

Electronic Thesis and Dissertation Repository

8-24-2015 12:00 AM

Tactics to Stay Alive: Predation Risk Alters Body Condition and Escape Behaviour

Benjamin T. Walters
The University of Western Ontario

Supervisor
Dr. Liana Zanette
The University of Western Ontario

Graduate Program in Biology

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
© Benjamin T. Walters 2015

Follow this and additional works at: <https://ir.lib.uwo.ca/etd>



Part of the [Ecology and Evolutionary Biology Commons](#)

Recommended Citation

Walters, Benjamin T., "Tactics to Stay Alive: Predation Risk Alters Body Condition and Escape Behaviour" (2015). *Electronic Thesis and Dissertation Repository*. 3112.
<https://ir.lib.uwo.ca/etd/3112>

This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact wlsadmin@uwo.ca.

Tactics to Stay Alive: Predation Risk Alters Body Condition and Escape Behaviour

(Thesis format: Integrated Article)

by

Benjamin Thomas Walters

Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

© Benjamin Walters 2015

Abstract

Recent manipulations show that when perceived predation risk is high, animals will chronically alter their escape behaviour, even if it affects physiological condition to such an extent that survival may be reduced. I tested the relationships amongst predation risk, mass change, and flying capacity in brown-headed cowbirds (*Molothrus ater*) housed in large outdoor aviaries. I measured changes in the birds' body condition, take-off behaviour and flying capacity after manipulating the ambient level of perceived predation risk in their environment using various predator or non-predator cues. Cowbirds exposed to predator stimuli gained, rather than lost, body mass and changed their take-off behaviour, by flying at higher angles and reduced speeds. Critically, flying capacity was not affected by any of these changes. I suggest these responses may be anti-predator tactics that birds utilize to reduce starvation risk and improve their chances of predator evasion when predation risk is high.

Keywords: predation risk, anti-predator response, escape behaviour, predator evasion, physiological condition, body mass, flight, take-off, flying capacity

Co-Authorship Statement

Dr. Liana Zanette will be the second co-author on the manuscript that is published from this thesis. Liana was central in the development and planning phase of this experiment. Her research expertise and fundamental knowledge of ecology played a key role in helping me accomplish this research. She also helped me design animal care protocols and provided feedback during data analysis and manuscript preparation. Her NSERC grants also funded my research.

Natalie Cheng will be the third co-author on the manuscript to be published from this thesis. Natalie aided in preparing the experimental site, conducting the predation risk manipulations, and collecting data during take-off behaviour trials.

Dr. Michael Clinchy will be the fourth co-author on the manuscript to be published from this thesis. Mike also played a key role in the design and development phase of this experiment. He helped construct the waterproof speaker systems and mount coverings we used during the predation risk manipulation. He also contributed by providing feedback regarding data analysis.

Acknowledgments

I have benefited from a tremendous amount of support from my family, mentors, peers, and volunteers during my time at Western. Without the encouragement that this wonderful group of people has provided me along the way, I would not have been able to accomplish this extremely challenging research.

First and foremost, I have to thank my family for the emotional support and guidance I received when times were tough. There are no words to explain how thankful I am to have them in my life. My mom, Janet Walters, and my sister, Lynn Walters, never hesitated to provide me with a boost of confidence when I was on the brink of insanity. I'm eternally grateful to my brother, John Walters, who brought me back down to earth more times than he brought my truck back to life. I also wouldn't have made it without my loving girlfriend, Emma Mullen, whose presence kept me grounded through it all.

My supervisors, Drs. Liana Zanette and Mike Clinchy, have taught me so many fundamental lessons about how to conduct quality research. In the past two years, they have challenged me to push the boundaries, think creatively, write more proficiently, conduct thorough analysis, and appreciate the priceless value of beer. I am incredibly grateful for their guidance, and the lessons I've learned from them have undoubtedly improved my character and skills as a researcher.

My lab-mates Natalie Cheng, Blair Dudeck, Emma Hobbs, Marek Allen, David Swan, Justin Suraci, Devin Roberts and Ashael Raveh have been an invaluable network of support. I have to give an especially big thanks to Natalie, who tolerated me through thick and thin during our collaboration at the aviaries. I've been spoiled to share my experiences

with such an intelligent and fun-loving group of people, and I have been lucky to develop such strong friendships that I'm sure will last long after these two years.

The list would not be complete without acknowledging the community of others that helped along the way. Peter Duenk and Caroline Rasenberg served as an invaluable source of help and advice when preparing for our research at ESW. The long hours invested by our volunteers, Jameson Barr, Shauna Taylor, and Aisha Agarwaal, kept the cogs turning throughout the entire field season. My advisory committee, Drs. David Sherry and Chris Guglielmo, provided valuable input and access to the tools I needed to complete such amazing research. Finally I'd like to thank a laundry list of others that have helped me out along the way including (but not limited to) Zander McKinnon, Scott Colbourne, Kayla Gradil, Malcolm Lau, Tim Hain, Andrew Gould, and Michela Rebuli.

Table of Contents

Abstract.....	ii
Co-Authorship Statement.....	iii
Acknowledgments.....	iv
List of Tables	viii
List of Figures.....	ix
List of Appendices	x
Chapter 1	1
General Introduction.....	1
1.1 Effects of predation risk on prey populations.....	1
1.2 Effects of predation risk on behaviour and physiology	2
1.3 Effects of predation risk on escape behaviour	6
1.4 Escape behaviour in avian species.....	9
1.5 Research objectives and hypotheses	14
1.6 Study species.....	15
1.7 References.....	16
Chapter 2.....	27
2.1 Introduction.....	27
2.2 Methods.....	31
2.2.1 Study site and species	31
2.2.2 Predation risk manipulation	31
2.2.3 Physiological assessment.....	33
2.2.4 Escape behaviour	36
2.2.5 Body mass manipulation.....	40
2.2.6 Statistical analyses	41
2.3 Results.....	44
2.3.1 Predation risk manipulation	44
2.3.2 Body mass manipulation.....	51
2.4 Discussion.....	54
2.5 References.....	63

Chapter 3.....	71
General Discussion	71
3.1 Tactics for survival in response to predation risk.....	71
3.2 Consequences for applied conservation.....	74
3.3 Challenges facing escape behaviour research.....	75
3.4 Future directions	77
3.5 References.....	80
Appendices.....	85
Curriculum vitae	89

List of Tables

Table 1. Matched calls used for predator and non-predator treatment playlists. Calls are matched based on broadcast time period and acoustic properties.....34

Table 2. Matched mounts displayed during predator and non-predator treatment periods. Mounts were matched for size and stance.....34

List of Figures

Figure 1. Change in cowbird body condition \pm SE between non-predator (blue) and predator (red) manipulations of (a) body mass: $F_{1,65.89} = 9.8$, $p = 0.003$ (b) fat mass: $F_{1,64.27} = 15.1$, $p = 0.001$ and (c) wet lean mass: $F_{1,65.42} = 0.003$ $p = 0.96$45

Figure 2. Change in cowbird take-off behaviour between non-predator (blue) and predator (red) manipulations of (a) take-off angle: $F_{1,50.68} = 6.5$, $p = 0.01$ and (b) take-off speed: $F_{1,40.90} = 4.8$, $p = 0.035$48

Figure 3. Relationship between percent change in vertical flying speed ($(\text{flying speed}_{\text{predator}} - \text{flying speed}_{\text{control}}) / \text{flying speed}_{\text{control}} * 100$) and percent change in body mass ($(\text{body mass}_{\text{predator}} - \text{body mass}_{\text{control}}) / \text{body mass}_{\text{control}} * 100$) in the predation risk manipulation for (a) female cowbirds: $R^2 = 0.19$, $F_{1,34} = 7.7$, $p = 0.009$ and (b) male cowbirds: $R^2 = 0.002$, $F_{1,25} = 0.04$, $p = 0.84$. The dotted line indicates no change in flying speed between the two manipulation periods. Each dot represents an individual cowbird.....50

Figure 4. Relationship between percent change in vertical flying speed ($(\text{flying speed}_{\text{post-manipulation}} - \text{flying speed}_{\text{baseline}}) / \text{flying speed}_{\text{baseline}} * 100$) and percent change in body mass ($(\text{body mass}_{\text{post-manipulation}} - \text{body mass}_{\text{baseline}}) / \text{body mass}_{\text{baseline}} * 100$) in the body mass manipulation for (a) female cowbirds: $R^2 = 0.30$, $F_{1,22} = 5.8$, $p = 0.025$ and (b) male cowbirds: $R^2 = 0.02$, $F_{1,21} = 0.009$, $p = 0.93$. The dotted line indicates no change in flying speed between the two manipulation periods. Each dot represents an individual cowbird.....53

List of Appendices

Appendix A. Picture of take-off measurement apparatus.....85

Appendix B. Picture of vertical flight chamber.....86

Appendix C. Table showing actual values of body mass, fat mass, wet lean mass, and vertical flying speed for the body mass manipulation between the baseline and post-manipulation periods for all treatment groups.....87

Appendix D. Ethics approval for animal use.....88

Chapter 1

General Introduction

1.1 Effects of predation risk on prey populations

Traditionally, predators were viewed simply as killers, limiting prey populations by catching and eating individuals (reviewed in Pritchard et al. 2006). Errington (1967) explained this as a common misconception, for people confused “the *fact* of predation with the *effect* of predation” (Errington 1967:235). We now understand that predators have a much more profound effect on prey populations, even to the degree that can shape the entire dynamics of an ecosystem (Estes et al. 2011). In addition to directly killing prey, the physiological, behavioural and morphological responses of prey that are associated to the risk of being killed can indirectly alter prey population dynamics (Preisser and Bolnick 2008). Even more striking are the vast number of empirical studies, conducted on a wide array of taxa, which indicate that predation risk effects may have an equal, if not greater, influence on prey populations than direct predation (Preisser et al. 2005).

Elucidating the population level effects that predation risk (i.e. the ambient level of risk that prey perceive in their environment) may have on prey can be a difficult task, as it requires the manipulation of predation risk in the absence of direct killing. A number of studies, however, have begun to quantify the effects of predation risk on prey survival. Several microcosm studies using aquatic and invertebrate species have adopted this design since the predators in these model systems can be directly manipulated, serving as threatening stimuli without being able to kill. For example, Schmitz et al.

(1997) compared the effects of predators on grasshopper nymph (*Melanoplus femurrubrum*) populations in two experiments. In the first experiment, they found that introducing predatory spiders (*Pisurina mira*), which were able to actively hunt and kill grasshoppers, resulted in a 29 % reduction in grasshopper population size. In the second, when the spiders were manipulated by gluing their mouthparts shut, so they could only threaten but not kill, grasshopper population size was still reduced by 20%. These experiments provide support that the risk of predation alone contributed greater effect on prey demography than direct killing. Manipulations in terrestrial vertebrate systems provide further evidence of the importance of risk effects on prey dynamics by examining their effects on reproduction. Sherrif et al. (2009) observed that pregnant snowshoe hares (*Lepus americanus*) exposed to a threatening live predator during parturition gave birth to poorer conditioned offspring and suffered a reduced birth rate. Furthermore, Zanette et al. (2011) reported a 40% reduction in the number of offspring produced per year in a population of wild female song sparrows when they experimentally increased the level of predation risk after controlling for direct nest predation. These experiments indicate that the effects of predation risk are indeed costly and need to be considered when determining the population level effects that predators have on prey.

1.2 Effects of predation risk on behaviour and physiology

While it is clear that predation risk has important implications for prey populations, these changes are likely a result of the costs of predation risk that prey face at an individual level. Prey are capable of perceiving the ambient level of predation risk that they face in an environment (reviewed in Caro 2005) and can respond to changes in risk by altering their behaviour (Schmitz et al. 1997; Lima 2009; Wirsing and Ripple

2011) and physiology (Hawlena and Schmitz 2010). These anti-predator responses come at a cost, however, as they mediate important functional trade-offs that can impact survival (Creel and Christianson 2008; Zanette et al. 2014).

Predators are lethal and present a clear challenge to prey survival, forcing prey to make decisions in order to minimize their risk of predation. Achieving this is not simple, as prey must perceive the level of risk and enlist a suite of anti-predator behaviours, which include changes in foraging, habitat use, and vigilance (Lima 1998; Caro 2005; Stankowich and Blumstein 2005). There are a vast number of documented cases in a wide array of taxa that illustrate how prey are sensitive to predators and will employ these sophisticated anti-predator behaviours in response to increased threat (Lima and Dill 1990; Apfelbach et al. 2005). For example, Bray and Nieh (2014) found that honey bees (*Apis mellifera*) chose to avoid foraging at a feeder with a live predator (i.e. a mantis (*Tenodera sinensis*)) 67 % of the time. In these cases, bees also reduced their foraging recruitment behaviour to conspecifics by 1.8 fold, suggesting that predator cues can cause a colony-wide shift in foraging behaviour. Additionally, when high abundances of tiger sharks (*Galeocerdo cuvier*) are present, dugongs (*Dugong dugon*) shift their foraging habitats into deeper waters that represent lower-quality foraging areas than the lush shallow banks that they prefer (Wirsing et al. 2007). Other studies have found prey to respond to heightened levels of predation risk by reducing activity and home range size (Borowski and Owadowska 2010), as well as by increasing vigilance (Cassini 1991; Morrison 2011). These studies, along with many others, reveal the impactful consequences of predator-mediated effects on prey behaviour and habitat use in

vertebrates and invertebrates alike (reviewed in Brown and Kotler 2004; Caro 2005; Schmitz et al. 2008).

Anti-predator behaviours may limit the probability that prey are killed, but they still carry significant costs. Reducing foraging activity and increasing vigilance in response to predators may limit the amount of time prey have available to obtain energy (Carey and Moore 1986, Brown et al. 1988, Childress and Lung 2003; Trussell et al. 2003), although prey may compensate by increasing their feeding intensity (McNamara et al. 2005). Furthermore, shifting to lower-quality foraging habitats in response to predation risk can result in changes in diet that affect nutrient uptake and energy budgets (Schmitz 2004). To illustrate, elk (*Cervus elaphus*) populations were found to switch from foraging in the high-quality open grasslands to the lower-quality, but safer, coniferous forest cover following the re-introduction of wolves into Yellowstone National Park (Creel et al. 2005). Therefore, under increasing threat, prey may opt to forgo foraging in an effort to limit their exposure to predators or constrain their foraging to times and places that are safer (MacLeod et al. 2007a). As predation risk increases, a prey's ability to achieve adequate nutrition may not be limited by the absolute quantity of resources available, but may result from prey either not being able to access food or because they must expend more energy to gain these resources (Krebs et al. 1995). As prey generally face two major sources of mortality, starvation and predation, they must balance their energy reserves to minimize the risk of death (Lima 1986, McNamara et al. 2005). Thus, it is evident that mortality, caused by starvation, and limited reproductive success, can be indirectly attributed to the predator-induced behavioural changes of prey (Boonstra et al. 1998, MacLeod et al. 2007b).

Prey also have physiological responses to predation risk that can increase their chance of survival. These responses may include changes in physiological traits that increase escape and enhance immune response (Irschick et al. 2007). For example, damselfly larvae (*Enallagma vesperum*) were found to swim faster and express a higher ATP-generating enzyme activity associated with escape speed when confronted with a live predator (i.e. a dragonfly) than those larvae that were only exposed to a caged predator (Strobbe et al. 2010). Additionally, larval exposure to predator cues was found to increase immune function and survival in adult wood frogs (*Lithobates sylvaticus*) (Groner et al. 2013), suggesting that exposure to predation risk at an early age can have fitness benefits later in life. Parents exposed to high predation risk may also bestow physiological advantages to their offspring to prepare them for a riskier environment. For instance, Coslovsky and Richner (2011) found that juvenile great tits (*Parus major*) showed accelerated wing growth, lowered wing-load, and longer wings at maturity when their mothers were exposed to high predation risk before and during ovulation. This suggests that mothers can give their offspring a selective advantage for predator evasion in preparation for a high-risk environment (Coslovsky and Richner 2011). Together, these studies illustrate how predators can shape prey physiology to where prey make an effort to improve their survival; however, responses to increased predation risk undoubtedly come with physiological costs as well.

Predator-induced stress responses can be either acute or chronic and have significant effects on physiology that have been frequently documented in a wide range of species (reviewed in Hawlena and Schmidtz 2010; Zanette et al. 2014). While stress responses can improve the chance of survival, they can also interfere with important

physiological functions (Hawlana and Schmitz 2010). Acute exposure to predators and predator cues can increase metabolic rate (Cooke et al. 2003), oxidative damage, and stress hormone levels (Slos and Stoks 2008). These can be quite costly physiological effects: higher metabolic rates increase heart rate and energy consumption in individuals (Chabot et al. 1996); oxidative damage can reduce life expectancy, growth rate, and reproductive output (Monaghan et al. 2009; Janssens and Stoks 2013); and high stress hormone levels can reduce gonadotropin function during reproduction (Sherrif et al. 2009). The chronic effects of predation risk have similar, long-term effects on physiology, including changes in body condition, immune function, and reproductive output (Boonstra et al. 1998; Clinchy et al. 2004, 2013; Zanette et al. 2011).

Despite the fact that prey exhibit a wide variety of costly behavioural and physiological responses to avoid predation, they are also faced with making immediate decisions of how to escape when confronted with the immediate threat of a predator. When a predator is detected, prey must decide if, when, and how to flee, and these decisions are vitally important in determining survival.

1.3 Effects of predation risk on escape behaviour

Escaping from predators is important for all prey species. Thus, it is expected that escape behaviour may vary widely among taxa, with differences hinging on the strategy and capacity of escape. Prey rely on sensory cues to detect oncoming predators (Stewart et al. 2014), and both the speed and direction of escape are highly important in determining a successful evasion (Katzir and Camhi 1993; Shiffman and Eilam 2004). It is reasonable to assume that prey escape at maximum speeds in order to distance

themselves from an attacking predator, yet it is evident that trajectory of an escape attempt is also considerably important in determining survival (reviewed in Domenici et al. 2011). Prior experience to predation risk can shape prey escape behaviour through learned responses (Adamo et al. 2013). Additionally, the physiological effects that predators elicit in prey may positively or negatively impact escape behaviour (Strobbe et al. 2010; Janssens et al. 2014); therefore, understanding how prey moderate their locomotor capacity in the presence of predation risk is crucial in identifying the effects of predators on escape behaviour.

Prey must first detect a predator before exhibiting any type of escape response, and they adopt the use of a variety of sensory cues to determine the presence of a predator that help them decide how and when to initiate escape (Fields and Yen 1997; Canfield 2003). The size, attack speed and attack orientation of a predator can also significantly alter escape behaviour (Webb 1982; Lind et al. 2002; Meager et al. 2006). For example, Seamone et al. (2014) found spiny dogfish sharks (*Squalus acanthias*) to show increased responsiveness to predator models with larger frontal profiles than smaller ones. Sharks also exhibited greater reaction distances and increased escape turning rates when predator models attacked with a head-on versus tail-on approach (Seamone et al. 2014). Therefore it is evident that the escape behaviours that prey adopt are dependent on the prevailing condition of an attack.

