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The Effects of Perceived Predation Threat on Stress Response and Memory in Birds

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Graduate Program in Neuroscience
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Abstract

This study examined how perception of predator cues, across three sensory modalities, affects physiology and behaviour of songbirds. I hypothesized that the perception of predator threat would elicit physiological and behavioural responses in both acute and chronic exposure conditions. My first study examined the responses of wild-caught black-capped chickadees (*Poecile atricapillus*) and house sparrows (*Passer domesticus*), as well as lab-bred zebra finches (*Taeniopygia guttata*), to acute predation cue exposure by coding video recorded behaviour and corticosterone analysis. My second study examined changes in black-capped chickadees' foraging behaviour and memory retention after chronic exposure to acoustic predation cues. There were no strong effects of the predator cue exposure on behaviour, corticosterone, or memory retention. This study expands on and fills gaps from the previous literature by examining different modalities of perception and predator effects on spatial memory abilities.

Keywords

Stress, Modality, Bird, Chronic Stress, Acute Stress, Perceived Predation Threat, Memory, Behaviour, Corticosterone

Co-Authorship Statement

I designed the studies, formulated the research questions, completed all data collection, sample preparations, and data analysis in regards to both studies.

Publications arising from chapter 2 will include Dr. Liana Zanette and Dr. Scott MacDougall-Shackleton as co-authors. Liana provided input on stimuli presentation and the resources to replicate (with modifications) the acoustic exposure. Scott provided a great deal of input in the development of my experimental design, as well as feedback on my data analysis and the writing of this thesis. Additionally, his NSERC funding to support this research, and the ethics approval for animal use. Assistance was provided by Dr. Elizabeth Hampson and Bavani Rajakumar for running the corticosterone assay.

Publication arising from chapter 3 will include Delaney Schofer and Dr. Scott MacDougall-Shackleton as co-authors. Delaney assisted with the data collection and animal care during the chickadee memory study. Delaney also examined flight path and flight distance data from the collected data set for an undergraduate thesis project, which will be incorporated into the manuscript. Scott provided a great deal of input in the development of my experimental design, as well as feedback on my data analysis and the writing of this thesis. Additionally, his NSERC funding to support this research, and the ethics approval for animal use. Assistance was provided by Dr. David Sherry and Dr. Caroline Strang in regards to input on experimental design, set up, and analysis.

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List of Abbreviations

BCCH	Black-capped chickadee
CORT	Corticosterone
HP	Hippocampus
HPA axis	Hypothalamic-Pituitary-Adrenal axis
HOSP	House sparrow
LMM	Linear Mixed Model
PCA	Principle Component Analysis
PC	Principle Component
RIA	Radioimmunoassay
SEM	Standard Error of the Mean
TnA	Nucleus taeniae of the amygdala
ZF	Zebra finch

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Chapter 1

1 Introduction

Predators are a ubiquitous threat in almost all environments, impacting the lives of organisms across a wide variety of taxa. Predators can affect prey populations both directly, through injury and mortality, and indirectly by altering demography through changes in behaviour and reproductive output (Bennett et al., 2016; Zanette, White, Allen, & Clinchy, 2011). A direct predator attack has an immediate impact on the individual, in which the animal is either killed or severely injured. Predation events are often quick with little build up or warning. However, animals that are able to narrowly avoid or evade an attack survive with a beneficial detect-and-avoid strategy when they encounter another predator in the future.

1.1 Direct Predation

Many different types of predators will attack adult birds, nestlings, or eggs. The predators capable of attack include: mammals, reptiles, brood parasite birds, and birds of prey. Nest predators are known to destroy nests along with breaking, eating, and/or removing eggs from nests thus contributing to reduced survival and hatchling success. Common nest predators include chipmunks, mice, squirrels, and snakes. Brood parasites, such as the brown-headed cowbird, are organisms that rely on others to raise their young in that they place their own eggs in the hosts nest and remove or kill the hosts eggs or hatchlings. Adult birds are also targeted by mammals and birds of prey; attacks can result in critical injuries or death to the bird thus eliminating them from the population. Free ranging domestic cats kill 1.3-4.0 billion birds annually, this includes owned outdoor cats and unowned cats (Loss et al., 2013). Many birds of prey are opportunistic and will eat or supplement their diet with small birds, smaller birds of prey are a higher threat as they have greater maneuverability and are more likely to expend effort in attacking small birds. There are well documented costs of high rates of predation from the aforementioned sources, but there are also costs associated with indirect predation.

1.2 Indirect Predation

An indirect predation event is when the animal perceives a predator through any one or multiple sensory cues and responds to the predation risk even though the animal is not in immediate danger. These cues can indicate that the animal should make a nest elsewhere, should abandon their nest, make fewer provisioning trips, or that the animal must stay vigilant and reduce time foraging. Preferences for safe breeding and foraging sites have been shown in fish, birds, and mammals (Lamanna & Martin, 2016). Therefore, variation in perceived risk can have influences on habitat preferences, distributions, behaviours, life-history traits, and offspring production in the absence of direct predation. The trade-offs of anti-predator behaviour and responses vary between taxa, species, and individuals. Simulated predator attack or predator presence cause animals to engage in anti-predator behaviours such as fleeing, freezing, avoiding the area, producing alarm calls, or increasing vigilance behaviour. There are long-term costs to anti-predator responses such as decreased foraging, abandoned nests, loss of prime habitat, or decreased provisioning of young.

1.2.1 Perception of Predation Threat

A simulated predator attack or simulated predator presence is only an effective method of studying predator-prey interactions and prey-specific responses if the prey is capable of perceiving the predation threat. The method used to present the predator cue may influence the detection and subsequent response from the subject. Studies vary widely in the measurements of detection, reactivity, and impacts on prey species using indirect predator threats.

There are a variety of ways to measure if, when, and how well animals are able to detect perceived predation threats. These consist of differences in physiological, behavioural, and fitness effects. Physiological measures such as increased neural activation, and increased levels of the hormone corticosterone (CORT) measured in feathers, hair, saliva, sweat, fecal matter, and/or blood plasma. Behavioural measures to distinguish when a threat has been perceived include: flight initiation distance, freezing or fleeing, avoidance, time spent foraging, amount of nestling provisioning and time incubating, alarm calls, and mobbing behaviour. Fitness measures can also be used to

assess impact of perceived predation threat such as time to returning to courtship, number of offspring produced, and clutch success.

