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Females in Control: Female Sensitivity to Predation Risk Affects Courtship and Reproductive Behaviours

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

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FEMALES IN CONTROL: FEMALE SENSITIVITY TO PREDATION RISK
AFFECTS COURTSHIP AND REPRODUCTIVE BEHAVIOURS

(Thesis format: Integrated Article)

by

Tin Nok Natalie Cheng

Graduate Program in Biology

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

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Abstract

Even in the absence of direct killing, predators have a pervasive effect on prey populations through costly anti-predator behavioural responses. In high risk environments, animals can reduce conspicuous behaviours such as courtship displays decreasing exposure at the cost of reproduction. Previous studies typically examined male behaviour immediately following a predator cue, thus, not considering temporal variations in risk or how female receptivity affects male courtship. I tested how courtship and reproductive behaviours of male and female brown-headed cowbirds (*Molothrus ater*) responded to chronic predation risk with temporal variations in risk. Under high predation risk, females performed fewer chatter calls, were more likely to reject courting males, and spent less time searching for host nests. Males greatly attenuated courtship displays, but male-male interactions were unaffected. I suggest that females perceive a greater risk associated with these reproductive behaviours than do males, and that females drive the predation risk effects on courtship.

Keywords

Predation risk, anti-predator behaviour, anti-predator behaviour costs, risk effects, sublethal effects, non-consumptive effects, courtship behaviour, female receptivity, risk allocation hypothesis

Co-Authorship Statement

I, Tin Nok Natalie Cheng, will be the first author of the manuscript. I conducted the literature review to help conceptualize and design the experiments. I worked in conjunction with Dr. Liana Zanette and Benjamin Walters to get approval for housing birds in the aviaries as well as setting up the aviaries and the predation risk manipulations. I conducted all the behavioural observations, transcriptions of the voice recordings, statistical analyses of the data, and writing of the manuscript.

Dr. Liana Zanette will be the second co-author on the manuscript. Liana played an integral part in helping to design the experimental protocol and providing the work environment (aviaries and lab space) and funding (NSERC) to make this experiment possible. She also helped with getting ethics approval for animal use and establishing animal care protocols. Furthermore, she contributed insight to the statistical analyses and writing of this thesis.

Benjamin Walters will be the third co-author of the manuscript that will be published from this thesis. Benjamin worked closely with me to set up the experiment site and conduct the predation risk manipulations. He also aided in the care of the animals and collecting video recordings of the birds.

Dr. Michael Clinchy will be the fourth co-author of the manuscript that will be published from this thesis. Michael helped in designing the experiment and setting up the experiment site. He also provided feedback for the data analyses and thesis writing.

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Table of Contents

Abstract.....	ii
Co-Authorship Statement.....	iii
Acknowledgments.....	iv
Table of Contents.....	v
List of Tables.....	vii
List of Figures.....	viii
List of Appendices.....	ix
Chapter 1: General Introduction.....	1
1.1 Predator Impacts on Prey Populations.....	1
1.2 The Benefits and Costs of Anti-Predator Responses.....	2
1.3 Predation Risk Effects on Reproductive Behaviours.....	6
1.4 Predation Risk Effects on Courtship Behaviour.....	7
1.5 Temporal Variations in Predation Risk.....	12
1.6 Factors Influencing the Sensitivity of Reproductive Behaviours to Risk.....	13
1.7 Research Objectives.....	14
1.8 Study Species.....	15
1.9 References.....	17
Chapter 2.....	30
2.1 Introduction.....	30
2.2 Methods.....	36
2.2.1 Animals & Housing.....	36
2.2.2 Predation Risk Manipulation.....	37
2.2.3 Quantifying Courtship, Mating and Other Reproductive Behaviours.....	40
2.2.4 Statistical Analysis.....	44

2.3 Results	47
2.4 Discussion	51
2.5 References	59
Chapter 3: General Discussion.....	68
3.1 Fitness Consequences of Reductions in Reproductive Behaviours	69
3.2 Adaptive Significance of Predation Risk Effects on Reproductive Traits	72
3.3 Study Limitations and Future Directions	74
3.4 Implications for Conservation and Management	75
3.5 Summary	76
3.6 References	78
Appendices.....	83
Curriculum Vitae	88

List of Tables

Table 1. List of species used for the auditory playbacks. Predator calls were matched with a non-predator based on their broadcast time and call acoustic characteristics..... 39

Table 2. List of species of the taxidermic mounts used as a visual predator or non-predator stimulus. Mounts were matched for size and stance. 41

List of Figures

Figure 1. Effect of perceived predation risk on (A) the percentage of rejections brown-headed cowbird (*Molothrus ater*) males received when displaying to females (n = 21), (B) the number of chatter calls produced by females per each aviary over an hour (n = 4), and (C) the number of artificial nest visits made by females over a 30 min nest inspection trial (n = 24) during the predator (red) and non-predator (blue) treatments. For (A) and (B), values presented are means \pm SE. For (C), medians are presented with 25th and 75th percentiles (boxes) and min and max values (bars). 48

Figure 2. Effect of perceived predation risk on the average number of displays brown-headed cowbird (*Molothrus ater*) males performed during a 5 min focal sample to (A) females (courtship, n = 29) and (B) other males (aggressive display, n = 35), during the predator (red) and non-predator (blue) treatments. Values presented are means \pm SE. ... 49

List of Appendices

Appendix A. Satellite view (Google Maps) of the four aviaries (12 x 30 x 60 feet) used to house the cowbirds. Dividers between aviaries 1 and 2 as well as between aviaries 3 and 4 visually isolated each social group.....	83
Appendix B. Creating Playbacks	84
Appendix C. Ethogram	86
Appendix D. Ethics Approval for Animal Use	87

Chapter 1: General Introduction

1.1 Predator Impacts on Prey Populations

Predators are traditionally thought to limit prey populations through the direct killing and eating of prey (Boutin 1995, Sinclair & Pech 1996, Preisser *et al.* 2005, Eberhardt *et al.* 2007). However, this view regarding predator-prey interactions severely underestimates the total effect predators can have on prey populations without considering that the risk of predation alone can influence prey population dynamics (Boonstra *et al.* 1998a, Hik 1995, Creel *et al.* 2007, Preisser & Bolnick 2008). One example of the effect predation risk can have on prey populations comes from studying snowshoe hare (*Lepus americanus*) population cycles. Direct predation is the proximate cause for the majority of snowshoe hare deaths during the declining phase of a cycle (Keith *et al.* 1977, Krebs *et al.* 1995). However, direct predation and food abundance cannot account for the reduced reproductive rate during the population decline or the lag after the decline in which hare populations remain low even when predator populations have declined (Cary & Keith 1979, Hik 1995, Boonstra *et al.* 1998b). Instead, these factors affecting population growth are attributed to predation risk effects on foraging behaviour (Hik 1995) and stress levels (as measured by cortisol levels, body condition, and other indices of stress physiology) affecting fecundity and resulting in long-lasting maternal effects (Boonstra *et al.* 1998b, Sheriff *et al.* 2010, Sheriff *et al.* 2015). Researchers have also documented up to a 43% reduction in pregnancy rates in elk (*Cervus elaphus*; Creel *et al.* 2011) and a 40% reduction in song sparrow (*Melospiza melodia*) offspring produced per year (Zanette *et al.* 2011) in response to increased predation risk.

Furthermore, these predation risk effects (also termed sublethal or non-consumptive effects) are thought to have an equal or even greater effect on prey populations than the direct effect predators have on mortality (Preisser *et al.* 2005, Creel & Christianson 2008). By surgically manipulating Damsel bugs (*Nabis* spp.) such that they could not consume prey but could still disturb prey, Nelson *et al.* (2004) were able to separate and compare the total effect and the risk effects of Damsel bugs predation on pea aphid (*Acyrtosiphon pisum*) population growth. They found that risk effects accounted for 39-80% of the total effect the Damsel bugs had on pea aphid population growth. Creel & Christianson (2008) gathered data from studies before and after reestablishment of the wolf population in Yellowstone National Park and radio-telemetry data of direct predation rates. Using this information, they determined that the risk effects on elk reproduction were approximately 2-3 times larger than direct predation alone. These studies also highlight the fact that quantifying the effects of predation risk requires careful manipulations in empirical studies or large amounts of hard to obtain observational data to separate risk effects from the effects of direct predation or food limitation (Creel & Christianson 2008). Nevertheless, predation risk alone in the absence of direct predation can have a strong impact on prey populations and is crucial to studying predator-prey interactions (Schmitz *et al.* 1997, Lima 1998, Creel & Christianson 2008).

1.2 The Benefits and Costs of Anti-Predator Responses

The mechanism by which predation risk can impact prey populations is through individual-level changes in which the presence of predators induces defensive responses in the prey that can be morphological (Tollrian & Dodson 1999), physiological, or

behavioural (Lima 1998, Preisser *et al.* 2005, Creel & Christianson 2008). Studies on a wide variety of taxa demonstrate that prey are able to assess the risk of predation in the environment and respond accordingly to avoid capture (reviewed in Lima 1998, Caro 2005). Prey can attend to visual (Blumstein *et al.* 2000), olfactory (Kats & Dill 1998, Herman & Valone 2000), and auditory cues (Pusenius & Ostfeld 2000) from their predators to know when and how to respond (Kelley & Magurran 2003, Martin *et al.* 2010). *Daphnia pulex* juveniles grow ‘neckteeth’, small protuberances around the neck reducing their vulnerability to predation in response to chemical cues from predatory *Chaoborus* larvae (Krueger & Dodson 1981). Female great tits (*Parus major*) exposed to perceived predation risk produced offspring that had larger wings and lowered body mass at maturity, which can be potentially advantageous for predator evasion (Coslovsky & Richner 2011). Beyond morphological and physiological responses, prey also exhibit behavioural flexibility to mitigate predation risk.

Behavioural changes in response to predation risk involve reducing conspicuous behaviours and engaging in behaviours that aid in the detection and avoidance of predators (Magnhagen 1991, Sih 1997, Lima 1998, Caro 2005). Birds can flock together (Caro 2005) and fish can form schools (Seghers 1974, Morgan & Godin 1985) in response to high predation risk. Large groups have the benefit of increasing vigilance, confusing the predator with multiple moving targets, and the ‘dilution effect’ where each individual has a reduced probability of being captured within a group (Morgan & Godin 1985, Magurran 1990, Lima 1995). Prey may also change their habitat use in response to predator cues (Lima 1998). When presented olfactory cues of northern pike (*Esox lucius*), juvenile roach (*Rutilus rutilus*) moved to open water, but when presented with visual cues

of pike, roach opted for structured habitat (Martin *et al.* 2010). Furthermore, individuals can reduce the chance of detection by predators by altering or stopping conspicuous behaviours. In the presence of a predator, willow ptarmigans (*Lagopus lagopus*) like many other prey species will remain motionless (i.e. freeze) since movement can aid a predator's search (Gabrielsen *et al.* 1985, Caro 2005). There is also a large body of literature that discusses foraging under predation risk since foraging involves reduced vigilance and increased movement (Godin & Smith 1988). Predation risk can impact where and when prey forage or even what they forage on (Lima & Dill 1990, Lima 1998, Lima & Bednekoff 1999, Brown & Kotler 2004, Christianson & Creel 2010). For example, gerbils (*Gerbillus allenbyi* and *Gerbillus pyramidum*) are captured more frequently in open areas compared to areas with shrub cover. Thus, both gerbil species under predation risk prefer foraging in bush microhabitats (Kotler *et al.* 1991).

While these changes in prey characteristics can help prey increase their chances of survival under predation threat, they also carry concomitant costs on survival and fitness (Lima 1998, Preisser *et al.* 2005, Creel & Christianson 2008). Investing in anti-predator responses can lead to reduced growth (Van Buskirk & Schmidt 2000) or increased susceptibility to other predators (Tollrian & Dodson 1999, Hoverman & Relyea 2009). Larval American bullfrog (*Rana catesbeiana*) and green frogs (*Rana clamitans*) experience lowered growth due to reduced activity levels and avoidance of predatory larval dragonflies (*Anax* spp.; Relyea & Werner 1999). Increased vigilance can negatively impact energy intake by reducing feeding efficiency (Fritz *et al.* 2002, Fortin *et al.* 2004). Reductions in energy intake can lead to death by starvation; however, individuals are typically able to balance the risk of starvation with the threat of predation

(Lima & Dill 1990, Lima 1998). Hungrier prey will be more willing to accept predation risk and forage more to prevent starvation (Bachman 1993, Koivula *et al.* 1995, Krause *et al.* 1998). Instead, reproduction is the other major cost of risk-sensitive foraging via ‘nutritionally mediated risk effects’ on the number or quality of offspring (Christianson & Creel 2010).

Reproduction has a great energy requirement (Drent & Daan 1980) and is also extremely nutritionally demanding for amino acids, vitamins, and minerals (Rose & Bradley 1998, Zanette *et al.* 2003). Thus, it is intuitive that changes in foraging behaviour that affect energy and nutrition intake would impact a female’s reproductive capabilities. In response to wolf predation risk, elks decreased their foraging and changed foraging habitats switching to a lower quality diet (Creel *et al.* 2005, Christianson & Creel 2008). To compensate for the large deficiencies, elk had to catabolize their own fat and muscle stores indicating a strong enough risk effect to impact reproduction (Christianson & Creel 2008, Christianson & Creel 2010, Creel *et al.* 2011). Risk effects on prey growth rate through lowered energy intake can also affect fitness since smaller individuals produce fewer eggs within some species (Williams 1966, Honěk 1993, Scrimgeour & Culp 1994). Reductions in parental provisioning due to changes in parental foraging as seen in stonechats (Scheuerlein & Gwinner 2006) and song sparrows (Zanette *et al.* 2011) can also stunt the growth and development and affect juvenile survival. Therefore, risk effects on foraging behaviour can have marked consequences for reproduction. Predation risk can also further affect reproductive success through changes in reproductive behaviours important for successfully finding a mate and ensuring offspring quality and survival.

1.3 Predation Risk Effects on Reproductive Behaviours

Engaging in reproduction incurs numerous physiological and behavioural costs that can shape an individual's investments into reproduction. Physiological costs of reproduction such as reduced growth (Fitch 1970) and decreased immune function against parasites (Lawniczak *et al.* 2007) or disease (Descamps *et al.* 2009) can impact future reproduction and affect how individuals may invest into current reproduction (reviewed in Reznick 1985, Reznick 1992). More importantly, the ultimate cost of reproduction is on survival as many reproductive behaviours inherently increase the susceptibility to predators (Lima & Dill 1990, Magnhagen, 1991, Sih 1994, Lima 2009). For example, increased movement involved with searching for and locating potential mates greatly increases the risk of encountering predators (Lima & Dill 1990, Sih 1994, Karino *et al.* 2000). Carrying eggs inhibits escape for gravid females as demonstrated in two species of Australian scincid lizards (*Leiopisma entrecasteauxii* and *Sphenomorphus tympanum*; Shine 1980) and starlings (*Sturnus vulgaris*; Lee *et al.* 1996). Copulation itself can also impair escape from predation as it reduces vigilance and manoeuvrability of prey (Magnhagen 1991, Sih 1994). Water striders (*Gerris remigis*) are placed under a greater risk during pre-mating struggles where males try to grasp females and also during copulation (Sih *et al.* 1990, Rowe 1994). It is clear that engaging in reproduction can greatly increase an individual's risk of predation.

In response to the survival cost of reproduction, prey can alter their reproductive behaviours to mitigate this risk. In an extreme example, bank voles (*Clethrionomys glareolus*) completely suppressed breeding after exposure to the odour of stoats (*Mustela ermine*; Ylönen & Ronkainen 1994). Predator risk effects on the number and duration of mating attempts has been demonstrated in water striders (Sih *et al.* 1990), wolf spiders

(*Pardosa milvina*; Taylor & Persons 2005), and an amphipod crustacean (*Gammarus duebeni*; Dunn *et al.* 2008). Intrasexual aggression related to gaining access to mates can also be influenced by predation risk; male guppies (*Poecilia reticulata*) reduced aggressive interactions with other male guppies in the presence of a predator (Kelly & Godin 2001). These examples demonstrate how risk effects can be mediated through changes in reproductive behaviours that have consequences for prey reproductive success. Another reproductive behaviour that can be risk sensitive is courtship which is typically necessary to gain mating opportunities but can be highly conspicuous (Magnhagen 1991, Hughes *et al.* 2012).

1.4 Predation Risk Effects on Courtship Behaviour

Courtship behaviour is a well-studied topic in a wide variety of taxa (Byers *et al.* 2010 and references therein) including different species of fish (Houde 1987, Hagedorn & Heiligenberg 1985), arthropods (Spieth 1974, Robinson & Robinson 1980), mammals (You & Jiang 2005, Musolf *et al.* 2010), reptiles (Crews 1975), amphibians (Wells 1978), and birds (Wood-Gush 1956, Morse 1970). Courtship involves using elaborate ornaments, dances, pheromones, or songs to attract potential mates and gain copulations. For example, to court females, male guppies vibrate stiffly to display his orange spots (Houde 1987). The superb bird of paradise (*Lophorina superba*) calls to attract the attention of a female then displays to her by thrusting out his breast shield showing off his iridescent crown (Frith & Frith 1988). Male wolf spiders (*Schizocosa crassipes*) use their forelegs to tap and wave creating tactile vibrations and a visual display for females (Stafstrom & Hebets 2013). Thus, courtship can be quite complex involving more than one sensory modality (Byers *et al.* 2010, Stafstrom & Hebets 2013).