Evasion success is likely determined by the adaptive escape behaviours that prey use when confronted with an attack by a predator. Flight initiation distance (FID), or the distance at which prey flee an attacking predator (Ydenburg and Dill 1986), has garnered considerable attention in this area, as FID may change depending on multiple factors: the

speed of an approaching predator (Cooper 2003), the availability of cover (Bonenfant and Kramer 1996), or alert distance of a predator (Blumstein et al. 2005). However, when predation is imminent, both escape speed and escape trajectory can be good predictors of whether or not a prey will successfully evade such an attempt on their life. To illustrate, Ilany and Eilam (2008) observed that spiny mice (*Acomys cahirinus*) were found to favor fleeing barn owl (*Tyto alba*) attacks in a sideways direction, which was found in a previous study to be a maneuver that decreased the owls' chances of a successful attack (Shifferman and Eilam, 2004). Escape speed was also found to be a good predictor of successful evasion, where faster mice increased their chances of survival (Ilany and Eilam, 2008). These companion studies elucidate the importance that both speed and direction have on survival when predation is looming.

Prey may learn from surviving a predator attack and can change their escape behaviour accordingly. Previous exposure to a predation risk can also cause changes in physiology that can alter a prey's locomotor capacity to aid them in escaping future attacks (Adamo and Baker 2011). In a study examining changes in escape strategy after repeated predator attacks in two grasshopper species, Bateman and Fleming (2014) found that *Schistocerca alutacea* fled further upon repeated approaches whereas *Psinidia fenestralis* opted to switch from fleeing from open sandy areas to grassy cover. While these responses differ, they both show changes in behaviour that should reduce predation risk by either distancing themselves or seeking refuge from predators (Bateman and Fleming 2014). In addition to making adjustments in behaviour, grasshoppers are also capable of altering their physiology to aid in escape when exposed to increased predation risk. Hawlena et al. (2011) found that grasshoppers (*Melanoplus femurrubrum*) raised in

areas of chronic predation risk improved their locomotor performance (i.e. take-off speed and jump distance) without expressing any conspicuous morphological changes when compared to those raised in a predator-free environment. Grasshoppers raised in a risky environment, however, were more physiologically stressed, as they exhibit elevated metabolism, changes in nutrient content, and reduced offspring quality when compared to the control group (Hawlena et al. 2011). Taken together, these studies suggest that predation risk can cause both behavioural and physiological shifts that affect escape behaviour. Therefore, it is important to take predation risk into account when monitoring changes in escape behaviour among prey species, as it may play a role in determining the actions that prey take in order to survive under different conditions.

1.4 Escape behaviour in avian species

A plethora of studies have examined escape behaviour in avian species, as it is evident that birds adopt a variety of escape strategies in response to a predator attack (Lima, 1993). Escape behaviour in birds has been quantified in a variety of perspectives, including changes in flight initiation distance (reviewed in Stankowich and Blumstein 2005), changes in behaviour and flying capacity at take-off (Kullberg et al. 1996, 1998; Lind et al. 2002, 2003), and theoretical models investigating escape behaviour after birds have become airborne (Hedenstrom and Rosen 2001; van den Hout et al. 2010). Similarly to other taxa, changes in locomotor capacity can affect a bird's ability to escape, especially during the physiologically-costly periods that many birds undergo that are linked to their annual life cycle (e.g. breeding (Kullberg et al. 2002); moulting (Swaddle and Witter 1997); and migration (Kullberg et al. 1996)). Surprisingly, despite all of this research, only studies on flight initiation distance have directly tested the

effects that environmental predation risk can have on avian escape behaviour (Møller 2008; Díaz et al. 2013). Thus, it is of biological importance that more integrative research must be applied to begin to further understand the effects that predators have on escape behaviour in birds.

Birds, and other organisms that can fly to escape predators, have an advantage over terrestrial prey species that are restricted to escaping in two dimensions. A vast number of studies have focused on the fleeing responses of birds to an approaching predator, with specific focus on flight initiation distance (the point at which a bird flees an oncoming predator) and alert distance (the point at which a bird becomes aware and monitors a predator) (Samia et al. 2013). Birds have been found to alter their decisions of when to flee in response to multiple factors, some of which include the start distance of the predator (Blumstein 2003), the distance at which the predator is detected (Samia and Blumstein 2015), and whether the predator is directly focused on the bird (Lee et al. 2013). Additionally, the level of predation risk that birds face in an environment can impact their decisions to flee (Møller 2008). For example, in a study examining average flight initiation distance in 159 European bird species, Díaz et al. (2013) found that birds generally flee at longer distances in areas of higher raptor abundance. These studies provide prime examples of how sensitive birds are to their environment, as there are several aspects that influence their escape behavior.

A growing body of work has also focused on changes in behaviour and flying capacity at take-off. Aerial predators tend to use surprise as a strategy when attacking small birds, and if birds can evade the initial strike by a predator its chances of survival are greatly increased (Cresswell 1996). Therefore, understanding take-off behaviour is

crucial, as it is the most common strategy of escape for birds when confronted with an attacking predator (Cresswell 1993). Take-off behaviour is generally quantified by measuring changes in take-off speed and take-off angle (Kullberg et al. 1996). Taking off at high speeds and steep angles is important for maximizing the chance of survival (Kenward 1978); however, birds face a trade-off between these two variables, as taking off at lower angles maximizes acceleration (Witter and Cuthill 1993). Consequently, take-off behaviour may be dependent on the situation that a bird is confronted with upon attack. Empirical evidence suggests that this is the case, as take-off behaviour changes in response to various prevailing conditions: the angle of attack by a predator (Kullberg et al. 1998; Lind et al. 2002), the attack speed of a predator (Lind et al. 2002), the detection distance of a predator (Lind et al. 2003), and the absence or presence of cover (Kullberg and Lafrenz 2007; Devereux et al. 2008). Many studies also quantify vertical flight speed as a measure of flying capacity (Veasey et al. 1998; Kullberg et al. 2002). Vertical flight speed is an important aspect of escape behaviour as it measures the bird's flying capacity when faced with the maximum challenge of flying directly against the force of gravity, such that reduced vertical flight speeds may serve as an indicator of an increased chance of predation (Veasey et al. 2000). For example, female blue tits (*Parus caeruleus*) face a reduction in vertical flight speed over the egg laying period, suggesting that physiological changes attributed to the breeding season compromise females' flight capacity and increase their susceptibility to a predator attack (Kullberg et al. 2002). Taken together, it is evident that birds face decisions in their escape strategies when confronted with an immediate predator attack, and quantifying both changes in take-off behaviour and flying

capacity together can be a useful tool in determining how birds change their escape behaviour in response to predators.

Birds face many physiological constraints over their annual cycle that can significantly alter their escape behaviour. These life-history challenges generally result in changes in wing-load (i.e. body mass to wing area ratio), which can be detrimental to flying capacity as higher wing loads are expected to reduce a bird's ability to create lift (Pennycuick 1989). Both the moulting and migration literature provide empirical examples of such instances, whereby birds that face either reduced wing area over the moulting period or large increases in mass due to migratory fuelling were shown to have reduced flying capacity (Kullberg et al. 1996; Swaddle and Witter 1997). Therefore, escape behavior may be hindered during exceptionally physiologically stressful conditions.

Nonetheless, birds may also make physiological changes in order to possibly enhance, or at least maintain, their escape behaviour in response to more risky conditions. For example, tree sparrows (*Passer montanus*) were found to concurrently reduce body mass and increase pectoral muscle size as a response to the decreased wing area they experienced over the moulting period (Lind and Jakobsson 2001), likely in an attempt to maintain their flying capacity. Similar physiological changes have been found in predation risk studies. When exposed to high levels of predation risk, birds have often been found to reduce their body mass (Gosler et al. 1995; Lilliendahl 1998; Gentle and Gosler 2001) and, in at least one case, increase their pectoral muscle size (van den Hout et al. 2006). These changes have often been interpreted as physiological adjustments that birds make in order to boost their flying capacity in a riskier environment (Witter and

Cuthill 1993; van den Hout et al. 2006), yet there is no empirical evidence that links these predator-induced changes in physiology to escape behaviour.

While it makes intuitive sense that losing mass may benefit escape behaviour by decreasing wing-load, there is also evidence that birds may gain mass in response to predation risk (Lilliendahl 1998; Pravosudov and Grubb 1998). Additionally, studies that examine changes in diurnal mass gain, which are similar to the levels of mass change caused by predation risk, show no such effects on flying capacity (Kullberg 1998; Kullberg et al. 1998; van der Veen and Sivars 2000; MacLeod 2006). This led MacLeod (2006) to suggest that there may be a magnitude of mass change (>10 %) needed to see significant effects on flying capacity. As these predator-induced mass changes fall well below the suggested threshold that are needed to affect flying capacity, it is clear that further experimentation is needed.

While it is evident that, like many other species, birds may change their escape behaviour in response to the ambient level of predation risk that they face, the only empirical evidence of this is found in the flight initiation distance literature (Møller 2008; Díaz et al. 2013). Thus, it is important that researchers employ a more integrative approach, by manipulating the level of ambient predation risk that birds perceive, to measure the behavioural and physiological responses that may affect escape behaviour. Only then can we begin to understand the total effect that predators have on escape behaviour in avian species.

1.5 Research objectives and hypotheses

As in all prey species, birds are highly sensitive to the conditions of their surrounding environment, yet little is known about how they alter their escape behaviour when confronted with a high level of predation risk. I hypothesize that if predation risk is increased, birds will exhibit anti-predator behavioural and physiological responses that may improve their evasion success, especially at the crucial point of take-off. Additionally, previous theory has only relied on assumptions that suggest birds may alter their body condition in response to increased predation risk as a tactic to improve flying capacity. I aim to integrate the components of predation risk, physiological change and flying capacity into one cohesive study to test the effects of predator-induced changes in body condition on flying capacity.

In Chapter 2, my objective is to determine how increased levels of perceived predation risk influence physiology and escape behaviour in the brown-headed cowbird (*Molothrus ater*) by manipulating the level of predation risk in the environment and measuring changes in physiological condition and escape behaviour. In Chapter 3, I discuss the broader biological significance of my findings and how they can be incorporated into existing theory of escape behaviour. As it is clear that the mere threat of predators alone has an overarching and ever-growing impact on prey populations and ecosystems, I also suggest several components of predation risk effects and escape behaviour that deserve further attention.

1.6 Study species

Brown-headed cowbirds are one of the most widespread blackbird species in North America, found across the entire United States, Southern Canada, and most of Mexico. They are a sexually dimorphic species that typically forages in open fields and short-grass areas near brushy edge habitats. Males are larger (42-50 g) than females (38-45 g) and have a shiny black body and brown head. Females have a uniform pale brown plumage.

Due to their unique reproductive strategy, cowbirds are a model species when investigating the effects of predation risk on body condition over the breeding season. They are obligate brood parasites, relying on a vast number of “host” species to incubate their eggs and raise their young (Woolfenden et al. 2002). This reproductive strategy effectively releases cowbirds from the physiologically stressful duties of parental care over the breeding season, such as nest building, incubation, and foraging for young (Lima 2009; Boyle et al. 2012) that may otherwise serve as confounding factors when investigating predator-induced changes in body condition over this period.

The individuals I studied were caught in the spring of 2014 from various locations around Southern Ontario. They typically breed from May to July; however, if they are housed in large aviaries in the onset of the breeding season, as they were for our study, copulations and egg-laying are scarce (David White, personal communication). During the breeding season, both males and females congregate together, as males present conspicuous singing and bowing displays to females.

1.7 References

- Adamo, S. A., J. L. Baker. 2011. Conserved features of chronic stress across phyla: the effects of long-term stress on behavior and the concentration of the neurohormone octopamine in the cricket, *Gryllus texensis*. *Hormones and Behavior* 60:478-483.
- Adamo, S. A., I. Kovalko, and B. Mosher. 2013. The behavioural effects of predator-induced stress responses in the cricket (*Gryllus texensis*): the upside of the stress response. *Journal of Experimental Biology* 216:4608-4614.
- Apfelbach, R., C. D. Blanchard, R. J. Blanchard, R. A. Hayes, and I. S. McGregor. 2005. The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neuroscience and Biobehavioral Reviews* 29:1123-1144.
- Bateman, P. W., P. A. Fleming. 2014. Switching to Plan B: changes in the escape tactics of two grasshopper species (Acrididae: Orthoptera) in response to repeated predatory approaches. *Behavioral Ecology and Sociobiology* 68:457-465.
- Blumstein, D. T. 2003. Flight-initiation distance in birds is dependent on intruder starting distance. *Journal of Wildlife Management* 67:852-857.
- Blumstein, D. T., E. Fernandez-Juricic, P. A. Zollner, and S. C. Garity. 2005. Inter-specific variation in avian responses to human disturbance. *Journal of Applied Ecology* 42:943-953.
- Bonenfant, M., D. L. Kramer. 1996. The influence of distance to burrow on flight initiation distance in the woodchuck, *Marmota monax*. *Behavioral Ecology* 7:299-303.
- Boonstra, R., D. Hik, G. R. Singleton, and A. Tinnikov. 1998. The impact of predator-induced stress on the snowshoe hare cycle. *Ecological Monographs* 68:371-394.
- Borowski, Z., E. Owadowska. 2010. Field vole (*Microtus agrestis*) seasonal spacing behavior: the effect of predation risk by mustelids. *Naturwissenschaften* 97:487-493.

- Boyle, W. A., D. W. Winkler, and C. G. Guglielmo. 2012. Rapid loss of fat but not lean mass prior to chick provisioning supports the flight efficiency hypothesis in tree swallows. *Functional Ecology* 26:895-903.
- Bray, A., J. Nieh. 2014. Non-consumptive predator effects shape honey bee foraging and recruitment dancing. *Plos One* 9:e87459.
- Brown, C. R. 1988. Social foraging in cliff swallows: local enhancement, risk sensitivity, competition and the avoidance of predators. *Animal Behaviour* 36:780-792.
- Brown, J. S., B. P. Kotler. 2004. Hazardous duty pay and the foraging cost of predation. *Ecology Letters* 7:999-1014.
- Canfield, J. G. 2003. Temporal constraints on visually directed c-start responses: behavioral and physiological correlates. *Brain Behavior and Evolution* 61:148-158.
- Carey, H. V., P. Moore. 1986. Foraging and predation risk in yellow-bellied marmots. *American Midland Naturalist* 116:267-275.
- Caro, T. 2005. Antipredator defenses in birds and mammals. University of Chicago Press, Chicago, USA
- Cassini, M. H. 1991. Foraging under predation risk in the wild guinea-pig *Cavia aperea*. *Oikos* 62:20-24.
- Chabot, D., P. Gagnon, and E. A. Dixon. 1996. Effect of predator odors on heart rate and metabolic rate of wapiti (*Cervus elaphus canadensis*). *Journal of Chemical Ecology* 22:839-868.
- Childress, M. J., M. A. Lung. 2003. Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Animal Behaviour* 66:389-398.

- Clinchy, M., L. Zanette, R. Boonstra, J. Wingfield, and J. Smith. 2004. Balancing food and predator pressure induces chronic stress in songbirds. *Proceedings of the Royal Society B-Biological Sciences* 271:2473-2479.
- Clinchy, M., M. J. Sheriff, and L. Y. Zanette. 2013. Predator-induced stress and the ecology of fear. *Functional Ecology* 27:56-65.
- Cooke, S. J., J. Steinmetz, J. F. Degner, E. C. Grant, and D. P. Philipp. 2003. Metabolic fright responses of different-sized largemouth bass (*Micropterus salmoides*) to two avian predators show variations in nonlethal energetic costs. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 81:699-709.
- Cooper, W. E. 2003. Effect of risk on aspects of escape behavior by a lizard, *Holbrookia propinqua*, in relation to optimal escape theory. *Ethology* 109:617-626.
- Coslovsky, M., H. Richner. 2011. Predation risk affects offspring growth via maternal effects. *Functional Ecology* 25:878-888.
- Creel, S., J. Winnie, B. Maxwell, K. Hamlin, and M. Creel. 2005. Elk alter habitat selection as an antipredator response to wolves. *Ecology* 86:3387-3397.
- Creel, S., D. Christianson. 2008. Relationships between direct predation and risk effects. *Trends in Ecology and Evolution* 23:194-201.
- Cresswell, W. 1996. Surprise as a winter hunting strategy in sparrowhawks *Accipiter nisus*, peregrines *Falco peregrinus* and merlins *F-columbarius*. *Ibis* 138:684-692.
- Cresswell, W. 1993. Escape responses by redshanks, *Tringa totanus*, on attack by avian predators. *Animal Behaviour* 46:609-611.
- Devereux, C. L., E. Fernandez-Juricic, J. R. Krebs, and M. J. Whittingham. 2008. Habitat affects escape behaviour and alarm calling in common starlings *Sturnus vulgaris*. *Ibis* 150:191-198.

- Diaz, M., A. P. Moller, E. Flensted-Jensen, T. Grim, J. Diego Ibanez-Alamo, J. Jokimaki, G. Marko, and P. Tryjanowski. 2013. The geography of fear: a latitudinal gradient in anti-predator escape distances of birds across Europe. *Plos One* 8:e64634.
- Domenici, P., J. M. Blagburn, and J. P. Bacon. 2011. Animal escapology II: escape trajectory case studies. *Journal of Experimental Biology* 214:2474-2494.
- Estes, J. A., J. Terborgh, J. S. Brashares, M. E. Power, J. Berger, W. J. Bond, S. R. Carpenter, T. E. Essington, R. D. Holt, J. B. C. Jackson, R. J. Marquis, L. Oksanen, T. Oksanen, R. T. Paine, E. K. Pikitch, W. J. Ripple, S. A. Sandin, M. Scheffer, T. W. Schoener, J. B. Shurin, A. R. E. Sinclair, M. E. Soule, R. Virtanen, and D. A. Wardle. 2011. Trophic downgrading of planet earth. *Science* 333:301-306.
- Errington, P.L. 1967. *Of predation and life*. Iowa State University, Ames, USA.
- Fields, D. M., J. Yen. 1997. The escape behavior of marine copepods in response to a quantifiable fluid mechanical disturbance. *Journal of Plankton Research* 19:1289-1304.
- Gentle, L., A. Gosler. 2001. Fat reserves and perceived predation risk in the great tit, *Parus major*. *Proceedings of the Royal Society B-Biological Sciences* 268:487-491.
- Gosler, A., J. Greenwood, and C. Perrins. 1995. Predation risk and the cost of being fat. *Nature* 377:621-623.
- Groner, M. L., J. C. Buck, S. Gervasi, A. R. Blaustein, L. K. Reinert, L. A. Rollins-Smith, M. E. Bier, J. Hempel, and R. A. Relyea. 2013. Larval exposure to predator cues alters immune function and response to a fungal pathogen in post-metamorphic wood frogs. *Ecological Applications* 23:1443-1454.
- Hawlena, D., O. J. Schmitz. 2010. Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *American Naturalist* 176:537-556.