Often studies will present the predator cues across one or more particular sense (modality). These modalities include, but are not limited to: visual, acoustic, or olfactory. There are also studies that use combinations of the aforementioned modality cues or use live presentations which might have a different impact than each modality presented separately. There is not enough consensus on the impact and response to any one modality to begin presenting them in conjunction.

1.2.2 Visually Perceived Predation Threat

Animals are able to detect predators through visual cues including shadows and the predator being visually detectable in the environment. This visual detection allows for prey species to respond with a variety of anti-predator responses when there is perceived visual evidence of a predator. For example, Vancouver Island marmots (*Marmota vancouverensis*) were found able to distinguish predator mounts from non-predator mounts, and captive-born animals responded similarly to wild-captured animals (Blumstein, Holland, & Daniel, 2006). The marmot predator response consisted of a decrease in the amount of time spent foraging and a decrease in the time spent within the burrow or vigilant at the burrow. The small difference for being captive-born is important because it suggests that animals reared at these facilities are likely to have adequate abilities to respond to predators upon release. After seeing a taxidermized mount of a fox, tamar wallabies (*Macropus eugenii*) responded by thumping their hind feet in alarm, suppressed foraging, and increased looking, similarly the sight of a taxidermized mount of a cat suppressed foraging and increased looking (Blumstein, Daniel, Griffin, & Evans, 2000). These responses to visual cues are not limited to mammals.

Research has also demonstrated that birds can perceive and react to visual predator cues that are simulating predator attacks or predator presence. These reactions can be physiological in nature or they can be behavioural as outlined in the examples of Table 1.1.. These studies suggest that not only is visual detection of a predator able to alter foraging behaviours but that head/face orientation of predators can also influence the predator risk assessment by prey species. Visual detection has also been found to not only

affect adult birds but nestlings as well, it has further been suggested that nestlings can demonstrate anti-predator behaviours. Static visual cues can evoke alarm calls, and nestlings will respond to these signals. Birds have been found to discriminate brood parasites mounts (which could potentially be a threat to the nest but not to the adults) from both a dangerous species mount (that are a threat to adults only) and an innocuous species mount (harmless control) (Welbergen & Davies, 2008). This adds to the growing evidence that birds are able to categorize predator threats from visual cues, the birds can even pass along this information to conspecifics. These studies taken together suggests that birds can transfer visual cues into acoustic information, which might increase the chance of avoidance or survival to those able to perceive and interpret that signal.

Table 1.1. Reference table of visual predator exposure effect on various bird subject species.

Reference	Subject species	Visual Predator	Measure & Effect
(Cantwell, Johnson, Kaschel, Love, & Freeberg, 2016)	Carolina chickadees (<i>Poecile carolinensis</i>) Tufted titmice (<i>Baeolophus bicolor</i>)	Snake model with head facing a feeder	Took fewer seeds More unsuccessful feeder visits
(Cockrem & Silverin, 2002)	Great tits (<i>Parus major</i>)	Taxidermized mount: Tegmalm's owl (<i>Aegolius funereus</i>)	Increased CORT
(Freeberg, Book, & Weiner, 2016)	Carolina chickadees (<i>Poecile carolinensis</i>)	Stuffed cat	Vigilant foraging Calling behaviour
(Grabarczyk & Ritchison, 2015)	Eastern bluebirds (<i>Sialia sialis</i>)	Raccoon mount (<i>Procyon lotor</i>)	Adults- calling behaviour
(Jones, Smith, Bebus, & Schoech, 2016)	European starlings (<i>Sturnus vulgaris</i>)	Raptor attack on a conspecific; Peregrine falcon (<i>Falco peregrinus</i>) Merlin (<i>Falco columbarius</i>) Cooper's hawk (<i>Accipiter cooperii</i>)	Increased CORT
(Soard & Ritchison, 2009)	Carolina chickadees (<i>Poecile carolinensis</i>),	Study skins of raptors;	Graded alarm calls (based on predator

	Black-capped chickadees (<i>Poecile atricapillus</i>)	Eastern screech-owl (<i>Megascops asio</i>) American kestrel (<i>Falco sparverius</i>) Sharp-shinned hawk (<i>Accipiter striatus</i>) Cooper's hawk (<i>Accipiter cooperii</i>) Great horned owl (<i>Bubo virginianus</i>) Red-tailed hawk (<i>Buteo jamaicensis</i>)	size and threat level) Stopped engaging in other activities (e.g. foraging)
(Vitousek, Jenkins, & Safran, 2014)	Barn swallows (<i>Hirundo rustica erythrogaster</i>)	Stuffed cat	Reduce provisioning of young
(Welbergen & Davies, 2008)	Reed warblers (<i>Acrocephalus scirpaceus</i>)	Taxidermized mounts; Common cuckoos (<i>Cuculus canorus</i>) Eurasian sparrowhawk (<i>Accipiter nisus</i>)	Mobbed cuckoos Graded alarm calls

1.2.3 Acoustically Perceived Predation Threat

Another primary way animals are able to detect predators is through acoustic cues, including predator calls and conspecific alarm calls. This detection of vocal signals allows for prey species to respond with a variety of anti-predator responses. Bipedal kangaroo rats are better at foraging in open areas because they are able to detect and escape predators, their adaptations over other rodents in the same environment include inflated auditory bullae which allows superior hearing and detection of approaching predators (Kotler, 1984). Month long playbacks of large carnivore vocalizations caused a reduction in raccoon (*Procyon lotor*) foraging, the raccoons spent less time in the intertidal area and less time feeding when the predator playbacks were present (Suraci, Clinchy, Dill, Roberts, & Zanette, 2016). Male wolf spiders (*Schizocosa ocreata*) responded to experimental playback of avian acoustic stimuli with antipredator behaviour significantly more often than to nonthreatening stimuli and took longer to return to courtship (Lohrey, Clark, Gordon, & Uetz, 2009). These examples illustrate that acoustic stimuli led to a perceived predation risk in mammals and invertebrates. This suggests that acoustic predator cues are a fundamental detection method for prey species.