The complexity or energetic costs of courtship signals makes them an honest signal of quality such that only males of high quality would be able produce the most desired form of the trait or display (Byers *et al.* 2010). Many aspects of courtship displays also reflect motor skills important for hunting or escaping predators like speed and manoeuvrability (Byers *et al.* 2010). Some studies have shown offspring had better survival, health and development when sired by males with more elaborate ornaments (Petrie 1994). Therefore, females can choose males using courtship as an honest signal of male genetic quality and proper development (Kodric-Brown & Brown 1984, Byers *et al.* 2010). Female selection for males that can provide her offspring with the most advantageous traits is especially important for species with males that only provide sperm without other benefits like parental care or defense (Trivers 1972). Some examples of female preferences for elaborate courtship signals include female guppies that prefer males with brighter orange spots (Houde 1987) and female wolf spiders that choose males who are able to perform more body shakes and leg raises (Rypstra *et al.* 2003). Male blue tits (*Cyanistes caeruleus*) that have more complex songs with highly consistent intersong intervals had greater reproductive success (Poesel *et al.* 2001). Although males should produce courtship signals of high intensity, they must balance the reproductive benefits with the cost of also being more conspicuous to predators (Magnhagen 1991).

Predators can easily eavesdrop in on prey courtship signals in order to locate and capture their prey (Burk 1982, Magnhagen 1991, Zuk & Kolluru 1998, Hughes *et al.* 2012). Fringe-lipped bats (*Trachops cirrhosus*) attend to playbacks of *Hyla boulengeri*, a species of frog they prey upon (Tuttle & Ryan 1981). Big brown bats (*Eptesicus fuscus*) locate katydids by listening to chorus calls (Buchler & Childs 1981). While it is typically

thought that males suffer the consequences of increased predation risk as the signaller, females are now known to also be at risk when attending to courting males (Hughes *et al.* 2012). Female wolf spiders (*Schizocosa uetzi*) were unable to detect a visual predator and captured more easily captured when attending to courtship vibrations (Hebets 2004). Geckos (*Hemidactylus tursicus*) wait outside the burrows of calling male field crickets (*Gryllobates suppicans*) in order to prey upon the female crickets attending to the courtship calls (Sakaluk & Belwood 1984). To alleviate the increased risk, individuals should alter when and how they engage in courtship.

Males have been found to reduce their courtship effort in response to predators by courting less or at a lower intensity (Magnhagen 1991). In response to frog-eating bats, tungara frogs (*Physalaemus pustulosus*) reduce the number of “chucks” they produce as part of their advertisement call (Ryan 1985). Mountain dusky salamanders (*Desmognathus orochrophaeus*) reduced their courtship behaviour when presented with a predator resulting in fewer spermatophore depositions (Uzendoski *et al.* 1993). Researchers examined changes in fiddler crab (*Uca beebei*) courtship behaviour in response to grackles (*Quiscalus mexicanus*) and found that males waved their claw less and built fewer mud pillars. Overall, fiddler crabs were observed to mate less in areas with more birds (Koga *et al.* 1998). Thus, males are able to assess the risk of engaging in courtship and adjust their behaviour to survive at the cost of reproductive success.

Females under elevated predation risk also change their behaviour in regards to courtship. However, this topic can benefit from further research as studies have traditionally focused on changes in male behaviour as the signaller (Hughes *et al.* 2012). Females may be less sexually receptive and less willing move around to sample males.

They may also actively avoid courting males so that they are not in close association with the conspicuous courtship signals (Sih 1994, Hughes *et al.* 2012). Fewer female fiddler crabs were willing to leave burrows to sample males in high risk environments (Koga *et al.* 1998) and female guppies spent less time near males after exposure to a predator stimulus (Godin & Briggs 1996). Studies have mainly focused on the consequences of these changes in mating behaviour in terms of mate choice. There may be less opportunity to choose the best quality male or females may choose based on safety instead of quality. Both guppies (Godin & Briggs 1996) and sand gobies (*Pomatoschistus minutus*; Forsgren 1992) no longer showed preference for more brightly coloured males in high risk environments. Female crickets typically prefer males with longer calls, but under predation risk, they chose males with shorter calls in safer locations (Hedrick & Dill 1993). Risk effects on female courtship behaviour can then theoretically lead to lost mating opportunities or lower quality offspring.

Another aspect of potential risk effects on courtship that requires more attention is the idea that changes in the behaviour of one sex should affect the other sex. Males are known to use cues from females to determine when or how vigorously to court. Male ring doves (*Streptopelia risoria*) responded to contact and auditory cues from females by engaging in more aggressive courtship (O'Connell *et al.* 1981). Male guppies only increased their courtship behaviour if presented with a receptive female, but not a non-receptive female (Guevara-Fiore *et al.* 2010). Unfortunately, studies that look at how risk effects on female behaviour can affect how males choose to engage in courtship are limited. Male jumping spiders (*Jacksonoides queenslandicus*; Su & Li 2006) and guppies (Dill *et al.* 1999) only reduced their courtship when females were exposed to the

predator, but not when only the males were exposed. While these studies demonstrate that females can drive changes in courtship, Su & Li (2006) and Dill and colleagues (1999) only measured male behaviour and did not directly identify what changes in female behaviour lead to the courtship reduction. Females may be actively avoiding males or males may choose to decrease their courtship effort if females are sexually unreceptive since courting would be lost energy and time (Evans *et al.* 2002, Hughes *et al.* 2012). Evans *et al.* (2002) found that presenting female guppies only with a predator resulted in male guppies switching from courtship to forced matings. He proposed from qualitative observations that this switch may be in response to females being less active and visually fixated on where the predator was last seen. Studies should quantify behaviours of both sexes to properly measure how risk may change courtship interactions.

My study uses an avian species and predation risk is a known cost of courtship in birds (Mougeot & Bretagnolle 2000, Hale 2004, Møller *et al.* 2006). While there is a lot known about courtship in birds such as male song complexity and repertoire size (Hasselquist *et al.* 1996, Reid *et al.* 2004), there are very few studies which look at risk effects on birdsong and displays (Lima 2009). One observational study found that northern cardinals (*Cardinalis cardinalis*) used less exposed perches to sing while near Cooper's hawk (*Accipiter cooperii*) nests (Duncan & Bednekoff 2006). Only recently was there an experimental study that found that veeries (*Catharus fuscescens*) sang fewer songs in the dusk chorus in response to owl playbacks (Schmidt *et al.* 2013). Further studies are needed to examine the behavioural flexibility of avian courtship under predation risk.

1.5 Temporal Variations in Predation Risk

Traditionally, studies measure anti-predator behaviour during or immediately after exposure to a predator or predator cue. Using this paradigm, prey would be expected to react strongly as they are under a dire threat and the results from these studies would suggest that lost foraging or mating opportunities would affect energy intake and fitness (Lima & Bednekoff 1999, Hughes *et al.* 2012). However, behavioural decisions that prey make also account for the fact that the level of predation risk varies temporally in a natural setting (Lima & Bednekoff 1999). Prey can compensate for periods of high risk by engaging more in the behaviours during times of low risk as described by the 'risk allocation hypothesis' (Lima & Bednekoff 1999). Tests of the risk allocation hypothesis do find that prey held in continual risk respond to temporal variations by being more active during periods of safety (Sih & McCarthy 2002, Creel *et al.* 2008).

This hypothesis helps to explain studies within the foraging literature that have inconsistent results on changes in body mass in response to predation threat. It was typically thought that body mass would be reduced due to lowered energy intake in risky situations. However, the 'interrupted foraging hypothesis', which relates to the risk allocation hypothesis, proposes that prey may greatly increase foraging in times of safety to prevent starvation when there are unpredictable periods of high risk thus maintaining or even exceeding their normal energy intake (Lima 1986, Lima & Bednekoff 1999, Macleod & Gosler 2006). Therefore, experimental studies that hold prey under constant safety then present them with a brief pulse of high risk may greatly overestimate the impacts of risk effects (Lima & Bednekoff 1999). Similarly, individuals may allocate their courtship behaviour to periods when they do not perceive any predators to maintain

their fitness. But there is a lack of experimental studies that consider temporal variations in risk when examining the impacts on courtship behaviour (Hughes *et al.* 2012).

1.6 Factors Influencing the Sensitivity of Reproductive Behaviours to Risk

Since changes in reproductive behaviours have direct fitness consequences, these behaviours may be less sensitive or not sensitive at all to predation risk if there are greater benefits to reproduction than the costs to survival. Prey must take into account the risk engaging in the behaviour poses and their residual reproductive value (i.e. their prospects for future reproduction) to make behavioural decisions that will optimize lifetime reproductive success (Lima & Dill 1990, Maghagen 1991). Although reproductive behaviours do typically increase prey exposure to predators, this is not necessarily true for every individual and the risk of predation may differ between individuals. Female jumping spiders (*J. queenslandicus*) are preferentially preyed upon by another species of jumping spiders (*Portia fimbriata*). *J. queenslandicus* males were found to be insensitive to increased predation risk displaying to females even when exposed to predators (Su & Li 2006). Male field crickets also showed differential response to predation risk depending on the length of their mating calls. Males with shorter calls were less sensitive to predation risk as measured by their how long they hid in a novel environment and their latency to resume calling after being disturbed by a predator (Hedrick 2000). Therefore, prey are able to assess their individual risk which influences how they might respond to elevated predation risk.

Life history traits also play a role in how prey respond to predation risk (Lima & Dill 1990, Maghagen 1991, Candolin 1998). Older individuals or individuals that only reproduce once should also be less sensitive to stressors (Clutton-Brock 1984, Wingfield

& Sapolsky 2003). The strategy in these situations should be to invest as much as possible into current reproduction even in the face of predation risk as there is little to no chance of reproducing in the future (Williams 1966). For prey that can reproduce multiple times, they have a greater residual reproductive value so the cost of predation on their fitness is greater since being killed by a predator negates any chance for future reproduction (Williams 1966, Kalas *et al.* 1995). In these cases, prey should reduce risky reproductive behaviours under increased predation risk to help them survive to reproduce in the future maximizing their lifetime reproductive success (Lima & Dill 1990, Maghagen 1991). These theories are demonstrated in a study by Candolin (1998) looking at threespine stickleback (*Gasterosteus aculeatus*) that reproduces multiple times during one breeding season. Male sticklebacks reduced reproductive efforts early on in the season in response to predation risk when there were still chances for reproduction later, but nearer the end of the season, they were willing to accept greater risks to reproduce more. Individual differences in sensitivity to predation risk make it hard to make generalized statements about risk effects on reproduction. Thus, more empirical studies are needed to further understand the intricacies of prey reproductive decisions under predation risk.

1.7 Research Objectives

While previous research has provided insights into the behavioural decisions prey make under predation risk regarding courtship, there are areas that require additional attention as discussed in this chapter that I aimed to address in the study presented in chapter two. I conducted an experimental study using an avian species to examine how courtship behaviour may respond to temporal changes in predation risk. I quantified

behaviours of both sexes to see how female behaviour can influence male courtship. I also measured the sensitivity of other reproductive behaviours such as female nest inspection and male dominance interactions to predation risk.

For this study, I manipulated the risk of predation in the environment using predator call playbacks and taxidermic mounts over a 10 d period. Although the birds were under chronic predation risk, there were periods of low risk in which no calls or mounts were presented thus altering the level of risk in the environment over time. I predicted that, if engaging in the courtship is risky, males should reduce their courtship effort and females would be less receptive to male courtship. If males reduced their courtship but not their other reproductive behaviours, this would suggest that male courtship behaviour may be influenced by the potential reduction in female receptivity. The birds may also allocate their courtship behaviour to periods of low risk to help alleviate any reductions in courtship during periods of high risk. Furthermore, I discuss how the results of my study can help to explain differences in the chances for future reproduction and the risks perceived between individuals.

1.8 Study Species

My study species is the brown-headed cowbird (*Molothrus ater*; herein referred to as ‘cowbirds’), a small icterid species. They are a wide-spread species found in northern Mexico, across the United States, and in southern Canada. Males have a brown coloured head with an iridescent black body while female plumage is brown throughout. Cowbirds are obligate brood parasites that lay their eggs in other birds’ nests and are known to parasitize over 220 species of birds (Friedman & Kiff 1985). Since they do not provide parental care, their reproductive success is mainly constrained by the number of eggs they

can produce or the availability of host nests (Scott & Ankney 1980). They are also able to spend much of their time during the breeding season engaging in courtship making them an ideal species to study chronic effects of predation risk on courtship behaviour.

The cowbird breeding season occurs between April and early July. In the mornings, females and males engage in courtship and mating behaviour around the forest edge near potential host nests. Females also use this time to inspect potential host nests (Thompson III 1994). Males court females by singing and performing a display that involves bowing with their wings spread. Males also use this display aggressively to other males. Female cowbirds are signallers themselves, producing chatter calls during the breeding season that males can orient to (Rothstein *et al.* 1988). During my study, I examined how predation risk affected the signalling behaviour of both males and females as well as how males were influenced by risk effects on female receiving behaviour.

1.9 References

- Bachman, G. C. (1993). The effect of body condition on the trade-off between vigilance and foraging in Belding's ground squirrels. *Animal Behaviour*, *46*(2), 233–244.
- Blumstein, D. T. (2000). Insular tammar wallabies (*Macropus eugenii*) respond to visual but not acoustic cues from predators. *Behavioral Ecology*, *11*(5), 528–535.
- Boonstra, R., Hik, D., Singleton, G. R., & Tinnikov, A. (1998a). The impact of predator-induced stress on the snowshoe hare cycle. *Ecological Monographs*, *68*(3), 371–394.
- Boonstra, R., Krebs, C. J., & Stenseth, N. C. (1998b). Population cycles in small mammals: The problem of explaining the low phase. *Ecology*, *79*(5), 1479–1488.
- Boutin, S. (1995). Testing predator-prey theory by studying fluctuating populations of small mammals. *Wildlife Research*, *22*(1), 89–99.
- Brown, J. S., & Kotler, B. P. (2004). Hazardous duty pay and the foraging cost of predation. *Ecology Letters*, *7*(10), 999–1014.
- Buchler, E. R., & Childs, S. B. (1981). Orientation to distant sounds by foraging big brown bats (*Eptesicus fuscus*). *Animal Behaviour*, *29*(2), 428–432.
- Burk, T. (1982). Evolutionary significance of predation on sexually signalling males. *The Florida Entomologist*, *65*(1), 90–104. Retrieved from
- Byers, J., Hebets, E., & Podos, J. (2010). Female mate choice based upon male motor performance. *Animal Behaviour*, *79*(4), 771–778.
- Candolin, U. (1998). Reproduction under predation risk and the trade-off between current and future reproduction in the threespine stickleback. *Proceedings of the Royal Society B: Biological Sciences*, *265*(1402), 1171–1175.
- Caro, T. (2005). *Antipredator Defenses in Birds and Mammals*. University of Chicago Press.

- Cary, J. R., & Keith, L. B. (1979). Reproductive change in the 10-year cycle of snowshoe hares. *Canadian Journal of Zoology*, *57*(2), 375–390.
- Christianson, D., & Creel, S. (2008). Risk effects in elk: Sex-specific responses in grazing and browsing due to predation risk from wolves. *Behavioral Ecology*, *19*(6), 1258–1266.
- Christianson, D., & Creel, S. (2010). A nutritionally mediated risk effect of wolves on elk. *Ecology*, *91*(4), 1184–1191.
- Clutton-Brock, T. H. (1984). Reproductive effort and terminal investment in iteroparous animals. *The American Naturalist*, *123*(2), 212–229.
- Coslovsky, M., & Richner, H. (2011). Predation risk affects offspring growth via maternal effects. *Functional Ecology*, *25*(4), 878–888.
- Creel, S., & Christianson, D. (2008). Relationships between direct predation and risk effects. *Trends in Ecology & Evolution*, *23*(4), 194–201.
- Creel, S., Christianson, D. A., & Winnie, J. A. (2011). A survey of the effects of wolf predation risk on pregnancy rates and calf recruitment in elk. *Ecological Applications*, *21*(8), 2847–2853.
- Creel, S., Christianson, D., Liley, S., & Winnie, J. A. (2007). Predation risk affects reproductive physiology and demography of elk. *Science (New York, N.Y.)*, *315*(5814), 960–960.
- Creel, S., & Winnie, J. A. (2005). Elk alter habitat selection as an antipredator response to wolves. *Ecology*, *86*(12), 3387–3397.
- Creel, S., Winnie, J. A., Christianson, D., & Liley, S. (2008). Time and space in general models of antipredator response: Tests with wolves and elk. *Animal Behaviour*, *76*(4), 1139–1146.

