lesser development of the brain and the syrinx (Chapter 5). This may account for my finding that birds in young forest are less able to maintain consistent frequency ratios within their songs during the chorus (Chapter 3). This difference in song consistency may be compounded by differences in habitat quality once the birds have settled (Chapter 5). At the time of chorusing, all breeding males start singing at dawn and this “dawn chorus” represents a vocal performance in term of *vocal vigour* and *vocal skills*. In fact, in terms of performance, the ability to maintain internal frequency ratios represents *vocal skill* (as defined by Byers et al. 2010), but it does not appear to reflect the immediate condition of the birds at the time of signal production (Chapter 3), but rather reflects their condition during the development during the song learning period (Chapter 5). On the other hand, once the birds enter their first breeding season, their song crystallizes, and their vocal skill is set for each individual. Another form of vocal performance reflects direct condition at the time of singing and is known as *vocal vigour* (song output). van Oort et al. (2006) showed that birds in young forest had lower song output than birds in mature forest. I demonstrated that song output at dawn is positively affected by short-term feeding (Chapter 2), but this increase in resources does not restore the song consistency differences between habitats (Chapter 3). The difference observed previously in song output across habitat can be attributed to lower food availability at the time of singing in young forest. In this case,

habitat quality has a short-term effect on the bird's condition and consequently on their vocal vigour. To conclude, while vocal skills may reflect the condition of the birds at the time of song learning (chapter 5), vocal vigour is known to reflect the bird's immediate condition at the time of vocal production (Chapter 2), and both of these measures of performance differ across habitat (van Oort et al. 2006, Grava et al. 2012, Chapter 3).

This habitat effect on song consistency is likely to alter the perception of the birds' signals relating to condition with other individuals across habitat. Females are known to attend to elements of the song structure related to male quality (Hoeschele et al. 2010) and dominant males tend to sing with higher consistency than subordinate males (Christie et al. 2004). My results show that the ability to sing with consistent internal song structure is used by males to assess other males' condition during diurnal interactions (Chapter 4). Dominant males in young forest are not considered as an equivalent threat compared to dominant males from mature forest, based only on the consistency of the broadcast songs. This reflects the long-term effect of habitat on the bird's ability to signal their status. No matter whether males in young forests are competitively similar to their rank counterparts in mature forest, young-forest males may be unable to signal this vocally, and thus may face greater numbers of challenges from intruders. From these results, I can anticipate that birds in young forest may be less able to defend their territories. In fact, Fort and Otter (2004) already showed that birds in young forest have a lower territorial behaviour than birds in mature forest. By showing that the relative perception of the males rank is condition- and habitat-dependent, my results give a good understanding of the territorial dynamics in the black-capped chickadee.

## **6.2. The effect of human activities on the production of sexual signal**

In this thesis, I explored the short-term and long-term effect of habitat quality, due to intensive forestry practices on a generalist songbird, the black-capped chickadee. Human activities, today more than ever, are responsible of a high degradation of the natural habitats of many bird species through a variety of activities. I focused my work on the effect of forest practices on vocal performance in the black-capped chickadee but there are a variety of examples that demonstrate how human activities affect individual condition and consequently the production of sexual signals that convey information about their quality.

Vocalizations are not the only sexual signals used by birds to convey information about their condition. In numerous bird species, visual signals are condition-dependent and are used by females to assess males' condition. For example, the expression of carotenoid-based plumage coloration reflects the food provisioning rates of the male blue tit and great tit (Hill 1991; Linville et al. 1998; Senar and Escobar 2002; Senar et al. 2002; Isakson et al. 2006). Many studies have also shown that females prefer males with brighter carotenoid-dependent colours (e.g. zebra finch, Burley and Coppersmith 1987; house finch, Hill 1991). At least in blue tits, the colour is assumed to be subject to sexual selection because it is correlated with male parental quality (Senar et al. 2002; Johnsen et al. 2005). One study in great tits has shown that such colour differences appear once the nestling hatch (phenotypic traits), and therefore reflect habitat quality rather than genetic quality (Hörak et al. 2000), while others have demonstrated both environmental and genetic effects in both tit species (Fitze et al. 2003a; Johnsen et al. 2003). As a result, habitat quality could have a profound impact on the expression of visual signals in those species. Yellow feathers of the underparts of blue tits and great tits are paler in a range of habitats assumed to be of poorer quality than

deciduous woodland (Ferns and Hinsley 2008). For example, Slagsvold and Lifjeld (1985) found that great tits are more yellow in deciduous than in coniferous forest, and it is known that birds in deciduous forest are bigger than birds in coniferous forest (Ulfstrand et al. 1981).

For acoustic signals, the relationship between forest structure and the production of acoustic signals is largely reviewed in the present work. Both song output and song consistency are higher in dominant than in subordinate males, suggesting the condition-dependency of those signals (Otter et al 1997; Christie et al. 2004). In this species, the study of the effect of forest structure on singing was based on a comparison of young and mature forest. van Oort et al. (2006) showed that birds in young forest had lower song output than birds in mature forest. My work showed that this difference likely results from a difference in condition across habitat at the time of singing (Grava et al. 2009, chapter 2), and may also reflect lower amounts of caterpillar prey in these habitats during the early part of the breeding season (as quantified by relative frass abundance - chapter 1). I also demonstrated that song consistency was affected by forest structure with birds in young forest having lower song consistency than birds in mature forest (Grava et al 2012, chapter 3). This difference is not alleviated by short-term food supplementation (Grava et al. 2012, chapter 3) and therefore more likely reflects the condition of the birds at an earlier period in their development prior to the breeding season, during the period of song learning (chapter 5).