- Hawlena, D., H. Kress, E. R. Dufresne, and O. J. Schmitz. 2011. Grasshoppers alter jumping biomechanics to enhance escape performance under chronic risk of spider predation. *Functional Ecology* 25:279-288.
- Hedenstrom, A., M. Rosen. 2001. Predator versus prey: on aerial hunting and escape strategies in birds. *Behavioral Ecology* 12:150-156.
- Ilany, A., D. Eilam. 2008. Wait before running for your life: defensive tactics of spiny mice (*Acomys cahirinus*) in evading barn owl (*Tyto alba*) attack. *Behavioral Ecology and Sociobiology* 62:923-933.
- Irschick, D., J. K. Bailey, J. A. Schweitzer, J. F. Husak, and J. J. Meyers. 2007. New directions for studying selection in nature studies of performance and communities. *Physiological and Biochemical Zoology* 80:557-567.
- Janssens, L., R. Stoks. 2014. Chronic predation risk reduces escape speed by increasing oxidative damage: a deadly cost of an adaptive antipredator response. *Plos One* 9:e101273.
- Janssens, L., R. Stoks. 2013. Predation risk causes oxidative damage in prey. *Biology Letters* 9:20130350.
- Katzir, G., J. M. Camhi. 1993. Escape response of the black mollies (*Poecilia sphenops*) to predatory dives of a pied kingfisher (*Ceryle rudis*). *Copeia* 2:549-553.
- Kenward, R. E. 1978. Hawks and doves: factors affecting success and selection in goshawk attacks on woodpigeons. *Journal of Animal Ecology* 47:449-460.
- Krebs, C. J., S. Boutin, R. Boonstra, A. R. E. Sinclair, J. N. M. Smith, M. R. T. Dale, K. Martin, and R. Turkington. 1995. Impact of food and predation on the snowshoe hare cycle. *Science* 269:1112-1115.
- Kullberg, C. 1998. Does diurnal variation in body mass affect take-off ability in wintering willow tits? *Animal Behaviour* 56:227-233.

- Kullberg, C., D. Houston, and N. Metcalfe. 2002. Impaired flight ability - a cost of reproduction in female blue tits. *Behavioral Ecology* 13:575-579.
- Kullberg, C., S. Jakobsson, and T. Fransson. 1998. Predator-induced take-off strategy in great tits (*Parus major*). *Proceedings of the Royal Society B-Biological Sciences* 265:1659-1664.
- Kullberg, C., T. Fransson, and S. Jakobsson. 1996. Impaired predator evasion in fat blackcaps (*Sylvia atricapilla*). *Proceedings of the Royal Society B-Biological Sciences* 263:1671-1675.
- Kullberg, C., M. Lafrenz. 2007. Escape take-off strategies in birds: the significance of protective cover. *Behavioral Ecology and Sociobiology* 61:1555-1560.
- Lee, S., S. Hwang, Y. Joe, H. Cha, G. Joo, H. Lee, J. Kim, and P. G. Jablonski. 2013. Direct look from a predator shortens the risk-assessment time by prey. *Plos One* 8:e64977.
- Lilliendahl, K. 1998. Yellowhammers get fatter in the presence of a predator. *Animal Behaviour* 55:1335-1340.
- Lilliendahl, K. 1997. The effect of predator presence on body mass in captive greenfinches. *Animal Behaviour* 53:75-81.
- Lima, S. L. 1998. Stress and decision making under the risk of predation: Recent developments from behavioral, reproductive, and ecological perspectives. *Stress and Behavior* 27:215-290.
- Lima, S. L., L. M. Dill. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 68:619-640.
- Lima, S. 1993. Ecological and evolutionary perspectives on escape from predatory attack - a survey of North-American Birds. *Wilson Bulletin* 105:1-47.

- Lima, S. 1986. Predation risk and unpredictable feeding conditions - determinants of body-mass in birds. *Ecology* 67:377-385.
- Lima, S. L. 2009. Predators and the breeding bird: behavioral and reproductive flexibility under the risk of predation. *Biological Reviews* 84:485-513.
- Lind, J., S. Jakobsson. 2001. Body building and concurrent mass loss: flight adaptations in tree sparrows. *Proceedings of the Royal Society B-Biological Sciences* 268:1915-1919.
- Lind, J., U. Kaby, and S. Jakobsson. 2002. Split-second escape decisions in blue tits (*Parus caeruleus*). *Naturwissenschaften* 89:420-423.
- Lind, J., L. Hollen, E. Smedberg, U. Svensson, A. Vallin, and S. Jakobsson. 2003. Detection distance influences escape behaviour in two parids, *Parus major* and *P-caeruleus*. *Journal of Avian Biology* 34:233-236.
- MacLeod, R. 2006. Why does diurnal mass change not appear to affect the flight performance of alarmed birds? *Animal Behaviour* 71:523-530.
- MacLeod, R., C. D. MacLeod, J. A. Learmonth, P. D. Jepson, R. J. Reid, R. Deaville, and G. J. Pierce. 2007b. Mass-dependent predation risk and lethal dolphin porpoise interactions. *Proceedings of the Royal Society B-Biological Sciences* 274:2587-2593.
- MacLeod, R., J. Lind, J. Clark, and W. Cresswell. 2007a. Mass regulation in response to predation risk can indicate population declines. *Ecology Letters* 10:945-955.
- McNamara, J. M., Z. Barta, A. I. Houston, and P. Race. 2005. A theoretical investigation of the effect of predators on foraging behaviour and energy reserves. *Proceedings of the Royal Society B-Biological Sciences* 272:929-934.
- Meager, J. J., P. Domenici, A. Shingles, and A. C. Utne-Palm. 2006. Escape responses in juvenile Atlantic cod *Gadus morhua* L.: the effects of turbidity and predator speed. *Journal of Experimental Biology* 209:4174-4184.

- Møller, A. P. 2008. Flight distance of urban birds, predation, and selection for urban life. *Behavioral Ecology and Sociobiology* 63:63-75.
- Monaghan, P., N. B. Metcalfe, and R. Torres. 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecology Letters* 12:75-92.
- Morrison, E. B. 2011. Vigilance behavior of a tropical bird in response to indirect and direct cues of predation risk. *Behaviour* 148:1067-1085.
- Pennycuik, C. J. 1989. Bird flight performance. A practical calculation manual. Oxford University Press, New York, USA
- Pravosudov, V. V., T. C. Grubb. 1998. Management of fat reserves in tufted titmice *Baeolophus bicolor* in relation to risk of predation. *Animal Behaviour* 56:49-54.
- Preisser, E., D. Bolnick, and M. Benard. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology* 86:501-509.
- Preisser, E. L., D. I. Bolnick. 2008. When predators don't eat their prey: nonconsumptive predator effects on prey dynamics. *Ecology* 89:2414-2415.
- Pritchard, J. A., D. M. Debinski, B. Olechnowski, and R. Vannimwegen. 2006. The landscape of Paul Errington's work. *Wildlife Society Bulletin* 34:1411-1416.
- Samia, D. S. M., D. T. Blumstein. 2015. Birds flush early and avoid the rush: an interspecific study. *Plos One* 10:e0119906.
- Samia, D. S. M., F. Nomura, and D. T. Blumstein. 2013. Do animals generally flush early and avoid the rush? a meta-analysis. *Biology Letters* 9:20130016.
- Schmitz, O. J. 2004. Perturbation and abrupt shift in trophic control of biodiversity and productivity. *Ecology Letters* 7:403-409.
- Schmitz, O., A. Beckerman, and K. OBrien. 1997. Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology* 78:1388-1399.

- Schmitz, O. J., J. H. Grabowski, B. L. Peckarsky, E. L. Preisser, G. C. Trussell, and J. R. Vonesh. 2008. From individuals to ecosystem function: toward an integration of evolutionary and ecosystem ecology. *Ecology* 89:2436-2445.
- Seamone, S., T. Blaine, and T. E. Higham. 2014. Sharks modulate their escape behavior in response to predator size, speed and approach orientation. *Zoology* 117:377-382.
- Sheriff, M. J., C. J. Krebs, and R. Boonstra. 2009. The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. *Journal of Animal Ecology* 78:1249-1258.
- Shifferman, E., D. Eilam. 2004. Movement and direction of movement of a simulated prey affect the success rate in barn owl *Tyto alba* attack. *Journal of Avian Biology* 35:111-116.
- Slos, S., R. Stoks. 2008. Predation risk induces stress proteins and reduces antioxidant defense. *Functional Ecology* 22:637-642.
- Stankowich, T., D. T. Blumstein. 2005. Fear in animals: a meta-analysis and review of risk assessment. *Proceedings of the Royal Society B-Biological Sciences* 272:2627-2634.
- Stewart, W. J., A. Nair, H. Jiang, and M. J. McHenry. 2014. Prey fish escape by sensing the bow wave of a predator. *Journal of Experimental Biology* 217:4328-4336.
- Strobbe, F., M. A. McPeck, M. De Block, and R. Stoks. 2010. Survival selection imposed by predation on a physiological trait underlying escape speed. *Functional Ecology* 24:1306-1312.
- Swaddle, J., M. Witter. 1997. The effects of molt on the flight performance, body mass, and behavior of European starlings (*Sturnus vulgaris*): an experimental approach. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 75:1135-1146.

- Trussell, G. C., P. J. Ewanchuk, and M. D. Bertness. 2003. Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. *Ecology* 84:629-640.
- van den Hout, P. J., K. J. Mathot, L. R. M. Maas, and T. Piersma. 2010. Predator escape tactics in birds: linking ecology and aerodynamics. *Behavioral Ecology* 21:16-25.
- van den Hout, P. J., T. Piersma, A. Dekinga, S. K. Lubbe, and G. H. Visser. 2006. Ruddy turnstones *Arenaria interpres* rapidly build pectoral muscle after raptor scares. *Journal of Avian Biology* 37:425-430.
- van der Veen, I., L. Sivars. 2000. Causes and consequences of mass loss upon predator encounter: feeding interruption, stress or fit-for-flight? *Functional Ecology* 14:638-644.
- Veasey, J. S., D. C. Houston, and N. B. Metcalfe. 2000. Flight muscle atrophy and predation risk in breeding birds. *Functional Ecology* 14:115-121.
- Veasey, J. S., N. B. Metcalfe, and D. C. Houston. 1998. A reassessment of the effect of body mass upon flight speed and predation risk in birds. *Animal Behaviour* 56:883-889.
- Webb, P. W. 1982. Avoidance responses of fathead minnow to strikes by 4 teleost predators. *Journal of Comparative Physiology* 147:371-378.
- Wirsing, A. J., W. J. Ripple. 2011. A comparison of shark and wolf research reveals similar behavioral responses by prey. *Frontiers in Ecology and the Environment* 9:335-341.
- Wirsing, A. J., M. R. Heithaus, and L. M. Dill. 2007. Fear factor: do dugongs (*Dugong dugon*) trade food for safety from tiger sharks (*Galeocerdo cuvier*)? *Oecologia* 153:1031-1040.

- Witter, M., I. Cuthill. 1993. The ecological costs of avian fat storage. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 340:73-92.
- Woolfenden, B. E., H. L. Gibbs, and S. G. Sealy. 2002. High opportunity for sexual selection in both sexes of an obligate brood parasitic bird, the brown-headed cowbird (*Molothrus ater*). *Behavioral Ecology and Sociobiology* 52:417-425.
- Ydenberg, R. C., L. M. Dill. 1986. The economics of fleeing from predators. *Advances in the Study of Behavior* 16:229-249.
- Zanette, L. Y., M. Clinchy, and J. P. Suraci. 2014. Diagnosing predation risk effects on demography: can measuring physiology provide the means? *Oecologia* 176:637-651.
- Zanette, L. Y., A. F. White, M. C. Allen, and M. Clinchy. 2011. Perceived predation risk reduces the number of offspring songbirds produce per year. *Science* 334:1398-1401.

Chapter 2

2.1 Introduction

Prior exposure to predators and predator cues has been shown to affect prey survival tactics through changes in anti-predator behaviour across multiple taxa (Dill 1974; Griffin et al. 2001; Eiben and Persons 2007; Lönnstedt et al. 2012; Gregory 2013). Experience with predators may increase a prey's ability to perceive risk and cause them to modify their escape behaviour, as is widely evident in studies examining the effect of pre-exposure to predators on flight initiation distance (Stankowich and Blumstein 2005), or the distance at which prey flee an approaching predator (Ydenberg and Dill 1986). Other aspects of escape behaviour, such as the changes in escape angle and escape speed that prey adopt when they flee, are less often quantified yet provide important insight into whether an individual will survive a predator attack. For example, Walker et al. (2005) provide evidence that guppies (*Poecilia reticulata*) which escape with higher maximum speeds and increased initial ascent angles have a higher chance of evading pike cichlid (*Crenicichla alta*) strikes.

Altering body condition may also affect the ability of prey to escape a predator attack. Support for this lies in the hypothesis that predation risk is mass-dependent (Witter and Cuthill 1993) and for prey, balancing energy stores helps to minimize the risk of starvation and predation (Lima 1986), whereby carrying larger energy reserves decreases the risk of starvation yet increases the vulnerability to predation (Lima 1986; Witter and Cuthill 1993; Witter et al. 1994). Mass-dependent predation risk theory predicts that predator-induced changes in mass should affect the efficiency at which an individual is able to escape predators (Witter and Cuthill 1993) since large gains in mass

have been found to reduce escape capacity (Witter et al. 1994, Kullberg et al. 1996, Lind et al. 1999; Burns and Ydenberg 2002, Roitberg et al. 2003). Therefore, in the context of altering physiology as an escape decision, prey should opt to adjust their body mass to levels that will provide insurance against starvation without compromising their ability to escape a predator attack.

Despite a vast literature on escape behaviour in birds, the effect of predation risk on behavioural decisions during flight, such as the crucial point of take-off, has not yet been investigated. Understanding take-off behaviour is crucial, as it is the most common strategy of escape for birds when confronted with an attacking predator (Cresswell 1993). While it is evident that birds will alter their take-off behaviour in response to the conditions they face when being attacked (Kullberg et al. 1998; Lind et al. 2002; Lind et al. 2003; Kullberg and Lafrenz 2007; Devereux et al. 2008), determining the effects of predation risk on take-off behaviour may reveal the tactics of escape that birds employ when they sense a greater risk. Take-off behaviour is quantified immediately after an alarmed take-off wherein both take-off angle and speed are included as performance measures (Witter et al. 1994; Kullberg et al. 1996, 1998; Burns and Ydenberg 2002). Birds face a trade-off between these two components as flying at steeper angles reduces linear acceleration (Witter and Cuthill 1993; Kullberg and Lafrenz 2007); thus, changes in take-off behaviour may not necessarily provide a measure of overall flying capacity because they are often not independent of one another. In such situations, it can be beneficial to quantify changes in the energy a bird generates to perform flight by measuring mechanical energy per unit mass to determine flying capacity (Swaddle et al. 1999; Williams and Swaddle 2003). Additionally, many studies also measure flying

capacity in terms of vertical flying speed (Veasey et al. 1998; Kullberg et al. 2002a, 2002b; Criscuolo et al. 2011), which is likely the most physically demanding type of flight as birds are forced to fly straight up against gravity (Kullberg et al. 2002a) and are not allowed to reduce their angle to maintain velocity (Witter and Cuthill 1993). Thus, integrating all of these flight measures into one study may be beneficial in determining the overall effects of predation risk on escape behaviour.

Much of the work focused on escape behaviour in birds is in the context of mass-dependent predation. Since low wing-loading (i.e. body mass/wing area) should maximize lift production by the wings, it is suggested that mass change may alter a bird's ability to escape a predator attack (Witter and Cuthill 1993). Mass-dependent predation risk theory is supported in the migratory fuelling literature, whereby depositing large fat loads in preparation for migration has been found to reduce the angle and/or speed of birds at take-off (Kullberg et al. 1996, 2000; Lind et al. 1999; Burns and Ydenberg 2002). However, studies that investigate mass change due to diurnal mass gain, which is marginal in comparison to migratory fuelling, report no such effects on flying capacity (Kullberg 1998; Kullberg et al. 1998; van der Veen and Sivars 2000; MacLeod 2006), which suggests that birds may be able to compensate for these smaller mass gains. This mounting evidence led MacLeod (2006) to propose that a mass change threshold of >10 % is needed to induce significant effects on flying capacity by affecting a bird's ability to create lift. Yet it is still undetermined as to whether predator-induced mass change, which has been found to fall below this 10 % threshold of body mass change in small birds (Gosler et al. 1995; Lilliendahl 1997; Lilliendahl 1998; Pravosudov and Grubb

1998; van der Veen and Sivars 2000; Gentle and Gosler 2001; Rands and Cuthill 2001), is used by birds as a tactic to enhance their ability to evade predators.

Here, we experimentally test whether brown-headed cowbirds (*Molothrus ater*) elicit changes in escape behaviour and physiology that may improve their probability of survival when subjected to experimentally increased levels of perceived predation risk. All subjects were housed in large outdoor aviaries that negated any chance of direct predation while we quantified changes in escape behaviour and physiological condition between high-risk and low-risk treatment periods. We report that cowbirds gained significant amounts of body mass, in the form of fat, and increased their pectoral muscle thickness in the high predation risk treatment. Cowbirds also changed their take-off behaviour by flying at higher take-off angles and reduced take-off speed when faced with a high-risk predator environment. Despite these predator-induced physiological and behavioural changes, the birds maintained their overall flying capacity between treatments, as they exhibited no changes in either mechanical energy gain at take-off or vertical flying speed. This predation risk manipulation fills an identified gap in the literature by providing the first experimental design that fully incorporates the effects of predator-induced mass change on escape behaviour in birds. We also report the results of a body mass manipulation in the laboratory where we find that the benefits of mass loss on flight capacity may be overestimated since no change in vertical flying speed was found after substantial levels of mass loss. Our results provide evidence that birds cue into the level of predation risk in their environment and enlist a suite of survival tactics that may decrease their probability of predation that should be included when considering the overall effects of predators on prey survival.

2.2 Methods

2.2.1 Study site and species

We captured 80 brown-headed cowbirds from various locations throughout Southern Ontario in April and May 2014 as they returned from migration. Upon capture, each bird was banded with a unique combination of coloured leg bands and tagged with a radio frequency identification (RFID) tag (or PIT, passive integrated transponder, tag) (Phidgets Inc.) fixed to a single leg band with epoxy and string. We housed 10 males and 10 females in each of four large outdoor aviaries (18.25 long x 9.15 wide x 3.65 tall m) located at the Environmental Sciences Western Field Station in London, ON. To control for environmental differences among groups, each of the four aviaries (labeled A, B, C, or D) had a similar landscape complete with multiple perches, shelters, watering and feeding stations, grass, and trees all configured in the same way. The aviaries provided a semi-natural environment in which the birds were free from direct predation. Each of two aviaries (A/B and C/D) were immediately adjacent to each other, but an opaque barrier prevented the birds from seeing one another. Aviaries A/B were separated from aviaries C/D by 150 m, isolating them both visually and acoustically. The birds had *ad libitum* access to a formulated dry diet version of the Bronx-Zoo diet for omnivorous birds (See White et al., 2007) and also foraged for food that is naturally present in the aviaries.