- Crews, D. (1975). Effects of different components of male courtship behaviour on environmentally induced ovarian recrudescence and mating preferences in the lizard, *Anolis carolinensis*. *Animal Behaviour*, 23(2), 349–356.
- Descamps, S., Gilchrist, H. G., Bety, J., Buttlar, E. I., & Forbes, M. R. (2009). Costs of reproduction in a long-lived bird: Large clutch size is associated with low survival in the presence of a highly virulent disease. *Biology Letters*, 5(2), 278–281.
- Dill, L. M., Hedrick, A. V., & Fraser, A. (1999). Male mating strategies under predation risk: Do females call the shots? *Behavioral Ecology*, 10(4), 452–461.
- Drent, R., & Daan, S. (1980). The prudent parent: Adjustments in avian breeding. *Ardea*, 68(1-4), 225–252.
- Duncan, W. J., & Bednekoff, P. A. (2006). Singing in the shade: Song and song posts of northern cardinals near nesting Cooper's hawks. *Canadian Journal of Zoology*, 84(6), 916–919.
- Dunn, A. M., Dick, J. T. A., & Hatcher, M. J. (2008). The less amorous Gammarus: Predation risk affects mating decisions in *Gammarus duebeni* (Amphipoda). *Animal Behaviour*, 76(4), 1289–1295.
- Eberhardt, L. L., White, P. J., Garrott, R. A., & Houston, D. B. (2007). A seventy-year history of trends in Yellowstone's northern elk herd. *Journal of Wildlife Management*, 71(2), 594–602.
- Evans, J., Kelley, J., Ramnarine, I., & Pilastro, A. (2002). Female behaviour mediates male courtship under predation risk in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, 52(6), 496–502.
- Fitch, H. S. (1970). *Reproductive cycles of lizards and snakes*. Lawrence, Kansas: University of Kansas, Museum of Natural History.
- Forsgren, E. (1992). Predation risk affects mate choice in a gobiid fish. *The American Naturalist*, 140(6), 1041–1049.

- Fortin, D., Boyce, M. S., Merrill, E. H., & Fryxell, J. M. (2004). Foraging costs of vigilance in large mammalian herbivores. *Oikos*, *107*(1), 172–180.
- Friedmann, H., & Kiff, L. F. (1985). The parasitic cowbirds and their hosts. *Proceedings of the Western Foundation of Vertebrate Zoology*, *2*(4), 1–79.
- Frith, D. W., & Frith, C. W. (1988). Courtship display and mating of the superb bird of paradise *Lophorina superba*. *Emu*, *88*(3), 183–188.
- Fritz, H., Guillemain, M., & Durant, D. (2002). The cost of vigilance for intake rate in the mallard (*Anas platyrhynchos*): An approach through foraging experiments. *Ethology Ecology & Evolution*, *14*(2), 91–97.
- Gabrielsen, G., Blix, A., & Ursin, H. (1985). Orienting and freezing responses in incubating ptarmigan hens. *Physiology & Behavior*, *34*(6), 925–934.
- Godin, J. G. J., & Smith, S. A. (1988). A fitness cost of foraging in the guppy. *Nature*, *333*(6168), 69–71.
- Godin, J.-G. J., & Briggs, S. E. (1996). Female mate choice under predation risk in the guppy. *Animal Behaviour*, *51*(1), 117–130.
- Guevara-Fiore, P., Stapley, J., & Watt, P. J. (2010). Mating effort and female receptivity: How do male guppies decide when to invest in sex? *Behavioral Ecology and Sociobiology*, *64*(10), 1665–1672.
- Hagedorn, M., & Heiligenberg, W. (1985). Court and spark: Electric signals in the courtship and mating of gymnotoid fish. *Animal Behaviour*, *33*(1), 254–265.
- Hale, A. M. (2004). Predation risk associated with group singing in a neotropical wood-quail. *The Wilson Bulletin*, *116*(2), 167–171.
- Hasselquist, D., Bensch, S., & von Schantz, T. (1996). Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature*, *381*(6579), 229–232.

- Hebets, E. A. (2004). Attention-altering signal interactions in the multimodal courtship display of the wolf spider *Schizocosa uetzi*. *Behavioral Ecology*, *16*(1), 75–82.
- Hedrick, A. V. (2000). Crickets with extravagant mating songs compensate for predation risk with extra caution. *Proceedings. Biological Sciences / The Royal Society*, *267*(1444), 671–675.
- Hedrick, A. V., & Dill, L. M. (1993). Mate choice by female crickets is influenced by predation risk. *Animal Behaviour*, *46*(1), 193–196.
- Herman, C. S., & Valone, T. J. (2000). The effect of mammalian predator scent on the foraging behavior of *Dipodomys merriami*. *Oikos*, *91*(1), 139–145.
- Hik, D. (1995). Does risk of predation influence population dynamics? Evidence from cyclic decline of snowshoe hares. *Wildlife Research*, *22*(1), 115–129.
- Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: A general relationship. *Oikos*, *66*(3), 483–492.
- Houde, A. E. (1987). Mate choice based upon naturally occurring color-pattern variation in a guppy population. *Evolution*, *41*(1), 1–10.
- Hoverman, J. T., & Relyea, R. A. (2009). Survival trade-offs associated with inducible defences in snails: The roles of multiple predators and developmental plasticity. *Functional Ecology*, *23*(6), 1179–1188.
- Hughes, N. K., Kelley, J. L., & Banks, P. B. (2012). Dangerous liaisons: The predation risks of receiving social signals. *Ecology Letters*, *15*(11), 1326–39.
- Kalas, J. A., Fisk, P., & Saether, S. A. (1995). The effect of mating probability on risk taking: An experimental study in lekking great snipe. *The American Naturalist*, *146*(1), 59–71.

- Karino, K., Kuwamura, T., Nakashima, Y., & Sakai, Y. (2000). Predation risk and the opportunity for female mate choice in a coral reef fish. *Journal of Ethology*, *18*(2), 109–114.
- Kats, L. B., & Dill, L. M. (1998). The scent of death: Chemosensory assessment of predation risk by prey animals. *Écoscience*, *5*(3), 361–394.
- Keith, L. B., Todd, A. W., Brand, C. J., Adamcik, R. S., & Rusch, D. H. (1977). An analysis of predation during a cyclic fluctuation of snowshoe hares. *Proceedings of the International Congress of Game Biologists*, *13*, 151–175.
- Kelley, J. L., & Magurran, A. E. (2003). Learned predator recognition and antipredator responses in fishes. *Fish and Fisheries*, *4*(3), 216–226.
- Kelly, C., & Godin, J.-G. (2001). Predation risk reduces male-male sexual competition in the Trinidadian guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, *51*(1), 95–100.
- Kodric-Brown, A., & Brown, J. H. (1984). Truth in advertising: The kinds of traits favored by sexual selection. *The American Naturalist*, *124*(3), 309–323.
- Koga, T., Backwell, P. R. Y., Jennions, M. D., & Christy, J. H. (1998). Elevated predation risk changes mating behaviour and courtship in a fiddler crab. *Proceedings of the Royal Society B: Biological Sciences*, *265*(1404), 1385–1390.
- Koivula, K., Rytokonen, S., & Orell, M. (1995). Willow Tits. *Ardea*, *83*, 397–404.
- Kotler, B. P., Brown, J. S., & Hasson, O. (1991). Factors affecting gerbil foraging behavior and rates of owl predation. *Ecology*, *72*(6), 2249–2260.
- Krause, J., Loader, S. P., McDermott, J., & Ruxton, G. D. (1998). Refuge use by fish as a function of body length-related metabolic expenditure and predation risks. *Proceedings of the Royal Society B: Biological Sciences*, *265*(1413), 2373–2379.

- Krebs, C. J., Boutin, S., Boonstra, R., Sinclair, A. R., Smith, J. N., Dale, M. R., ... Turkington, R. (1995). Impact of food and predation on the snowshoe hare cycle. *Science (New York, N.Y.)*, 269(5227), 1112–1115.
- Krueger, D. A., & Dodson, S. I. (1981). Embryological induction and predation ecology in *Daphnia pulex*. *Limnology and Oceanography*, 26(2), 219–223.
- Lawniczak, M., Barnes, A., Linklater, J., Boone, J., Wigby, S., & Chapman, T. (2007). Mating and immunity in invertebrates. *Trends in Ecology & Evolution*, 22(1), 48–55.
- Lee, S. J., Witter, M. S., Cuthill, I. C., & Goldsmith, A. R. (1996). Reduction in escape performance as a cost of reproduction in gravid starlings (*Sturnus vulgaris*). *Proceedings of the Royal Society B: Biological Sciences*, 263(1370), 619–623.
- Lima, S. L. (1986). Predation risk and unpredictable feeding conditions: Determinants of body mass in birds. *Ecology*, 67(2), 377–385.
- Lima, S. L. (1995). Back to the basics of anti-predatory vigilance: The group-size effect. *Animal Behaviour*, 49(1), 11–20.
- Lima, S. L. (1998). Nonlethal effects in the ecology of predator-prey interactions. *BioScience*, 48(1), 25–34.
- Lima, S. L. (2009). Predators and the breeding bird: Behavioral and reproductive flexibility under the risk of predation. *Biological Reviews*, 84(3), 485–513.
- Lima, S. L., & Bednekoff, P. A. (1999). Temporal variation in danger drives antipredator behavior: The predation risk allocation hypothesis. *The American Naturalist*, 153(6), 649–659.
- Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, 68(4), 619–640.

- Macleod, R., & Gosler, A. G. (2006). Capture and mass change: Perceived predation risk or interrupted foraging? *Animal Behaviour*, *71*(5), 1081–1087.
- Magnhagen, C. (1991). Predation risk as a cost of reproduction. *Trends in Ecology & Evolution*, *6*(6), 183–186.
- Magurran, A. E. (1990). The adaptive significance of schooling as an anti-predator defence in fish. *Annales Zoologici Fennici*, *27*(2), 51–66.
- Martin, C. W., Fodrie, F. J., Heck, K. L., & Mattila, J. (2010). Differential habitat use and antipredator response of juvenile roach (*Rutilus rutilus*) to olfactory and visual cues from multiple predators. *Oecologia*, *162*(4), 893–902.
- Møller, A. P. (2005). Song post exposure, song features, and predation risk. *Behavioral Ecology*, *17*(2), 155–163.
- Morgan, M. J., & Godin, J. J. (1985). Antipredator benefits of schooling behaviour in a Cyprinodontid fish, the banded killifish (*Fundulus diaphanus*). *Zeitschrift Für Tierpsychologie*, *70*(3), 236–246.
- Morse, D. H. (1970). Territorial and courtship songs of birds. *Nature*, *226*(5246), 659–661.
- Mougeot, F., & Bretagnolle, V. (2000). Predation as a cost of sexual communication in nocturnal seabirds: An experimental approach using acoustic signals. *Animal Behaviour*, *60*(5), 647–656.
- Musolf, K., Hoffmann, F., & Penn, D. J. (2010). Ultrasonic courtship vocalizations in wild house mice, *Mus musculus musculus*. *Animal Behaviour*, *79*(3), 757–764.
- Nelson, E. H., Matthews, C. E., & Rosenheim, J. A. (2004). Predators reduce prey population growth by inducing changes in prey behaviour. *Ecology*, *85*(7), 1853–1858.

- O'Connell, M. E., Reboulleau, C., Feder, H. H., & Silver, R. (1981). Social interactions and androgen levels in birds. *General and Comparative Endocrinology*, *44*(4), 454–463.
- Petrie, M. (1994). Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature*, *371*(6498), 598–599.
- Poesel, A., Foerster, K., & Kempenaers, B. (2001). The dawn song of the blue tit *Parus caeruleus* and its role in sexual selection. *Ethology*, *107*(6), 521–531.
- Preisser, E. L., & Bolnick, D. I. (2008). When predators don't eat their prey: Nonconsumptive predator effects on prey dynamics. *Ecology*, *89*(9), 2414–2415.
- Preisser, E. L., Bolnick, D. I., & Benard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, *86*(2), 501–509.
- Pusenius, J., & Ostfeld, R. S. (2000). Effects of stoat's presence and auditory cues indicating its presence on tree seedling predation by meadow voles. *Oikos*, *91*(1), 123–130.
- Reid, J. M., Arcese, P., Cassidy, A. L. E. V., Hiebert, S. M., Smith, J. N. M., Stoddard, P. K., ... Keller, L. F. (2004). Song repertoire size predicts initial mating success in male song sparrows, *Melospiza melodia*. *Animal Behaviour*, *68*(5), 1055–1063.
- Relyea, R. A., & Werner, E. E. (1999). Quantifying the relation between predator-induced behavior and growth performance in larval anurans. *Ecology*, *80*(6), 2117–2124.
- Remick, D. (1992). Measuring the costs of reproduction. *Trends in Ecology & Evolution*, *7*(2), 42–45.
- Reznick, D. (1985). Costs of reproduction: An evaluation of the empirical evidence. *Oikos*, *44*(2), 257–267.

- Robinson, M. H., & Robinson, B. (1980). *Comparative studies of the courtship and mating behavior of tropical araneid spiders (Pacific Insects Monograph)*. Honolulu, Hawai'i: Dept. of Entomology, Bishop Museum.
- Rose, M. R., & Bradley, T. J. (1998). Evolutionary physiology of the cost of reproduction. *Oikos*, 83(3), 443–451.
- Rothstein, S. I., Yokel, D. A., & Fleischer, R. C. (1988). The agonistic and sexual functions of vocalizations of male brown-headed cowbirds, *Molothrus ater*. *Animal Behaviour*, 36(1), 73–86.
- Rowe, L. (1994). The costs of mating and mate choice in water striders. *Animal Behaviour*, 48(5), 1049–1056.
- Ryan, M. J. (1985). *The tungara frog: A study in sexual selection and communication*. Chicago, Illinois: University of Chicago Press.
- Rypstra, A. L., Wieg, C., Walker, S. E., & Persons, M. H. (2003). Mutual mate assessment in wolf spiders: Differences in the cues used by males and females. *Ethology*, 109(4), 315–325.
- Sakaluk, S. K., & Belwood, J. J. (1984). Gecko phonotaxis to cricket calling song: A case of satellite predation. *Animal Behaviour*, 32(3), 659–662.
- Scheuerlein, A., & Gwinner, E. (2006). Reduced nestling growth of East African stonechats *Saxicola torquata axillaris* in the presence of a predator. *Ibis*, 148(3), 468–476.
- Schmidt, K. A., & Belinsky, K. L. (2013). Voices in the dark: Predation risk by owls influences dusk singing in a diurnal passerine. *Behavioral Ecology and Sociobiology*, 67(11), 1837–1843.
- Schmitz, O. J., Beckerman, A. P., & O'Brien, K. M. (1997). Behaviorally mediated trophic cascades: Effects of predation risk on food web interactions. *Ecology*, 78(5), 1388–1399.

- Scott, D. M., & Ankney, D. (1980). Fecundity of the brown-headed cowbird in southern Ontario. *The Auk*, 97(4), 677–683.
- Scrimgeour, G. J., & Culp, J. M. (1994). Feeding while evading predators by a lotic mayfly: Linking short-term foraging behaviours to long-term fitness consequences. *Oecologia*, 100-100(1-2), 128–134.
- Seghers, B. H. (1974). Schooling Behavior in the Guppy (*Poecilia reticulata*): An Evolutionary Response to Predation. *Evolution*, 28(3), 486–489.
- Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2010). The ghosts of predators past: Population cycles and the role of maternal programming under fluctuating predation risk. *Ecology*, 91(10), 2983–2994.
- Sheriff, M. J., McMahon, E. K., Krebs, C. J., & Boonstra, R. (2015). Predator-induced maternal stress and population demography in snowshoe hares: The more severe the risk, the longer the generational effect. *Journal of Zoology*, 296(4), 305–310.
- Shine, R. (1980). “Costs” of reproduction in reptiles. *Oecologia*, 46(1), 92–100.
- Sih, A. (1994). Predation risk and the evolutionary ecology of reproductive behaviour. *Journal of Fish Biology*, 45(sA), 111–130.
- Sih, A. (1997). To hide or not to hide? Refuge use in a fluctuating environment. *Trends in Ecology & Evolution*, 12(10), 375–376.
- Sih, A., Krupa, J., & Travers, S. (1990). An experimental study on the effects of predation risk and feeding regime on the mating behavior of the water strider. *The American Naturalist*, 135(2), 284–290.
- Sih, A., & McCarthy, T. M. (2002). Prey responses to pulses of risk and safety: Testing the risk allocation hypothesis. *Animal Behaviour*, 63(3), 437–443.
- Sinclair, A. R. E., & Pech, R. P. (1996). Density dependence, stochasticity, compensation and predator regulation. *Oikos*, 75(2), 164–173.

- Spieth, H. T. (1974). Courtship behavior in *Drosophila*. *Annual Review of Entomology*, 19(1), 385–405.
- Stafstrom, J. A., & Eileen A. Hebets. (2013). Female mate choice for multimodal courtship and the importance of the signaling background for selection on male ornamentation. *Current Zoology*, 59(2), 200–209.
- Su, K. F. Y., & Li, D. (2006). Female-biased predation risk and its differential effect on the male and female courtship behaviour of jumping spiders. *Animal Behaviour*, 71(3), 531–537.
- Taylor, A. R., Persons, M. H., & Rypstra, A. L. (2005). The effect of perceived predation risk on male courtship and copulatory behavior in the wolf spider *Pardosa milvina* (Araneae, Lycosidae). *Journal of Arachnology*, 33(1), 76–81.
- Thompson III, F. R. (1994). Temporal and spatial patterns of breeding brown-headed cowbirds in the midwestern United States. *The Auk*, 111(4), 979–990.
- Tollrian, R., & Dodson, S. I. (1999). Inducible defences in cladocera: Constraints, costs, and multipredator environments. In R. Tollrian & C. D. Harvell (Eds.), *The Ecology and Evolution of Inducible Defenses* (pp. 177–202). Princeton, New Jersey: Princeton University Press.
- Trivers, R. L. (1972). Parental investment and sexual selection. In B. Campbell (Ed.), *Sexual Selection and the Descent of Man, 1871–1971* (pp. 136–179). Chicago, Illinois: Aldine-Atherton.
- Tuttle, M. D., & Ryan, M. J. (1981). Bat predation and the evolution of frog vocalizations in the Neotropics. *Science (Washington)*, 214(4521), 677–678.
- Uzendoski, K., Maksymovitch, E., & Verrell, P. (1993). Do the risks of predation and intermale competition affect courtship behavior in the salamander *Desmognathus ochrophaeus*? *Behavioral Ecology and Sociobiology*, 32(6), 421–427.