In other species, habitat fragmentation alters repertoire size (e.g. blue tits, Doutrelant et al. 2000; house sparrow, Reid et al. 2005), a characteristic of signalling that is known to be associated with a better fitness in numerous birds' species. By increasing the distance between neighboring males, habitat fragmentation might affect song variation in some bird

species (white crowned sparrow *Zonotrichia leucophrys nuttalli*, Trainer 1983; corn bunting *Miliaria calandra*, Holland et al., 1996). Studies on Dupont's lark (*Chersophilus duponti*; Laiolo and Tella 2005; 2007) found that isolation caused song repertoires to decrease drastically in fragmented habitats. The authors suggest that cultural diversity should be taken into consideration the same way as biodiversity when looking at the effect of landscape alteration on bird populations (Laiolo and Tella 2007).

By affecting the environment (food quality and/or availability) and the physiology of the organisms, heavy metal pollutant released in the environment by industry can have a profound effect on a bird's condition and production of both visual and acoustic signal. Eeva et al. (1998) compared plumage of birds living close to an air pollution source to those of birds living further from the source. They found that the plumage brightness was negatively affected by air pollution, and that this reflects lower food abundance in the polluted area. Also, aberrant tail morphology in barn swallow is another visual signal associated with a decrease in condition. For example, males with increased ectoparasitism show higher asymmetry in the length of outer tail feathers (Moller 1992b) than other birds. Moller (1993) studied the effect of the highly contaminated site of Chernobyl on fluctuating asymmetry (perturbations in symmetry of bilaterally-symmetrical traits) in the barn swallow. He compared birds sampled from the contaminated site to birds sampled both from the site before the catastrophe and museum specimens from the area. After the catastrophe, the level of asymmetry in male tail length was higher than before the catastrophe. Similarly, Gorissen et al. (2005) found that great tits from sites polluted with heavy metal sang less at dawn and had a smaller repertoire of song types than birds from the non-polluted area. The authors

suggest that long-term effect of heavy metal exposure during song learning could alter the development of the birds and consequently their song complexity.

The effect of human activities on natural landscape can also be considered in terms of urban and agricultural activities. For the past 10,000 years, humanity has been transforming the landscape through development of both agriculture and urban areas. The relative quality of those environments from a bird's perspective is still under investigation, as generalization about the relative quality of urban vs. rural environment is extremely difficult and therefore studies hard to compare. For example, great tits in mixed forest have higher carotenoid level, which are typically associated with higher condition, than birds in sub-urban area (Figuerola and Senar 2005), demonstrating that the latter areas are perceived as lower quality than natural environment. However, the study of plumage colour in house finch show that birds from sub-urban areas are more colourful than birds nesting further into rural areas with intensive agriculture, suggesting that sub-urban areas might hold more food resources than polluted rural areas (Hill 1995). The study of the effect of urban area on birdsong to date has focused primarily on the effect of urban noise on birdsong frequency. An increased in minimum frequency of the songs of birds has been detected in response to urban noise (Luther and Baptista 2009; Hu and Cardoso 2010). Slabbekoorn and Peet (2003) showed that birds in urban areas sang at higher frequency to ensure their song would transmit through the urban noise. In a 30-years study, Luther and Baptista (2009) showed that the songs of the white-crowned sparrow increase in frequency along with the increase of urban noise.

### 6.3. Implications of my results on bird's life history and conservation

My results show the implications that habitat quality has on the bird's life history. Previous research showed that habitat had a direct effect on the bird's territoriality (Fort and Otter 2004a), reproduction (Fort and Otter 2004b, Otter et al. 2007) and signalling (van Oort 2006). My results demonstrate the developmental and ecological mechanisms linking those differences. First, I demonstrated that habitat has a direct effect on chickadee *behaviour* and reproduction via resource limitation during the breeding season. I showed in this thesis that the difference in song output across habitat observed previously (van Oort et al. 2006) is directly related to a difference in food availability at the time of chorusing (Grava et al. 2009, Chapter 2). My results also demonstrate that the difference in food availability during the breeding season does differ across habitat (Chapter 1). This difference in food availability can explain other differences in life history across habitat. All behaviour that represents non-maintenance activities requires extra energetic investment. As a result, it will be undertaken only if the bird has enough energy to sustain its other primary life-sustaining activities (Godfrey 2003). If birds in young forest have access to scarcer resources during the breeding season than birds in mature forest, it could explain why they do not invest as much energy in non-maintenance activities. Those activities range from singing, but also breeding and defending territory. It has been demonstrated that birds in mature forests have higher reproductive success, at the UNBC study sites (Fort and Otter 2004b) but also at the JPRF study sites (unpublished data). In my sites, the low reproductive success was particularly due to high nest abandon during the early breeding season. I showed in chapter 1 that the caterpillar population is significantly lower in young forest during the incubation period, which correspond to first stage of breeding. The scarcer food resources during the early

breeding season could therefore help understanding the low reproductive success observed across habitat. Territoriality is another non-maintenance activities that birds will invest into if they have extra energy. It has been shown that birds in young forest are less territorial than birds in mature forest at the UNBC study sites (Fort and Otter 2004b), and this result has been confirmed by personal observations for four consecutive field seasons at the JPRF study sites. Again, the low resource access in young forest that I demonstrated interfere with chorusing (Chapter 2), could similarly explain the low territorial behaviour across habitat.