There is also evidence that birds can detect and perceive acoustic cues simulating predation risk as well as produce acoustic responses to perceived predation threats. As outlined in Table 1.2 many bird species have been investigated in regards to the effect of perceived predation threat through acoustic cues by exposing the subjects to playlists of calls, or conspecific mobbing/alarm calls on a variety of outcomes. It has been demonstrated that birds can have physiological and neural changes that occur in the brain in response to perceived predator cues. Furthermore, research suggests that the nucleus taeniae of the amygdala (TnA) and the hippocampus (Hp) are not only important in the perception of predation risk but also for retaining information about previous predation events. There are also examples of behavioural and demographic impacts of acoustic predator threats, because even when direct predation has been eliminated, the perception of predation alone is enough to impact clutch success. These findings highlight the importance of studying nest predation from the offspring's perspective for a more accurate picture of predator-prey interaction, because it is not only the parent that can adjust behaviour and respond hormonally to predation risk. Birds can also vary the characteristics of their alarm calls based on predator size and level of perceived threat, this suggest that an alarm call with graded signalling informs conspecifics about the presence and behaviour of a predator and the degree to which it poses a threat. Demographic cost from behavioural responses to increases in perceived predation threat suggests a strong selection for animals to choose safe breeding and foraging sites when encountering variation in perceived risk. Also, this demonstrates why it is vital for birds to be able to detect and assess risk with any and all senses. Despite extensive use of acoustic predator calls investigating behavioural responses and the effects on demographics, we know relatively less about the hormonal changes that occur in response to acoustic stimuli in birds.

Table 1.2. Reference table of acoustic predator exposure effect on various bird subject species.

Reference	Subject species	Acoustic Predator Cue	Measure & Effect
(Avey, Hoeschele, Moscicki,	Black-capped chickadee (<i>Poecile atricapillus</i>)	Mobbing calls Northern saw-whet owl (<i>Aegolius acadicus</i>)	Increased ZENK expression in caudomedial

Bloomfield, & Sturdy, 2011)		Great-horned owl (<i>Bubo virginianus</i>)	mesopallium and caudomedial nidopallium
(Billings, Greene, & De La Lucia Jensen, 2015)	Black-capped chickadees (<i>Poecile atricapillus</i>) Mountain chickadees (<i>Poecile gambeli</i>),	Northern pygmy-owl (<i>Glaucidium gnoma</i>) Sharp-shinned hawk (<i>Accipiter striatus</i>) Northern goshawk (<i>Accipiter gentilis</i>)	Called more in response to the calls of smaller more dangerous raptors than to larger raptors
(Eggers, Griesser, Nystrand, & Ekman, 2006)	Siberian jays (<i>Perisoreus infaustus</i>)	Eurasian jay (<i>Garrulus glandarius</i>) Hooded crow (<i>Corvus cornix</i>) Common raven (<i>Corvus corax</i>)	Produced smaller clutches
(Grabarczyk & Ritchison, 2015)	Eastern bluebirds (<i>Sialia sialis</i>)	Adult Eastern bluebirds in response to a raccoon mount (<i>Procyon lotor</i>)	Nestlings crouched
(Hobbs, 2015)	Black-capped chickadees (<i>Poecile atricapillus</i>)	Mobbing calls High zee calls Cooper's hawk (<i>Accipiter cooperii</i>) American crow (<i>Corvus brachyrhynchos</i>) Red-tailed hawk (<i>Buteo jamaicensis</i>) Barred owl (<i>Strix varia</i>) Sharp-shinned hawk (<i>Accipiter striatus</i>) Northern saw-whet owl (<i>Aegolius acadicus</i>) Merlin (<i>Falco columbarius</i>)	Short-term activation in both the nucleus taeniae of the amygdala and the hippocampus Long-term activation in both the nucleus taeniae of the amygdala and the hippocampus
(Ibáñez-Álamo, Chastel, & Soler, 2011)	Common blackbird (<i>Turdus merula</i>)	Magpie (<i>Pica pica</i>)	Nestlings change corticosterone levels
(Lamanna & Martin, 2016)	American robin (<i>Turdus migratorius</i>) Warbling vireo (<i>Vireo gilvus</i>)	Red squirrel (<i>Tamiasciurus hudsonicus</i>) Chipmunk (<i>Tamias spp.</i>) Gray jay (<i>Perisoreus canadensis</i>)	Reduced time incubating Changed the egg size

	Dusky flycatcher (<i>Empidonax oberholseri</i>) Chipping sparrow (<i>Spizella passerine</i>) Dark-eyed junco (<i>Junco hyemalis</i>) Lincoln's sparrow (<i>Melospiza lincolnii</i>) White-crowned sparrow (<i>Zonotrichia leucophrys</i>) Swainson's Thrush (<i>Catharus ustulatus</i>) MacGillivray's warbler (<i>Geothlypis tolmiei</i>) Lazuli bunting (<i>Passerina amoena</i>)	Steller's jay (<i>Cyanocitta stelleri</i>) Common raven (<i>Corvus corax</i>)	Decrease provisioning rates Reduced hatch success
(Soard & Ritchison, 2009)	Carolina chickadees (<i>Poecile carolinensis</i>)	Conspecific alarm call in response to smaller predators (e.g. Eastern screech-owl, <i>Megascops asio</i>)	Produced more calls
(Witterick, 2017)	Black-capped chickadees (<i>Poecile atricapillus</i>)	Cooper's hawk (<i>Accipiter cooperii</i>) American crow (<i>Corvus brachyrhynchos</i>) Red-tailed hawk (<i>Buteo jamaicensis</i>) Barred owl (<i>Strix varia</i>) Sharp-shinned hawk (<i>Accipiter striatus</i>) Northern saw-whet owl (<i>Aegolius acadicus</i>) Merlin (<i>Falco columbarius</i>)	Decrease in the number of location movements Dendritic morphology changes and inhibited neurogenesis in both the nucleus taeniae of the amygdala and the hippocampus
(Zanette, White, Allen, & Clinchy, 2011)	Song sparrows (<i>Melospiza melodia</i>)	Corvid Hawk Owl Raccoon (<i>Procyon lotor</i>) Brown-headed cowbird (<i>Molothrus ater</i>)	Reduced the number of offspring by 40%