- Van Buskirk, J., & Schmidt, B. R. (2000). Predator-induced phenotypic plasticity in larval newts: Trade-offs, selection, and variation in nature. *Ecology*, *81*(11), 3009–3028.
- Wells, K. D. (1978). Courtship and parental behavior in a Panamanian poison-arrow frog (*Dendrobates auratus*). *Herpetologica*, *34*(2), 148–155.
- Williams, G. C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's principle. *The American Naturalist*, *100*(916), 687–690.
- Wingfield, J. C., & Sapolsky, R. M. (2003). Reproduction and resistance to stress: When and how. *Journal of Neuroendocrinology*, *15*(8), 711–724.
- Wood-Gush, D. G. M. (1956). The agonistic and courtship behaviour of the brown leghorn cock. *The British Journal of Animal Behaviour*, *4*(4), 133–142.
- Ylönen, H., & Ronkainen, H. (1994). Breeding suppression in the bank vole as antipredatory adaptation in a predictable environment. *Evolutionary Ecology*, *8*(6), 658–666.
- You, Z., & Jiang, Z. (2005). Courtship and mating behaviors in Przewalski's gazelle (*Procapra przewalskii*). *Acta zoologica Sinica*, *51*(2), 187–194.
- Zanette, L., Smith, J. N. M., van Oort, H., & Clinchy, M. (2003). Synergistic effects of food and predators on annual reproductive success in song sparrows. *Proceedings. Biological Sciences / The Royal Society*, *270*(1517), 799–803.
- Zanette, L. Y., White, A. F., Allen, M. C., & Clinchy, M. (2011). Perceived predation risk reduces the number of offspring songbirds produce per year. *Science*, *334*(6061), 1398–401.
- Zuk, M., & Gita R. Kolluru. (1998). Exploitation of sexual signals by predators and parasitoids. *The Quarterly Review of Biology*, *73*(4), 415–438.

Chapter 2

2.1 Introduction

Predators have a powerful effect on their prey populations beyond direct predation (e.g. killing and eating prey) by shaping changes in prey behaviour (Preisser *et al.* 2005, Creel & Christianson 2008, Estes *et al.* 2011). The mere risk of predation leads to anti-predator responses such as engaging in avoidance behaviours (i.e. seeking refuge, increasing vigilance) and limiting or stopping conspicuous behaviours in order to reduce the chance of encountering a predator (Lima 1998). As discussed in chapter one, many reproductive behaviours are well-established to be sensitive to predation risk since many traits that benefit an individual's reproductive success can also increase the risk of predation by increasing conspicuousness (Endler 1982, Ryan *et al.* 1982, Svensson 1988, Hughes *et al.* 2012) and decreasing escape ability (Shine 1980, Rowe 1994, Lee *et al.* 1996; further reviewed in Lima & Dill 1990, Magnhagen 1991, Sih 1994). Thus, exposure to elevated predation risk has been found to halt reproduction (Magnhagen 1990, Ylönen 1994) or lead to various changes in reproduction including, but not limited to the timing of reproduction (Reznick & Endler 1982, Endler 1987, Stibor 1992), parental care (Badyaev & Ghalambor 2001, Eggers *et al.* 2005, Zanette *et al.* 2011), and investments into eggs (e.g. number or content; Fraser & Gilliam 1992, Coslovsky & Richner 2011, Zanette *et al.* 2011; also see Lima 2009).

Courtship behaviour is another aspect of reproduction that can place individuals at greater risk of predation. The function of courtship is to attract the attention of potential mates using physical characteristics like ornaments and bright colours or conspicuous courtship behaviours like singing and dancing (Andrew 1961, Byers *et al.* 2010).

Predators can also easily eavesdrop on these signals to locate their prey, thus making courtship behaviour risky (Helfman 1986, Magnhagen 1991, Lima 2009, Hughes *et al.* 2012). Both males and females have been demonstrated to respond to predation risk by altering their behaviours as described in chapter one. Such reductions in courtship effort, whether signalling or receiving, can impact current reproductive success through lost mating opportunities. However, more work is needed regarding research looking at how predation risk affects courtship behaviour as there is surprisingly little work done using avian species although birdsong and displays are known to increase predation risk (Lima 2009). Furthermore, while females are known to be at risk when attending to courtship displays (Hughes *et al.* 2012), there still remains few studies that examine the changes in female behaviour in response to courtship under predation risk.

Two other considerations that require much more attention are the ideas that changes in the behaviour of one sex can affect the other and that temporal variations in risk can affect how individuals respond to predation risk in nature. Males may further alter their courtship effort if females are less willing to receive and respond to courtship signals under predation risk. But this idea still remains largely unexplored as many studies do not examine the behaviour of both sexes at the same time and it is unclear what changes in female behaviour the males may respond to. There are four known studies that expose both males and females to predation risk when measuring changes in courtship behaviour (Magurran & Nowak 1991, Dill *et al.* 1999, Evans *et al.* 2002, Su & Li 2006). However, two of these studies only looked at changes in male behaviour and found that exposing only female spiders (Su & Li 2006) or guppies (Dill *et al.* 1999) caused a reduction in male courtship. Thus, these two studies did not directly measure

what changes in female behaviour the males cued in on. Magurran & Nowak (1991) and Evans *et al.* (2002) did examine both female and male guppy behaviour in response to predation risk, but their results had some discrepancies that may be due to differences in their female guppies being virgins or the guppies being laboratory raised or wild caught. Magurran & Nowak (1991) indicated that the increase in female schooling behaviour or predator inspection may be what leads to the changes in male courtship; however, Evans *et al.* (2002) found no differences in schooling behaviour in their female guppies but suggest from qualitative observations that it may be due to the females being fixated on the predator rendering courtship from males ineffective. Therefore, it is clear that more work looking at both the sexes and using a greater variety of species should be done to fully understand the extent of the impact risk effects on courtship behaviour.

The other important factor that should be considered is fact that prey species in nature are under chronic predation risk and that the level risk of predation varies temporally (Lima & Bednekoff 1999). Traditionally, experimental paradigms house individuals in low risk after a period of time before presenting them with a predator cue (Lima & Bednekoff 1999). Individuals typically respond to the high risk period by greatly reducing or stopping their behaviours. Measuring the changes in behaviour between being housed in low risk and the brief pulse of high risk can overestimate the total effect predation risk can have on behaviours. This is because individuals in a natural setting may not have the luxury of drastically reducing important behaviours if periods of high risk are frequent or prolonged or they may compensate for the lost opportunities by significantly increasing behaviours during periods of lower risk (Lima & Bednekoff 1999, Wiedenmayer 2004). For example, under immediate threat, prey may cease

foraging, but predation risk may not necessarily negatively impact their overall energy intake if prey greatly increase their foraging during times of low risk to prevent starvation according to the ‘interrupted foraging hypothesis’ (Lima 1986, Lima & Bednekoff 1999). This idea is part of ‘risk allocation hypothesis’ in the foraging literature which describes how prey can change the timing in which they feed according to temporal variations in the level of predation risk (Lima & Bednekoff 1999). Similarly, to mitigate the risks of courtship, individuals can alter when they signal or receive signals (Endler 1980, Sih 1987, Lima & Dill 1990, Hughes *et al.* 2012). However, temporal variations in risk have yet to be considered in studies examining predation risk effects on courtship behaviour (Hughes *et al.* 2012). In a separate paper, we presented results demonstrating that the birds in this study gained mass under predation risk, which can be attributed to large increases in foraging during low risk periods as per the ‘interrupted foraging hypothesis’ (Walters 2015). The birds in this study may also adjust their courtship behaviour to court more during times of low risk such that overall levels of courtship may not be affected by predation risk. Thus, testing whether predation risk actually affects courtship behaviour to an extent that can affect reproductive success in nature requires presenting prey with predation risk that varies temporally over an extended period of time as I have aimed to do so in the study presented here.

To fill the gaps in knowledge I have just discussed, I experimentally tested whether perceived predation risk affects courtship, mating and other reproductive behaviours in brown-headed cowbirds (*Molothrus ater*, hereafter ‘cowbirds’). By using an avian species, my study expand on the current knowledge of risk effects on courtship as the majority of the literature only uses invertebrates and aquatic species (Lima 2009).

Cowbirds are obligate broods parasites that lay their eggs in nests of other birds and exhibit no parental care. Female reproduction in cowbirds involves evaluating courting males and finding an appropriate host nest to lay her eggs in. Female cowbirds also produce loud chatter calls used to signal their receptivity to males (Rothstein *et al.* 1988). Male cowbirds' reproductive success is dependent on the number of displays he produces (White *et al.* 2010). Males court females by directing songs to her that is accompanied with a visual display involving fluffed feathers, spread wings and bowing. By singing, the male may make himself conspicuous to eavesdropping predators; moreover, the bowing display that accompanies the song may prevent him from being able to be vigilant for predators and impede his ability to fly away if necessary placing males at even greater risk while courting. Not only do males display to females, but they also display to other males as an aggressive gesture in regards to dominance (White *et al.* 2010) and aggressive intra-sexual signals can typically increase an individual's susceptibility to predation as well (Jakobsson *et al.* 1995, Kelly & Godin 2001). A male's dominance status greatly affects his reproductive success as subordinate males receive little to no access to females (Darley 1982).

The first objective of this study was to manipulate the perceived predation risk to document potential changes in the reproductive behaviours described above. However, instead of only comparing behaviours between the presence vs. absence of a predator cue as is traditionally done, the birds in my study were placed under chronic predation risk in which the level of risk varied temporally. As cowbirds are iteroparous thus have chances for future reproduction, I expect that they would respond to elevated predation risk by adjusting the reproductive behaviours that increase their susceptibility to predation.

However, if they reduce their courtship effort during times of high risk and increase their effort during times of low risk then the overall levels of their reproductive behaviours may not be affected by the elevated predation risk. By using both male and female cowbirds, this allowed me to further examine receiver behaviour under elevated predation risk and to fulfill the second objective of my study which was to address the gaps in knowledge regarding how changes in behaviour of one sex can affect the other in regards to courtship behaviour. Since male cowbirds display to both sexes, I could assess whether males are responding to the level of predation risk in the environment or any potential changes in female receptivity. If females are less receptive under predation risk and males reduce their courtship effort but not their male-to-male displays, this would suggest that the males are responding to changes in female receptivity, but not the predation risk. Using these results, I also discuss any potential sex differences in residual reproductive output and the risk perceived by engaging in reproductive behaviours.

2.2 Methods

This study was conducted to examine how male and female brown-headed cowbirds' reproductive behaviour responded to perceived predation risk that varied temporally over an extended period of time. I manipulated the perceived predation risk using auditory playbacks and taxidermy mounts of predators and non-predators presented to wild-caught cowbirds housed in large, semi-natural outdoor aviaries. Playbacks were presented with periods of calls and silences in randomized intervals and mounts were presented at randomized times such that the level of risk varied temporally. Each predator and non-predator treatment lasted 10 d and all birds received both treatments in a repeated measures design. During the treatments, I measured female cowbirds' receptivity to courtship and host nest inspection behaviour. I also examined male cowbirds' courtship behaviour, aggressive male-male interactions associated with dominance, and their dominance hierarchy.

2.2.1 Animals & Housing

I captured cowbirds at banding stations between April 11 and May 4, 2014 in south western Ontario as they returned from migration. All birds were colour banded for unique identification. I placed 10 male and 10 female brown-headed cowbirds in each of four, large outdoor aviaries (3.66 m x 9.14 m x 18.29 m). The aviaries were outfitted with grass, perches, trees, an a-frame shelter, automated water containers and feeding stations from which they were provided with *ad-libitum* food supply of modified Bronx Zoo diet which included white millet, protein content (Mazuri Gamebird Starter, Purina Dog Chow, Purina Mills Aquamax Grower 600), oyster shell, whole egg powder, and carotenoids (Kermin Oro Glo). The cowbirds were able to see and hear the environment

outside of the aviaries but were protected from any predator attacks. Each of two aviaries were immediately adjacent to one another with an opaque wall in between to prevent the two groups of birds from seeing each other. The two pairs of aviaries were separated by a distance of 136 m thereby being visually and acoustically isolated.

2.2.2 Predation Risk Manipulation

I tested for predation risk effects on courtship and other reproductive behaviours by manipulating the perceived level of risk. I exposed the birds to auditory playbacks and taxidermic mounts of predators and non-predators between June 10 and July 6, 2014 (during the cowbird breeding season). This allowed at least one month between capture and the manipulation for the birds to acclimate. I applied a repeated measures design wherein birds in each aviary received one treatment (either predator or non-predator) lasting 10 d with a 5 d break in between before they would receive the other 10 d treatment for temporal and additional spatial replication. To avoid sound contamination, birds in aviaries immediately adjacent to one another each experienced the same treatments at the same time. Aviaries 1 and 2 received the predator treatment first while aviaries 3 and 4 received the non-predator treatment first. Treatments between pairs of aviaries were staggered (i.e. aviaries 1 and 2 started on June 10th while aviaries 3 and 4 started on June 12th) to allow behavioural observations between pairs of aviaries to be made on the same day into the treatment and at the same time of day. To prevent habituation, the stimuli were presented on an on-off cycle wherein stimuli were presented on days 1-4 and 7-8 without any stimuli presentation on days 5-6 and 9-10 (following Zanette *et al.* 2011).

Sounds of predators or non-predators were broadcasted from playback units consisting of a pair of speakers (Logitech Z130 Speakers) and a MP3 player (Hipstreet 4GB MP3 Player). Each aviary contained two playback units housed in custom weatherproof boxes mounted 2.4 m high on the columns of the aviaries and placed at least 12 m apart from each other. To help prevent habituation during each 10 d trial, the speakers were moved to different locations in the aviary every 2 d. Playbacks were also randomized between the two speaker boxes such that only one speaker was broadcasting at a time in each aviary. The sounds consisted of the calls of eight different predator species known to prey upon cowbirds which were paired with eight different non-predator species that cowbirds would encounter in southern Ontario (Table 1; Cornell Lab of Ornithology, online data). I included both diurnal and nocturnal species and played each species' calls at the appropriate time. Each predator species was paired with a non-predator species by subjectively matching their calls and then testing the differences in the frequency characteristics between their calls (following Zanette *et al.* 2011). For nocturnal species, the choices of species were limited so predator and non-predator species were matched even if they did not sound subjectively similar. Paired species calls were not statistically different in peak frequency ($t_7 = -1.2$, $p = 0.26$), minimum frequency ($t_7 = -0.3$, $p = 0.80$), maximum frequency ($t_7 = -1.6$, $p = 0.16$), and frequency range ($t_7 = 0.3$, $p = 0.75$). Five to ten exemplars of calls from each species were taken from The Cornell Lab of Ornithology and Borror Laboratory of Bioacoustics. The exemplars were randomized to create two 24 h playlists for each treatment used on alternating days. Playbacks were adjusted to broadcast at 80 dB from 1 m away and

Table 1. List of species used for the auditory playbacks. Predator calls were matched with a non-predator based on their broadcast time and call acoustic characteristics.

Time	Matched Species	
	Predators	Non-predators
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>Dendroica coronate</i>)
Day	American kestrel (<i>Falco sparverius</i>)	Cedar waxwing (<i>Bombycilla cedrorum</i>)
Night	Eastern screech owl (<i>Megascops kennicottii</i>)	Common loon (<i>Gavia immer</i>)
Night	Northern saw-whet owl (<i>Aegolius arcadius</i>)	Wood frog (<i>Rana sylvatica</i>)
Night	Barred Owl (<i>Strix varia</i>)	Northern Leopard Frog (<i>Lithobates pipiens</i>)

included a call-to-silence ratio of 1:1.5 during the day and 1:2.3 during the night (following Zanette *et al.* 2011). The interspersed calls and silences simulated temporal variations in predation risk.

On days 1-4 as well as 7-8 of the manipulations, I also presented the birds with taxidermic mounts of two different predator or non-predator species for each respective treatment (Table 2). Two different mounts were each presented each day, once at a randomized time between 1100 and 1400h and the second at a randomized time between 1400 and 1700h. I altered the location of the mount each day to prevent habituation. Mounts were initially hidden below an opaque box then revealed for 5 min.

2.2.3 *Quantifying Courtship, Mating and Other Reproductive Behaviours*

All behavioural observations were conducted solely by TC and recorded onto a voice recorder (Sony ICD-PX333) for later transcription. I assessed the responses of females to predation risk in two ways by examining whether females were (i) receptive to courtship and (ii) willing to engage in host nest inspection. For the first, I measured how the females responded to male courtship displays and the females' propensity to emit chatter calls. Whenever a focal male (see below) performed a courtship display, I quantified the response of the female as either receptive or a rejection. A 'receptive' response was considered to have occurred when the female remained in close proximity (less than 0.6 m) to the male after 1 s of the display, while a rejection occurred if the female flew at least 0.6 m away from the male within 1 s of his display (following David White, personal communication). Males may display multiple times in a row to females that remain thus females that are unreceptive to courtship should move away from the male. Typically, if the female rejected a male display, she would leave before she could

Table 2. List of species of the taxidermic mounts used as a visual predator or non-predator stimulus. Mounts were matched for size and stance.