On the other hand, my results also showed that habitat quality can indirectly impact the bird's behaviour. I showed that through habitat selection during flock establishment and effect of habitat on brain and syrinx development, males black-capped chickadee in young forest have a lower ability to maintain internal song structure than do birds in mature forest (Chapter 3 and 5). Through altering their song structure, habitat quality can consequently impact male's reproductive success. If males occupying poor-quality habitats produce songs with lower consistency, poorer renditions of song-types, or decreased repertoires (globally considered as decreased "vocal skill"), they may have more difficult time attracting mates or extra-pair partners or have to expend extra effort defending their territories from intruding males. Maintenance of internal structure of notes within and between songs (song consistency) is known to accurately reflect male social rank in chickadees, and is known to be attended to by female within this species (Hoeschele, 2010). I also demonstrated that song consistency is a condition-dependent signal used by males to assess the condition of male intruders and that, based on this signal, dominant birds in mature forest do not consider dominant birds from young forest as equally ranked than dominant birds from mature forest (Chapter 4). This relative perception of the bird's rank across habitat can potentially interfere

with the bird's territorial behaviour, with birds from young forest being less able to defend their territory against intruder than birds in mature forest. Also, if song consistency is a vocal skill used by the female to assess male quality, males with high song consistency should be preferred over males with low consistency. Byers (2006) demonstrated such an association in chestnut-sided warblers (*Dendroica pensylvanica*), where males exhibiting a high song consistency have higher access to extra-pair copulations. Similarly, Botero et al. (2009) demonstrated that highly-consistent male mocking birds have a better reproductive success than less-accurate singers. If the singer's vocal skill reflects the inherent quality of the singer, it could act as a reliable signal with which females may be selecting among males. Thus, reduction in the ability of males to maintain consistent song structure when occupying young forests may compromise their perceived attractiveness, and thus their reproductive potential.

The results of my thesis can help investigating the use of the *fee-bee* song as a measure of habitat quality. If song output and structure is affected by habitat quality, it seems possible to use those signals to assess the relative quality of habitat, as measured through its short-term and long-term effects on resident species. Godfrey (2003) suggested that condition-dependent signals can be used as a measure of habitat quality. In the black-capped chickadee, song output gives a measure of the birds' condition (Otter et al. 1997) and this signal is affected by the quality of the habitat (van Oort et al. 2006). My work demonstrates that song output is directly related to food limitation of the signaller at the time of singing (Grava et al. 2009; Chapter 2). This signal gives a proxy of the perceived environment by males. By comparing the song output at dawn of male black-capped chickadees of the same rank in different habitat, I should be able to detect whether a difference in quality exists between the two habitats. The ability to sing with consistency, on the other hand, gives me

long-term information on the conditions available to birds during their early developmental period. My results suggest that song consistency reflect the conditions faced by birds during the learning period, which occurs from when the birds are still nestlings to the time of the first settlement (Chapter 5). The birds with lower feather CORT, during the learning period, indicative of lower condition in other species (Fairhurst et al. 2012), are more likely to settle in low-quality habitat. This habitat may then compound effects that resulted in these birds having lower neural development of song centers and syringeal mass when it was time for song production (Chapter 5), and consequently lower ability to sing with high consistency (Chapter 3). As a result, the ability to sing with consistent internal frequency ratios could give me additional information about the quality of the habitat in which a bird is recorded.

By combining the study of the song output with the study of song consistency across habitats that may differ in quality, one may be able to compare the quality of the habitat as perceived by the birds at multiple times in its life history and therefore in the year. For example, I could consider two adjacent habitats that differ in structure, such as two forest types differing in tree species, in which black-capped chickadees are present and breed (a scenario close to the study done on blue tit in France by Blondel et al. (1993) who showed that the *behaviour* of birds in evergreen oak (*Quercus ilex*) forest differ from birds in deciduous forest (*Quercus pubescens*) due to difference in food availability). Those habitats might be of very different quality for the birds if the amount of food available for breeding is lower in one habitat compare to the other (e.g. lower amount of caterpillar). At the time of singing, birds recorded in low-food habitat would have a lower song output than birds recorded in the rich-environment. However, those habitats might not differ in quality to birds during the song learning period. The amount of food available when the birds are dispersing

and learning their song may be similar (e.g. same amount of seed/insects during the fall and winter), which could result in birds recorded in both habitats showing no difference in the ability to maintain internal frequency ratios of their songs. This example demonstrates that the signals entrenched into the *fee-bee* song of the black-capped chickadee gives two measures of habitat quality that can help biologists to assess the relative quality of habitat over two independent time periods in the bird's annual cycle.

Finally, the study of the consequences of habitat quality on condition and behaviour in generalist species is part of a new concept in conservation biology. Scientists are now not only taking into consideration the diversity of species in an ecosystem, but also the abundance and health of individuals within a population. While diversity is important, as it provides information about the importance to protect specific environments, the presence and condition of generalist species populations give reliable information about the quality of those environments. I now realize that the ecological services provided by species to humanity are mostly provided by the mass of individuals in generalist species rather than by the few individuals in rare species populations.

#### **6. 4. Future research**

My thesis demonstrates the effect of habitat quality on vocal performance and explores the mechanisms through which habitat affects the information entrenched within the *fee-bee* song of the black-capped chickadee. I suggest that the *fee-bee* song can be used as a measure of signaller-condition by the receiver through two means: the song output at dawn gives information about the condition of the signaller at the time of singing, whereas the ability to maintain internal frequency ratios provides information on the condition of the

birds during the song learning period. This thesis brings a holistic approach to understand the link between the production of signals, the condition of the birds and the quality of the habitat in which the birds is settling and/or breeding.