1.2.4 Olfactory Perceived Predation Threat

Olfactory or chemical cue predator detection have been observed in many invertebrates, fish (Chivers & Smith, 1998), reptiles and amphibians (Ferrer & Zimmer, 2007), and mammals (Apfelbach, Blanchard, Blanchard, Hayes, & McGregor, 2005). Typical sources of such odours include predator skin and fur, urine, feces, and anal glands secretions. Odours from a variety of carnivores when presented to rats and mice elicited an innate avoidance response as well as activations of carnivore odour-selective sensory neurons (Ferrero et al., 2011). In response to odours of mammalian predators, bank voles (*Clethrionomys glareolus*) significantly avoided or decreased utilization of the pen that the scent was present in by 50-90% of initial numbers (Jedrzejewski, Rychlik, & Jedrzejewska, 1993). Though owl and rabbit scents did not change voles' distribution in the terrarium. Larvae of the California newt (*Taricha torosa*), exhibited predator-avoidance behaviour in response to a chemical cue produced by cannibalistic adults but the anti-predator behaviour was suppressed when other prey was present (Ferrer & Zimmer, 2007). This demonstrates that even larvae amphibians are able to detect and respond appropriately to perceived predator risk. While there is extensive research in a wide variety of taxa there is surprisingly little research on avian detection of predator olfactory cues.

Olfactory information and chemical communication is important for recognising nests, discriminating partners, and other social behaviours. The detection of chemical cues or chemical communication have been studied in a wide variety of taxa but is often neglected in birds. There is evidence that birds can not only detect chemical and olfactory cues but can use them to perceive and avoid predators strategically. As outlined in Table 1.3 only a few bird species have been investigated in regards to the effect of perceived predation threat through olfactory cues and most have focused on avoidance of nest boxes or areas where the scent is present. This behavioural adjustment of predator avoidance provides evidence that birds may use olfactory cues to perceive and avoid threats. Behaviours and roosting choices may differ depending on relative abundances, types, and presence of predators, suggesting that birds can not only perceive odours but use olfaction to assess the environment and estimate nest site quality. There are also studies that do not demonstrate any difference between a non-predator and predator

condition, this suggests that the birds were either unable to detect the predator cue or the snake scent did not ultimately impact their selection of a nest site.

Table 1.3. Reference table of olfactory predator exposure effect on various bird subject species.

Reference	Subject species	Olfactory Predator Cue	Measure & Effect
(Amo, Galván, Tomás, & Sanz, 2008)	Blue tits (<i>Cyanistes caeruleus</i>)	Urine and gland secretion: Ferret (<i>Mustela furo</i>)	Avoid nest boxes Delayed and refused to enter the entry into the nest-box Decreased the time spent inside the nest box when feeding nestlings
(Amo, Visser, & Oers, 2011).	Great tits (<i>Parus major</i>)	Urine: Ferret (<i>Mustela furo</i>)	Both lab-bred and predator naïve birds avoid nest boxes
(Godard, Bowers, & Morgan Wilson, 2007).	Eastern bluebirds (<i>Sialia sialis</i>)	Skin chemical cues and waste byproducts: Black rat snake (<i>Elaphe obsoleta</i>)	Did not avoid nest boxes
(Griggio, Fracasso, Mahr, & Hoi, 2016).	House sparrows (<i>Passer domesticus</i>)	Urine: Mouse (<i>Mus musculus domesticus</i>)	Avoided area with predator scent
(Roth, Cox, & Lima, 2008)	House finches (<i>Carpodacus mexicanus</i>)	Feces: House cat	Responded to both a non-predator or predator scent cue by: Delaying their first feeding Spent less time on the feeder Reduced feeding bout length (particularly pronounced in the predator treatment)

It can be further generalised that birds have innate chemical detection abilities. Five species of passerines (European goldfinches (*Carduelis carduelis*), great tits (*Parus major*), gray catbirds (*Dumetella carolinensis*), eastern phoebes (*Sayornis phoebe*) and black-capped chickadees (*Poecile atricapillus*)) were evaluated for their ability to form conditioned responses to odour stimuli (Clark, Avilova, & Bean, 1993). Within passerines there was no correlation between olfactory acuity and relative size of the olfactory bulb, however, there is a correlation across orders of birds. The threshold detection level for cyclo-hexanone was within the range 0.3-0.7 ppm, this range is comparable to other passerines, and for other reagents in pigeons, chickens and quail. This range of sensitivity to reagents is similar to values of reagents reported for mammalian species such as rats and rabbits. These findings support the idea that birds possess an adequate sense of smell. There is some evidence that suggests larger olfactory bulb size improves olfaction, foraging, or navigational skills (Khan et al., 2015). Differences in the olfactory abilities among birds reflect diverse specialized functions, such as foraging, orientation/navigation, homing, nesting, activity pattern, and individual recognition. All aforementioned examples in each modality follow very different exposure durations, thus there is no consensus between responses to acute durations of perceived predation threat and responses to chronic durations of perceived predation threat.

1.3 Stress, Hypothalamic-Pituitary-Adrenal Axis, and Corticosterone

Stress in general is a strain or tension resulting from adverse or demanding circumstances. Physiological stress is an organism's response to a stressor, in which the body is reacting to a good or bad experience, a threat or challenge. Stress responses function as a way for the organism to maintain homeostasis, a stable equilibrium, when not experiencing a demanding circumstance. A strong measure of stress response is the maximum concentration of the hormone corticosterone (CORT). Research has demonstrated that CORT levels begin to increase around 3 min after initial disturbance and are maximal in blood collected 30 min post-capture or post-exposure to a stressor (Baugh, van Oers, Naguib, & Hau, 2013; Clinchy, Zanette, Boonstra, Wingfield, &

Smith, 2004; Jones et al., 2016; Wingfield, 2005). Predator-induced stress has been used to exemplify the concept of stress for close to a century because it is a universally understood concept that frightening stimuli triggers an immediate response (Clinchy, Sheriff, & Zanette, 2013). Yet, there are still unclear questions about how birds perceive and respond to stressors and the long-term impacts of predator-prey interactions.