Taxidermic Mount Species	
Predators	Non-predators
Red-shouldered hawk (<i>Buteo lineatus</i>)	Northern Pintail (<i>Anas acuta</i>)
Cooper's hawk (<i>Accipiter cooperii</i>)	Northern Flicker (<i>Colaptes auratus</i>)

be identified. Therefore, female responses to male courtship were compared as the proportion of rejections each male received. Chatter calls are emitted by receptive females and can function to entice them to court the females (Rothstein *et al.* 1988). It was rarely possible for me to identify which female called because my attention was on the focal male. Therefore, I tallied the number of chatter calls per aviary per 1 h trial for analysis. I assessed the tendency of females to engage in reproductive behaviours other than courtship by measuring host nest inspection effort. To do so, a 30 min trial was conducted on day 1 of each treatment in which I presented females in each aviary with two artificial nests made from raffia containing four plasticine eggs moulded to a length of 2.3 cm and width of 1.6 cm to approximate the size and shape of a larger end song sparrow (*Melospiza melodia*) egg or a smaller end red-winged blackbird (*Agelaius phoeniceus*) egg, both of which are common host species for cowbirds. The two artificial nests were secured onto a branch of two different cedars and the location of the nests was the same in both treatments. I recorded the identity of each female that visited each nest and the length of each visit.

I assessed the effects of perceived predation risk on males by conducting a 5 min focal sampling of all males on every day of the treatment period. I randomized the order upon which each male per aviary would be sampled each day and alternated the order of the aviaries per day. I repeated this same order for each day of the opposite risk manipulation treatment of each aviary. All observations occurred between 0600 and 1100 h when cowbirds most rigorously engage in courtship and mating (White *et al.* 2002). Observations were conducted atop a 1.2 m platform and began after waiting 15 min to allow the birds to habituate to the observer's presence.

I tested whether males would attenuate their courtship displays to females when I elevated the level of predation risk in the environment. A courtship display occurred when a male directed a song toward a female no more than 0.6 m away at an angle of 45° or less (following White *et al.* 2002). Songs are always accompanied by a visual display ('song spread' display) when directed at another cowbird (O'Loughlen & Rothstein 2010a). I scored each display as low intensity (puffed feathers and outstretched head), medium intensity (slight spread of wings and slight head bow toward the ground), and high intensity (fully-spread wings and head fully bowed so that the male's head was completely oriented toward the ground; Dufty 1986). I assumed that the highest visual display intensity would confer the greatest risk to a male. Therefore, males may also potentially alleviate the risk of predation beyond reducing the number of displays by attenuating the intensity of their displays.

I also examined the effect of perceived predation risk on four aspects of male-male interactions. I quantified the number of displays directed to males and the intensity of those displays because male-to-male displays are involved with establishing and maintaining a male dominance hierarchy in cowbirds. The dominance status of a male cowbird affects his reproductive success whereby subordinates typically receive little to no access to females (Darley 1982). I also recorded the number of male-male aggressive physical interactions (pecks, attacks, and displacements), which is also related to dominance, initiated by each male. These interactions were tallied during the male focal samples, along with 4 h recordings using a digital video recorder (Swann DVR4-3425) at feeders. Since these aggressive behaviours are conspicuous and energetically costly, males may reduce these behaviours under predation risk which can lead to changes in the

dominance structure. Each male was also given a dominance score calculated using the formula for David's score (DS) that uses the weighted and unweighted proportion of wins and losses during agonistic male interactions (David 1987), which included male-to-male displays, physical attacks and displacements in this case. DS was chosen as the index for calculating dominance scores since it is better at handling reversals between pairs (Bang *et al.* 2010). Reversals are defined as when the subordinate individual wins some interactions against the dominant individual within a male pair; the dominant individual would win 100% of the interactions within a pair that didn't show reversals. If the males do not maintain their dominance interactions while under predation risk, this may lead to higher frequency of reversals due to greater instability within the dominance structures. Therefore, the number of male pairs that showed reversals was tallied to examine whether there is a change in the frequency of reversals between predation risk treatments.

2.2.4 Statistical Analysis

The proportion of female rejections an individual male received was calculated by dividing the number of times a female rejected the male by the total number of times he displayed to females. I conducted a linear mixed model (LMM) analysis to compare the proportion of rejections with treatment (predator vs. non-predator) as the repeated measures factor. The number of female chatter calls produced in each aviary was square root transformed and compared using a repeated measures ANOVA. Female nest inspection behaviour was quantified as the number of visits to the artificial nests, the cumulative amount of time females spent on the nests, and the average time spent per nest visit over the 30 min trial. I used Wilcoxon signed-rank tests to compare the number of visits and average time spent per nest visit between treatments. The cumulative amount

of time females spent on the nest was square-root transformed and compared using a paired sample t-test.

I tested for differences in the number of courtship displays between treatments performing a LMM analysis on log-transformed data. The proportion of each courtship display intensity was compared between treatments using Wilcoxon sign-rank tests. Male cowbirds tend to use lower intensity displays when courting females (Dufty 1986). In accordance to this, I also found that they rarely used high intensity displays (median = 0% and mean = 5.79% of all displays) to females. Therefore, I only compared the proportions of the low and medium display intensities for courtship displays.

I also used a LMM to compare the number of male-to-male displays and the proportion of medium and high display intensities were compared using a Wilcoxon sign-rank test since males typically use higher intensity displays as an aggressive signal to other males (Dufty 1986). In my study, males rarely used low intensity displays to other males (average of 0.32%, median of 0%). The number of aggressive interactions initiated by each male and their dominance scores were compared using a Wilcoxon sign-ranked test. I conducted a McNemar's test to determine whether the frequency of reversals was higher in one treatment compared to the other.

Data were transformed using log or square-root transformations for homogeneity of variances and normality when necessary; however, all results are presented as untransformed values for ease of interpretation. If the assumptions for using parametric tests could not be met even after transformation, a non-parametric test was performed instead. The LMM analyses used individual identity nested within aviary as a random effect to account for potential variances between the four different aviaries and thus the

order of treatment, but there was no significant random effect ($p \geq 0.536$) in any of the models. Statistical analyses were performed using SPSS v.20 (IBM, Armonk, NY, USA) or Statistica v. 12 (StatSoft, Tulsa, Oklahoma, U.S.A). Tests were two-tailed with p values less than 0.05 considered significant.

Three males and two females that died during the first round of manipulations or between rounds were removed from all analyses. Individuals were removed from the respective analyses if they did not perform that specific behaviour in both treatment rounds (i.e. a male that never sang to females during the entire study was not included in the data analysis involving male calls to females).

2.3 Results

Females significantly reduced their receptivity to male courtship behaviour when I elevated the level of perceived predation risk in the environment. Males received a larger proportion of rejections from females in response to their courtship displays (Figure 1A; LMM: Treatment, $F_{1,19} = 6.7$, $p = 0.018$), and females emitted significantly fewer chatter calls as counted per aviary (Figure 1B; repeated measures ANOVA: Treatment, $F_{1,3} = 28.1$, $p = 0.013$) when exposed to the predator stimuli. High predation risk also affected another aspect of female reproductive behaviour, their nest inspection effort. Females inspected artificial nests fewer times during the predator treatment than the non-predator treatment (Figure 1C; Wilcoxon sign-ranked test, $Z = 2.3$, $p = 0.022$) and tended to spend less total time inspecting nests when exposed to predator (53.70 ± 8.52 s) versus non-predator stimuli (77.50 ± 13.11 s), although the differences were not statistically significant (paired sample t-test, $t_{23} = -1.9$, $p = 0.072$). The average amount of time a female spent on the nests per visit did not differ between treatments (median = 18.0 vs. 21.6 s; Wilcoxon sign-ranked test, $Z = 0.1$, $p = 0.94$).

On the other hand, males seemed to perceive and respond to the level of predation risk differently than females. Males did reduce their courtship displays (Figure 2A; LMM: Treatment, $F_{1,28} = 5.8$, $p = 0.023$), performing approximately 11 fewer displays per h on average in the predator than the non-predator treatment. However, this reduction in the number of courtship displays seems mainly driven by the reduction in female receptivity as males continued to display frequently to other males regardless of predation risk. Males maintained the number of male-to-male displays whether predation risk was high or low (Figure 2B; LMM: Treatment, $F_{1,34} = 1.0$, $p = 0.336$). Furthermore, males did

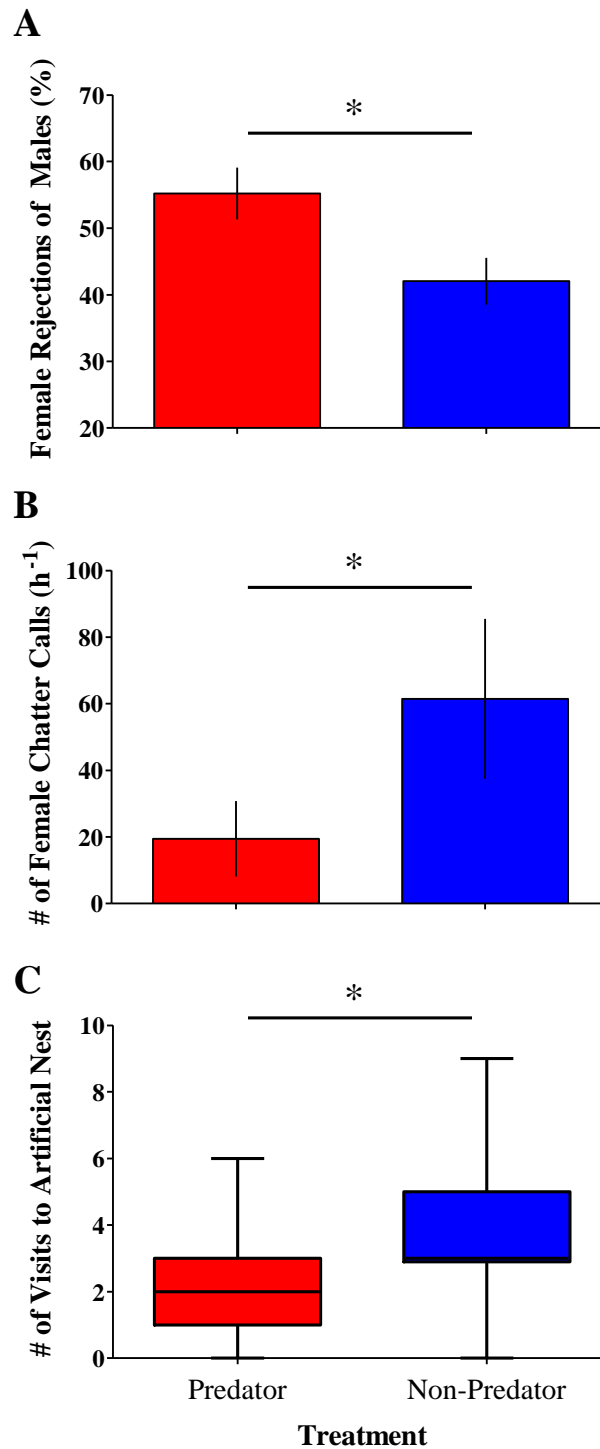


Figure 1. Effect of perceived predation risk on (A) the percentage of rejections brown-headed cowbird (*Molothrus ater*) males received when displaying to females ($n = 21$), (B) the number of chatter calls produced by females per each aviary over an hour ($n = 4$), and (C) the number of artificial nest visits made by females over a 30 min nest inspection trial ($n = 24$) during the predator (red) and non-predator (blue) treatments. For (A) and (B), values presented are means \pm SE. For (C), medians are presented with 25th and 75th percentiles (boxes) and min and max values (bars).

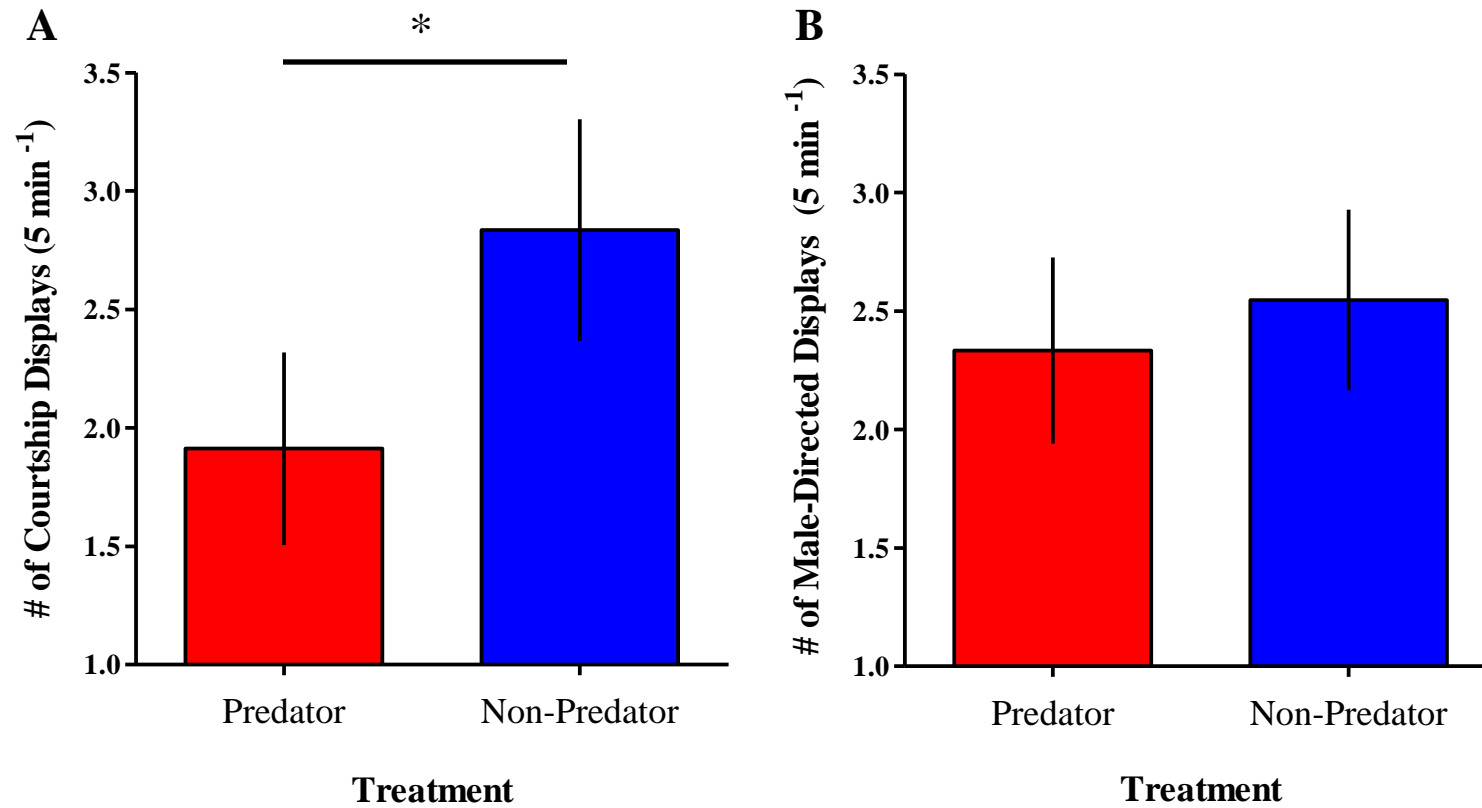


Figure 2. Effect of perceived predation risk on the average number of displays brown-headed cowbird (*Molothrus ater*) males performed during a 5 min focal sample to (A) females (courtship, $n = 29$) and (B) other males (aggressive display, $n = 35$), during the predator (red) and non-predator (blue) treatments. Values presented are means \pm SE.

not change the proportion of each display intensity to either females (low intensity displays: median = 18.52% vs. 15.63%; Wilcoxon sign-ranked test, $Z = 1.6$, $p = 0.11$; medium intensity displays: median = 75.00% vs. 76.19%; $Z = 0.7$, $p = 0.46$; predator vs. non-predator treatment) or males (medium intensity displays: median = 8.22% vs. 7.69%; Wilcoxon sign-ranked test, $Z = 1.8$, $p = 0.078$; high intensity displays: median = 91.49% vs. 92.31%; $Z = 1.8$, $p = 0.074$; predator vs. non-predator treatment).

To provide further evidence that male reproductive behaviours were less risk sensitive, males also did not alter the number of aggressive encounters they initiated between treatments (median = 1.00 vs. 0.50, predator vs. non-predator treatment; Wilcoxon sign-ranked test, $Z = 1.6$, $p = 0.11$), and the perceived predation risk had no effect on the males' dominance structure. Average dominance scores per male were similar between the predator (median = 3.67) and non-predator (0.28; Wilcoxon sign-ranked test, $Z = 0.6$, $p = 0.53$) treatments, and I found the exact same proportion of reversals in dominance interactions between pairs in both playback treatments (31.82%; McNemar's test, $X^2 = 0.0$, $p = 1.00$).