However, we are still investigating whether food availability differs naturally across habitat during the breeding season. Analysis of frass data, presented in Chapter 1, suggests that differences in caterpillar abundance differs between years, but more importantly to my thesis, also differs between habitat classes during the incubation period following the dawn chorus. I suggest in Chapter 2 that birds in young forest sing less than in mature forest because they have access to less food resources, but I could not directly quantify the amount of food available at this time of the year. Effectively, the chorus period typically occurs prior to the leaf burst period, so it is difficult to monitor caterpillar frass between habitats at this time of the year. Birds during this period tend to forage on trunk invertebrates, which I was unable to quantify. However, looking at patterns of prey availability in general during the spring may provide insight into habitat differences. During the breeding season, the black-capped chickadee relies mostly on caterpillar resources they find under the tree leaves, as they represent a high-protein resource the birds need to sustain their energetic cost while providing sufficient food for the nestlings. It has been shown in a wild population of blue tits living in two habitats where evergreen (*Quercus ilex*) or deciduous oak (*Q. Pubescens*) predominate, that the main difference in resources during the breeding season occurs because the caterpillar peaks in evergreen oak forest was delayed of few weeks (Blondel et al. 1993). We are currently monitoring the relative abundance of caterpillars in both habitats to see whether a difference exists in the absolute amount of caterpillar during the peak or whether the hatching peak is delayed between habitats. Preliminary results tend to show that the

caterpillar peak seems to happen at the same time in both habitats, but that the amount of caterpillar available in young forest is lower than in mature forest. More data are needed to detail the exact caterpillar population trend in both habitats.

Also, although I demonstrated that birds differ in measures associated with condition prior to settlement, and my results suggest that this can explain the lower ability to maintain consistent frequency ratio in the song of birds in young forest, I need more information about the exact links between the condition of the juvenile birds at the time of song learning and their singing ability. More data are needed to monitor the birds' condition in the first fall and winter and consequently their song structure, and such studies may need to focus on a single long-term population to track cohorts over a number of years.

Finally, it seems that habitat quality differs on a temporal scale. Birds tend to perceive the quality of their environment differently during years with particularly harsh or mild winters. It seems plausible that food availability (i.e. caterpillar abundance) depends largely on winter temperature or snow condition. Models predicting future climate change anticipate higher winter a decrease in temperature in North-Central BC, which would tend to increase insect winter survival. However, an increase in snow fall is also predicted, which would potentially increase the length of time the ground is snow-covered and make seeds and insects less available during late winter and early spring. As forest structure is different between habitats, it is very likely that climate change will differentially affect my forest classes, and create complex interactions on bird behaviour and condition. Using meteorological stations at multiple spots across habitats may help clarify the relationship between the effect of climate change and future bird populations.

## REFERENCES

- Airey D.C., Buchanan K.L., Szekely T., Catchpole C.K. and DeVoogd T.J. 2000. Song, sexual selection, and a song control nucleus (HVC) in the brains of European sedge warblers. *Journal of Neurobiology*. 44:1–6.
- Airey D.C., DeVoogd T.J. 2000. Greater song complexity is associated with augmented song system anatomy in zebra finches. *Neuroreport*. 11:2339–2344.
- Alatalo, R.V., Glynn C. and Lundberg A. 1990. Singing rate and female attraction in the pied flycatcher: an experiment. *Animal Behavior*. 39:601–603.
- Arlettaz R., Patthey P., Baltic M, Leu T., Schaub M., Palme R. and Jenni-Eiermann S. 2007. Spreading free-riding snow sports represent a novel serious threat for wildlife. *Proceedings of the Royal Society of London B*. 274:1219-1224.
- Baker M.C., Baker M.S.A and Gammon D.E. 2003. Vocal ontogeny of nestling and fledgling black-capped chickadees (*Poecile atricapilla*) in natural populations. – *Bioacoustics*. 13:265-296.
- Blondel J., Dias P.C., Maistre M. and Perret P. 1993. Habitat heterogeneity and life history variation of Mediterranean Blue Tits (*Parus caeruleus*). *Auk*. 110:511–520.
- Bolhuis J.J. and Gahr M. 2006. Neural mechanisms of birdsong memory. *Nature Reviews Neuroscience*. 7:347-357.
- Bolhuis J.J., Okanoya K. and Scharff C. 2010. Twitter evolution: Converging mechanisms in birdsong and human speech. *Nature Reviews Neuroscience*. 11:747-759.

- Bonier F., Moore I.T., Martin P.R. and Robertson R.J. 2009. The relationship between fitness and baseline glucocorticoids in a passerine bird. *General and Comparative Endocrinology*. 163:208–213
- Bortolotti G.R. 2010. Flaws and pitfalls in the chemical analysis of feathers: bad news-good news for avian chemoecology and toxicology. *Ecological applications*. 20:1766-1774.
- Bortolotti G.R., Marchant T., Blas J. and Cabezas S. 2009a. Tracking stress: localisation, deposition and stability of corticosterone in feathers. *Journal of Experimental Biology*. 212:1477-1482.
- Bortolotti G.R., Marchant T.A., Blas J. and German T. 2008 Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Functional Ecology*. 22:494-500.
- Bortolotti G.R., Mougeot F., Martinez-Padilla J., Webster L.M.I. and Piertney S.B. 2009b. Physiological Stress Mediates the Honesty of Social Signals. *Plos One* 4 (3): e4983. doi:10.1371/journal.pone.0004983.
- Botero C.A., Rossman R.J., Caro L.M., Stenzler L.M., Lovette I.J., de Kort S.R. and Vehrencamp S.L. 2009. Syllable type consistency is related to age, social status and reproductive success in the tropical mockingbird. *Animal Behavior*. 77:701-706.
- Brenowitz E.A., Margoliash D, and Nordeen K.W. 1997. An introduction to birdsong and the avian song system. *Journal of Neurobiology*. 33:495-500.