Most predator-prey interaction research that measures CORT focuses on endogenous CORT, that which the organism has produced within itself, as a means to assess that animals reactivity to the stressor (Breuner, Patterson, & Hahn, 2008; Sopinka et al., 2015). CORT can also be manipulated in subjects through CORT implants or CORT infused diets, in which the increased CORT is exogenous because it originated from outside the organism. This method can be effective in impacting the organism but could be argued to be less biologically relevant as the organism is not producing its natural level of CORT. This study is investigating the effect of endogenous CORT on behavioural measures.

Corticosterone (CORT; the dominant avian glucocorticoid) is secreted after an activation of the hypothalamic–pituitary–adrenal (HPA) axis (Baugh et al., 2013; Hegab & Wei, 2014). This endocrine axis is essential for coping with demanding circumstances and stressful events. The stress response consists of multiple components. First, the baseline levels of glucocorticoids are maintained at a day-to-day energy homeostatic balance. Second, the response is initiated within a few minutes after a stimulus (stressor, e.g. predator) is perceived, then through cascading activation the adrenal glands secrete glucocorticoids, a class of steroid hormones, above baseline concentrations. Third, this level of glucocorticoid continues to increase in the blood until it reaches a peak concentration. Fourth, a process of negative feedback reduces the circulating glucocorticoid levels allowing the baseline level to be re-achieved, enabling the animal to respond to future challenges. Like other steroid hormones, CORT can affect diverse regulatory and behavioural processes simultaneously.

An increase in plasma CORT can be used to indicate when and to what degree a bird is experiencing stress (Cockrem, 2007). The integration of the HPA axis and the limbic system through glucocorticoid signalling is imperative in initiating and regulating a suitable stress response following real or perceived threats (Caudle, 2016). Variation in

the initiation of the stress response might play a role in acute coping behaviour, while the magnitude, duration, and amount of activation might have longer term consequences including how effectively an individual can endure future stressors and which individuals will survive stressful natural events (Baugh et al., 2013). There can be acute and chronic threats that can be perceived as a stressor to the prey and have been found to elicit behavioural responses, physiological responses, and impact cognitive abilities.

1.4 Acute Stress and Acute Perceived Predation Threat

Predator attacks or presentations of predator stimuli are often acute events that are short in duration lasting seconds (Jones et al., 2016), minutes (Roth et al., 2008), or hours (Ibáñez-Álamo et al., 2011). Studies across all three previously mentioned sensory modalities have used acute predator presentations to investigate the behavioural, physiological, or cognitive changes that occur in response to an acute perceived predation threat. Specifically, during the hour that mustelid scent was presented on a nest box great tits avoided the nest box (Amo et al., 2011). Other behavioural effects of acute predator presentation could include the example of snake models with heads facing the feeder being presented for one minute to a mixed species flocks of Carolina chickadee and tufted titmice that resulted in the birds taking fewer seeds and having more unsuccessful feeder visits (Cantwell et al., 2016). Acute visual presentations of a predator threat are capable of activating a stress response. For example, European starlings witnessing an attack on a conspecific, where the attack lasted from 2-8sec, increased the level of CORT in the observing birds (Jones et al., 2016).

Acute stress has also been found to mediate cognitive abilities through hormones. There are some indications that a short-term elevation in CORT may result in a better memory for caches. When mountain chickadees were treated 5 min prior to retrieval with exogenous CORT, through injected wax moth larvae, the birds recovered more seeds and tended to visit more cache-related sites than controls during retrieval following a caching trial (Saldanha, Schlinger, & Clayton, 2000). In contrast, when zebra finches that were selectively bred to respond to an acute stressor with high plasma CORT were compared to a random-bred control the high CORT birds performed less well on the spatial task after a 20 min restraint than the controls (Hodgson et al., 2007). This suggests that CORT

can have different effects on cognitive abilities, though it may depend on the species, the type of test used, and/or the method of eliciting a stress response in the bird.

Nevertheless, these findings provide evidence to suggest that stress hormones have important regulatory roles in avian spatial cognition.

1.5 Chronic Perceived Predation Threat

Living in an environment with high predator threat, reoccurring threats, or urbanization can be long-lasting and unpredictable leading to chronic stress. Presentations of chronic threats or predator stimuli are often prolonged events that are long in duration lasting days (Zanette et al., 2011), weeks (Figueiredo, Bodie, Tauchi, Dolgas, & Herman, 2003), months (Suraci et al., 2016), or multiple months (Pravosudov, Kitaysky, Wingfield, & Clayton, 2001). Studies across the sensory modalities have used chronic predator presentations to investigate the behavioural, physiological, or cognitive changes that occur in response to a chronic perceived predation threat. Specifically, when song sparrows were exposed to predator playbacks that played a call every few minutes for 24 h on a 4-day-on-4-day-off cycle for 130 days, there was a reduction in offspring by 40% (Zanette et al., 2011). This chronic predator threat has also been found to impact behaviour of mammals. During playbacks of large carnivores that played 24 h (20% of the time with a call playing) for 28 days there was a reduction in foraging and feeding in raccoons (Suraci et al., 2016). An organism is chronically stressed when there is a long-term activation of the HPA axis this can be caused by unpredictable factors in the environment. The baseline levels of CORT were significantly higher in birds that were food-restricted for 94 days than in birds maintained on ad libitum food (Pravosudov, Kitaysky, Wingfield, & Clayton, 2001). The overall increase in CORT induced by chronic stress is also supported by the finding that CORT detected in the daily feces collection of adult male mice is increased after five weeks of chronic mild stress (Melo, Drews, Zimmer, & Bilkei-Gorzo, 2014). Elevated CORT levels, generally occurring after stressful events, has been found to impair cognition, memory, and reduce the structural and functional plasticity of the brain (Lupien, McEwen, Gunnar, & Heim, 2009; McEwen, 2007). There is not sufficient information about how birds respond to predator threats across different sensory systems via CORT despite there being evidence that

CORT is involved after stressful events. There is also evidence that stressful circumstances can impact neural and cognitive processes in birds but little is known about the specific impact of perceived predator stress.