2.4 Discussion

By experimentally manipulating the level of perceived predation risk in the environment, my study demonstrates that prey will adjust their reproductive behaviours in response to elevated risk. Even when presented with temporal variations in the predation risk including periods of low risk, female cowbirds responded strongly to the chronically elevated predation risk such that they were more likely to reject a male courtship display and produced fewer chatter calls. Perceived predation risk also affected the effort female cowbirds spent on inspecting potential host nests resulting in a reduction in the number of artificial nest visits and the amount of time females spent on the artificial nests. These results help to corroborate previous research supporting the idea that prey can attend to and respond to elevated predation risk by investing more into anti-predator responses instead of current reproduction in order to survive and reproduce in future years maximizing their lifetime reproductive success (Lima & Dill 1990, Magnhagen 1991, Lima 1998). While male cowbirds did reduce the number of courtship displays produced during the predator treatment, the changes in male courtship seem to be mainly driven by the reduction in female receptiveness rather than the elevated predation risk since the males' behaviour was otherwise unchanged. Males did not alter the intensity of their displays nor did they change their behaviour when interacting with other males (i.e displays, other aggressive interactions, dominance structure) in response to the predation treatment. Thus, female cowbirds seem to be more sensitive to the elevated predation risk than the males, and furthermore, the changes in female receptivity influenced male courtship behaviour. These sex differences in the response to elevated predation risk may reflect differences in the amount of perceived risk, chances for future reproduction, and strategies in dealing with temporal variations in risk.

Female cowbirds were less receptive to males and actively avoided courting males during the predator treatment. This avoidance helps to alleviate predation risk because being in close association with and actively attending to courtship displays increases the receiver's exposure to predators (Dawkins & Guilford 1991, Hughes *et al.* 2012). Females also produced fewer chatter calls which can help minimize predation risk in two ways. Firstly, females draw less attention from males because chatter calls can be used by female cowbirds to signal their receptive status to males (Rothstein *et al.* 1988). Secondly, chatter calls are loud and long; therefore, reducing this behaviour would reduce the chance that an eavesdropping predator may detect her signal. Under predation risk, a reduction in interactions between males and females and the unwillingness to evaluate males can lead to a reduction in choosiness in females (Crowley *et al.* 1991, Sih 1994). The importance of female choice in mating systems has been widely reviewed, and in reproductive systems where the males only provide sperm, without territory or parental care, as they do in cowbirds, females should choose males with the most advantageous genes as indicated by their sexual displays (Fisher 1930, Kirkpatrick 1982, Hedrick 1988, Eberhard 1996, Andersson & Simmon 2006). Sand goby females under predation risk show less preference for large males under predation risk, but larger males have a greater advantage in male-male competition and have higher reproductive success than smaller males (Forsgren 1992). Therefore, if female cowbirds are less choosy due to the reduction in receptivity to courtship in response to predation risk, they may not be selecting for the best quality males potentially reducing her offspring's fitness.

Females also responded to the elevated predation risk by visiting the artificial nests fewer times and spending less time at the artificial nests. This reduction in activity

can help females be less conspicuous to predators. Obligate brood parasites like cowbirds inspect nests by pecking eggs to help determine the age of the nest in order to gauge the optimal time to lay her eggs (Massoni & Reboreda 1999, Swan *et al.* 2015). Shiny cowbird (*Molothrus bonariensis*) chicks had lower survival when they hatched after the host or if no eggs were removed (Fiorini *et al.* 2009). Cowbirds can also purposely destroy host eggs to induce the host to abandon the nest to re-nest (as per the farming hypothesis) if the nest was not at the appropriate stage to parasitize (Arcese *et al.* 1996). Pecking and removal of host eggs can also benefit the incubation of the cowbird egg according to the host incubation limit and efficiency hypothesis which suggests that larger clutches may affect the incubation of parasitic eggs (Davies & Brooke 1988, Peer & Bollinger 2000). Thus, a reduction in nest inspection effort in response to perceived predation risk can potentially impact offspring hatching success and survival.

Male cowbirds performed fewer courtship displays during the predator treatment. This seems consistent with previous studies that have shown a reduction in courtship behaviour under predation risk in a variety of species such as fiddler crabs (Koga *et al.* 1998), tungara frogs (Ryan 1985), threespine sticklebacks (*Gasterosteus aculeatus*; Candolin 1997), and Allegheny Mountain dusky salamanders (*Desmognathus ochrophaeus*; Uzendoski *et al.* 1993). However, since male cowbirds also perform a similar, but more intense display as an aggressive act to other males (Dufty 1986), I expected that males would also reduce the number of male-to-male displays. Contrary to this, males did not reduce the number of male-to-male displays nor did they change the intensity at which they displayed to either sex. I suggest then that the reduction in courtship effort is in response to changes in female receptivity and not directly due to

predation risk. This may be a result of females limiting the opportunities males have to court them as well as the lowered benefit of trying to court females who are not sexually receptive. My results lend support to the few studies that find that males change their mating behaviour in response to females being exposed to predators. For example, male guppies that were not exposed to a predator model changed their mating tactics by increasing the proportion of forced mating attempts after female guppies were exposed to a predator model (Evans *et al.* 2002). Similarly, Su & Li (2006) found that male jumping spiders (*Jacksonoides queenslandicus*) displayed less to females only if the females were also exposed to the predator. These previous studies were unable to pinpoint or did not directly measure the changes in female behaviour that the males responded to, but my study demonstrates that male courtship behaviour can be influenced by females actively avoiding males while under predation risk. The reduction in courtship has implications for reproductive success as cowbirds' courtship song and display typically precedes copulation (Rothstein *et al.* 1988) as is the case in many other species (Andrew 2008, Jackson 1978, Van Den Assem 1975). White *et al.* (2009) also found that male cowbird copulation success is positively correlated with the number of female-directed displays the male cowbirds produce. Therefore, changes in female receptiveness in response to predation risk can change courtship dynamics and hold consequences for their fitness.

To provide further support for the idea that male reproductive behaviours were not sensitive to predation risk itself, there were also no changes in the number of aggressive interactions, dominance score, and the frequency of reversals. This is contrary to studies that find that male competition and aggression leads to an increased risk of predation (Jakobsson *et al.* 1995) and should therefore be minimized under high

predation risk as seen in guppies (Kelly & Godin 2001) and Coho salmon (*Oncorhynchus kisutch*; Martel & Dill 1993). However, I know that the male cowbirds in this study did attend to the risk of predation through changes in physiological measures of mass and pectoral muscle thickness as well as flight behaviour during take-off (Walters 2015). The increase in mass during the predator treatment was attributed to cowbirds compensating for lack of foraging during periods of high risk by feeding more during low risk periods (Walters 2015). Therefore, males may employ the risk allocation strategy for reproductive behaviours and engage more in dominance-related behaviours during the times of low risk such that the overall levels of these behaviours are unchanged. These results may also indicate that maintaining dominance status is highly beneficial to the males especially when females are unreceptive to courtship. As dominance status is integral to gaining access to females (Dufty 1986), cowbirds should maintain their rank in order to gain access to females when possible.

I also postulate that the lack of sensitivity of the males' reproductive behaviours may indicate that the behaviours measured in this study do not significantly increase the risk for the males or the males have a low chance for reproduction in the future. In my study, females perceived a greater risk in receiving courtship displays although males were willing to continue to display. This may suggest there is a female-biased mortality due to predation as is documented in other species such as spiders (Li & Jackson 1996, Su & Li 2006) and crickets (Sakaluk & Belwood 1984). Also producing and carrying eggs can also increase the risk for females (Shine 1980, Lee *et al.* 1996) which can help explain why female cowbirds are more sensitive to predation risk since they have been documented to be able to produce up to 40 eggs over an eight week period (Scott &

Ankney 1980). Additionally, I suggest that male cowbird reproductive behaviours may be less conspicuous and risky compared to female behaviour. Male cowbird displays are short-range signals directed to another individual usually no further than 0.6 m away (White *et al.* 2010, O’Loghlen & Rothstein 2010b). On the other hand, female cowbirds signal using chatter calls, which are loud and long, and used over longer distances (Friedmann 1929). These differences in call characteristics make chatter calls riskier than male displays (Hughes *et al.* 2012). This would contribute to female cowbirds being more cautious under predation risk than males are similar to how male crickets with shorter calls are less sensitive to predation risk than males with longer calls (Hedrick 2000). Male-male aggressive behaviour such as pecks or displacements are again short-range interactions and typically occur when the males are in a group. The anti-predator benefits of being in a flock due to increased vigilance by group members are well established (Lima & Dill 1990). However, females spend much of their time alone searching for host nests which also requires a lot of movement and attention further increasing her risk. Beyond differences in risk, the males being willing to continue to invest in current reproduction can also indicate that the males have a low prospect for future reproduction (Sargent & Gross 1986, Candolin 1998, Lima 2009). Therefore, males may not be expected to modulate their reproductive behaviours under predation risk or they can employ different strategies that females under much greater risk cannot such as engaging more in reproduction during low risk periods.

Previous studies have found that when prey are presented with a predator stimulus and are under immediate risk, they will reduce their activity and courtship effort. However, in a natural setting where the risk of predation varies temporally, prey can

engage more in reproductive behaviours during low risk periods to compensate for the lost opportunities when they are in immediate danger. Therefore, to determine whether predation risk can affect courtship levels to an extent that affects fitness in a natural setting, the birds in my study were placed under chronically elevated predation risk but were presented with periods of predator calls and periods of silences to simulate temporal variations in the level of risk. I found that despite having periods of low risk, female cowbirds were highly sensitive to the elevated risk in the environment becoming less receptive to courtship and putting less effort into host nest inspection. In response to this, although male reproductive behaviours were otherwise unaffected by the elevated predation risk, I determined that male courtship behaviour was influenced by the females actively avoiding the males. Also by examining the behaviours of both sexes under predation risk, I found sex differences in the sensitivity of reproductive behaviours to predation risk reflect differences in risk and prospects for future reproductive success leading to different behavioural decisions in optimizing lifetime reproduction success. Thus, my results highlight the fact that individual characteristics, whether within- or between-species, contribute to differences in how prey may respond to predation risk. Although I suggest that these changes in courtship behaviour can negatively impact reproductive success, a subsequent study will be needed to determine how these changes might actually affect reproductive success in the form of copulation success and number of viable eggs laid. Nonetheless, my study provides further evidence that predators can impact prey even in the absence of direct predation through risk effects on reproductive behaviours. This corroborates previous studies on predator-prey interactions that examine sub-lethal effects of predation on prey behaviour (Lima & Dill 1990, Lima 1998, Lima

2009) and further stresses the importance considering these risk effects as part of the total impact predators can have on prey populations.

2.5 References

- Andersson, M. & Simmons, L. W. (2006). Sexual selection and mate choice. *Trends in Ecology & Evolution*, 21(6), 296–302.
- Andrew, R. J. (2008). The displays given by passerines in courtship and reproductive fighting: A review. *Ibis*, 103a(3), 315–348.
- Arcese, P., Smith, J. N. & Hatch, M. I. (1996). Nest predation by cowbirds and its consequences for passerine demography. *Proceedings of the National Academy of Sciences*, 93(10), 4608–4611.
- Badyaev, A. V. & Ghalambor, C. K. (2001). Evolution of life histories along elevational gradients: Trade-off between parental care and fecundity. *Ecology*, 82(10), 2948–2960.
- Bang, A., Deshpande, S., Sumana, A. & Gadagkar, R. (2010). Choosing an appropriate index to construct dominance hierarchies in animal societies: a comparison of three indices. *Animal Behaviour*, 79(3), 631–636.
- Brown, J. S. (1999). Vigilance, patch use and habitat selection: Foraging under predation risk. *Evolutionary Ecology Research*, 1(1), 49–71.
- Byers, J., Hebets, E. & Podos, J. (2010). Female mate choice based upon male motor performance. *Animal Behaviour*, 79(4), 771–778.
- Candolin, U. (1997). Predation risk affects courtship and attractiveness of competing threespine stickleback males. *Behavioral Ecology and Sociobiology*, 41(2), 81–87.
- Candolin, U. (1998). Reproduction under predation risk and the trade-off between current and future reproduction in the threespine stickleback. *Proceedings of the Royal Society B: Biological Sciences*, 265(1402), 1171–1175.
- Caro, T. (2005). *Antipredator Defenses in Birds and Mammals*. Chicago, IL: University of Chicago Press.

- Coslovsky, M. & Richner, H. (2011). Predation risk affects offspring growth via maternal effects. *Functional Ecology*, 25(4), 878–888.
- Creel, S. & Christianson, D. (2008). Relationships between direct predation and risk effects. *Trends in Ecology & Evolution*, 23(4), 194–201.
- Crowley, P. H., Travers, S. E., Linton, M. C., Cohn, S. L., Sih, A. & Sargent, R. C. (1991). Mate density, predation risk, and the seasonal sequence of mate choices: A dynamic game. *The American Naturalist*, 137(4), 567–596.
- Daly, M. (1978). The cost of mating. *The American Naturalist*, 112(986), 771–774.
- Darley, J. A. (1982). Territoriality and mating behavior of male brown-headed cowbird. *The Condor*, 84(1), 15–21.
- David, H. A. (1987). Ranking from unbalanced paired-comparison data. *Biometrika*, 74(2), 432–436.
- Davies, N. B. & Brooke, M. de L. (1988). Cuckoos versus reed warblers: Adaptations and counteradaptations. *Animal Behaviour*, 36(1), 262–284.
- Dawkins, M. S. & Guilford, T. (1991). The corruption of honest signalling. *Animal Behaviour*, 41(5), 865–873.
- Dill, L. M., Hedrick, A. V & Fraser, A. (1999). Male mating strategies under predation risk: Do females call the shots? *Behavioral Ecology*, 10(4), 452–461.
- Dufty, A. M. (1986). Singing and the establishment and maintenance of dominance hierarchies in captive brown-headed cowbirds. *Behavioral Ecology and Sociobiology*, 19(1), 49–55.
- Eberhard, W. G. (1996). *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton, NJ: Princeton University Press.
- Eggers, S. (2004). Predator-induced plasticity in nest visitation rates in the Siberian jay (*Perisoreus infaustus*). *Behavioral Ecology*, 16(1), 309–315.

- Endler, J. A. (1982). Convergent and divergent effects of natural selection on color patterns in two fish faunas. *Evolution*, 36(1), 178–188.
- Endler, J. A. (1987). Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Animal Behaviour*, 35(5), 1376–1385.
- Estes, J. A., Terborgh, J., Brashares, J. S., Power, M. E., Berger, J., Bond, W. J., Carpenter, S. R., Essington, T. E., Holt, R. D., Jackson, J. B. C., Marquis, R. J., Oksanen, L., Oksanen, T., Paine, R. T., Pickett, E. K., Ripple, W. J., Sandin, S. A., Scheffer, M., Schoener, T. W., Shurin, J. B., Sinclair, A. R. E., Soule, M. E., Virtanen, R. & Wardle, D. A. (2011). Trophic downgrading of planet Earth. *Science*, 333(6040), 301–6.
- Evans, J., Kelley, J., Ramnarine, I. & Pilastro, A. (2002). Female behaviour mediates male courtship under predation risk in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, 52(6), 496–502.
- Fiorini, V. D., Tuero, D. T. & Rebores, J. C. (2009). Shiny cowbirds synchronize parasitism with host laying and puncture host eggs according to host characteristics. *Animal Behaviour*, 77(3), 561–568.
- Fisher, R. A. (1930). *The Genetical Theory of Natural Selection: A Complete Variorum Edition*. Oxford, UK: Oxford University Press.
- Forsgren, E. (1992). Predation risk affects mate choice in a gobiid fish. *The American Naturalist*, 140(6), 1041–1049.
- Fraser, D. F. & Gilliam, J. F. (1992). Nonlethal impacts of predator invasion: Facultative suppression of growth and reproduction. *Ecology*, 73(3), 959.
- Friedmann, H. (1929). *The Cowbirds: A Study in the Biology of Social Parasitism*. Springfield, Illinois: Charles C. Thomas.
- Hedrick, A. V. (1988). Female choice and the heritability of attractive male traits: An empirical study. *The American Naturalist*, 132(2), 267–276.

- Hedrick, A. V. (2000). Crickets with extravagant mating songs compensate for predation risk with extra caution. *Proceedings of the Royal Society of London B: Biological Sciences*, 267(1444), 671–5.
- Helfman, G. S. (1986). Behavioral response of prey fishes during predator-prey interactions. In G. S. Helfman (Ed.), *Predator-Prey Relationships* (pp. 135–156). Chicago, IL: Chicago University Press.
- Hoefler, C. D., Persons, M. H. & Rypstra, A. L. (2008). Evolutionarily costly courtship displays in a wolf spider: a test of viability indicator theory. *Behavioral Ecology*, 19(5), 974–979.
- Hughes, N. K., Kelley, J. L. & Banks, P. B. (2012). Dangerous liaisons: The predation risks of receiving social signals. *Ecology Letters*, 15(11), 1326–39.
- Jackson, R. R. (1978). The mating strategy of *Phidippus johnsoni* (Araneae, Salticidae). *Behavioral Ecology and Sociobiology*, 4(2), 123–132.
- Jakobsson, S., Brick, O. & Kullberg, C. (1995). Escalated fighting behaviour incurs increased predation risk. *Animal Behaviour*, 49(1), 235–239.
- Kelly, C. & Godin, J.-G. (2001). Predation risk reduces male-male sexual competition in the Trinidadian guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, 51(1), 95–100.
- Kirkpatrick, M. (1982). Sexual Selection and the Evolution of Female Choice. *Evolution*, 36(1), 1–12.
- Koga, T., Backwell, P. R. Y., Jennions, M. D. & Christy, J. H. (1998). Elevated predation risk changes mating behaviour and courtship in a fiddler crab. *Proceedings of the Royal Society B: Biological Sciences*, 265(1404), 1385–1390.
- Lee, S. J., Witter, M. S., Cuthill, I. C. & Goldsmith, A. R. (1996). Reduction in escape performance as a cost of reproduction in gravid starlings (*Sturnus vulgaris*). *Proceedings of the Royal Society B: Biological Sciences*, 263(1370), 619–623.