- Breuner C.W., Patterson S.H. and Hahn T.P. 2008. In search of relationships between the acute adrenocortical response and fitness. *General and Comparative Endocrinology*. 157:288-295.
- Buchanan K.L., Spencer K.A., Goldsmith A.R. and Catchpole C.K. 2003. Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). *Proceeding of the Royal Society of London B*. 270:1149–1156.
- Burt J.M. and Vehrencamp S.L. 2005. Dawn chorus as an interactive communication network. – In: Animal communication networks (McGregor P.K., ed.). Cambridge University Press, Cambridge, UK. p. 320–343.
- Byers B.E. 2007. Extrapair paternity in chestnut-sided warblers is correlated with consistent vocal performance. *Behavioural Ecology*. 18:130-136.
- Byers J., Hebets E. And Podos J. 2010. Female mate choice based upon male motor performance. *Animal Behaviour*. 79:771-778.
- Carlson A. 1998. Territory Quality and Feather Growth in the White-Backed Woodpecker *Dendrocopos leucotos*. *Journal of Avian Biology*. 29(2):205-207.
- Carlson A. 1998. The effect of habitat loss on a deciduous forest specialist species: the White-backed Woodpecker (*Dendrocopos leucotos*). *Forest Ecology and Management*. 131:215-221.

- Chin E.H., Love O.P., Verspoor J.J, Williams T.D., Rowley K. and Burness G. 2009. Juveniles exposed to embryonic corticosterone have enhanced flight performance. *Proceedings of the Royal Society of London B*. 276:499–505.
- Christie P.J., Mennill D.J. and Ratcliffe L.M. 2004. Pitch shifts and song structure indicate male quality in the dawn chorus of black-capped chickadees. *Behavioral Ecology and Sociobiology*. 55:341–348.
- Cohen J. 1988. *Statistical Power Analysis for the Behavioral Sciences*. 2nd edn. Lawrence Erlbaum, Hillsdale, NJ.
- Cuthill I.C. and Macdonald W.A. 1990. Experimental manipulation of the dawn and dusk chorus in the Blackbird *Turdus merula*. *Behavioral Ecology and Sociobiology* 26:209–216.
- Desrochers A. 1989. Sex, Dominance, and Microhabitat use in Wintering Black-Capped Chickadees: A Field Experiment. *Ecology*. 70:636–645.
- Desrochers A. 1990. Sex determination of black-capped chickadees with a discriminant analysis. *Journal of Field Ornithology*. 61:79-84.
- DeVoogd T.J., Krebs J.R., Healy S.D. and Purvis A. 1993. Relations between song repertoire size and the volume of brain nuclei related to song—comparative evolutionary analyses amongst oscine birds. *Proceeding of the Royal Society of London B Biological Science*. 254:75–82.
- Doutrelant C., Blondel J., Perret P. and Lambrechts M.M. 2000. Blue tit song repertoire size, male quality and interspecific competition. *Journal of Avian Biology*. 31:360–366.

- Eeva T., Lehikoinen E. and Nikinmaa M. 2003. Pollution-induced nutritional stress in birds: an experimental study of direct and indirect effects. *Ecological Applications*. 13:1242–124.
- Ekman J. 1987. Exposure and time use in willow tit flocks: the cost of subordination. *Animal Behaviour*. 35:445-452.
- Ekman J.B. and Askenmo C.E.H. 1984. Social rank and habitat use in willow tit groups. *Animal Behaviour*. 32(2):508-514.
- Ellis L. 1995. Dominance and Reproductive Success Among Nonhuman Animals: A Cross-Species Comparison. *Ethology and Sociobiology*. 16:257-333.
- Fairhurst G.D., Navarro J., González-Solís J., Marchant T.A. and Bortolotti G.R. 2012. Feather corticosterone of a nestling seabird reveals consequences of sex-specific parental investment. *Proceeding of the Royal Society of London B*. 279:177-184.
- Fickens M.S., Weise C.M. and Popp J.W. 1990. Dominance rank and resource access in winter flocks of black-capped chickadees. *Wilson Bulletin*. 102:623–633.
- Foote J.R., Mennill D.J., Ratcliffe L.M. and Smith S.M. 2010. Black-capped chickadee (*Poecille atricapillus*). - In: The Birds of North America Online (Poole A., ed.). Ithaca, Cornell Lab of Ornithology.
- Foote J.R., Fitzsimmons L.P., Mennill D.J. and Ratcliffe L.M. 2008. Male chickadees match neighbors interactively at dawn: support for the social dynamics hypothesis. *Behavioral Ecology*. 19:1192-1199.

- Forstmeier W., Kempenaers B., Meyer A. and Leisler B. 2002. A novel song parameter correlates with extra-pair paternity and reflects male longevity. *Proceeding of the Royal Society of London B*. 269:1479-1485.
- Fort K.T. and Otter K.A. 2004a. Territorial breakdown of black-capped chickadees *Poecile atricapillus*, in disturbed habitats? *Animal Behavior*. 68:407–415.
- Fort K.T. and Otter K.A. 2004b. Effects of habitat disturbance on reproduction in black-capped chickadees (*Poecile atricapillus*) in Northern British Columbia. *Auk*. 121:1070–1080.
- Gahr M., Metzdorf R., Schmidl D. and Wickler W. 2008. Bi-directional sexual dimorphisms of the song control nucleus HVC in a songbird with unison song. *PLoS One*. 3:e3073.
- Gammon D.E. 2004. Black-capped chickadee dawn chorus and subsequent sexual activity. *Wilson Bulletin*. 116:252–256.
- Garamszegi L.Z. and Eens M. 2004. Brain space for a learned task: strong intraspecific evidence for neural correlates of singing behavior in songbirds. *Brain Research Reviews*. 44:187–193.
- Godfrey J.D. 2003. Potential use of energy expenditure of individual birds to assess quality of their habitats. – In: Conservation applications of measuring energy expenditure of New Zealand birds: Assessing habitat quality and costs of carrying radio transmitters (Williams M., Comp.) *Science for Conservation*, p. 11–24.
- Gorissen L., Snoeijs T., Van Duyse E. and Eens M. 2005. Heavy metal pollution affects dawn singing behavior in a small passerine bird. *Oecologia*. 145:504–509.