2.2.2 Predation risk manipulation

We manipulated the ambient level of predation risk in the environment using both acoustic and visual stimuli and tested for effects on various components of physiological

condition and escape behaviour. Birds spent one month acclimatizing to the aviaries before we started the predation risk manipulations, which we conducted in June 2014. Birds in each aviary were exposed to either a predator or non-predator treatment for 10 d, followed by 5 d of rest which was followed by the opposite 10 d treatment providing us with a repeated measures design, providing temporal and additional spatial replication. Each pair of aviaries was exposed to the same treatment at the same time.

Acoustic stimuli consisted of audio playback calls of predator or non-predator species, broadcast 24 h per day from two weatherproofed speakers that were mounted to columns inside each aviary. Each treatment was assigned two alternating 24 h playlists, containing randomized compilations of multiple unique calls from eight different species of either predators or non-predators known to occur in Southern Ontario during the breeding season (Table 1; Cornell Lab of Ornithology, online data). We paired calls from each predator species with that of a non-predator species such that there were no significant differences in frequency characteristics (peak: $t_{1,7} = -1.22$, $p = 0.26$; maximum: $t_{1,7} = -0.26$, $p = 0.8$; minimum: $t_{1,7} = -1.56$, $p = 0.16$; range: $t_{1,7} = 0.32$, $p = 0.75$), and the volume of calls were standardized to 80 dB at 1 m. Calls were broadcast at the appropriate time of the day (e.g. daytime for diurnal species), and each audio clip of a species calling at their natural rate was interspersed with a period of silence at a ratio of 1:1.5 (call : silence) during the day and 1:2.3 during the night, to prevent habituation (following Zanette et al. 2011). We also presented visual stimuli consisting of taxidermic mounts of two different predator or non-predator species, which were matched for size and stance (Table 2). The birds in each aviary were exposed to the two different mounts at a randomized time each day during the manipulation period, once between 1100 -

1400h and the other between 1400 - 1700h. Before each presentation, we concealed the mount under an opaque box inside the aviary attached to a pulley and cord leading to a blind positioned outside of the aviary. A researcher located behind the blind would pull the cord to reveal the mount for a 5 min period before re-covering it with the box. The cowbirds clearly reacted to the predator mounts by flying, perching up high away from the mount, and refraining from foraging during presentations.

To further prevent habituation to acoustic and visual stimuli, we repositioned the speakers and mounts to new locations (speakers every 2 d, mounts every 1 d), and presented the stimuli on an “on” versus “off” rotation (following Zanette et al. 2011) wherein stimuli were presented on days 1-4 and 7-8 with off periods interspersed on days 5-6 and 9-10.

2.2.3 Physiological assessment

We offset the start of the manipulation between pairs of aviaries by 2 d, which allowed us to collect our physiological measures on treatment day 9 and 10 for all birds. To do so, we caught the birds in one aviary between 930 h and 1600 h using potter and house traps and transported them via individual cloth bird bags to the field station laboratory. This procedure was repeated on day 10 for the other aviary in the pair. All birds were placed in a small holding cage within 15 min of capture, processed for physiological data, and immediately returned to their aviaries. We quantified measures likely to affect flight including body mass, fat mass, total wet lean mass, pectoral muscle thickness, haematocrit, and glucose levels for each bird. Changes in body mass due to

Table 1 Matched calls used for predator and non-predator treatment playlists. Calls are matched based on broadcast time period and acoustic properties.

Time when broadcast	Matched calls	
	Predator group	Non-predator group
Day	Sharp-shinned hawk (<i>Accipiter striatus</i>)	Killdeer (<i>Charadrius vociferous</i>)
Day	Cooper's hawk (<i>Accipiter cooperii</i>)	Northern flicker (<i>Colaptes auratus</i>)
Day	Red-shouldered hawk (<i>Buteo lineatus</i>)	American robin (<i>Turdus migratorius</i>)
Day	Red-tailed hawk (<i>Buteo jamaicensis</i>)	Yellow-rumped warbler (<i>Setophaga coronata</i>)
Day	American kestrel (<i>Falco sparverius</i>)	Cedar waxwing (<i>Bombycilla cedrorum</i>)
Night	Eastern screech-owl (<i>Megascops asio</i>)	Common loon (<i>Gavia immer</i>)
Night	Northern saw-whet owl (<i>Aegolius acadicus</i>)	Wood frog (<i>Lithobates sylvatica</i>)
Night	Barred owl (<i>Strix varia</i>)	Northern leopard frog (<i>Rana pipiens</i>)

Table 2 Matched mounts displayed during predator and non-predator treatment periods. Mounts were matched for size and stance.

Matched mounts	
Predator group	Non-predator group
Cooper's hawk (<i>Accipiter cooperii</i>)	Northern flicker (<i>Colaptes auratus</i>)
Red-shouldered hawk (<i>Buteo lineatus</i>)	Northern Pintail (<i>Anas acuta</i>)

increases in predation threat are typically ascribed (though have not been directly measured) to changes in fat stores (Gentle and Gosler 2001), which are assumed to affect a bird's flying capacity. Pectoral muscle mass, but not lean mass, has been shown to increase in birds faced with heightened predation risk (van den Hout et al. 2006). Haematocrit levels may decrease in response to increased predation risk (Clinchy et al. 2004); however, Saino et al. (1997) found higher hematocrit levels in birds that were faced with increased energetic demands of flight. Blood glucose levels may be expected to increase during times of higher predation risk to help power muscles during take-off flight because stressed animals should have a greater ability to mobilize energy for immediate muscle use (Boonstra et al. 1998).

We calculated average body mass to 0.5 g using a spring scale (Lightline Spring Pesola Scale, 100g). Total fat and wet lean mass were measured using Quantitative Magnetic Resonance (hereafter QMR; Echo MRI-B; Echo-Medical Systems, Houston, TX, USA; Guglielmo et al. 2011). The QMR scanner was calibrated before experiment initiation, and scanner performance was verified at the beginning of each treatment day (following Guglielmo et al. 2011). Two scans were made using the two accumulation and 'small bird' scanning settings of the QMR (2 min duration per scan) and were averaged to obtain a single measurement of wet lean mass and fat mass for each bird. After scanning, a digital photograph of the right wing (positioned against a scaled board) was taken. Wing area was calculated in cm^2 from the images using the software program ImageJ (Rogers 2015) in order to calculate wing-load (i.e. body mass/wing area; expressed as g/cm^2) for each bird. Subsequently, 75 μL of blood was collected from the brachial vein and glucose was immediately measured using a glucose meter (Contour

Next EZ meter; Bayer AG, Leverkusen, Germany; Clinchy et al. 2004). The time between capture and blood collection was 30 min on average and hence unlikely to affect blood glucose levels, as Ramage-Healey and Romero (2000) found no effect on glucose levels even 30 min after a stress response during daytime in the summer photoperiod. Following collection, blood samples were stored in a cooler for transport. Haematocrit was measured from each sample after centrifugation. We calculated average pectoral muscle thickness from two measurements taken of the left pectoral muscle using a portable ultrasound (LOGIC Book XP Vet; GE Healthcare, Milwaukee, WI, USA) with an 8L-RS linear probe at 10 MHz. Using scanning gel as a medium between the probe and the skin of the bird, the probe was placed transversally on the left pectoral muscle 90° from the rostral top of the sternum to the shoulder (Dietz et al. 1999). Pectoral muscle thickness was measured at a 45° angle from the sternal keel, starting at the junction of the horizontal sternum and the sternal keel to the top of the pectoral muscle (0.1 mm) (Swanson and Merkord 2013).

2.2.4 Escape behaviour

We examined escape behaviour in two separate contexts. We measured the take-off behaviour and flying capacity of the birds in the aviaries after they faced a simulated predator attack while feeding at a specially designed take-off apparatus. Additionally, we estimated a bird's flying capacity by measuring flying speed in a vertical flight chamber in the laboratory before their physiological condition was assessed at the end of each treatment.

Fleeing from a predator: take-off behaviour and flying capacity

We measured take-off behaviour and flying capacity in the aviaries during two trial periods, once on day 4 and once on day 5 of the treatment period. To do so, we designed an automated feeder system situated within our take-off apparatus (see below). Whenever a bird landed on a wooden perch to feed, an RFID reader (RFID Read-Write; Phidgets Inc., Calgary, AB, Canada) would record the individual's identity and its body mass was logged and recorded by a micro load cell (0-780 g; Phidgets Inc., Calgary, AB, Canada) attached to the perch. Each device was calibrated immediately before take-off trials. This automated system effectively eliminated the need for recapture and handling, which can cause unwanted shifts in body mass (MacLeod and Gosler 2006) during the take-off measurement period. We confirmed that the body mass levels estimated with our automated system on days 4 and 5 of each treatment period showed a strong and significant relationship with the body mass measurements we took at the end of both the control (linear regression: $R^2 = 0.92$, $F_{1,48} = 530.2$, $p < 0.001$) and the predator treatments (linear regression: $R^2 = 0.82$, $F_{1,46} = 221.7$, $p < 0.001$) on days 9-10.

We conducted take-off behaviour trials using specially designed apparatuses constructed of two parallel vertical 1-m² walls attached perpendicularly to a 1-m² wooden base (see Appendix A for picture of setup). The walls were placed 45 cm apart to ensure that the birds would engage in straight-line horizontal and vertical flight. The back wall was painted white and mounted with the automated feeder system. The front wall consisted of a transparent acrylic sheet marked every 2.54 cm to serve as a scale for measuring vertical and horizontal displacement during flight. When a bird landed on the perch to feed, a researcher positioned behind a blind outside of the aviary would pull a

string to raise a spring-loaded flag, which was attached to the side of the apparatus, towards the bird thereby initiating take-off.

Take-off events were recorded using digital video recorders (Swann DVR4-3425, 30 frames/s) positioned perpendicular to each flight apparatus. We analyzed the first 0.2 s (6 frames) of each take-off event (Kullberg 1998; MacLeod 2006). Vertical and horizontal displacements (to the nearest 1.27 cm) and associated time (frame count) were measured relative to the 2.54 cm transparent grid using the center of the head as the reference point for all calculations (Chin et al. 2009). We calculated three measurements of escape behaviour for the first 0.2 s of flight that described the birds' take-off behaviour and flying capacity. Take-off behaviour was assessed by measuring both take-off angle ($^{\circ}$) and speed (m/s). Take-off flying capacity was assessed by measuring mechanical energy change per unit mass E (J/kg), which estimates the energy (in the Newtonian sense) expended by a bird in order to power flight. Mechanical energy change is composed of kinetic and potential energy taking into account, in one measure, the height gained during flight and the vertical and horizontal components of flight velocity (Swaddle et al. 1999). We calculated this measure using the equation from Williams and Swaddle (2003), $E = \frac{1}{2} (V_x^2 + V_z^2) + gz$, where V_x and V_z are the vertical and horizontal units of flight velocity, respectively, g is the acceleration due to gravity, and z is height (Williams and Swaddle 2003). As it suggests a higher input of energy, relatively higher levels of mechanical energy gain indicate stronger flying capacity. We used the fastest flight performed by each bird (following Chin et al. 2009).

Outdistancing a predator: flying capacity as measured by vertical flying speed

We assessed the birds' capacity to maintain its distance from an attacking predator by measuring their vertical flying speed when required to fly directly against the force of gravity at an angle of 180°. We reasoned that this would represent the ultimate challenge of flight and would reveal a bird's current capacity to escape a predator, because any reduction in flying speed would be indicative of poorer flying capacity and, consequently, an increase in predation risk. Since individuals are not able to adjust their angle of flight under these conditions, any change in vertical flying speed results in an equal change in mechanical energy. Therefore, vertical flying speed is, in fact, a measure of mechanical energy whereby relatively high speeds equal relatively more energy generated to power flight.

We measured flying speed prior to physiological assessment on treatment days 9 and 10 using a vertical flight chamber (see Appendix B for setup), which we built by integrating the designs of Kullberg et al. (2002a) and Chin et al. (2009). The vertical chamber consisted of a metal frame (200 cm x 40 cm x 40 cm) enclosed by white wallboard and a transparent acrylic sheet — permitting observation through the front. A perch was inserted into a box topped with fine netting and placed atop the chamber where the birds were collected after each flight. A 2.54 cm grid was superimposed onto the acrylic surface to create a scale of reference to measure vertical displacement during flights. Cowbirds were introduced into the chamber from the bottom, via a tube (approximately 30 cm in length and 10 cm in diameter), emerging at an upward angle of approximately 30° (Chin et al. 2009). We flew each bird twice, with a 2 min rest period in between, and used the fastest measure in our analyses (Following Chin et al. 2009).

All vertical flying speed trials were recorded using a digital video recorder (Swann DVR4-3425, 30 frames/s) placed perpendicular to the flight chamber. We measured flying speed starting 21 cm upwards from where the birds emerged (i.e. 50 cm from the bottom of the chamber), which is equivalent to the length of approximately one wing beat (Chin et al. 2009). Using the center of the head as a reference point (Chin et al. 2009), we counted the number of frames it took for each bird to reach a vertical displacement of 100 cm from the starting point. We ceased measurement 50 cm from the top of the chamber to ensure that birds were not decelerating at the end of flight, which is conservative for such measures (Kullberg et al. 2002a). We used these parameters to calculate flying speed (m/s) for the middle 100 cm section of the chamber.

2.2.5 Body mass manipulation

MacLeod (2006) speculated that large changes in mass (>10 %), whether increases or decreases, are required before flying capacity would be significantly affected. Studies investigating natural gains in mass (e.g. diurnal mass gain, mass gain in preparation for migration) support this threshold effect; however, no study has investigated whether this threshold holds up when mass is lost, thereby determining whether mass loss does indeed provide birds with more lift. We tested whether mass losses of over 10 % could indeed affect flight by conducting a body mass manipulation of captive birds kept at the Advanced Facility for Avian Research, University of Western Ontario. Sixty cowbirds were transported from the outdoor aviaries, housed in individual cages (46 long x 76 wide x 46 tall cm), and divided into three treatment groups with equal sex ratios: (i) a control where the body mass of birds was maintained (N = 20); (ii) a 5 % body mass loss group (N = 20), which is similar to mass changes commonly

experienced by birds from dawn to dusk (MacLeod 2006), and below the threshold hypothesized as being necessary to affect flying capacity, and; (iii) a 15 % body mass loss group (N = 20), which was above the hypothesized threshold.

We initially fed all the birds *ad libitum* until they had acclimated to the lab and their mass had stabilized. At this point, individuals were removed from their cage, and we obtained basal, pre-manipulation measures of vertical flying speed, body mass, total fat mass and wet lean mass following the same protocols as in the predation risk experiment. At this point, we continued to provide *ad libitum* food for the control group, but reduced mass for the two other treatment groups by gradually reducing the quantity of food that birds received each day. If a bird reached their target mass before others, its mass was maintained until all individuals in the experiment had reached their targeted body mass. At this time, birds were re-assessed for post-manipulation levels of flying capacity by measuring flying speed in the vertical chamber and total fat and wet lean mass with QMR. All baseline and post manipulation measures were assessed for all birds on the same day. After completion, birds were fed *ad-libitum* for 5 d and released.

2.2.6 Statistical analyses

All physiological and escape behaviour measures were analyzed using linear mixed models (LMM) that included treatment as a repeated measures term and sex as a fixed factor with individual identity nested within aviary as a random effect. For all physiological variables and vertical flying speed, our sample size was 75 as we were unable to capture 5 birds for processing. To examine whether take-off behaviour and its associated mechanical energy gain were related to physiology, we re-ran the LMMs but

included body mass, wing-loading and pectoral muscle thickness as covariates and examined the relationships amongst these physiological components and all take-off flight variables.

We examined whether an individual's mass change was related to variation in its vertical flying speed between treatments by testing the covariate relationships between changes in flying speed (i.e. $\% \text{ Change Flight} = ((\text{flying speed}_{\text{predator}} - \text{flying speed}_{\text{control}}) / \text{flying speed}_{\text{control}}) * 100$) and changes in body mass (i.e. $\% \text{ Change Mass} = ((\text{body mass}_{\text{predator}} - \text{body mass}_{\text{control}}) / \text{body mass}_{\text{control}}) * 100$). We tested this relationship by conducting an ANCOVA with sex as a fixed factor and both $\% \text{ Change Mass}$ and $\% \text{ Change Mass}^2$ as covariates. We conducted an identical analysis examining the relationship between changes in flying speed and changes in pectoral muscle thickness. We report any significant or meaningful covariate results with R^2 values, and if a significant $\% \text{ Change Mass} \times \text{Sex}$ interaction term was reported, we ran analyses on each sex individually. To further investigate the relationship between flying speed and pectoral muscle thickness, we used Spearman rank correlations to compare pectoral muscle thickness with flying speed within each treatment.

For the body mass manipulation, we analyzed the percentage change in all physiological variables and flying speed (e.g. $\% \text{ Change Fat} = ((\text{fat mass}_{\text{post-manipulation}} - \text{fat mass}_{\text{baseline}}) / \text{fat mass}_{\text{baseline}}) * 100$) between the three manipulation treatments (i.e. control, 5 % mass loss, and 15 % mass loss) using two-way ANOVAs that included sex and manipulation treatment as fixed factors. Four birds died during the manipulation due to causes unrelated to food manipulation, resulting in final sample sizes of $N = 17, 19, 20$ in the control, 5 % mass loss and 15 % mass loss treatments, respectively. Five

individuals did not fly straight up in the vertical flight chamber during vertical flight trials and were removed from the flying speed analysis.

As with the predation risk manipulation, we examined whether an individual's mass change was related to variation in its vertical flying speed between baseline and post-manipulation periods by testing the covariate relationships between changes in flying speed (i.e. $\% \text{ Change Flight} = ((\text{flying speed}_{\text{post-manipulation}} - \text{flying speed}_{\text{baseline}}) / \text{flying speed}_{\text{baseline}}) * 100$) and changes in body mass (i.e. $\% \text{ Change Mass} = ((\text{body mass}_{\text{post-manipulation}} - \text{body mass}_{\text{baseline}}) / \text{body mass}_{\text{baseline}}) * 100$). We tested this relationship by conducting an ANCOVA with sex as a fixed factor and both $\% \text{ Change Mass}$ and $\% \text{ Change Mass}^2$ as covariates. Again we report our covariate results with R^2 values, and if a significant $\% \text{ Change Mass} \times \text{Sex}$ interaction term was reported, we ran analyses on each sex individually.