- Li, D. & Jackson, R. R. (1996). Prey preferences of *Portia fimbriata*, an araneophagic, web-building jumping spider (Araneae: Salticidae) from Queensland. *Journal of Insect Behavior*, 9(4), 613–642.
- Lima, S. L. (1986). Predation risk and unpredictable feeding conditions: Determinants of body mass in birds. *Ecology*, 67(2), 377–385.
- Lima, S. L. (1998). Nonlethal effects in the ecology of predator-prey interactions. *BioScience*, 48(1), 25–34.
- Lima, S. L. (2009). Predators and the breeding bird: Behavioral and reproductive flexibility under the risk of predation. *Biological Reviews*, 84(3), 485–513.
- Lima, S. L. & Bednekoff, P. A. (1999). Temporal variation in danger drives antipredator behavior: The predation risk allocation hypothesis. *The American Naturalist*, 153(6), 649–659.
- Lima, S. L. & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, 68(4), 619–640.
- Magnhagen, C. (1990). Reproduction under predation risk in the sand goby, *Pomatoschistus minutes*, and the black goby, *Gobius niger*: The effect of age and longevity. *Behavioral Ecology and Sociobiology*, 26(5), 331–335.
- Magnhagen, C. (1991). Predation risk as a cost of reproduction. *Trends in Ecology & Evolution*, 6(6), 183–186.
- Magurran, A. E. & Garcia, C. M. (2000). Sex differences in behaviour as an indirect consequence of mating system. *Journal of Fish Biology*, 57(4), 839–857.
- Martel, G. & Dill, L. (1993). Feeding and aggressive behaviours in juvenile coho salmon (*Oncorhynchus kisutch*) under chemically-mediated risk of predation. *Behavioral Ecology and Sociobiology*, 32(6), 365–370.

- Massoni, V. & Reboreda, J. C. (1999). Egg puncture allows shiny cowbirds to assess host egg development and suitability for parasitism. *Proceedings of the Royal Society B: Biological Sciences*, 266(1431), 1871–1874.
- Moore, M. C. (2009). Effect of female sexual displays on the endocrine physiology and behaviour of male white-crowned sparrows, *Zonotrichia leucophrys*. *Journal of Zoology*, 199(2), 137–148.
- O’Loghlen, A. L. & Rothstein, S. I. (2010a). Multimodal signalling in a songbird: Male audiovisual displays vary significantly by social context in brown-headed cowbirds. *Animal Behaviour*, 79(6), 1285–1292.
- O’Loghlen, A. L., & Rothstein, S. I. (2010b). It’s not just the song: Male visual displays enhance female sexual responses to song in brown-headed cowbirds. *The Condor*, 112(3), 615–621.
- Peer, B. D. & Bollinger, E. K. (2000). Why do female brown-headed cowbirds remove host eggs? A test of the incubation efficiency hypothesis. In J. N. M. Smith, T. L. Cook, S. I. Rothstein, S. K. Robinson & S. G. Sealy (Eds.), *Ecology and Management of Cowbirds and Their Hosts: Studies in the Conservation of North American Passerine Birds* (pp. 187–192). Austin, TX: University of Texas Press.
- Polis, G. a, Barnes, J. D., Seely, M. K., Henschel, J. R. & Enders, M. M. (2009). Predation as a major cost of reproduction in Namib Desert tenebrionid beetles. *Ecology*, 79(7), 2560–2566.
- Preisser, E. L., Bolnick, D. I. & Benard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, 86(2), 501–509.
- Reznick, D. & Endler, J. A. (1982). The impact of predation on life history evolution in Trinidadian Guppies (*Poecilia reticulata*). *Evolution*, 36(1), 160–177.

- Rothstein, S. I., Yokel, D. A. & Fleischer, R. C. (1988). The agonistic and sexual functions of vocalizations of male brown-headed cowbirds, *Molothrus ater*. *Animal Behaviour*, 36(1), 73–86.
- Rowe, L. (1994). The costs of mating and mate choice in water striders. *Animal Behaviour*, 48(5), 1049–1056.
- Ryan, M. J. (1985). *The Tungara Frog: A Study in Sexual Selection and Communication*. Chicago, IL: University of Chicago Press.
- Ryan, M. J., Tuttle, M. D. & Stanley Rand, A. (1982). Bat Predation and Sexual Advertisement in a Neotropical Anuran. *The American Naturalist*, 119(1), 136–139.
- Sakaluk, S. K. & Belwood, J. J. (1984). Gecko phonotaxis to cricket calling song: A case of satellite predation. *Animal Behaviour*, 32(3), 659–662.
- Sargent, R. C. & Gross, M. R. (1986). Williams principle: An explanation of parental care in teleost fishes. In T. J. Pitcher (Ed.), *The Behaviour of Teleost Fishes* (pp. 275–293). Boston, MA: Springer US.
- Scott, D. M. & Ankney, D. (1980). Fecundity of the brown-headed cowbird in southern Ontario. *The Auk*, 97(4), 677–683.
- Shine, R. (1980). “Costs” of reproduction in reptiles. *Oecologia*, 46(1), 92–100.
- Sih, A. (1987). Predators and prey lifestyles: an evolutionary and ecological overview. In W. C. Kerfoot & A. Sih (Eds.), *Predation: Direct and Indirect Impacts on Aquatic Communities* (pp. 203–224). Hanover, NH: University Press of New England.
- Sih, A. (1994). Predation risk and the evolutionary ecology of reproductive behaviour. *Journal of Fish Biology*. 45(sA), 111–130.

- Sih, A., Krupa, J. & Travers, S. (1990). An experimental study on the effects of predation risk and feeding regime on the mating behavior of the water strider. *The American Naturalist*, 135(2), 284–290.
- Stibor, H. (1992). Predator induced life-history shifts in a freshwater cladoceran. *Oecologia*, 92(2), 162–165.
- Su, K. F. Y. & Li, D. (2006). Female-biased predation risk and its differential effect on the male and female courtship behaviour of jumping spiders. *Animal Behaviour*, 71(3), 531–537.
- Svensson, I. (1988). Reproductive costs in two sex-role reversed pipefish species (Syngnathidae). *Journal of Animal Ecology*, 57(3), 929–942.
- Swan, D. C., Zanette, L. Y. & Clinchy, M. (2015). Brood parasites manipulate their hosts: experimental evidence for the farming hypothesis. *Animal Behaviour*, 105, 29–35.
- Uzendoski, K., Maksymovitch, E. & Verrell, P. (1993). Do the risks of predation and intermale competition affect courtship behavior in the salamander *Desmognathus ochrophaeus*? *Behavioral Ecology and Sociobiology*, 32(6), 421–427.
- Van Den Assem, J. (1975). Male courtship behaviour, female receptivity signal, and size differences between the sexes in Pteromalinae (Hym., Chalcidoidea Pteromalidae), and comparative notes on other chalcidoids. *Netherlands Journal of Zoology*, 26(4), 535–548.
- Veasey, J. S., Houston, D. C. & Metcalfe, N. B. (2000). Flight muscle atrophy and predation risk in breeding birds. *Functional Ecology*, 14(1), 115–121.
- Walters, B. (2015). *Tactics to stay alive: Predation risk alters body condition and escape behaviour* (Master's thesis). University of Western Ontario, London, Ontario.

- White, D. J., Gersick, A. S., Freed-Brown, G. & Snyder-Mackler, N. (2010). The ontogeny of social skills: Experimental increases in social complexity enhance reproductive success in adult cowbirds. *Animal Behaviour*, 79(2), 385–390.
- White, D. J., King, A. P. & West, M. J. (2002). Facultative development of courtship and communication in juvenile male cowbirds (*Molothrus ater*). *Behavioral Ecology*, 13(4), 487–496.
- White, D. J., King, A. P., West, M. J., Gros-Louis, J. & Tuttle, E. M. (2009). Effects of singing on copulation success and egg production in brown-headed cowbirds, *Molothrus ater*. *Behavioral Ecology*, 21(2), 211–218.
- Wiedenmayer, C. P. (2004). Adaptations or pathologies? Long-term changes in brain and behavior after a single exposure to severe threat. *Neuroscience and Biobehavioral Reviews*, 28(1), 1–12.
- Ylönen, H. (1994). Vole cycles and antipredatory behaviour. *Trends in Ecology & Evolution*, 9(11), 426–30.
- Zanette, L. Y., White, A. F., Allen, M. C. & Clinchy, M. (2011). Perceived predation risk reduces the number of offspring songbirds produce per year. *Science*, 334(6061), 1398–401.

Chapter 3: General Discussion

Prey must consider a complex combination of perceived risk and potential for current and future reproduction in order to make behavioural decisions to optimize their lifetime reproductive success. I presented in chapter one a review of the importance of studying risk effects in regards to predator-prey interactions and discussed how risk effects are mediated through costly anti-predator responses. As part of this discussion, I provided evidence from previous research that risk effects on courtship behaviour is one of the many ways the mere presence of predators can lead to significant impacts on prey population dynamics. However, there are other studies that do not find an impact of predation risk on courtship, thus empirical studies are needed to understand how prey courtship behaviour may respond to predation risk since the costs to survival and benefits to reproduction can differ between individuals.

In chapter two, I presented a study where I measured reproductive behaviours of brown-headed cowbirds in response to chronically elevated predation risk. Mainly the females were most sensitive to the risk manipulation. Female cowbirds were more likely to reject courting males, reduced their chatter calls, and spent less time inspecting host nests. I found these significant effects irrespective of incorporating temporal variations in risk in which females could have used the periods of low risk to compensate for times of high risk. On the other hand, males only reduced their courtship displays in response to the lowered receptivity of the females and did not change the intensity of their displays or male-male interactions. The sex differences in sensitivity of reproductive behaviours to predation risk may reflect differences in the risk perceived by the sexes and the chances for future reproduction. Males may also be engaging more in these behaviours during

periods of low risk as a strategy to maintain dominance. My study, therefore, addressed the gaps in knowledge in the current literature regarding temporal variations in risk, the sexes influence on each other, and the lack of avian studies. These findings also support previous research that have demonstrated that predation risk effects on prey populations can be mediated through changes in individual prey behaviour that have fitness consequences.

3.1 Fitness Consequences of Reductions in Reproductive Behaviours

Courtship is an important part of reproductive success as its role is two-fold: one, courtship is typically required to induce females to be sexually receptive for copulation (Van Den Assem 1975, Jackson 1978, Andrew 2008), and two, females use courtship to choose the best quality mate (Kodric-Brown & Brown 1984, Byers *et al.* 2010). Female receptivity is important in many species where copulation can only be successful if the female allows the males to mate with her (Van Den Assem 1973, Halliday 1990, Vinnedge & Verrell 1998). Male parasitic wasps of the family Pteromlidae only stop their courtship to move into copulation position when given an antennal gesture from females signalling her receptivity (Van Den Assem 1973). Spermatophore deposition in the plethodontid salamander (*Desmognathus ocoee*) is only possible if males make females completely sexually responsive using courtship (Halliday 1990, Vinnedge & Verrell 1998). Similarly, a cowbird male's copulation success is correlated with the number of songs directed to females (White *et al.* 2009) and a female solicitation display is required for males to copulate successfully (Yokel & Rothstein 1991). In my study, I found an overall reduction in courtship interactions due to females being less receptive when cowbirds were exposed to perceived predation risk. Fewer courtship attempts would lead

to fewer interactions and less chances for copulation. Moreover, the lowered receptivity of female cowbirds suggests that even when courtship occurred it may not lead to copulation. Thus, a reduction in courtship persistence can have strong consequences for reproductive success.

Although copulation success is related to courtship displays from cowbird males, a study of the cowbird mating system found that the number of eggs produced in a flock was more related the number of displays between males (White *et al.* 2009). The mechanism behind this is unclear, but it is suggested that male competition may stimulate the females' reproductive hormones (White *et al.* 2009). I found that male cowbirds maintained their male-to-male displays despite the increased perceived predation risk. However, this relationship between male competition and egg production may not hold true under predation risk if the females are unwilling to attend to male-to-male displays much like they are unwilling to attend to courtship displays. A reduction in copulation success may also result in a lower number of viable eggs if they are not fertilized. Furthermore, egg production has also been attributed to environmental factors including predation risk (Carey 1996, Eggers *et al.* 2006, Zanette *et al.* 2011). Thus, female cowbirds have a large amount of control over reproductive success through copulations and egg production. The great sensitivity of female cowbirds to predation risk would suggest then that cowbird reproduction would be affected by risk regardless of the maintenance of male competition.

The reduction in female cowbird receptivity holds further consequences for fitness if their mate choice is affected. Since females were more likely to move away from courting males in the predator treatment, it can be inferred that females perceived a

large risk to being in close association with males and assessing their courtship displays. A combination of increased risk of evaluating males and lowered encounters between pairs may result in less discrimination between males or choosing ‘safer’ males that may not be normally preferred (Sih 1994, Jennions & Petrie 1997). A reduction in choosiness has been demonstrated in guppies (Godin & Briggs 1996, Godin & McDonough 2003), sand gobies (Forsgren 1992), and swordtails (Johnson & Basolo 2003). If male courtship reflects a male’s contribution through sperm quality or quantity (Matthews et al 1997) or her offspring’s genetic quality (Williams 1966), changes in female mate choice under predation risk can affect a female’s fitness.

As an obligate brood parasite, female cowbirds are also responsible for finding suitable host nests in which to lay her eggs. Cowbirds are able to determine when a host nest is appropriate for egg laying by pecking eggs (Massoni & Reboresda 1999, Swan *et al.* 2015). The age of the nest is important information for cowbirds since her egg must hatch around the same time as the host eggs or else there is less chance of survival for the cowbird chick (Fiorini *et al.* 2009). The destruction of host eggs can also serve two other purposes. First of all, host nests that are past the appropriate time to lay can be destroyed to force the host to re-nest providing the cowbird with another opportunity; this is termed ‘the farming hypothesis’ (Arcese *et al.* 1996). Secondly, parasitic eggs are more likely to hatch if a host egg is removed since the host may not be able to incubate the whole clutch according to the ‘the incubation limit hypothesis’ (Davies & Brooke 1988). Also, ‘the incubation efficiency hypothesis’ states that a large clutch can adversely affect the incubation efficiency of a cowbird egg (Peer & Bollinger 2000). Therefore, female cowbirds should remove host eggs to ensure adequate incubation of her egg. A reduction

in nest inspection behaviour as demonstrated in my study under predation risk can prevent females from finding an appropriate nest and impact a cowbird offspring's chances for survival. In sum, my study supports the theory that risk effects on prey populations can be mediated through changes in courtship and other reproductive behaviours impacting the birth rate and offspring quality and survival.

3.2 Adaptive Significance of Predation Risk Effects on Reproductive Traits

While anti-predator responses are costly in terms of fitness, they are actually adaptive in high risk situation to ensure survival and reflect a prey's 'best case scenario' for optimizing their lifetime reproductive success. Therefore, predation pressure can lead to directional selection on traits that would infer a much lower reproductive success in a low risk environment, but allows prey to avoid predators with as much lifetime reproductive success as possible in a high risk environment (Endler 1980). For example, great tits (Coslovsky & Richner 2011) and 12 other passerine species (Cheng & Martin 2012) produced offspring with smaller body mass but larger wings in response to predation risk. Thus, there was a trade-off between larger wings for better escape from predators and body mass, which affects reproductive success since smaller individuals may be less competitive than other larger males. Over evolutionary time, if the predation pressure maintains, these traits would be continually selected for and be more represented in the population. Female guppies from populations under greater predation were found to prefer drabber males (Stoner & Breden 1988) and are genetically differentiated from females in populations with lowered predation risk (Breden & Stoner 1987). The combination of female preference and natural selection against brightly coloured males then leads to a greater selection for dull-coloured males in high risk populations (Stoner

& Breden 1988). In a more drastic example, 90% of male field crickets (*Teleogryllus oceanicus*) under threat by parasitoids lost their ability to call due to a change in wing morphology over 20 generations (Zuk *et al.* 2006).

For the cowbirds in my study, predation can lead to selection for females and males who are able to exert behavioural flexibility under predation risk. Females were the most sensitive to predation risk which seems like they are potentially preferentially captured by predators. Therefore, under predation risk, selection would favour females who can attend to the presence of predators and reduce those risky reproductive behaviours even at the cost of current reproduction in order to maximize her reproductive lifespan. In low risk environments, males who court frequently have the greater reproductive success (White *et al.* 2009), but in high risk environments, males that continue to court frequently may not be preferred by females but males that completely stop courting would also receive no copulations. Therefore, males that exhibit greater behavioural flexibility and are able to attend to predator cues and female receptivity to know how much or when to court would be at an advantage. Predation risk may also lead to non-assortative mating in cowbirds if females no longer show any form of preference for males (Crespi 1989). However, this seems unlikely as male cowbirds maintained their dominance status even under predation risk. Even if females have no preference, dominant males will outcompete subordinate males to maintain access to females (Dufty 1986). Overall, predation risk can alter not only the number of offspring produced, but the gene flow between generations potentially promoting characteristics that may not otherwise be advantageous in low risk environments.