- Gosler A.G. and Carruthers T. 1999. Body reserves and social dominance in the great tit *Parus major* in relation to weather in southwest Ireland. *Journal of Avian Biology* 30:447–45.
- Gosler A.G. 1996. Environmental and social determinants of winter fat storage in the great tit *Parus major*. *Journal of Animal Ecology*. 65:1-17.
- Grava T., Grava A. and Otter K.A. 2012. Vocal performance varies with habitat quality in black-capped chickadees (*Poecile atricapillus*). *Behaviour*. 149: 35–50.
- Grava T., Grava A. and Otter K.A. 2009. Habitat quality affects the perception of dominant males based on their relative song consistency. In preparation.
- Grava T., Grava A. and Otter K.A. 2009. Supplemental feeding and dawn singing in black-capped chickadees. *Condor*. 111:560–564.
- Grubb T.C. Jr. 1989. Ptilochronology: feather growth bars as indicators of nutritional status. *Auk*. 106:314-320.
- Grubb T.C. and Yosef R. 1994. Habitat-specific nutritional condition in Loggerhead Shrikes (*Lanius ludovicianus*): evidence from ptilochronology. *Auk*. 111:756-759.
- Grubb T.C. Jr. 2006 (ed). Ptilochronology: feather time and the biology of birds. Oxford University Press.
- Harms N.J., Fairhurst G.D., Bortolotti G.R. and Smits J.E.G. 2010. Variation in immune function, body condition, and feather corticosterone in nestling Tree Swallows (*Tachycineta bicolor*) on reclaimed wetlands in the Athabasca oil sands, Alberta, Canada. *Environmental Pollution*. 158: 841-848.

- Ho R. 2006. Handbook of univariate and multivariate data analysis and interpretation with SPSS. Boca Raton, FL: Chapman and Hall/CRC.
- Hoeschele M., Moscicki M.K., Otter K.A., van Oort H., Fort K.T., Farrell T.M., Lee H., Robson S.W.J. and Sturdy C.B. 2010. Dominance signalled in an acoustic ornament. *Animal Behaviour*. 79:657–664.
- Hultsch H. and Todt D. 2004. Chapter 3 - Learning to sing. - In: Nature's Music (Marler P. and Slabbekoorn H., eds.), Academic Press, San Diego, p. 80-107.
- Hunter A.F. and Elkinton J.S. 2000. Effects of synchrony with host plant on populations of a spring-feeding lepidopteran. *Ecology*. 81:1248–1261.
- Jackson D.A. 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology*. 74:2204–2214.
- Kitaysky A.S., Romano M.D., Piatt J.F., Wingfield J.C. and Kikuchi M. 2005. The adrenocortical response of tufted puffin chicks to nutritional deficits. *Hormones and Behavior*. 47:606–619.
- Koivula K. 1994. Social dominance in willow tit (*Parus montanus*) flocks: causes and effects of resource access, survival, and reproduction. *Acta Univ Ouluensis A*. 263:1:36.
- Lambrechts M.M., Caro S., Charmantier A., Gross N., Galan M.J., Perret P., Cartan-Son M., Dias P.C., Blondel J. and Thomas D.W. 2004. Habitat quality as a predictor of spatial variation in blue tit reproductive performance: a multi-plot analysis in a heterogeneous landscape. *Oecologia*. 141:555-561.
- Lampe H.M. and Espmark Y.O. 1994. Song structure reflects male quality in pied flycatchers *Ficedula hypoleuca*. *Animal Behavior*. 47:869-876.

- Legendre P. and Legendre L. (eds) 1998. Numerical ecology: developments in environmental modelling. 2nd ed. Elsevier Science, Amsterdam.
- Lucas J.R., Freeberg T.M., Egbert J. and Schwabl H. 2006. Fecal corticosterone, body mass, and caching rates of Carolina chickadees (*Poecile carolinensis*) from disturbed and undisturbed sites. *Hormones and Behavior*. 49:634 – 643.
- Lucas J.R., Schraeder A. and Jackson C. 1999. Carolina Chickadee (Aves, Paridae, *Poecile carolinensis*) vocalization rates: effects of body mass and food availability under aviary conditions. *Ethology*. 105:503–520.
- Marra P.P. and Holberton R.L. 1998. Corticosterone levels as indicators of habitat quality: effects of habitat segregation in a migratory bird during the non-breeding season. *Oecologia* 116:284-292.
- Meigs J.B., Smith D.C. and van Buskirk J. 1983. Age determination of Black-capped chickadees. *Journal of Field Ornithology*. 54:283–286.
- Mennill D.J. and Otter K.A. 2007. Status signalling and communication networks in black-capped chickadees: Complex communication with a simple song. – In: Ecology and Behavior of Chickadees and Titmice: an Integrated Approach (Otter, K.A., ed.). Oxford, Oxford University Press, p. 215-233.
- Mooney R. 2009. Neurobiology of song learning. *Current Opinion in Neurobiology*. 19:654-660.
- Müller C., Jenni-Eiermann S., Blondel J., Perret P., Caro S.P., Lambrechts M.M. and Jenni L. 2007. Circulating corticosterone levels in breeding *Parus caeruleus* differ between island and mainland populations and between habitats. *General and Comparative Endocrinology*. 154:128–136.