We used parametric tests on data that had homogeneous variances and normal error distributions, and applied Box Cox transformations when necessary. Otherwise non-parametric tests were used. All statistical analyses were conducted using Statistica 6.0 (StatSoft, Tulsa, Oklahoma, U.S.A) and SPSS Statistics for Macintosh 22.0 (IBM, Armonk, NY, USA). All figures and statistical tests report means of untransformed data \pm 1 standard error.

2.3 Results

2.3.1 Predation risk manipulation

Physiological responses

Exposure to elevated predation risk substantially affected the physiological condition of the cowbirds (Fig 1). Cowbirds carried a significantly greater amount of body mass when in the high predator risk environment compared to the non-predator control (Fig 1a; LMM: Treatment, $F_{1,65.89} = 9.8$, $p = 0.003$). Both sexes showed the same pattern of response to predation risk (Treatment x Sex, $F_{1,65.89} = 0.6$, $p = 0.44$), though as expected, males were significantly heavier ($50.49 \text{ g} \pm 0.44$) than were females overall ($40.84 \text{ g} \pm 0.41$; Sex; $F_{1,71.98} = 258.9$, $p = 0.001$). This mass gain response to predation risk led to a significant increase in wing-loading ($0.461 \pm 0.006 \text{ g/cm}^2$) compared to the non-predator control ($0.452 \pm 0.005 \text{ g/cm}^2$; LMM: Treatment, $F_{1,63.16} = 11.2$, $p = 0.001$; Sex, $F_{1,69.87} = 0.3$, $p = 0.61$; Treatment x Sex, $F_{1,63.16} = 1.0$, $p = 0.3$), which would be expected to render flight more difficult for the birds experiencing high predation risk.

The predator-induced mass gains that the cowbirds exhibited were due to increased body fat and not changes in lean tissue. Cowbirds gained a significant amount of fat when exposed to the high rather than low predator risk environment (Fig 1b; LMM: Treatment, $F_{1,64.27} = 15.1$, $p = 0.001$), regardless of sex (Treatment x Sex, $F_{1,64.27} = 0.01$, $p = 0.91$; Sex, $F_{1,61.13} = 0.1$, $p = 0.81$). By contrast, total wet lean mass remained constant regardless of the level of predation risk (Fig 1c; LMM: Treatment, $F_{1,65.42} = 0.003$, $p = 0.96$) for both sexes (Treatment x Sex, $F_{1,65.42} = 1.4$, $p = 0.24$), though

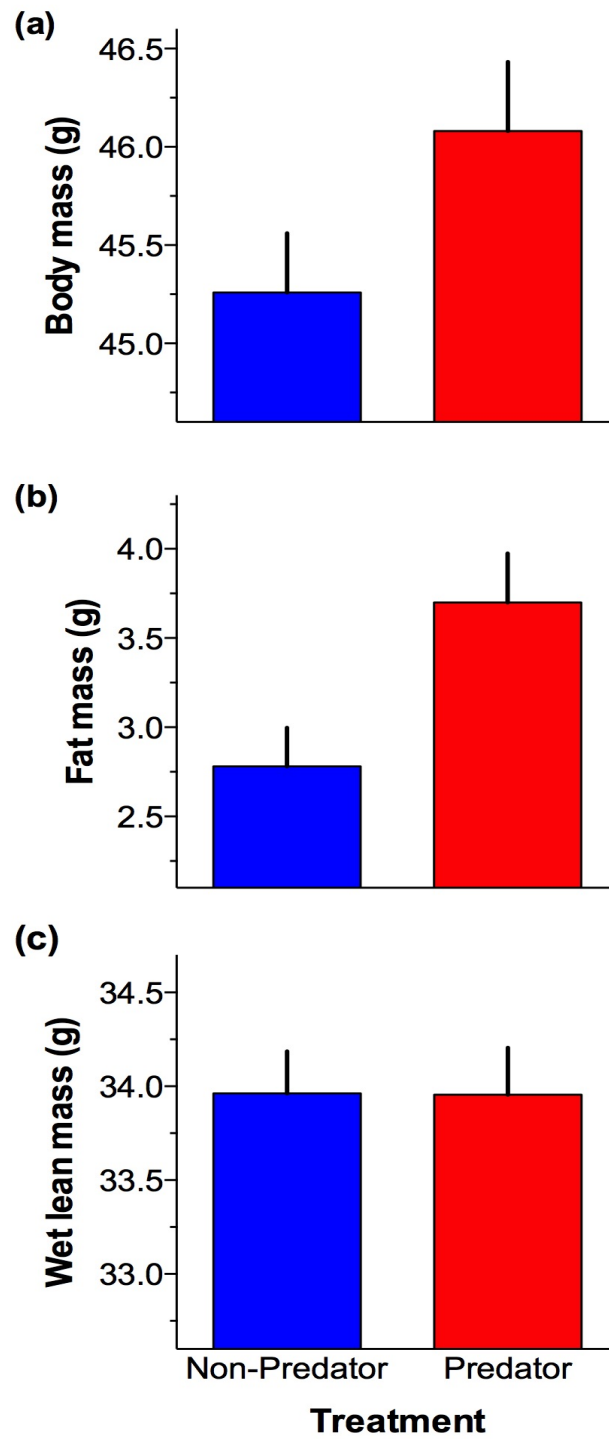


Figure 1 Change in cowbird body condition \pm SE between non-predator (blue) and predator (red) manipulations of (a) body mass: $F_{1,65.89} = 9.8$, $p = 0.003$ (b) fat mass: $F_{1,64.27} = 15.1$, $p = 0.001$ and (c) wet lean mass: $F_{1,65.42} = 0.003$ $p = 0.96$

males carried a significantly higher total wet lean mass (37.89 ± 0.33 g) than did females overall (30.02 ± 0.32 g; LMM: Sex, $F_{1,72.58} = 294.1$, $p = 0.001$).

Despite the lack of a treatment effect on total wet lean mass, pectoral muscle thickness significantly increased in the high predator risk environment (7.72 ± 0.06 mm) compared to the control (7.57 ± 0.05 mm; LMM: Treatment, $F_{1,68.64} = 5.6$, $p = 0.02$), with both sexes displaying similar gains (Treatment x Sex, $F_{1,68.64} = 0.3$, $p = 0.61$). Overall, males had thicker pectoral muscles (7.88 ± 0.06 mm) than females (7.41 ± 0.06 mm; LMM: Sex, $F_{1,71.89} = 33.4$, $p = 0.001$). Elevating perceived predation risk did not lead to significant changes in either haematocrit (LMM: Treatment, $F_{1,66.66} = 0.4$, $p = 0.53$; Sex, $F_{1,70.93} = 0.5$, $p = 0.48$; Treatment x Sex, $F_{1,66.66} = 0.2$, $p = 0.66$) or blood glucose (LMM: Treatment, $F_{1,67.67} = 0.2$, $p = 0.66$; Sex, $F_{1,72.71} = 0.5$, $p = 0.47$; Treatment x Sex, $F_{1,67.67} = 0.4$, $p = 0.53$).

Fleeing from a predator: take-off behaviour and flying capacity

Our two distinct predation risk environments affected the take-off behaviour of cowbirds when they were confronted with an immediate threat (i.e. a flag being raised). When in a high predation risk environment, cowbirds took-off at a significantly steeper angle than when they experienced a low predation risk environment (Fig 2a; LMM: Treatment, $F_{1,50.68} = 6.5$, $p = 0.01$), regardless of sex (Sex, $F_{1,53.44} = 1.9$, $p = 0.18$; Treatment x Sex, $F_{1,50.68} = 1.1$, $p = 0.30$). Consistent with a trade-off between take-off angle and take-off speed, both male and female cowbirds took-off at a significantly reduced speed in the predator treatment compared to the non-predator control (Fig 2b; LMM: Treatment, $F_{1,40.90} = 4.8$, $p = 0.035$; Sex, $F_{1,49.76} = 0.05$, $p = 0.83$; Treatment x

Sex, $F_{1,40.90} = 2.4$, $p = 0.13$). While cowbirds changed their take-off behaviour, they retained their flying capacity at take-off, as we found no significant effects on the mechanical energy generated to power flight when birds experienced the predator (3.35 ± 0.13 J/kg) versus the non-predator treatment (3.21 ± 0.15 J/kg; LMM: Treatment, $F_{1,37.02} = 0.608$, $p = 0.440$; Sex, $F_{1,51.44} = 1.121$, $p = 0.295$; Treatment \times Sex, $F_{1,37.02} = 0.004$, $p = 0.951$). Body mass, wing-loading, and pectoral muscle thickness were not associated with an individual's take-off angle, take-off speed, or mechanical energy change (Covariate relationship, $p > 0.10$ in all cases).

Outdistancing a predator: flying capacity as measured by vertical flying speed

Our assessment of a cowbird's ability to outdistance a predator revealed that they were able to maintain their flying capacity regardless of the level of predation risk they had experienced. When required to fly straight up, cowbirds flew at nearly identical speeds in both the high (1.82 ± 0.03 m/s) and low (1.81 ± 0.03 m/s) predator risk environments (LMM: Treatment, $F_{1,63.99} = 0.02$, $p = 0.90$; Sex, $F_{1,69.83} = 0.6$, $p = 0.43$; Treatment \times Sex, $F_{1,63.99} = 1.2$, $p = 0.28$). Therefore, while increases in predation risk did lead to an increase in body mass, and thus wing-loading, flying capacity, as measured by vertical flying speed, was not compromised. To further investigate this surprising finding, we examined whether the degree to which an individual altered their body mass in response to elevated risk (% Change Mass) was related to how fast they flew (% Change Flight). We found a negative trend in this relationship (% Mass Change, $R^2 = 0.12$, $F_{1,59} = 3.2$, $p = 0.08$), with mass change significantly affecting one sex differently than the other (% Mass Change \times Sex, $F_{1,59} = 4.34776$, $p = 0.041$).

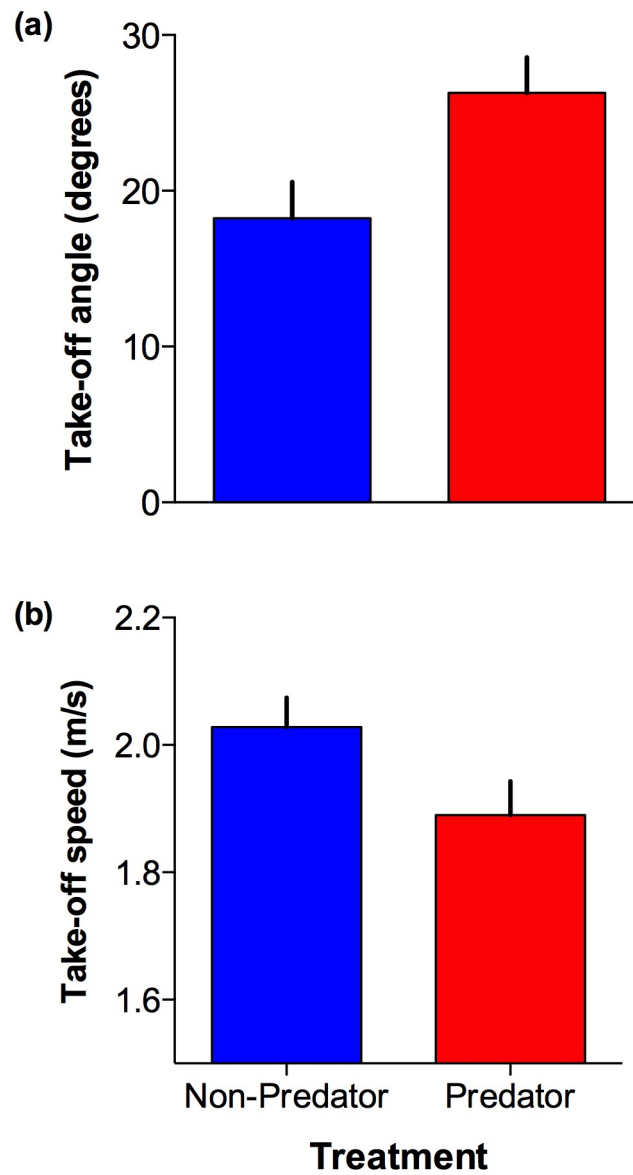


Figure 2 Change in cowbird take-off behaviour between non-predator (blue) and predator (red) manipulations of (a) take-off angle: $F_{1,50.68} = 6.5$, $p = 0.01$ and (b) take-off speed: $F_{1,40.90} = 4.8$, $p = 0.035$

Specifically, when faced with vertical flight, females that gained the most body mass in the predator treatment flew worse, showing a significantly reduced flying capacity as measured by their vertical flying speed (Fig 3a; % Mass Change, $R^2 = 0.19$, $F_{1,34} = 7.7$, $p = 0.009$). Male cowbirds, however, showed no such relationship (Fig 3b; % Mass Change, $R^2 = 0.002$, $F_{1,25} = 0.04$, $p = 0.84$), as their vertical flying speed was completely unaffected by how much mass they gained or lost. No relationship between changes in flying speed and pectoral muscle thickness were found (% PMT Change, $F_{1,58} = 1.4$, $p = 0.23$; Sex, $F_{1,58} = 0.02$, $p = 0.88$; % PMT Change x Sex, $F_{1,58} = 0.6$, $p = 0.43$). Additionally, pectoral muscle thickness was not related to vertical flying speed in either the predator (Spearman $r = 0.17$, $t_{66} = 1.46$, $p = 0.15$) or control treatment (Spearman $r = -0.13$, $t_{67} = -0.80$, $p = 0.34$).

Our results thus far indicate that the cowbirds increase their mass significantly when predation risk is heightened, and such increases do compromise vertical flying speed at least for females. Nonetheless, our predation risk treatments had no overall effect on vertical flying speed whatsoever. MacLeod (2006) speculated that only changes in body mass that exceeded 10 % would translate into effects on flight. The magnitude of the body mass change that we found between our treatments fell below this threshold, as we found increases in body mass by 2.54 % for females and 1.22 % for males. Therefore, we conducted a body mass manipulation experiment to test whether a greater perturbation in mass would indeed affect flight.

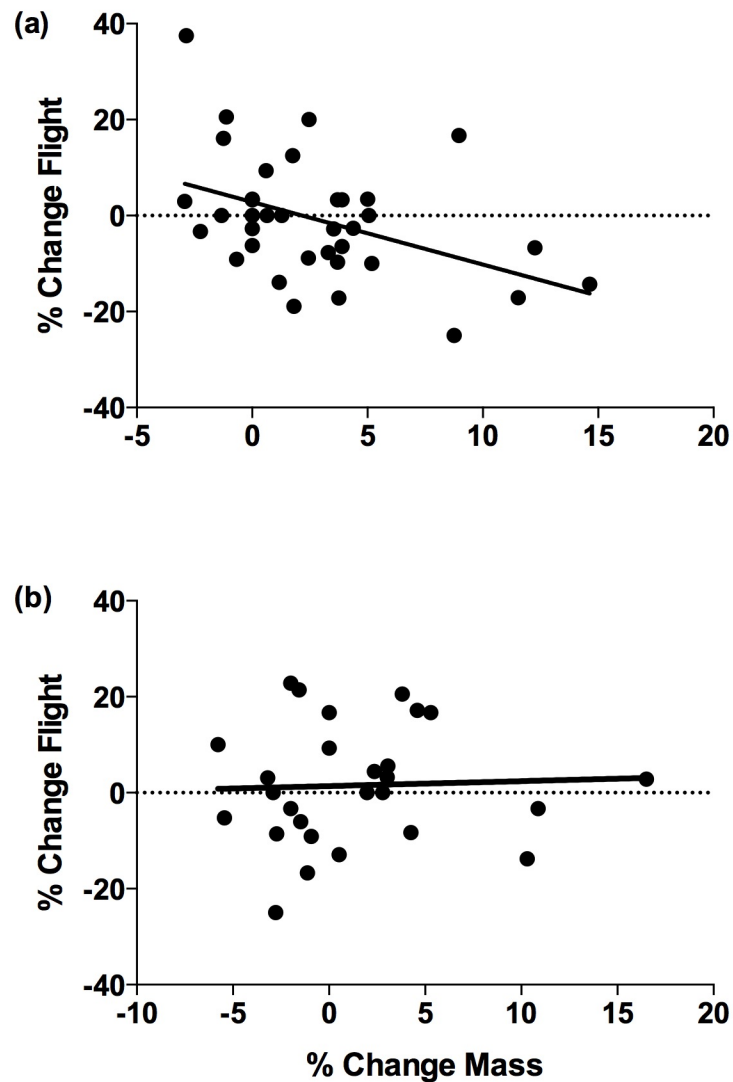


Figure 3 Relationship between percent change in vertical flying speed ($(\text{flying speed}_{\text{predator}} - \text{flying speed}_{\text{control}}) / \text{flying speed}_{\text{control}} * 100$) and percent change in body mass ($(\text{body mass}_{\text{predator}} - \text{body mass}_{\text{control}}) / \text{body mass}_{\text{control}} * 100$) in the predation risk manipulation for (a) female cowbirds: $R^2 = 0.19$, $F_{1,34} = 7.7$, $p = 0.009$ and (b) male cowbirds: $R^2 = 0.002$, $F_{1,25} = 0.04$, $p = 0.84$. The dotted line indicates no change in flying speed between the two manipulation periods. Each dot represents an individual cowbird.

2.3.2 *Body mass manipulation*

Physiological responses

Our three distinct body mass manipulation treatments led to considerable effects on the physiology of the cowbirds. Compared to the baseline, our control birds showed a slight increase in mass (2.34 ± 1.02 %) after the manipulation period, while the 5 % mass loss group experienced a moderate body mass reduction (-6.42 ± 0.97 %), and the 15 % mass loss group experienced a large body mass reduction (-16.93 ± 0.94 %; Two-way ANOVA: Treatment, $F_{2,50} = 96.5$, $p < 0.01$; Sex, $F_{1,50} = 0.000$, $p = 1.00$; Treatment x Sex, $F_{2,50} = 1.4$, $p = 0.25$; see Appendix C for a table showing actual values for the body mass manipulation). In terms of fat mass, the control group showed a gain of 63.47 ± 15.4 %, those in the 5 % mass loss group moderately decreased fat by -11.30 ± 14.58 %, while birds in the 15% mass loss group experienced the largest decrease in fat mass (-62.79 ± 14.17 %; Two-way ANOVA: Treatment, $F_{2,50} = 18.2$, $p < 0.01$; Sex, $F_{1,50} = 0.8$, $p = 0.39$; Treatment x Sex, $F_{2,50} = 0.8$, $p = 0.42$). Total wet lean mass was also affected as the control group lost a small amount of lean mass over the manipulation period (-2.72 ± 0.89 %), the birds in the 5 % mass loss group experienced a moderate loss (-7.91 ± 0.85 %), and the 15 % mass loss group lost the highest percentage of lean mass (-11.38 ± 0.82 %; Two-way ANOVA: Treatment, $F_{2,50} = 25.2$, $p < 0.01$; Sex, $F_{1,50} = 0.3$, $p = 0.88$; Treatment x Sex, $F_{2,50} = 0.2$, $p = 0.81$).