1.6 Spatial Memory and Neural Changes

Cognition is process of acquiring knowledge through experience and the senses, resulting in perception and sensation (Morand-Ferron, Cole, & Quinn, 2016). Memory is the ability of the brain to take experiences and perceptions and encode, store, and retrieve that information. Memory is vital over time to influence and guide future actions.

Memory in food-storing birds is particularly important because they require the ability to retrieve food from a wide variety of stores (also referred to as caches) over varying amounts of time after storage. Spatial memory refers to specific memories for spatial information, such as a geographical layout or positional layout (Morand-Ferron et al., 2016). Spatial memory abilities allow animals to retain and cognitively manipulate and retrieve information about their spatial environment. For food-storing birds like the black-capped chickadee, successful cache retrieval to a certain extent depends on an accurate, long-lasting memory for individual cache sites. Spatial memory in the case of food-storing birds refers to the retention, success of collection, cache retrieval, use of information about the environment, and evaluating relationships between different locations. As food-caching animals rely on their caches for overwinter survival, spatial memory may be critical for survival (Croston et al., 2016; Herz et al., 1994; Sherry & Vaccarino, 1989; Sherry, Vaccarino, Buckenham, & Herz, 1989).

Spatial memory and learning abilities are dependent upon neural structures, such as the hippocampus, the frontal lobes, and the amygdala (O'Connell & Hofmann, 2011). Due to its liposoluble characteristics, CORT can easily cross the blood–brain barrier and access the brain where there are receptors to bind to (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). The hippocampus, the frontal lobes, and the amygdala have been shown to be influenced by elevated CORT, because they contain glucocorticoid receptors. Among birds that store food, their hippocampus is enlarged relative to brain and body size when compared with non-storers, as well as an increased volume of one of the major afferent-efferent pathways (the septo-hippocampal pathway) (Krebs, 1990).

Hippocampal damage has been found to disrupt forms of spatial memory in food-storing species suggesting a functional similarity to the mammalian hippocampus though they are structurally distinct (Clayton & Krebs, 1995). Hippocampal lesions have been found to impair spatial memory, but not other types of memory (Hampton & Shettleworth, 1996), and impair the ability to find hidden food caches in food storing birds (Sherry & Vaccarino, 1989). Black-capped chickadees exposed to predator playbacks showed a significant changes in activation, lasting dendritic morphology changes, and inhibited neurogenesis in both the TnA and Hp (Hobbs, 2015; Witterick, 2017). This suggests that perceived acoustic predator threat can effect neural structures important for learning and memory, thus if presented chronically it is reasonable to assume that perceived predator threat could impact a food-storing birds spatial memory ability.

1.7 Research Objectives

In this thesis, I explore both the immediate impact and the long lasting effects of perceived predation risk on avian physiology and behaviour. My research aims to answer the question of how perceived predation risk can immediately impact avian behaviour and physiology as well as the long term effects of perceived predation risks on cognitive related behaviours. My first objective is to determine if perceived predation risk induces changes in corticosterone levels after a short term exposure and changes in behaviour during the exposure. My second objective is to test for lasting impacts of the perceived predation risk on the spatial memory ability of food-storing birds. Ecological considerations motivated the selection of species (a food-storer), the type of stressor (perceived predation threat), and the selection of the task (spatial memory). The black-capped chickadee is a model species for testing ideas concerning the neurobiology of spatial memory and its interaction with hormones, caching behaviour, and environmental stress (Brodin & Urhan, 2014; Clayton & Emery, 2015). Black-capped chickadees have been used for multiple decades to investigate memory, caching, and spatial memory abilities, however, currently there are not any investigations of chronic predator stress impacts on food retrieval abilities in black-capped chickadees.

I hypothesize that under increased perceived predation risk, birds will show behavioural and physiological changes. I predicted that I would see increased

corticosterone levels but reduced grooming, feeding, and movement behaviour. Additionally, I predicted that the wild birds would follow this pattern and the predator-naïve birds would not have increased corticosterone levels and would continue grooming, feeding, and moving in all exposure conditions. Furthermore, I predicted that birds exposed to increased perceived predation risk would take longer to solve a spatial memory task and would be less accurate when solving the task.

In Chapter 2 my objective was to assess the effects of acute perceived predation across corticosterone levels and behaviours displayed by zebra finches (*Taeniopygia guttata*), black-capped chickadees (*Poecile atricapillus*), and house sparrows (*Passer domesticus*) tested in acoustic isolation in the lab. In Chapter 3, my objective was to assess the effects of chronic perceived predation on spatial memory ability black-capped chickadees (*Poecile atricapillus*), tested in an indoor experimental room with artificial trees. In Chapter 4, I discuss the broader ecological and methodological significance of my findings, and how they can expand our knowledge of the effects of perceived predation risk on the brain.

1.8 References

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Chapter 2

2 Effect of Acute Perceived Predation Threat on Behaviour and Corticosterone

2.1 Introduction

Predator cues are increasingly being used to acquire fundamental information about how the behaviour, brain, and endocrine system of birds respond during acute or chronic stress (Apfelbach et al., 2005; Dantzer, Fletcher, Boonstra, & Sheriff, 2014). Acute stress is defined as being of short duration (minutes to hours) and chronic stress is a longer duration (days to weeks) (Boonstra, 2013). Acute stress event examples could include being pursued by a predator or detecting the scent of a predator on a nest box. Examples of chronic stress events could include long-term food shortage or building a nest next to a busy highway. An acute predator attack or a predation threat can be perceived as a stressor to the prey and has been found to elicit behavioural and physiological responses.

To perceive a predator threat the stimuli must first be detected in the surrounding environment. When these stimuli are perceived as threatening, the stress responses is initiated (Cockrem, 2007). In response to a stressor the hypothalamic–pituitary–adrenal (HPA) axis triggers a rapid release of glucocorticoids from the adrenal glands into the bloodstream (Bennett et al., 2016; Clinchy et al., 2013). This increases the circulating glucocorticoids, including corticosterone (CORT), in the bloodstream. An increase in plasma CORT can be used to indicate if and to what degree the birds is experiencing an acute stressor. This elevation of CORT is combined with a suppression of behavioural and physiological processes that are not immediately essential for survival (Vitousek et al., 2014). Behaviours such as foraging, mating, and grooming are suppressed in favour of freezing, fleeing, and vigilance which promote survival through avoiding or evading the predator threat.