- Murphy M.T., Sexton K., Dolan A.C. and Redmond L.J. 2008. Dawn song of the Eastern Kingbird: an honest signal of male quality? *Animal Behavior*. 75:1075–1084.
- Naguib M., and Mennill D. 2010. The signal value of birdsong: empirical evidence suggests song overlapping is a signal. *Animal Behaviour*. 80:e11-e15.
- Nowicki S., Hasselquist D., Bensch S. and Peters, S. 2000. Nestling growth and song repertoire size in great reed warblers: evidence for song learning as an indicator mechanism in mate choice. *Proceedings of the Royal Society of London, Series B*. 267, 2419e2424.
- Nowicki S., Searcy W.A. and Peters S. 2002. Brain development, song learning and mate choice in birds: a review and experimental test of the “nutritional stress hypothesis”. *Journal of Comparative Physiology*. 188: 1003–1014.
- Otter K.A, van Oort H. and Fort K.T. 2007b. Habitat quality and reproductive behavior in chickadees and tits: potential for habitat matrix use in forest generalists. – In: *Ecology and Behavior of Chickadees and Titmice: an Integrated Approach* (Otter, K.A., ed.). Oxford University Press, Oxford, p. 277–291.
- Otter K., Chruszcz B. and Ratcliffe L. 1997. Honest advertisement and singing during the dawn chorus of black-capped chickadees, *Parus atricapillus*. *Behavioral Ecology* 8: 167-173.
- Otter K., Ratcliffe L., Michaud D. and Boag P.T. 1998. Do female black-capped chickadees prefer high ranking males as extra-pair partners? *Behavioral Ecology and Sociobiology*. 43:25-36.

- Otter K.A., Atherton S.E. and van Oort H. 2007a. Female food solicitation calling, hunger levels and habitat differences in the black-capped chickadee. *Animal Behavior*. 74:847-853.
- Otter K.A. and Ratcliffe L. 2005. Enlightened decisions: female assessment and communication networks. – In: *Animal communication networks* (McGregor P.K., ed.). Cambridge University Press, Cambridge, UK, p. 133–151.
- Otter K.A., Ratcliffe L., Michaud D. and Boag P.T. 1998. Do female black-capped chickadees prefer high-ranking males as extra-pair partners? *Behavioral Ecology and Sociobiology*. 43:25–36.
- Otter K.A., Ramsay S.M. and Ratcliffe L. 1999. Enhanced reproductive success of female black-capped chickadees mated to high-ranking males. *Auk*. 116:345–354.
- Otter K.A., van Oort H. and Fort K.T. 2007b. Habitat quality and reproductive behavior in chickadees and tits: potential for habitat matrix use in forest generalists. – In: *Ecology and Behavior of Chickadees and Titmice: an Integrated Approach* (Otter, K.A., ed.). Oxford University Press, Oxford, p. 277–291.
- Pfaff J.A., Zann L., MacDougall-Shackleton S.A. and MacDougall-Shackleton E.A. 2007. Song repertoire size varies with HVC volume and is indicative of male quality in song sparrows (*Melospiza melodia*). *Proceeding of the Royal Society of London series B*. 274:2035–2040.
- Podos J., Lahti D.C. and Moseley D.L. 2009. Vocal performance and sensorimotor learning in songbirds. *Advances in the Study of Behavior*. 40:159-195.

- Poesel A., Dabelsteen T. and Pedersen S.B. 2004. Dawn song of male Blue Tits as a predictor of competitiveness in midmorning singing interactions. *Acta Ethologica*. 6:65–71.
- Ratcliffe L., Mennill D.J. and Schubert K.A. 2007. Social dominance and fitness in black-capped chickadees. – In: Ecology and Behavior of Chickadees and Titmice: an Integrated Approach (Otter, K.A., ed.). Oxford University Press, Oxford, p. 131–146.
- Reid J.M., Arcese P., Cassidy A.L.E.V., Hiebert S M., Smith J.N.M., Stoddard P. K., Marr A.B. and Keller L.F. 2004. Song repertoire size predicts initial mating success in male Song Sparrows, *Melospiza melodia*. *Animal Behavior*. 68:1055–1063.
- Reid J.M., Arcese P., Cassidy A.L.E.V., Hiebert S M., Smith J.N.M., Stoddard P. K., Marr A.B. and Keller L.F. 2005. Fitness Correlates of Song Repertoire Size in Free-Living Song Sparrows (*Melospiza melodia*). *The American Naturalist*. 165:299-310
- Reijnen R. and Foppen R. 1994. The Effects of Car Traffic on Breeding Bird Populations in Woodland. I. Evidence of Reduced Habitat Quality for Willow Warblers (*Phylloscopus trochilus*) Breeding Close to a Highway. *Journal of Applied Ecology*. 31:85-94.
- Ritschard M., Riebel K. and Brumm H. (2010). Female zebra finches prefer high-amplitude song. *Animal Behavior*. 79:877-883.
- Sanz J.J., Garcia-Navas V. and Ruiz-Peinado J.V. 2010. Effect of habitat type and nest site features on the breeding performance of great and blue tits in a Mediterranean landscape. *Ornis Fennica*. 87:41–51.

- Sapolsky R.M, Romero L.M. and Munck A.U. 2000. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions. *Endocrine Reviews*. 21:55-89.
- Schmidt K.M., Furlongera A.A., Lapierrera J.M, MacDougall-Shackletona E.A. and MacDougall-Shackleton S.A. 2012. Regulation of the HPAaxis is related to songcomplexity and measures of phenotypic quality in song sparrows. *Hormones and Behavior*. 61:652–659.
- Searcy W.A. and Beecher M.D. 2009. Song as an aggressive signal in songbirds. *Animal Behaviour*. 78:1281-1292.
- Searcy W.A. and Beecher M.D. 2011. Continued scepticism that song overlapping is a signal. *Animal Behaviour*. 81:e1-e4.
- Shackleton S.A. and Ratcliffe L. 1993. Development of Song in Hand-Reared Black-Capped Chickadees. *The Wilson Bulletin*. 105:637-644.
- Silverin B. 1997. The stress response and autumn dispersal behaviour in willow tits. *Animal Behavior*. 53:451–459.
- Smith S.M. 1991. The black-capped chickadee: behavioral ecology and natural history. Cornell University Press, Ithaca, NY.
- Smulders T., Lisi M., Tricomi E., Otter K., Chruczcz B., Ratcliffe L. and DeVogd T. 2006. Failure to Detect Seasonal Changes in Song System Nuclei of the black-capped chickadee. *Journal of Neurobiology*. 66: 991-1001.