Vertical flying speed

Even though we altered the body mass of cowbirds by up to 15 % on average, vertical flying speed was not affected, as no significant changes were found between the

baseline and post-manipulation periods in the control ($-0.43 \pm 5.08\%$), the 5% reduction ($0.48 \pm 4.54\%$) and the 15% reduction groups ($2.12 \pm 4.56\%$; Two-way ANOVA: Treatment, $F_{2,45} = 0.1$, $p = 0.93$; Treatment x Sex, $F_{2,45} = 1.2$, $p = 0.32$). Males did tend to show an increase in their flying speed ($5.72 \pm 3.78\%$) and females a decrease between the baseline and post-manipulation periods ($-4.29 \pm 3.94\%$), but the differences were not statistically significant (Sex, $F_{2,45} = 3.4$, $p = 0.07$). At the individual level, we found associations between percent changes in body mass and flying speed that are comparable to our predation risk manipulation. Percent change in body mass over the manipulation period showed a significant and negative relationship with flying speed (% Mass Change, $R^2 = 0.24$, $F_{1,43} = 5.8$, $p = 0.02$), and once again affected females and males differently (% Mass Change x Sex, $F_{1,43} = 6.4$, $p = 0.02$). Changes in body mass and flying speed exhibited a quadratic relationship for females (Fig 4a; % Mass Change, $F_{1,22} = 9.1$, $p = 0.006$; % Mass Change², $R^2 = 0.30$, $F_{1,22} = 5.8$, $p = 0.025$) but no relationship for males (Fig 4b; % Mass Change, $R^2 = 0.02$, $F_{1,21} = 0.009$, $p = 0.93$). Figure 4a indicates that for females, no amount of mass loss, even up to a 20% reduction, led to an appreciable change in vertical flying speed. As females increasingly gained mass, however, their vertical flying speed was increasingly compromised.

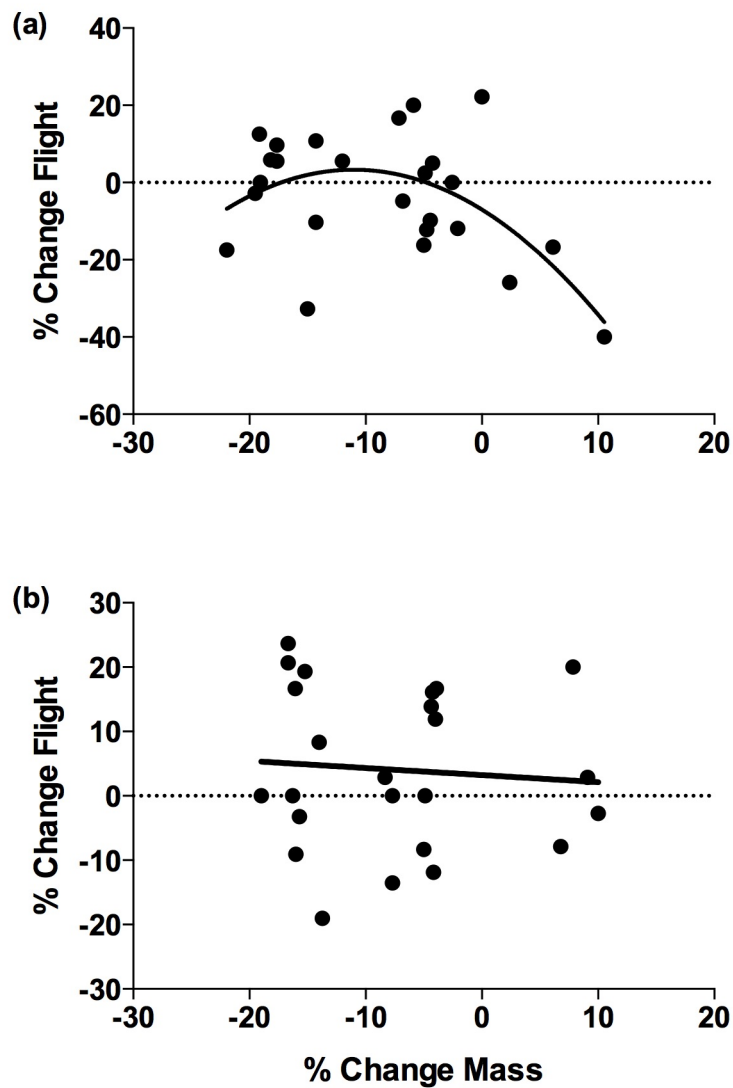


Figure 4 Relationship between percent change in vertical flying speed ($(\text{flying speed}_{\text{post-manipulation}} - \text{flying speed}_{\text{baseline}}) / \text{flying speed}_{\text{baseline}} * 100$) and percent change in body mass ($(\text{body mass}_{\text{post-manipulation}} - \text{body mass}_{\text{baseline}}) / \text{body mass}_{\text{baseline}} * 100$) in the body mass manipulation for (a) female cowbirds: $R^2 = 0.30$, $F_{1,22} = 5.8$, $p = 0.025$ and (b) male cowbirds: $R^2 = 0.02$, $F_{1,21} = 0.009$, $p = 0.93$. The dotted line indicates no change in flying speed between the two manipulation periods. Each dot represents an individual cowbird.

2.4 Discussion

Our experimental results demonstrate, for the first time, that an increase in predation risk causes physiological changes in birds and alters their take-off behaviour while escaping from a predator without compromising their overall flying capacity. The high predation risk environment induced a significant change in body condition, as cowbirds gained body mass, through gains in fat (Fig 1), and increased their pectoral muscle thickness. Experiencing a high level of predation risk also caused the birds to flee at a much steeper angle (nearly 10° steeper) but at reduced speeds (Fig 2). The fact that neither take-off angle nor speed was associated with any predator-induced physiological responses suggests that these shifts in escape behaviour were dependent on the level of perceived predation risk alone. When subjected to high predation risk the birds retained their flying capacity, maintaining both their ability to power their flight at take-off (i.e. mechanical energy gain) and to power their vertical flying speed. Additionally, our body mass manipulation demonstrated that no amount of mass loss provided birds with better lift, as we saw no changes in flying capacity as measured by vertical flying speed even after a 15 % loss in body mass. Even though we found no overall effects of mass change on flying capacity in either manipulation, mass gain did affect vertical flying speeds at an individual level. Females that gained relatively large amounts of mass experienced a reduction in vertical flying speed in both manipulations (Figs 3a, 4a). Males, however, flew with the same speed regardless of whether they increased or decreased mass (Figs 3b, 4b).

The relationships among predation risk, predator-induced physiological changes, and escape behaviour that we report here suggest that birds may adopt at least three

tactics to enhance their chances of survival in response to heightened predation risk. First, mass gain is expected to reduce starvation risk when the foraging environment becomes more unpredictable due to the presence of predators (Lilliendahl 1998; McNamara et al. 2005). Second, a steeper take-off angle is known to be effective in diverting away from the attack trajectory of a predator, likely enhancing the chance of escape when faced with a surprise attack (Shifferman and Eilam 2004). Third, birds temper their behavioural and physiological responses to predation risk in order avoid compromising their flying capacity while simultaneously limiting their risk of starvation. Furthermore, the results from our body mass manipulation suggest that mass loss is unlikely to be a survival tactic that birds use to improve their chances of evading predators since it likely results in a increased risk of starvation without providing any benefit flying capacity. Rather, when birds decrease their mass in response to increased predation risk, it is likely a consequence of other predator-induced behavioral and physiological changes.

Regarding the first survival tactic, gaining mass may be a general and likely approach that birds use when faced with increased predation risk to buffer their energy reserves when predators impose limitations on their foraging opportunities (MacLeod et al. 2007), thereby adopting an “interrupted foraging” response to reduce starvation risk in an unpredictable foraging environment. Predators limit the access prey have to food by causing prey to avoid foraging in times and places of increased risk (McNamara et al. 2005; MacLeod et al. 2007). This predator-induced reduction in foraging promotes an increase in starvation risk. In this case, an increase in body mass due to increases in energy stores (i.e. fat) would allow animals to ensure they have enough resources to

prevent starvation when their foraging may be frequently interrupted by predators (Lima 1986; Houston and McNamara 1993; McNamara et al. 2005, MacLeod et al. 2007). In our manipulation, birds in the high-risk predator treatment gained body mass in the form of fat rather than wet lean mass (Fig 1), which indicates that they invested more in gaining energy than lean tissue. This predator-induced mass response is consistent with other experimental studies that have found birds to gain mass when predation risk is heightened (Lilliendahl 1998; Pravosudov and Grubb 1998), but while others have suggested that the predator-induced mass gains were due to fat, it was not directly measured.

It is evident that birds exhibit varying responses of mass change when predation risk is heightened, as some studies report significant gains in mass (Lilliendahl 1998; Pravosudov and Grubb 1998) while others observe significant losses (Gosler et al., 1995; Lilliendahl, 1997; van der Veen and Sivars, 2000). MacLeod et al. (2007) suggested that the quality of a bird's foraging environment could be the general determining factor of whether mass is gained or lost, whereby a food-rich environment is likely needed to permit birds to gain mass when their foraging is made more unpredictable by the presence of predators (i.e. interrupted foraging response). Because the birds in our predation risk manipulation were supplied with food *ad libitum*, food quantity could explain our results. However, Zanette et al. (2013) report that female song sparrows ate less from supplemental food-rich sources and suffered poorer physiological condition when they experienced frequent experimental nest predation, suggesting that predation risk may alter food intake even when food is unlimited. One possibility is that the sparrows in this study viewed this supplemental food as "risky" foraging option and

opted to feed at safer, lower-quality sources. While it is not explicitly clear how the cowbirds went about gaining mass in the high predation risk treatment, the feeders in the aviaries provided the primary source of food. Therefore, they may have gained mass by increasing their foraging intensity in between predator stimuli exposures to compensate for their reduced foraging time (McNamara et al. 2005), or it is also possible that they viewed the feeders in the aviaries as “safe”, food-rich foraging options from where they could forage without exposing themselves to a greater risk of predation (Searle et al. 2008). Nonetheless, further experimentation is needed to determine what changes in foraging behaviour may be the mechanism behind this mass gain response.

Regarding the second survival tactic, the effect of elevated predation risk alone caused the birds to flee with an increase in take-off angle but at reduced speeds, which suggests that they invested more in outmaneuvering, rather than outrunning, a fast-attacking predator (Lind et al. 2002). Increases in take-off angle can force predators to make abrupt changes in their attack trajectory, likely reducing capture probability (Howland 1974; Andersson and Norberg 1981; Hendenstrom and Rosen 2001; Lind et al. 2002; Shifferman and Eilam, 2004). The birds displayed a nearly perfect trade-off of angle and speed as they flew with the same overall flight capacity (i.e. mechanical energy gain) in each treatment. Trading-off angle and speed is characteristic of birds (Kullberg et al. 1998; Kullberg and Lafrenz 2007), and many studies provide evidence that birds will cue into the varying conditions they face upon attack and adjust their take-off behaviour accordingly (Kullberg et al., 1998; Lind et al., 2002; Lind et al., 2003; Kullberg and Lafrenz, 2007; Devereux et al., 2008). To our knowledge, researchers do not often measure take-off angle, speed, and mechanical energy gain together (although see

Swaddle et al. 1999; Willams and Swaddle 2003); however, doing so provides a more comprehensive way to quantify take-off flight, since it not only provides information on take-off behaviour, but also summarizes the overall effect any changes in angle and speed may have on flying capacity. Our results are complemented by studies that report birds may exhibit other changes in escape behaviour, such as increasing flight initiation distance, when predation risk is increased (Zanette et al. 2011, Díaz et al. 2013), which, when taken together, help reveal the significant effect that predators have on avian escape behaviour.

Regarding the third survival tactic, we found that while birds gained mass to provide a buffer against starvation risk when they faced elevated levels of predation risk, they temper this response to ensure their overall flying capacity (i.e. mechanical energy gain, vertical flying speed) is not compromised. This response is contradictory to the mass-dependent predation risk theory, which proposes that predator-induced mass changes should affect flying capacity by altering wing-load (Witter and Cuthill, 1993). While it makes intuitive sense that mass change may affect a bird's ability to create lift, many studies have found no reduction in flying capacity from smaller magnitudes of mass gain (i.e. mass gain over the day; Kullberg 1998; Kullberg et al. 1998; Veasey et al. 1998; van der Veen and Lindstrom 2000; MacLeod 2006). The negative relationship between predator-induced mass gain and vertical flying speed we reported for females at an individual level (Fig 3a) indicates large mass gains may indeed be detrimental to flying capacity since females that gained comparatively large amounts of mass suffered a reduced vertical flying speed. However, we did not find an overall treatment effect on flying capacity because females only exhibited about a 2 % mass gain on average, which

is consistent with the speculation of MacLeod et al. (2006) that mass changes greater than 10 % are needed to elicit significant effects on flying capacity. Thus, predator-induced gains in mass, which have been found to fall well below this 10 % threshold (Lilliendahl, 1998; Pravosudov and Grubb, 1998; this study), are unlikely to be a factor affecting survival through reduced flight capacity as previously proposed (Hendenstrom 1992; Witter and Cuthill 1993; Witter et al. 1994; Metcalfe and Ure 1995) because birds limit their mass gain to retain their ability to evade predators.

Interestingly, unlike females, the vertical flying speed of male cowbirds was completely unaffected by either mass gains or losses. Why mass gains of up to 17 % have no effect on flying capacity is unknown. Sexual differences in escape behaviour have been documented in other taxa such as mammals (Stankowich and Cross 2005; Lea and Blumstein 2011) and reptiles (Lailvoux et al. 2003; Shine et al. 2003). It is possible that the males' larger structural size, greater amount of lean tissue, and larger pectoral muscles may enable them to be less constrained than females to mass changes when escaping. Additionally, male cowbirds exhibit much more conspicuous behaviour over the breeding season, displaying both mating calls to court females and aggressive male-to-male counter singing in order to preserve their dominance structure (White et al. 2010). Consequently, males may be more vulnerable to eavesdropping predators and need to maintain their flying capacity to a greater extent than females.

Our body mass manipulation suggests that losing mass likely increased the birds' risk of starvation, but did not affect flying capacity. Therefore, predator-induced mass loss is not likely a tactic that birds use to improve their chances of escape, but instead it could be a byproduct of suppressed foraging from the presence of predators (Carrascal

and Polo 1999; van der Veen and Sivars 2000) or predation stress in general (van der Veen and Sivars 2000; Clinchy et al. 2004). These results contradict the suggestion of the mass-dependent predation risk hypothesis that mass loss should reduce the risk of predation by improving flying capacity because lighter birds have lower wing-loads and hence create more lift (Witter and Cuthill, 1993; Witter et al., 1994; Gosler et al., 1995). Even though the 15 % mass loss group surpassed the >10 % threshold, the birds exhibited no change in vertical flying speed, suggesting this threshold may only apply to mass gains. The significant quadratic relationship between body mass change and vertical flying speed in females (Fig 4a) supports this claim. Individual-level analysis demonstrates that females' flying capacity is unaffected by mass losses of up to 20 %, yet we find that larger mass gains begin to compromise flying capacity, exhibiting a nearly identical trend as our predation risk manipulation (Fig 3a). Additionally, males also displayed similar trends to the predation risk manipulation (Fig 3b) whereby their vertical flying speed is once again unaffected by mass change (Fig 4b). These striking similarities between our two experiments also provide further evidence that moderate mass gains, not mass loss, may be the best strategy for birds faced with high predation risk, as such a response presumably reduces their starvation risk and allows them to maintain the same flying capacity regardless of mass change.

Our results suggest that flying capacity will be compromised only when birds gain considerable amounts of mass. Birds preparing for migration are most likely to suffer an increased risk of predation since many species have been shown to carry up to an extra 40 % of their body mass in fat during this period (Blem 1976). Many studies have shown that flying capacity is compromised by these extreme mass gains (Kullberg et al. 1996,

2000; Lind et al. 1999; Burns and Ydenberg 2002), suggesting that birds must limit their risk of predation in other ways. Ydenberg et al. (2004) found that migratory birds adjust their migration tactics, by reducing stopover times in predator-heavy areas, to limit their exposure to predators. This observation suggests that when reduced flying capacity is unavoidable birds may have to resort to other anti-predator behaviours to limit their risk predation.

Even though lean mass remained unchanged between predation risk treatments we found an increase in pectoral muscle thickness by 1.9 % on average in the predator treatment, although no relationship was found between either pectoral muscle thickness or changes in pectoral muscle thickness and flying capacity. While this physiological change is similar to the predator-induced gains in pectoral muscle thickness (4.1 %) found in ruddy turnstones (*Arenaria interpres*) by van den Hout et al. (2006), our results contradict their suggestion that predator-induced gains in pectoral muscle may be a physiological investment to improve flying capacity when risk is high (van den Hout et al. 2006). While our results support that birds can show rapid physiological flexibility in organ size (Piersma et al. 1999; Lind and Jakobsson 2001) and suggest that increases in pectoral muscle thickness may come at the expense of other lean tissues since wet lean mass remained unchanged (van den Hout et al. 2006), they provide no support that predator-induced increases in pectoral muscle thickness will boost flying capacity. The magnitude of gain in pectoral muscle thickness in our experiment, however, may have simply been too small to significantly affect flying capacity; thus, we propose that future studies continue to quantify pectoral muscle size and flying capacity together to help further reveal this relationship.

In all, our results demonstrate that, while increased predation risk causes birds to exhibit behavioral and physiological changes in an overall strategy to survive, they maintain their flying capacity regardless of their environment. Studies focused on juvenile development have also shown support for the importance of preserving flying capacity whereby birds will forgo developing important factors such as immune function and structural size in order to prioritize structures associated with flight (Bize et al. 2003; Wright et al. 2006, Verspoor et al. 2007; Miller 2010; Miller 2011). These results, together with ours, suggest that birds preserve their flying capacity at all costs because evading predators is too important for their survival.

2.5 References

- Andersson, M., R. A. Norberg. 1981. Evolution of reversed sexual size dimorphism and role partitioning among predatory birds, with a size scaling of flight performance. *Biological Journal of the Linnean Society* 15:105-130.
- Bize, P., A. Roulin, L. F. Bersier, D. Pfluger, and H. Richner. 2003. Parasitism and developmental plasticity in Alpine swift nestlings. *Journal of Animal Ecology* 72:633-639.
- Blem, C. 1976. Patterns of lipid storage and utilization in birds. *American Zoologist* 16:671-684.
- Boonstra, R., D. Hik, G. R. Singleton, and A. Tinnikov. 1998. The impact of predator-induced stress on the snowshoe hare cycle. *Ecological Monographs* 68:371-394.
- Burns, J., R. Ydenberg. 2002. The effects of wing loading and gender on the escape flights of least sandpipers (*Calidris minutilla*) and western sandpipers (*Calidris mauri*). *Behavioral Ecology and Sociobiology* 52:128-136.
- Butler, P. J., C. M. Bishop. 2000. Flight. Pages 391-435 in G. C. Whittow, editor: *Sturkie's Avian Physiology*, 5th edition. Academic Press, San Diego, USA
- Carrascal, L. M., V. Polo. 1999. Coal tits, *Parus ater*, lose weight in response to chases by predators. *Animal Behaviour* 58:281-285.
- Chin, E. H., O. P. Love, J. J. Verspoor, T. D. Williams, K. Rowley, and G. Burness. 2009. Juveniles exposed to embryonic corticosterone have enhanced flight performance. *Proceedings of the Royal Society B-Biological Sciences* 276:499-505.
- Clinchy, M., L. Zanette, R. Boonstra, J. Wingfield, and J. Smith. 2004. Balancing food and predator pressure induces chronic stress in songbirds. *Proceedings of the Royal Society B-Biological Sciences* 271:2473-2479.