3.3 Study Limitations and Future Directions

My study supports the idea that predation risk affects reproductive behaviours in a way that can affect fitness. We must be cautious in interpreting these results to be definitively applicable to birds in the wild since my birds were held in captivity. I made a great effort to maintain the aviaries as a semi-natural environment, but in the wild, there are other environmental factors like the amount of shelter that can contribute to how the birds respond to predation risk. However, in combination with previous observational studies of prey populations in high risk environments, it is reasonable to interpret my results as providing further evidence for the connection between predation risk and changes in reproductive behaviour.

I documented changes in female receptivity and the number of courtship interactions, which I speculate will impact fitness based on previous knowledge of mating systems. But further studies are needed to measure the actual fitness consequences of these changes by measuring copulation success and the number of eggs produced. Observations of the cowbird mating system in similar outdoor aviary conditions finds that on average 0.2 copulations would be observed for each male per hour of observation (White *et al.* 2009). The large number of hours of observations needed to measure changes in copulation success was not possible with one observer and the limited time the cowbirds courted each morning. It would also be difficult to prevent the birds habituating to the predator manipulations over a much longer period if the birds continued to be exposed to predator cues without an actual predator attack. I was also unable to collect eggs since cowbirds that are placed in captive environments shortly before breeding season typically do not lay eggs that season (David White, personal communication). A future study should collect eggs and determine whether they are

viable as a measure of offspring production under predation risk. Microsatellite DNA analysis can also be used to determine the parentage of the eggs to examine the relationships between courtship, female mate choice, copulation success and reproductive output in a high predation risk environment.

I also found that females were more sensitive to the predation risk manipulation than the males were. This suggests that females may undertake a greater risk engaging in reproductive behaviours than males do. Studies to determine whether females are preferentially captured when engaging in courtship such as is the case for spiders (Hebets 2004) and crickets (Sakaluk & Belwood 1984) would help to understand the differential reproductive strategies between males and females under predation risk. Another strategy that should be further explored is how prey can attend to the temporal variations in risk so that they can compensate during low risk periods for the lost mating opportunities during high risk periods. The female cowbirds in my study had an overall reduction in reproductive behaviours regardless of low risk periods, but males may be using the low risk periods to maintain their dominance interactions. As the use of temporal variations in predation risk in signalling behaviours has yet to be tested, this avenue of research would be a large contribution to our understanding of the different strategies in response to predation risk when courting in natural environments (Hughes *et al.* 2012).

3.4 Implications for Conservation and Management

Predation risk effects have clear fitness consequences for prey and must be considered when conservation and management of a prey species is necessary (Creel & Christianson 2008, Preisser & Bolnick 2008). Not only does predation risk affect a prey's current reproductive success, but risk effects can last multiple generations through

maternal effects. If the offspring experience the same high risk environment, traits that allow the parents to survive and have some reproductive success would be adaptive. However, if the environment between the parents and the offspring are mismatched, these characteristics expressed in the offspring due to changes in reproductive behaviours of the parents would be less favourable. This can contribute to long-lasting effects of predation risk causing a lag in prey population recovery even after the predator populations decline as discussed in chapter one with the snowshoe hares (Boonstra *et al.* 1998, Sheriff *et al.* 2010, Sheriff *et al.* 2015). Conservation and management must take into account predation risk effects since only eliminating direct predation may not necessarily help prey population recovery immediately. Research is needed to examine the mechanisms behind risk effects on prey population dynamics to pinpoint which prey characteristics are most susceptible to risk effects and should be targeted for conservation efforts.

3.5 Summary

Prey attend to the level of risk in the environment and adjust their courtship behaviour accordingly. The behavioural strategies exhibited under predation risk can have costs on current reproductive success, but can help prey survive in order to maximize their reproductive lifespan. My study demonstrated that brown-headed cowbird females responded to predation risk in a way that suggests they are aware of the risks associated with signalling, attending to male courtship, and host nest inspection. Subsequently, their reduction in receptivity to courtship resulted in a fewer number of courtship attempts by males. My results highlight the importance of examining both males and females to get an accurate indication of how a behaviour that involves both sexes may change under predation risk. Even when presenting the birds with periods of

low risk to simulate temporal variations in risk much like in a natural setting, perceived predation risk still significantly impacted the reproductive behaviours measured to an extent that can potentially have fitness consequences. In closing, the influence of predators on prey populations is pervasive extending beyond consumptive effects such that the overall picture of predator-prey interactions would not be complete without considering risk effects on prey populations.

3.6 References

- Andrew, R. J. (2008). The displays given by passerines in courtship and reproductive fighting: A review. *Ibis*, *103a*(3), 315–348.
- Arcese, P., Smith, J. N., & Hatch, M. I. (1996). Nest predation by cowbirds and its consequences for passerine demography. *Proceedings of the National Academy of Sciences*, *93*(10), 4608–4611.
- Boonstra, R., Krebs, C. J., & Stenseth, N. C. (1998). Population cycles in small mammals: The problem of explaining the low phase. *Ecology*, *79*(5), 1479–1488.
- Breden, F., & Stoner, G. (1987). Male predation risk determines female preference in the Trinidad guppy. *Nature*, *329*(6142), 831–833.
- Byers, J., Hebets, E., & Podos, J. (2010). Female mate choice based upon male motor performance. *Animal Behaviour*, *79*(4), 771–778.
- Carey, C. (1996). Avian energetics and nutritional ecology. In C. Carey (Ed.), *Avian Energetics and Nutritional Ecology* (pp. 324–374). Boston, MA: Springer US.
- Cheng, Y.-R., & Martin, T. E. (2012). Nest predation risk and growth strategies of passerine species: Grow fast or develop traits to escape risk? *The American Naturalist*, *180*(3), 285–295.
- Coslovsky, M., & Richner, H. (2011). Predation risk affects offspring growth via maternal effects. *Functional Ecology*, *25*(4), 878–888.
- Creel, S., & Christianson, D. (2008). Relationships between direct predation and risk effects. *Trends in Ecology & Evolution*, *23*(4), 194–201.
- Crespi, B. J. (1989). Causes of assortative mating in arthropods. *Animal Behaviour*, *38*(6), 980–1000.
- Davies, N. B., & Brooke, M. de L. (1988). Cuckoos versus reed warblers: Adaptations and counteradaptations. *Animal Behaviour*, *36*(1), 262–284.

- Dufty, A. M. (1986). Singing and the establishment and maintenance of dominance hierarchies in captive brown-headed cowbirds. *Behavioral Ecology and Sociobiology*, *19*(1), 49–55.
- Eggers, S., Griesser, M., Nystrand, M., & Ekman, J. (2006). Predation risk induces changes in nest-site selection and clutch size in the Siberian jay. *Proceedings. Biological Sciences / The Royal Society*, *273*(1587), 701–706.
- Endler, J. A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, *34*(1), 76–91.
- Fiorini, V. D., Tuero, D. T., & Reboreda, J. C. (2009). Shiny cowbirds synchronize parasitism with host laying and puncture host eggs according to host characteristics. *Animal Behaviour*, *77*(3), 561–568.
- Forsgren, E. (1992). Predation risk affects mate choice in a gobiid fish. *The American Naturalist*, *140*(6), 1041–1049.
- Godin, J. G. J., & McDonough, H. E. (2003). Predator preference for brightly colored males in the guppy: A viability cost for a sexually selected trait. *Behavioral Ecology*, *14*(2), 194–200.
- Godin, J.-G. J., & Briggs, S. E. (1996). Female mate choice under predation risk in the guppy. *Animal Behaviour*, *51*(1), 117–130.
- Halliday, T. R. (1990). The evolution of courtship behavior in newts and salamanders. In *Advances in the Study of Behavior, Volume 19* (pp. 137–169). Academic Press.
- Hebets, E. A. (2004). Attention-altering signal interactions in the multimodal courtship display of the wolf spider *Schizocosa uetzi*. *Behavioral Ecology*, *16*(1), 75–82.
- Hughes, N. K., Kelley, J. L., & Banks, P. B. (2012). Dangerous liaisons: The predation risks of receiving social signals. *Ecology Letters*, *15*(11), 1326–39.

- Jackson, R. R. (1978). The mating strategy of *Phidippus johnsoni* (Araneae, Salticidae). *Behavioral Ecology and Sociobiology*, 4(2), 123–132.
- Jennions, M. D., & Petrie, M. (2007). Variation in mate choice and mating preferences: A review of causes and consequences. *Biological Reviews*, 72(2), 283–327.
- Johnson, J. B., & Basolo, A. L. (2003). Predator exposure alters female mate choice in the green swordtail. *Behavioral Ecology*, 14(5), 619–625.
- Kodric-Brown, A., & Brown, J. H. (1984). Truth in advertising: The kinds of traits favored by sexual selection. *The American Naturalist*, 124(3), 309–323.
- Massoni, V., & Reboreda, J. C. (1999). Egg puncture allows shiny cowbirds to assess host egg development and suitability for parasitism. *Proceedings of the Royal Society B: Biological Sciences*, 266(1431), 1871–1874.
- Matthews, I. M., Evans, J. P., & Magurran, A. E. (1997). Male display rate reveals ejaculate characteristics in the Trinidadian guppy, *Poecilia reticulata*. *Proceedings of the Royal Society B: Biological Sciences*, 264(1382), 695–700.
- Peer, B. D., & Bollinger, E. K. (2000). Why do female brown-headed cowbirds remove host eggs? A test of the incubation efficiency hypothesis. In J. N. M. Smith, T. L. Cook, S. I. Rothstein, S. K. Robinson, & S. G. Sealy (Eds.), *Ecology and Management of Cowbirds and Their Hosts: Studies in the Conservation of North American Passerine Birds* (pp. 187–192). Austin: University of Texas Press.
- Preisser, E. L., & Bolnick, D. I. (2008). When predators don't eat their prey: Nonconsumptive predator effects on prey dynamics. *Ecology*, 89(9), 2414–2415.
- Sakaluk, S. K., & Belwood, J. J. (1984). Gecko phonotaxis to cricket calling song: A case of satellite predation. *Animal Behaviour*, 32(3), 659–662.
- Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2010). The ghosts of predators past: Population cycles and the role of maternal programming under fluctuating predation risk. *Ecology*, 91(10), 2983–2994.

- Sheriff, M. J., McMahon, E. K., Krebs, C. J., & Boonstra, R. (2015). Predator-induced maternal stress and population demography in snowshoe hares: The more severe the risk, the longer the generational effect. *Journal of Zoology*, 296(4), 305–310.
- Sih, A. (1994). Predation risk and the evolutionary ecology of reproductive behaviour. *Journal of Fish Biology*, 45(sA), 111–130.
- Stoner, G., & Breden, F. (1988). Phenotypic differentiation in female preference related to geographic variation in male predation risk in the Trinidad guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, 22(4), 285–291.
- Swan, D. C., Zanette, L. Y., & Clinchy, M. (2015). Brood parasites manipulate their hosts: experimental evidence for the farming hypothesis. *Animal Behaviour*, 105, 29–35.
- Van Den Assem, J. (1973). Male courtship patterns and female receptivity signal of Pteromalinae (Hym., Pteromalidae), with a consideration of some evolutionary trends and a comment on the taxonomic position of *Pachycrepoideus vindemiae*. *Netherlands Journal of Zoology*, 24(3), 253–278.
- Van Den Assem, J. (1975). Male courtship behaviour, female receptivity signal, and size differences between the sexes in Pteromalinae (Hym., Chalcidoidea Pteromalidae), and comparative notes on other chalcidoids. *Netherlands Journal of Zoology*, 26(4), 535–548.
- Vinnedge, B., & Verrell, P. (1998). Variance in male mating success and female choice for persuasive courtship displays. *Animal Behaviour*, 56(2), 443–448.
- White, D. J., King, A. P., West, M. J., Gros-Louis, J., & Tuttle, E. M. (2009). Effects of singing on copulation success and egg production in brown-headed cowbirds, *Molothrus ater*. *Behavioral Ecology*, 21(2), 211–218.
- Williams, G. C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's principle. *The American Naturalist*, 100(916), 687–690.

- Yokel, D. A., & Rothstein, S. I. (1991). The basis for female choice in an avian brood parasite. *Behavioral Ecology and Sociobiology*, 29(1), 39–45.
- Zanette, L. Y., White, A. F., Allen, M. C., & Clinchy, M. (2011). Perceived predation risk reduces the number of offspring songbirds produce per year. *Science*, 334(6061), 1398–401.
- Zuk, M., Rotenberry, J. T., & Tinghitella, R. M. (2006). Silent night: Adaptive disappearance of a sexual signal in a parasitized population of field crickets. *Biology Letters*, 2(4), 521–524.

Appendices

Appendix A. Satellite view (Google Maps) of the four aviaries (12 x 30 x 60 feet) used to house the cowbirds. Dividers between aviaries 1 and 2 as well as between aviaries 3 and 4 visually isolated each social group.



Appendix B. Creating Playbacks

Selecting Predator and Non-Predator Species

Predator and non-predators were chosen and matched to create high risk and low risk playbacks

- Diurnal and nocturnal predator and non-predator species were chosen with the following selection criteria:
 - o Known to interact with the study species
 - o Present at the geographical location and time of year of the study
- Samples of calls were collected from online resources such as:
 - o The Cornell Lab of Ornithology's Macaulay Library (<http://www.birds.cornell.edu>)
 - o Borror Laboratory of Bioacoustics (<https://blb.osu.edu/>)
- Spectral analysis of the frequency properties of calls was done using Audacity (<http://audacityteam.org>)
 - o An alternative program is The Cornell Lab of Ornithology's Raven (<http://www.birds.cornell.edu/brp/raven/RavenOverview.html>)
- Predator and non-predator species were matched by comparing their calls:
 - o Subjectively
 - o According to time of day (diurnal and nocturnal)
 - o Using acoustic properties of their calls to statistically test differences between:
 - Minimum/maximum frequency
 - Peak frequency (frequency with the greatest amplitude)
 - Frequency range (maximum frequency – minimum frequency)

Create the Exemplars

Sound files of calls from each of the predator and non-predator species were selected and edited to create an extensive list of exemplars to use

- ~10 exemplars were collected for each predator and non-predator species
 - o Ideal exemplars had minimal background noise and were not from juveniles
- Exemplars were cleaned up using Audacity by removing background noise where possible and minimizing noise between calls
 - o To ensure acoustic properties of the calls were not affected visual examination of the spectrogram was conducted while editing the exemplars
- MP3 player and speakers were selected that could broadcast the range of frequencies present in the calls

- All exemplars were adjusted such that they broadcasted at 80 dB from 1 m away using a sound pressure level meter
- Silences were added to the exemplars such that the diurnal exemplars had a call-to-silence ratio of 1:1.5 and the nocturnal exemplars had a ratio of 1:2.3

Building the Playlists

Two 24-hour playlists were created by randomizing the presentation of all the exemplars. Each hour of playback was played from either one of two speakers so that the playbacks would be randomized between two locations.

- Exemplars were randomized into a list rotating through each predator species to build one hour sound files
- The number of diurnal and nocturnal sound files was determined according to when sunrise and sunset were during the study period
 - o An additional of one hour crepuscular period (mixture of diurnal and nocturnal species) per sunrise and sunset where a mixture of diurnal and nocturnal species calls were played
 - o For example, if there were 15 hours of daylight, the playlist included 14 hours of diurnal exemplars, 2 hours of mixed diurnal and nocturnal exemplars, and 8 hours of nocturnal exemplars
- This process of making one hour sound files was repeated until 48 hours of playbacks were created
- The order of predator exemplars was used to make the matching non-predator playlist such that the matched predator and non-predator species would be played at the same time
- Each one hour sound file was loaded onto either of two MP3 players such that the playbacks would be randomly broadcasted from one of two speaker box locations
- Note: All randomizations were done by numbering each option (i.e. each exemplar, one hour sound file, or playlist) and using a random number generator

Appendix C. Ethogram

Type of Behaviour	Behaviour	Description
Male Display	Courtship Display	A display and song from a male where the male bows and spreads his wings directed to a female no greater than 0.6 m away at an angle of 45° or less; each courtship display is also scored based on the level of intensity (see below)
Male Display	Male-to-Male Display	A display and song from a male where the male bows and spreads his wings directed to a male no greater than 0.6 m away at an angle of 45° or less; each courtship display is also scored based on the level of intensity (see below)
Aggressive Interaction	Displacement	One male approaches another and leaves such that the first male typically takes the spot of the male that is displaced
Aggressive Interaction	Peck	A quick, forward jabbing motion with the beak at another individual
Aggressive Interaction	Attack	Any other motion that makes aggressive physical contact with another individual
Female Response	Non-Receptive Response	After a male courtship display is directed at a female, the female moves at least 0.6 m away from the male within 1 second of the display
Female Response	Receptive Response	After a male courtship display is directed at a female, the female remains at least 0.6 m away from the male within 1 second of the display
Type of Variation	Variation	Description
Display Intensity Level	Low	A male display in which the male only slightly puffs up his feathers with an outstretched head
Display Intensity Level	Medium	A male display in which the male slightly spreads his wings with a slight head bow towards the ground
Display Intensity Level	High	A male display in which the male fully spreads his wings and bows looking completely down towards the ground

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

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- Females in control: Female sensitivity to predation risk affects courtship and reproductive behaviours.** (Oral Presentation)
Ontario Ecology, Ethology and Evolution Colloquium
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- 2013 The Abe Black Memorial Prize, McMaster University
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