The indirect effects of acute perceived predation risk have been associated with changes in CORT levels and behavioural changes. There is evidence that birds can perceive threats through a variety of different sensory systems. Experimentally manipulating how birds are presented with stimuli can help elucidate how relevant each sensory system is to detecting threats or distinguishing threats from non-threats. The

exposures and cues used when attempting to create the perception of threat range from live predators to synthetic representations such as synthetic spray scents or polyester fur with plush material predator models. The measures used to indicate behavioural and physiological responses to the presented stimuli also vary widely between studies; studies measure CORT, other studies measure individual brain activation, and some measure clutch size or population levels effects of perceived predation risk. The main connection between previous studies that have investigated predator-prey interaction is that birds are able to detect and distinguish perceived predator threats in many different sensory systems. All sensory systems are important but they have advantages and disadvantages; the visual sensory system may provide unambiguous information about the identity, direction, distance, movement, and general behaviour (actively hunting, preening, lounging, etc.) of predators, whereas information associated with olfactory cues about predators is more ambiguous as there is less certainty about a predator's identity, location, movement and behavioural state. Acoustic information allows for birds to hear in all directions and perceive stimuli from behind visual barriers this allows them to be less visually vigilant when foraging and allows them to detect if a predator is nearby but not yet visible. Olfactory information makes the bird aware that a predator was previously in the area or is still near-by which gives the bird a signal for risk assessment of how safe the area is.

Vision is a very important sensory system for gathering visual information about the environment and useful for predator detection as the more quickly and accurately one can locate and identify the predator the higher chance of survival one has. Research has demonstrated that birds can perceive and react to visual predator cues as outlined in Table 1.1 in Chapter 1. Adding to the growing evidence that birds are able to categorize predator threats and convey that information to conspecifics it has been found that mobbing behaviour and calling take visual information perceived by an individual and share it to any surrounding conspecifics, nestlings, or similar species. Of particular importance to this study static visual cues have been found to evoke alarm calls. Visual detection of a predator has been able to alter foraging behaviours. However, the head/face orientation of predators can influence the predator risk assessment by prey species, which is supported by studies using snake models and stuffed cats. There is also evidence that a

visual stimuli can be detected and initiate a stress response. For example, witnessing a raptor attack on a conspecific triggered an increased CORT response in the observing bird, and CORT has been found to be increased in a subject bird when exposed to a taxidermized predator specimen. CORT was also higher in birds that could not immediately fly away from the predator. These findings support the idea that birds can distinguish visually between animals/objects that are potential predators and animals/objects that are not; while also demonstrating that visual predator stimuli can cause behavioural changes (e.g. mobbing calls or decreased foraging) and physiological responses specifically by increasing CORT levels.

Auditory processing of acoustic information is also essential for birds to interpret their surroundings and to detect stimuli that may not be visually obvious. Research has demonstrated that birds can perceive and react to acoustic predator cues, either from the predators themselves or from alarm calls. Birds have demonstrated to respond to perceived acoustic predators behaviourally through calling and reducing movement, as well as physiologically through adjusting hormones and activations within the brain as outlined in Table 1.2 in Chapter 1. Chickadees in particular are known to be highly vigilant, susceptible to several avian predator species, can discriminate different predator species by sight, and can encode information to others about predator threat levels to other birds through calls. Despite extensive use of acoustic predator calls investigating behavioural responses and the effects on demographics, we know relatively little about the hormonal changes that occur in response to acoustic stimuli in birds. There is evidence of acoustic predator threat activating relevant areas in the avian brain, including lasting dendritic morphology changes and inhibited neurogenesis in both the TnA and the Hp. These findings support the idea that birds can detect, interpret, and react to acoustic stimuli, however, there is still questions about whether acute acoustic predator stimuli can elevate CORT levels in adult birds.

The olfactory sensory system and the ability to sense chemicals in the environment is important for recognising nests, discriminating partners, and other social behaviours. The detection of chemical cues have been studied in a wide variety of taxa but is often neglected in birds. There are relatively few studies that investigate olfaction in birds, most studies focusing on predator detection examine avoidance and to the best

of my knowledge there have been no studies on acute olfactory predator threat presentations and CORT changes. There is some evidence that birds can not only detect chemical cues but can use them to detect and avoid predators, however, there may be differences in the olfactory abilities among birds that reflects the diverse specialized functions, such as foraging, orientation/navigation, homing, nesting, activity pattern, and individual recognition. As outlined in Table 1.3 in Chapter 1 only a few bird species have been investigated in regards to the effect of perceived predation threat through olfactory cues and most have focused on avoidance of nest boxes or areas where the scent is present. Great tits that were lab-bred and predator naïve still avoided the predator scent suggesting that birds may have innate chemical detection abilities (Amo, Visser, & Oers, 2011). Recognition is an advantage for early detection/assessment of predation risk but it can also lead to an overestimation of risk if the predator is no longer present. When house sparrows (*Passer domesticus*) were presented with mouse urine (representing a possible competitor and a threat to eggs and hatchlings), males but not females preferred to spend significantly more time in front of the hay odour, than in front of the scent of mouse urine (Griggio et al., 2016). The results strengthen the hypothesis that birds can not only perceive odours but also use olfaction to assess the environment and estimate nest site quality. Overall, these results suggest that birds can perceive odours and use olfaction to assess the environment.

This study is an investigation of the effects of acute exposure to predator cues via different sensory modalities on physiological and behavioural responses in birds. While it has been demonstrated that birds will react, both physiologically and behaviourally, to acute exposures of perceived predation threats this study will be the first to examine how different birds perceive predators through different sensory systems. To meet these objectives I exposed three species of birds to different levels of threat (control, non-predator, and predator) across three modalities (visual, acoustic, and olfactory) and recorded behaviour as well as the CORT response via a blood sample. Thus, this is the first study that compares the differences in behaviour and CORT levels after exposure to different sensory systems using a standardized methodology. This study is also novel in using both wild and lab-bred birds to gauge the reactions to acoustic, visual, and olfactory predator threat cues. Do responses to predators depend on previous exposure or

experience? Will the different bird species react to the presented cues based on previous exposure events? Will there be differences in hormone levels based on the type of stimuli presented?