- Criscuolo, F., P. Monaghan, A. Proust, J. Skorpilova, J. Laurie, and N. B. Metcalfe. 2011. Costs of compensation: effect of early life conditions and reproduction on flight performance in zebra finches. *Oecologia* 167:315-323.
- Devereux, C. L., E. Fernandez-Juricic, J. R. Krebs, and M. J. Whittingham. 2008. Habitat affects escape behaviour and alarm calling in common starlings *Sturnus vulgaris*. *Ibis* 150:191-198.
- Diaz, M., A. P. Møller, E. Flensted-Jensen, T. Grim, J. Diego Ibanez-Alamo, J. Jokimaki, G. Marko, and P. Tryjanowski. 2013. The geography of fear: a latitudinal gradient in anti-predator escape distances of birds across Europe. *Plos One* 8:e64634.
- Dietz, M. W., A. Dekinga, T. Piersma, and S. Verhulst. 1999. Estimating organ size in small migrating shorebirds with ultrasonography: an intercalibration exercise. *Physiological and Biochemical Zoology* 72:28-37.
- Dill, L. M. 1974. Escape response of zebra danio (*Brachydanio-Rerio*). 2. effect of experience. *Animal Behaviour* 22:723-730.
- Eiben, B., M. Persons. 2007. The effect of prior exposure to predator cues on chemically-mediated defensive behavior and survival in the wolf spider *Rabidosa rabida* (Araneae : Lycosidae). *Behaviour* 144:889-906.
- Gentle, L., A. Gosler. 2001. Fat reserves and perceived predation risk in the great tit, *Parus major*. *Proceedings of the Royal Society B-Biological Sciences* 268:487-491.
- Gosler, A., J. Greenwood, and C. Perrins. 1995. Predation risk and the cost of being fat. *Nature* 377:621-623.
- Gregory, P. T. 2013. Once bitten, twice shy: does previous experience influence behavioural decisions of snakes in encounters with predators? *Ethology* 119:919-925.
- Griffin, A. S., C. S. Evans, and D. T. Blumstein. 2001. Learning specificity in acquired predator recognition. *Animal Behaviour* 62:577-589.

- Guglielmo, C. G., L. P. McGuire, A. R. Gerson, and C. L. Seewagen. 2011. Simple, rapid, and non-invasive measurement of fat, lean, and total water masses of live birds using quantitative magnetic resonance. *Journal of Ornithology* 152:75-85.
- Hedenstrom, A. 1992. Flight performance in relation to fuel load in birds. *Journal of Theoretical Biology* 158:535-537.
- Hedenstrom, A., M. Rosen. 2001. Predator versus prey: on aerial hunting and escape strategies in birds. *Behavioral Ecology* 12:150-156.
- Houston, A. I., J. M. Mcnamara. 1993. A theoretical investigation of the fat reserves and mortality levels of small birds in winter. *Ornis Scandinavica* 24:205-219.
- Howland, H. C. 1974. Optimal strategies for predator avoidance - relative importance of speed and maneuverability. *Journal of Theoretical Biology* 47:333-350.
- Kullberg, C. 1998. Does diurnal variation in body mass affect take-off ability in wintering willow tits? *Animal Behaviour* 56:227-233.
- Kullberg, C., D. Houston, and N. Metcalfe. 2002a. Impaired flight ability - a cost of reproduction in female blue tits. *Behavioral Ecology* 13:575-579.
- Kullberg, C., N. B. Metcalfe, and D. C. Houston. 2002b. Impaired flight ability during incubation in the pied flycatcher. *Journal of Avian Biology* 33:179-183.
- Kullberg, C., S. Jakobsson, and T. Fransson. 2000. High migratory fuel loads impair predator evasion in sedge warblers. *Auk* 117:1034-1038.
- Kullberg, C., S. Jakobsson, and T. Fransson. 1998. Predator-induced take-off strategy in great tits (*Parus major*). *Proceedings of the Royal Society B-Biological Sciences* 265:1659-1664.
- Kullberg, C., T. Fransson, and S. Jakobsson. 1996. Impaired predator evasion in fat blackcaps (*Sylvia atricapilla*). *Proceedings of the Royal Society B-Biological Sciences* 263:1671-1675.

- Kullberg, C., M. Lafrenz. 2007. Escape take-off strategies in birds: the significance of protective cover. *Behavioral Ecology and Sociobiology* 61:1555-1560.
- Lilliendahl, K. 1998. Yellowhammers get fatter in the presence of a predator. *Animal Behaviour* 55:1335-1340.
- Lilliendahl, K. 1997. The effect of predator presence on body mass in captive greenfinches. *Animal Behaviour* 53:75-81.
- Lima, S. 1986. Predation risk and unpredictable feeding conditions: determinants of body mass in birds. *Ecology* 67:377-385.
- Lind, J., S. Jakobsson. 2001. Body building and concurrent mass loss: flight adaptations in tree sparrows. *Proceedings of the Royal Society B-Biological Sciences* 268:1915-1919.
- Lind, J., U. Kaby, and S. Jakobsson. 2002. Split-second escape decisions in blue tits (*Parus caeruleus*). *Naturwissenschaften* 89:420-423.
- Lind, J., T. Fransson, S. Jakobsson, and C. Kullberg. 1999. Reduced take-off ability in robins (*Erithacus rubecula*) due to migratory fuel load. *Behavioral Ecology and Sociobiology* 46:65-70.
- Lind, J., L. Hollen, E. Smedberg, U. Svensson, A. Vallin, and S. Jakobsson. 2003. Detection distance influences escape behaviour in two parids, *Parus major* and *P-caeruleus*. *Journal of Avian Biology* 34:233-236.
- Loennstedt, O. M., M. I. McCormick, M. G. Meekan, M. C. O. Ferrari, and D. P. Chivers. 2012. Learn and live: predator experience and feeding history determines prey behaviour and survival. *Proceedings of the Royal Society B-Biological Sciences* 279:2091-2098.
- MacLeod, R. 2006. Why does diurnal mass change not appear to affect the flight performance of alarmed birds? *Animal Behaviour* 71:523-530.

- MacLeod, R., A. G. Gosler. 2006. Capture and mass change: perceived predation risk or interrupted foraging? *Animal Behaviour* 71:1081-1087.
- MacLeod, R., J. Lind, J. Clark, and W. Cresswell. 2007. Mass regulation in response to predation risk can indicate population declines. *Ecology Letters* 10:945-955.
- McNamara, J. M., Z. Barta, A. I. Houston, and P. Race. 2005. A theoretical investigation of the effect of predators on foraging behaviour and energy reserves. *Proceedings of the Royal Society B-Biological Sciences* 272:929-934.
- Metcalfe, N. B., S. E. Ure. 1995. Diurnal variation in-flight performance and hence potential predation risk in small birds. *Proceedings of the Royal Society B-Biological Sciences* 261:395-400.
- Miller, D. A. 2011. Immediate and delayed effects of poor developmental conditions on growth and flight ability of juvenile mourning doves *Zenaida macroura*. *Journal of Avian Biology* 42:151-158.
- Miller, D. A. 2010. Morphological plasticity reduces the effect of poor developmental conditions on fledging age in mourning doves. *Proceedings of the Royal Society B-Biological Sciences* 277:1659-1665.
- Payne, R. B. 1973. Breeding season of a parasitic bird - brown-headed cowbird, in Central California. *Condor* 75:80-99.
- Piersma, T., G. Gudmundsson, and K. Lilliendahl. 1999. Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. *Physiological and Biochemical Zoology* 72:405-415.
- Pravosudov, V. V., T. C. Grubb. 1998. Management of fat reserves in tufted titmice *Baeolophus bicolor* in relation to risk of predation. *Animal Behaviour* 56:49-54.
- Rands, S., I. Cuthill. 2001. Separating the effects of predation risk and interrupted foraging upon mass changes in the blue tit *Parus caeruleus*. *Proceedings of the Royal Society B-Biological Sciences* 268:1783-1790.

- Remage-Healey, L., L.M. Romero. 2000. Daily and seasonal response to stress in captive starlings (*Sturnus vulgaris*): Glucose. *General and Comparative Endocrinology* 119: 60-68.
- Rogers, C. M. 2015. Testing optimal body mass theory: evidence for cost of fat in wintering birds. *Ecosphere* 6:art55
- Roitberg, B., E. Mondor, and J. Tyerman. 2003. Pouncing spider, flying mosquito: blood acquisition increases predation risk in mosquitoes. *Behavioral Ecology* 14:736-740.
- Saino, N., J. Cuervo, M. Krivacek, F. deLope, and A. Moller. 1997. Experimental manipulation of tail ornament size affects the hematocrit of male barn swallows (*Hirundo rustica*). *Oecologia* 110:186-190.
- Searle, K. R., C. J. Stokes, and I. J. Gordon. 2008. When foraging and fear meet: using foraging hierarchies to inform assessments of landscapes of fear. *Behavioral Ecology* 19:475-482.
- Shifferman, E., D. Eilam. 2004. Movement and direction of movement of a simulated prey affect the success rate in barn owl *Tyto alba* attack. *Journal of Avian Biology* 35:111-116.
- Shine, R., H. G. Cogger, R. R. Reed, S. Shetty, and X. Bonnet. 2003. Aquatic and terrestrial locomotor speeds of amphibious sea-snakes (Serpentes, Laticaudidae). *Journal of Zoology* 259:261-268.
- Stankowich, T., D. T. Blumstein. 2005. Fear in animals: a meta-analysis and review of risk assessment. *Proceedings of the Royal Society B-Biological Sciences* 272:2627-2634.
- Swaddle, J., E. Williams, and J. Rayner. 1999. The effect of simulated flight feather moult on escape take-off performance in starlings. *Journal of Avian Biology* 30:351-358.

- Swanson, D. L., C. Merkord. 2013. Seasonal phenotypic flexibility of flight muscle size in small birds: a comparison of ultrasonography and tissue mass measurements. *Journal of Ornithology* 154:119-127.
- van den Hout, P. J., T. Piersma, A. Dekinga, S. K. Lubbe, and G. H. Visser. 2006. Ruddy turnstones *Arenaria interpres* rapidly build pectoral muscle after raptor scares. *Journal of Avian Biology* 37:425-430.
- van der Veen, I., L. Sivars. 2000. Causes and consequences of mass loss upon predator encounter: feeding interruption, stress or fit-for-flight? *Functional Ecology* 14:638-644.
- van der Veen, I., K. Lindstrom. 2000. Escape flights of yellowhammers and greenfinches: more than just physics. *Animal Behaviour* 59:593-601.
- Veasey, J. S., N. B. Metcalfe, and D. C. Houston. 1998. A reassessment of the effect of body mass upon flight speed and predation risk in birds. *Animal Behaviour* 56:883-889.
- Verspoor, J. J., O. P. Love, E. Rowland, E. H. Chin, and T. D. Williams. 2007. Sex-specific development of avian flight performance under experimentally altered rearing conditions. *Behavioral Ecology* 18:967-973.
- Walker, J., C. Ghalambor, O. Griset, D. McKenney, and D. Reznick. 2005. Do faster starts increase the probability of evading predators? *Functional Ecology* 19:808-815.
- White, D. J., L. Ho, G. de los Santos, and I. Godoy. 2007. An experimental test of preferences for nest contents in an obligate brood parasite, *Molothrus ater*. *Behavioral Ecology* 18:922-928.
- Williams, E., J. Swaddle. 2003. Moulting, flight performance and wingbeat kinematics during take-off in European starlings *Sturnus vulgaris*. *Journal of Avian Biology* 34:371-378.

- Witter, M. S., I. C. Cuthill, and R. H. C. Bonser. 1994. Experimental investigations of mass-dependent predation risk in the European starling, *Sturnus vulgaris*. *Animal Behaviour* 48:201-222.
- Witter, M., I. Cuthill. 1993. The ecological costs of avian fat storage. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 340:73-92.
- Wright, J., S. Markman, and S. M. Denney. 2006. Facultative adjustment of pre-fledging mass loss by nestling swifts preparing for flight. *Proceedings of the Royal Society B-Biological Sciences* 273:1895-1900.
- Ydenberg, R. C., R. Butler, D. Lank, B. Smith, and J. Ireland. 2004. Western sandpipers have altered migration tactics as peregrine falcon populations have recovered. *Proceedings of the Royal Society B-Biological Sciences* 271:1263-1269.
- Ydenberg, R. C., L. M. Dill. 1986. The economics of fleeing from predators. *Advances in the Study of Behavior* 16:229-249.
- Yokel, D. A., S. I. Rothstein. 1991. The basis for female choice in an avian brood parasite. *Behavioral Ecology and Sociobiology* 29:39-45.
- Zanette, L. Y., A. F. White, M. C. Allen, and M. Clinchy. 2011. Perceived predation risk reduces the number of offspring songbirds produce per year. *Science* 334:1398-1401.
- Zanette, L. Y., K. A. Hobson, M. Clinchy, M. Travers, and T. D. Williams. 2013. Food use is affected by the experience of nest predation: implications for indirect predator effects on clutch size. *Oecologia* 172:1031-1039.

Chapter 3

General Discussion

The goal of this thesis was to further explore the sometimes profound effects that perceived predation risk alone can have on prey species. In Chapter 1, I reviewed how predation risk can have an overarching impact on prey demography that ranges from the population to the individual level. I provided a specific focus on predator-induced effects in escape behaviour and highlight areas in need of further exploration in avian species. In Chapter 2, I explored the effects of predation risk on at the individual level through an experiment using the brown-headed cowbird as a model species. I manipulated predation risk using visual and auditory stimuli and measured changes in physiological condition (i.e. body mass, fat mass, lean mass, pectoral muscle thickness) and escape behaviour (i.e. take-off speed and angle, mechanical energy gain, vertical flying speed). As a result, I integrated the components of predation risk, physiological change, and escape behaviour into a cohesive study to better understand their relationships and to determine the underlying mechanisms that may affect cowbirds' survival. In this final chapter, I aim to summarize the broader biological significance of my findings and discuss the impact of predation risk on conservation management. I also outline some of the challenges facing escape behaviour research and provide some future directions to further explore when researching escape behaviour in avian species.

3.1 Tactics for survival in response to predation risk

I tested to what extent the threat of predation induces physiological effects and changes in escape behaviour in an avian species and, in turn, whether such predator-

induced physiological changes impact the capacity to escape from predators. In my predation risk manipulation, I suggested that cowbirds exhibited multiple tactics to enhance survival when they perceived a higher risk of predation. They gained body mass and altered their escape behaviour at take-off, yet these mass gains did not affect the birds' ability to escape a predator as they displayed no changes in flying capacity between the low and high predator risk environments. While predator-induced mass gain had no effects on flying capacity, my body mass manipulation in the lab showed reductions in body mass from basal levels also led to no changes in flying capacity.

My results are contradictory to the long held assumption that predator-induced mass changes may be a tactic used by prey to improve their flying capacity for predator avoidance (Witter and Cuthill 1993). My results are, however, consistent with other experimental evidence that also found no effects of proportionately similar increases in mass (i.e. due to diurnal mass gain) on flying capacity (Kullberg 1998; MacLeod 2006). Collectively, this suggests that the effects of predator-induced mass change on flying capacity are not as unyielding as previously proposed and should not be included as a mechanism that determines an individual's chance of survival (Creel and Christensen 2008).

My results indicate birds do respond both physiologically and behaviourally to predators in ways that could scale up to population level effects on prey demography. First, an increased threat of predation caused the cowbirds to increase their mass to levels that did not compromise their flying capacity. At an individual level, starvation-predation risk theory focuses on how prey balance their energy reserves in response to the varying types of risk to limit their chance of mortality (Houston and McNamara 1993). If

conditions permit, prey may increase their energy stores (i.e. fat) and thereby reduce their starvation risk (Lilliendahl 1998; McNamara et al. 2005). Incidentally, if birds gain too much fat it may reduce their capacity to escape (Kullberg et al. 1996). Prey that are able to balance their energy stores by adding enough fat to reduce starvation risk, but not gaining too much to compromise their capacity to escape predators, may effectively increase their probability of survival. The increased fat and unchanged flying capacity of the cowbirds in my predation risk manipulation suggest this may be a tactic that birds are using to increase their probability of survival. If applied to the population level, this finding could provide an additional factor to MacLeod et al.'s (2007) suggestion that mass gain in response to predation risk is a good indicator of increasing bird populations since they are not only able to buffer their energy stores against the risk of starvation, but do so without sacrificing their ability to escape predators.

I also found that cowbirds changed their escape behaviour when predation risk was high by increasing their take-off angle at the cost of lower speed. This change in behaviour may serve as a beneficial evasion technique that will increase an individual's chance of survival, as diverting from the attack trajectory of an oncoming predator, rather than trying to outrun it, has been found to decrease capture rate (Shifferman and Eilam 2004). My findings also suggest that birds invest more in the vertical component of flight by increasing their take-off angles. Møller et al. (2010) observed that species that adopt a relatively greater vertical component of flight when escaping have a greater adult survival rate. Thus, this change in escape behaviour when under a higher risk of predation could also have possible population level implications on the survival rate of adult cowbirds.

In all, my research provides clarifications to the effects of predators on avian escape behaviour and contributes to the growing knowledge base of predator-prey dynamics. I suggest that the behavioural and physiological responses to predation risk that are presented in the study be considered as mechanisms that may impact the chances of prey survival. It is evident from my findings that the effects of predation risk on the escape behaviour are substantial and should be considered in general ecological theory, as well as in the development of conservation and management planning.

3.2 Consequences for applied conservation

In the context of a diverse and ever-changing ecological system, recent research has made it apparent that the effects of predation risk play a key role in shaping prey demography and ecosystem function (Preisser and Bolnick 2008; Sih et al. 2010; MacLeod et al. 2014). While it is true that predators often contribute to the decline of prey populations (Salo et al. 2007), many management plans resort to simply protecting prey from direct predation and ignore the effects of predation risk altogether (Isaksson et al. 2007). Ignoring the contributing factors of predation risk often results in a vast underestimation of the overall effects that predators have on prey (Peckarsky et al. 2008); thus, when a population is threatened, limiting direct killing may not be enough to promote population recovery. Limiting predator exposure altogether may be the most effective mode of action as it limits both the direct and risk effects of predators. Additionally, since both food and predators have a synergistic effect on prey populations (Zanette et al. 2003), it may be beneficial to adopt conservation measures that simultaneously provide supplemental food while limiting predator numbers when prey populations become threatened by predators.