- Solis M.M. and Doupe A.J. 2000. Compromised Neural Selectivity for Song in Birds with Impaired Sensorimotor Learning. *Neuron*. 25:109–121.
- Spencer K.A., Buchanan K.L., Goldsmith A.R. and Catchpole C.K. 2004. Developmental stress, social rank and song complexity in the European starling (*Sturnus vulgaris*). *Proceedings of the Royal Society of London B*. 271:121–123.
- Spencer K.A., Buchanan K.L., Goldsmith A.R. and Catchpole C.K. 2003. Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). *Hormones and Behavior*. 44:132–139.
- Spencer K.A., Buchanan K.L., Leitner S., Goldsmith A.R. and Catchpole C.K. 2005a. Parasites affect song complexity and neural development in a songbird. *Proceedings of the Royal Society of London B Biological Sciences*. 272:2037–2043.
- Staicer C.A., Spector D.A and Horn A.G. 1996. The dawn chorus and other diel patterns in acoustic signalling. – In: Ecology and evolution of acoustic communication in birds (Kroodsma D.E. and Miller E.H. eds.). Cornell University Press, Ithaca, NY, p. 426–453.
- Strong A.M and Sherry T.W. 2000. Habitat- specific effects of food abundance on the condition of Ovenbirds wintering in Jamaica. *Journal of Animal Ecology*. 69:883-895.
- Suthers R.A., Goller F. and Pytte C. 1999. The neuromuscular control of birdsong. *Philosophical Transactions of the Royal Society of London series B*. 354:927–939.
- Sueur J., Aubin T. and Simonis C. 2008. Seewave: a free modular tool for sound analysis and synthesis. *Bioacoustics*. 18:213-226.

- Suorsa P., Huhta E., Nikula A., Nikinmaa M., Jäntti A., Helle H., Hakkarainen H. 2003. Forest management is associated with physiological stress in an old-growth forest passerine. *Proceedings of the Royal Society of London B*. 270:963–969.
- Szekely T., Catchpole C.K., DeVoogd A., Marchl Z. and DeVoogd T.J. 1996. Evolutionary changes in a song control area of the brain (HVC) are associated with evolutionary changes in song repertoire among European warblers (Sylviidae). *Proceedings of the Royal Society of London B Biological Sciences*. 263:607–610.
- Takaki Y., Eguchi K. and Nagata H. 2001. The growth bars on tail feathers in the male Styan's Grasshopper Warbler may indicate quality. *Journal of Avian Biology*. 32:319–325.
- Thomas R.J. 1999. The effect of variability in the food supply on the daily singing routines of European Robins: a test of a stochastic dynamic programming model. *Animal Behavior*. 57:365–369.
- Tremblay I., Thomas D.W., Blondel J., Perret P. and Lambrechts M.M. 2005. The effect of habitat quality on foraging patterns, provisioning rate and nestling growth in Corsican Blue Tits *Parus caeruleus*. *Ibis*. 147:17–24.
- Tremblay I., Thomas D.W., Lambrechts M.M., Blondel J. and Perret P. 2003. Variation in Blue Tit breeding performance across gradients in habitat richness. *Ecology*. 84:3033–3043.

- van Oort H., Otter K.A., Fort K. and Holschuh C.I. 2006. Habitat quality affects the reliability of a condition-dependent signal in black-capped chickadees. *Ethology*. 112:772-778.
- van Oort H. and Otter K.A. 2005. Natal nutrition and the habitat distributions of male and female black-capped chickadees. *Canadian Journal of Zoology*. 83:1495-1501.
- van Oort H., Otter K.A., Fort K.T. and McDonnell Z. 2007. Habitat, dominance, and the phenotypic quality of male black-capped chickadees. *Condor*. 109:88–96.
- Walker B.G, Boersma P.D and Wingfield J.C. 2005. Field Endocrinology and Conservation Biology. *Integrative Comparative Biology*. 45: 12-18.
- Walters J.R. 1998. The ecological basis of avian sensitivity to habitat fragmentation. - In: Avian conservation: research and management (Marzluff J.M and Sallabanks R eds.), American Ornithologists' Union, Raptor Research Foundation.
- Wayland M., Gilchrist H.G., Marchant T., Keating J. and Smits J.E. 2002. Immune function, stress response, and body condition in arctic-breeding common eiders in relation to cadmium, mercury, and selenium concentrations. *Environmental Research*. 90:47-60.
- Welling P.P., Rytönen S.O., Koivula K.T. and Orell M.I. 1997. Song rate correlates with paternal care and survival in Willow Tits: advertisement of male quality? *Behavior*. 134:891–904.
- Westneat M.W., Long J., John H., Hoese W. and Nowicki S. 1993. Kinematics of birdsong: functional correlation of cranial movements and acoustic features in sparrows. *Journal of Experimental Biology*. 182:147-171.

Zann R. and Cash E. 2008. Developmental stress impairs song complexity but not learning accuracy in non-domesticated zebra finches (*Taeniopygia guttata*). *Behavioural Ecology and Sociobiology* 62:391–400.

Zahavi A. 1975. Mate selection - a selection for a handicap. *Journal of Theoretical Biology*. 53:205-214.