Estimating the potential population level effects of predation risk is a daunting task that involves analyzing several elements that may affect prey demography (Liley and Creel 2008; MacLeod et al. 2014). As mortality can result from factors related to anti-predator behaviour (i.e. starvation), it is important to understand the functional traits of prey and how they may help predict predation risk effects on a population level (Creel 2011). However, it is often difficult to gauge how predation risk effects might alter the survival patterns of prey because of the strategic anti-predator responses that prey adopt (MacLeod et al. 2014). The results from my experiment, while not directly measuring survival, help provide insight on the anti-predator defenses of avian species. As it is clear that conservation and management programs must be altered to include the indirect effects of predation, these strategic responses to predators must be taken into account when determining the survival probabilities of prey populations.

3.3 Challenges facing escape behaviour research

Escape behaviour is broad term that spans beyond the actions of prey when confronted with the immediate threat of a predator. In addition to deciding when, where, and how to flee an impending predator attack, escape behaviour includes the many decisions that prey make to avoid detection and the defenses that prey employ when they are overtaken by a predator (reviewed in Ruxton et al. 2004; Caro 2005). Many studies have examined how perceived predation risk may affect a prey's decisions to avoid predation (Lima & Dill, 1990). Much less research, however, has focused how increasing predation risk may alter the escape tactics prey use when fleeing a predator (although see Hawlena et al. 2011; Díaz et al. 2013).

Once prey begin to actively flee from an oncoming predator, it is ideal that both speed and direction of escape are quantified as vectors, yet studies generally only report directionality without any quantification of escape speed (Cooper and Blumstein 2015). Integrating escape speed measures into these study designs is important as they can be a good predictor of whether or not prey successfully escape an attack (Ilany and Eilam 2008). Combining these measures with the use of live predators and prey are necessary to assess the value of survival that can be attributed to different escape tactics that prey adopt when confronted with an immediate threat. Aquatic and invertebrate microcosm studies have been successful using these methods (Strobbe et al. 2003; Walker et al. 2005), but more studies in terrestrial vertebrates are needed to further understand effective measures of escape behaviour (for example, Ilany and Eilam 2008).

When the use of live predators is not a viable option, one challenging aspect of determining the effects of predation risk on escape behaviour is to accurately reproduce the risk of a predator. As prey assess their risk of predation by using their sensory functions, manipulating the level of risk using predator cues, such as audio playbacks of predator calls, predator models, and predator odors, has been widely successful in provoking anti-predator responses in prey (Lilliendahl 1998, Apfelbach et al. 2005, Hettner et al. 2014). My study, for instance, encompassed multiple aspects of predation risk through auditory (broadcasting predator calls with speakers) and visual (presenting taxidermic models of predators) stimuli. Nonetheless, it is possible that these cues may not elicit the full fear response from prey that, for example, a hawk swooping overhead may evoke. Prey can detect very subtle cues of predation risk, such as distinguishing whether or not a predator is looking at them, and they vary their fear response as a result

(Carter et al. 2008). Although my results provide evidence that the cowbirds in my study did detect some degree of predation risk, these subtle cues illustrate the difficulty of replicating predation risk experiments in controlled conditions. Ensuring studies are as realistic to nature as possible is critical to capturing the full suite of escape behaviours that prey exhibit.

3.4 Future directions

Avian species significantly vary in their size, shape, behaviour and ecological distribution. The recent increase in studies focused on the effects of predation risk has revealed that they likely play a role in every ecological system, affecting prey at both the individual and population level. My research has shown that certain aspects of escape behaviour are affected by perceived predation risk, yet there is still much to be determined, as little research has investigated the escape behaviour of birds in mid-flight. Additionally, experiments that integrate predation risk, physiological change and escape behaviour in other environmental contexts and life-history stages are needed to further elucidate the anti-predator strategies of birds.

When analyzing escape behaviour in avian species, most experimental studies focus on how birds escape from a stationary position, measuring variation in take-off angle and speed after a simulated predator attack (see Kullberg et al. 1996, 1998; MacLeod 2006). While these measures are important since they focus on the crucial point of escape for many avian species (i.e. take-off; Cresswell 1993), much less attention has been given to the important aspects of escape flight after take-off, such as mid-flight maneuverability (although see Warrick 1998). Research that integrates direct

manipulations of predation risk along with measuring both physiological change and escape behaviour with this focus, while logistically challenging, would deliver biologically meaningful results. Additionally, quantifying predator-induced changes in socially coordinated escape behaviour in the field would provide further insight into understanding the overall effects of predators and group size on escape behaviour in birds (van den Hout et al. 2010). These experiments could be achieved using a trained predator, such as a raptor (Kenward 1978); however, ethical concerns may rise from such methods.

Climatic conditions and resource availability may also exacerbate the effects of predation risk (Priesser et al. 2009). Studies have illustrated that birds exhibit physiological changes in response to both abiotic factors, such as temperature (Vézina et al. 2006) and food availability (Pierce and McWilliams 2004). Consequently, these factors may play a key role in determining a bird's escape capacity. My study controlled for environmental stochasticity by conducting the experiment in identical semi-natural settings over the same seasonal period, but different seasons provide birds with new challenges. For example, birds face colder temperatures and lower quality foraging environments over winter and generally keep relatively high body mass levels (Lima 1986), which could force them to adopt different survival tactics than they would otherwise. Further research should include multi-season experiments to evaluate the effects of environmental condition on physiology and escape behaviour.

Birds also face many physiologically trying periods in their annual life cycle (e.g. migration and moulting). While studies have shown that physiological changes during these periods may negatively affect escape behaviour (Kullberg et al. 1996; Swaddle and

Witter 1997), integrating predation risk into these contexts may reveal interesting and biologically important information into how predators affect escape behaviour when birds are physiologically constrained.

Lastly, population level studies could be used to determine whether the physiological and behavioural responses of birds to predators represent plasticity or adaptation. Coslovsky and Richner (2011) found that predation risk may elicit adaptive maternal effects that provide a selective advantage to offspring reared in a dangerous environment by selecting for traits that may enhance predator evasion. However, it would be interesting to explore if birds reared for generations in areas of high predation exhibit traits, such as increased take-off angle or increased pectoral mass, that are heritable to their offspring. Assessing whether birds reared in high predation environments are genetically different from other populations would be very interesting from a conservation and evolutionary standpoint.

In general, the evidence from my experiment reveals important information about the relationship between predation risk, physiological condition, and escape behaviour. As researchers have moved beyond thinking about predators as simple killers to being fundamental components of the ecological structure whose mere presence can impact entire prey populations, it is evident that predation risk effects need to be thoroughly considered when investigating populations and ecosystems. Further research will provide additional support for including the predator-induced effects on escape behaviour as a mechanism for determining survival in prey species.

3.5 References

- Apfelbach, R., C. D. Blanchard, R. J. Blanchard, R. A. Hayes, and I. S. McGregor. 2005. The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neuroscience and Biobehavioral Reviews* 29:1123-1144.
- Caro, T. 2005. *Antipredator defenses in birds and mammals*. University of Chicago Press, Chicago, USA
- Carter, J., N. J. Lyons, H. L. Cole, and A. R. Goldsmith. 2008. Subtle cues of predation risk: starlings respond to a predator's direction of eye-gaze. *Proceedings of the Royal Society B-Biological Sciences* 275:1709-1715.
- Creel, S. 2011. Toward a predictive theory of risk effects: hypotheses for prey attributes and compensatory mortality. *Ecology* 92:2190-2195.
- Creel, S., D. Christianson. 2008. Relationships between direct predation and risk effects. *Trends in Ecology & Evolution* 23:194-201.
- Cresswell, W. 1993. Escape responses by redshanks, *Tringa totanus*, on attack by avian predators. *Animal Behaviour* 46:609-611.
- Coslovsky, M., H. Richner. 2011. Predation risk affects offspring growth via maternal effects. *Functional Ecology* 25:878-888.
- Diaz, M., A. P. Moller, E. Flensted-Jensen, T. Grim, J. Diego Ibanez-Alamo, J. Jokimaki, G. Marko, and P. Tryjanowski. 2013. The geography of fear: a latitudinal gradient in anti-predator escape distances of birds across Europe. *Plos One* 8:e64634.
- Hettena, A. M., N. Munoz, and D. T. Blumstein. 2014. Prey responses to predator's sounds: a review and empirical study. *Ethology* 120:427-452.
- Hawlena, D., H. Kress, E. R. Dufresne, and O. J. Schmitz. 2011. Grasshoppers alter jumping biomechanics to enhance escape performance under chronic risk of spider predation. *Functional Ecology* 25:279-288.

- Houston, A. I., J. M. McNamara, and J. M. C. Hutchinson. 1993. General results concerning the trade-off between gaining energy and avoiding predation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 341:375-397.
- Ilany, A., D. Eilam. 2008. Wait before running for your life: defensive tactics of spiny mice (*Acomys cahirinus*) in evading barn owl (*Tyto alba*) attack. *Behavioral Ecology and Sociobiology* 62:923-933.
- Isaksson, D., J. Wallander, and M. Larsson. 2007. Managing predation on ground-nesting birds: The effectiveness of nest exclosures. *Biological Conservation* 136:136-142.
- Kenward, R. E. 1978. Hawks and doves - factors affecting success and selection in goshawk attacks on woodpigeons. *Journal of Animal Ecology* 47:449-460.
- Kullberg, C. 1998. Does diurnal variation in body mass affect take-off ability in wintering willow tits? *Animal Behaviour* 56:227-233.
- Kullberg, C., T. Fransson, and S. Jakobsson. 1996. Impaired predator evasion in fat blackcaps (*Sylvia atricapilla*). *Proceedings of the Royal Society B-Biological Sciences* 263:1671-1675.
- Liley, S., S. Creel. 2008. What best explains vigilance in elk: characteristics of prey, predators, or the environment? *Behavioral Ecology* 19:245-254.
- Lilliendahl, K. 1998. Yellowhammers get fatter in the presence of a predator. *Animal Behaviour* 55:1335-1340.
- Lima, S. L., L. M. Dill. 1990. Behavioral decisions made under the risk of predation - a review and prospectus. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 68:619-640.
- Lima, S. 1986. Predation risk and unpredictable feeding conditions - determinants of body mass in birds. *Ecology* 67:377-385.

- MacLeod, C. D., R. MacLeod, J. A. Learmonth, W. Cresswell, and G. J. Pierce. 2014. Predicting population-level risk effects of predation from the responses of individuals. *Ecology* 95:2006-2015.
- MacLeod, R. 2006. Why does diurnal mass change not appear to affect the flight performance of alarmed birds? *Animal Behaviour* 71:523-530.
- MacLeod, R., J. Lind, J. Clark, and W. Cresswell. 2007. Mass regulation in response to predation risk can indicate population declines. *Ecology Letters* 10:945-955.
- McNamara, J. M., Z. Barta, A. I. Houston, and P. Race. 2005. A theoretical investigation of the effect of predators on foraging behaviour and energy reserves. *Proceedings of the Royal Society B-Biological Sciences* 272:929-934.
- Møller, A. P. 2010. Up, up, and away: relative importance of horizontal and vertical escape from predators for survival and senescence. *Journal of Evolutionary Biology* 23:1689-1698.
- Peckarsky, B. L., P. A. Abrams, D. I. Bolnick, L. M. Dill, J. H. Grabowski, B. Luttbeg, J. L. Orrock, S. D. Peacor, E. L. Preisser, O. J. Schmitz, and G. C. Trussell. 2008. Revisiting the classics: considering nonconsumptive effects in textbook examples of predator-prey interactions. *Ecology* 89:2416-2425.
- Pierce, B. J., S. R. McWilliams. 2004. Diet quality and food limitation affect the dynamics of body composition and digestive organs in a migratory songbird (*Zonotrichia albicollis*). *Physiological and Biochemical Zoology* 77:471-483.
- Preisser, E. L., D. I. Bolnick. 2008. The many faces of fear: comparing the pathways and impacts of nonconsumptive predator effects on prey populations. *Plos One* 3:e2465.
- Preisser, E. L., D. I. Bolnick, and J. H. Grabowski. 2009. Resource dynamics influence the strength of non-consumptive predator effects on prey. *Ecology Letters* 12:315-323.

- Ruxton, G. D., T. N. Sherratt, and M. P. Speed. 2004. Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry. .
- Salo, P., E. Korpimäki, P. B. Banks, M. Nordstrom, and C. R. Dickman. 2007. Alien predators are more dangerous than native predators to prey populations. *Proceedings of the Royal Society B-Biological Sciences* 274:1237-1243.
- Shifferman, E., D. Eilam. 2004. Movement and direction of movement of a simulated prey affect the success rate in barn owl *Tyto alba* attack. *Journal of Avian Biology* 35:111-116.
- Sih, A., D. I. Bolnick, B. Luttbeg, J. L. Orrock, S. D. Peacor, L. M. Pintor, E. Preisser, J. S. Rehage, and J. R. Vonesh. 2010. Predator-prey naivete, antipredator behavior, and the ecology of predator invasions. *Oikos* 119:610-621.
- Strobbe, F., M. A. McPeck, M. De Block, and R. Stoks. 2010. Survival selection imposed by predation on a physiological trait underlying escape speed. *Functional Ecology* 24:1306-1312.
- Swaddle, J., M. Witter. 1997. The effects of molt on the flight performance, body mass, and behavior of European starlings (*Sturnus vulgaris*): an experimental approach. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 75:1135-1146.
- van den Hout, P. J., K. J. Mathot, L. R. M. Maas, and T. Piersma. 2010. Predator escape tactics in birds: linking ecology and aerodynamics. *Behavioral Ecology* 21:16-25.
- Vezina, F., K. M. Jalvingh, A. Dekinga, and T. Piersma. 2006. Acclimation to different thermal conditions in a northerly wintering shorebird is driven by body mass-related changes in organ size (vol 209, pg 3141, 2006). *Journal of Experimental Biology* 209:3952-3952.
- Walker, J., C. Ghalambor, O. Griset, D. McKenney, and D. Reznick. 2005. Do faster starts increase the probability of evading predators? *Functional Ecology* 19:808-815.

- Warrick, D. R., K. P. Dial. 1998. Kinematic, aerodynamic and anatomical mechanisms in the slow, maneuvering flight of pigeons. *Journal of Experimental Biology* 201:655-672.
- Witter, M., I. Cuthill. 1993. The ecological costs of avian fat storage. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 340:73-92.
- Zanette, L., J. N. M. Smith, H. van Oort, and M. Clinchy. 2003. Synergistic effects of food and predators on annual reproductive success in song sparrows. *Proceedings of the Royal Society B-Biological Sciences* 270:799-803.

Appendices

Appendix A. Picture of take-off measurement apparatus.



Appendix B. Picture of vertical flight chamber.



Appendix C. Table showing actual values of body mass, fat mass, wet lean mass, and vertical flying speed for the body mass manipulation between the baseline and post-manipulation periods for all treatment groups.

Variable	Treatment group	Manipulation period			
		Baseline		Post-manipulation	
		Mean	±SE	Mean	±SE
Body Mass (g)	Control	45.83	1.19	46.85	1.16
	5 % Loss	44.58	1.13	41.67	1.09
	15 % Loss	47.05	1.10	39.10	1.07
Fat Mass (g)	Control	5.51	0.80	6.97	0.67
	5 % Loss	5.16	0.76	4.55	0.63
	15 % Loss	5.76	0.74	2.15	0.61
Wet Lean Mass (g)	Control	30.32	0.54	29.53	0.59
	5 % Loss	29.49	0.51	27.16	0.56
	15 % Loss	30.74	0.50	27.23	0.55
Flying Speed (m/s)	Control	1.54	0.06	1.50	0.07
	5% Loss	1.66	0.06	1.66	0.07
	15% Loss	1.67	0.06	1.69	0.07

Appendix D. Ethics approval for animal use.**AUP Number:** 2010-024**PI Name:** Zanette, Liana**AUP Title:** The Effects Of Predators And Predator Risk On Prey: From Genes To Ecosystems**Approval Date:** 04/04/2014

Official Notice of Animal Use Subcommittee (AUS) Approval: Your new Animal Use Protocol (AUP) entitled "The Effects Of Predators And Predator Risk On Prey: From Genes To Ecosystems" has been APPROVED by the Animal Use Subcommittee of the University Council on Animal Care. This approval, although valid for four years, and is subject to annual Protocol Renewal. 2010-0245

This AUP number must be indicated when ordering animals for this project. Animals for other projects may not be ordered under this AUP number. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Copeman, Laura on behalf of the Animal Use Subcommittee University Council on Animal Care

Curriculum vitae

Benjamin Thomas Walters
 Department of Biology
 University of Western Ontario
 London, ON N6A 5B7

Education

M.Sc. Candidate (Biology) 2015
 University of Western Ontario, London, ON

B.Sc. (Animal Science) 2011
 University of Nebraska, Lincoln, NE

Research Experience

Graduate Student and Research Assistant 2013-2015
 University of Western Ontario
 Department of Biology, Liana Zanette PhD

Research Technician 2009-2012
 University of Nebraska
 UNL Center for Avian Cognition, Alan Bond PhD

Laboratory Assistant 2011
 University of Nebraska
 UNL Reproductive Physiology Lab, Jill Kerl MSc

Teaching Experience

Graduate Teaching Assistant
 University of Western Ontario
 Department of Biology

- BIO 2290G: Scientific Methods in Biology 2015
- BIO 2483A: Ecology 2014
- BIO 2471B: Vertebrate Biology 2014
- BIO 3440A: Ecology of Populations 2013

Undergraduate Teaching Assistant
 University of Nebraska
 Department of Agriculture and Natural Resources

- ASCI 171: Human-Companion Animal Interactions 2011
- ASCI 251: Intro to Companion Animals 2011

Awards and Scholarships

Canadian Society of Zoologists Travel Grant	2015
College of Agriculture and Natural Resources Dean's List	2010-2011
Nebraska Farm Bureau Scholarship	2008

Conferences and Presentations

May 2015. *A tactic to stay alive: high risk environments affect physiological condition and enhance escape behaviour.* Canadian Society of Zoologists, Calgary AB

May 2015. *A tactic to stay alive: anti-predator behavioural responses affect physiology but not flight performance.* Ontario Ecology, Ethology, and Evolution Colloquium, Toronto ON

March 2015. *Brown-headed cowbirds: a real menace?* Retiring with Strong Minds Student Outreach Session, London ON

January 2015. *The effect of perceived predation risk on body condition and flight ability.* Friday Philosophical Graduate Seminar Series, London, ON

October 2014. *Does this predator make me look fat? – The effect of predation risk on body condition,* Biology Graduate Research Forum, London ON

Memberships and Affiliations

Animal Behaviour Society
 Canadian Society of Zoologists
 Alpha Zeta Agricultural Fraternity