If birds are able to detect acute predator threats using different sensory systems then sensory specific stimuli should elicit physiological and behavioural responses. Specifically, predator exposed birds should be more vigilant, move less, and have higher corticosterone levels than non-predator exposed, control exposed, or baseline birds. Furthermore, I would predict that this elicited response would be stronger in the wild birds (house sparrow, *Passer domesticus* and black-capped chickadee, *Poecile atricapillus*) than the lab-bred predator-naïve zebra finches (*Taeniopygia guttata*). Given the evidence that birds are able to perceive predators from acoustic, visual, and olfactory cues I would therefore predict that the trends should not differ based on the sensory system the bird is tested in.

2.2 Methods

2.2.1 Overview

I examined the stress response of songbirds to acute predator threats that were perceived through one of three sensory modalities. I used multiple species of songbirds: wild-caught black-capped chickadees (BCCH) and house sparrows (HOSP), and lab-bred zebra finches (ZF). Birds were assigned to one of three experimental conditions in which they were exposed to one of visual, acoustic, or olfactory stimuli. Each bird was exposed to a predator, a non-predator, and a control stimulus in randomized order across three acute exposure events lasting 30 minutes each. Video recordings were taken prior to and during the exposure to record the birds' behaviour. Blood samples were obtained immediately following exposure for corticosterone analysis.

2.2.2 Subjects

2.2.2.1 Zebra Finches

Twenty-nine zebra finches were bred for a different project in January 2017 at the Advanced Facility for Avian Research, University of Western Ontario. After the study

concluded the birds were either euthanized or transferred to Trent University for other studies.

Thirty-two zebra finches were transferred to this project in February 2017 and April 2017, at the beginning of the experiment the birds were sexually mature (older than three months) and had no previous experience in any behavioural study. The zebra finches were housed in groups at the Advanced Facility for Avian Research, University of Western Ontario in either a 60×40×45 cm cage or in a 80×40×45 cm cage supplied with enrichment materials. The zebra finches were maintained at a temperature of 20–24 °C and a photoperiod of 14L:10D. The zebra finches were provided with a commercial tropical seed mixture for finches (Hagen, Living World, Quebec, Canada), water, shell grit, and cuttlefish bone ad libitum. The zebra finches were also given supplementary egg food (blended bread and hard-boiled eggs) daily. When randomly assigning the zebra finches to the modality conditions, I also ensured balanced sex and temperature ratios from the previous study they were bred in.

2.2.2.2 Black-capped Chickadees

Between November 2016 and March 2017, 12 black-capped chickadees were captured using seed-baited Potter traps from several sites at the University of Western Ontario in London, Ontario, Canada (43°00'37" N, 81°16'47" W). Nine of the birds were used in a spatial memory behavioural study prior to this study; the others had not been used in any prior studies. Blood was collected and stored for genetic sexing. Birds were quarantined at the Advanced Facility for Avian Research, University of Western Ontario for a period of three weeks following the newest addition. Birds were housed indoors and individually in either a 60×40×45 cm cage or in a 80×40×45 cm cage supplied with enrichment materials. The chickadees were maintained at a temperature of 20–24 °C and a natural light cycle photoperiod of roughly 12L:12D. Chickadees were provided Mazuri small bird diet (catalogue# 56A6; PMI Nutrition International, LLC, Brentwood, MO), black oil sunflower seeds, and water ad libitum throughout the experiment. Visual inspection throughout the experiment revealed that all birds had large bulging furcular fat deposits (fat scores of 4 on a 0 to 5 scale). The experiment was conducted between July 2017 and December 2017. After the study concluded the birds were held until release.

2.2.2.3 House Sparrows

During September 2017, 31 house sparrows were captured using seed-baited Potter traps and mist nets from private residences and several sites at the University of Western Ontario in London, Ontario, Canada (43°00'37" N, 81°16'47" W). Birds were quarantined at the Advanced Facility for Avian Research, University of Western Ontario for a period of three weeks following the newest addition. The house sparrows were housed indoors at the Advanced Facility for Avian Research, University of Western Ontario in either a 60×40×45 cm cage or in an 80×40×45 cm cage supplied with enrichment materials. The house sparrows were maintained at a temperature of 20–24 °C and a photoperiod of 13L:11D. House sparrows were provided Mazuri small bird diet (catalogue # 56A6; PMI Nutrition International, LLC, Brentwood, MO), commercial seed mixture for budgies (Hagen, Living World, Quebec, Canada), and water ad libitum throughout the experiment. Visual inspection throughout the experiment revealed that all birds had large bulging furcular fat deposits (fat scores of 4 on a 0 to 5 scale), nails were clipped as needed. When randomly assigning the house sparrows to the modality conditions I also ensured balanced sex ratios.

The experiment was conducted between September 2017 and December 2017. After the study concluded the birds were euthanized and brains were extracted for a related project.

2.2.3 Experimental Design

Prior to the predator cue exposures a baseline blood sample was taken in the home room. The birds were moved to single cages (40×25×30 cm) 24 h prior to the baseline bleed. The blood was collected in under three minutes of researchers entering the room.

Approximately 12 days after the baseline blood sampling the first experimental exposure set-up occurred. For each exposure set-up I first put the bird in the single-housing cage, I then moved that cage into a sound-attenuating chamber (50x70x50 cm, Industrial Acoustics Company Inc., Bronx, NY) overnight with ad libitum access to food and water. The next day between 09:00 and 12:00 I placed the video camera (ACTIVEON CX Action Camera) for 10 minutes prior to the stimulus presentation; for the exposure I then placed the stimulus in the chamber for 30 minutes. Following

exposure I took a blood sample within three minutes of opening the chamber and returned the bird to its home-cage. This process was repeated two more times for each bird, with more than two weeks separating exposure events (Figure 2.1). A final baseline blood sample was collected from the zebra finches and the black-capped chickadees, but not the house sparrows, a week after the third and final exposure event.

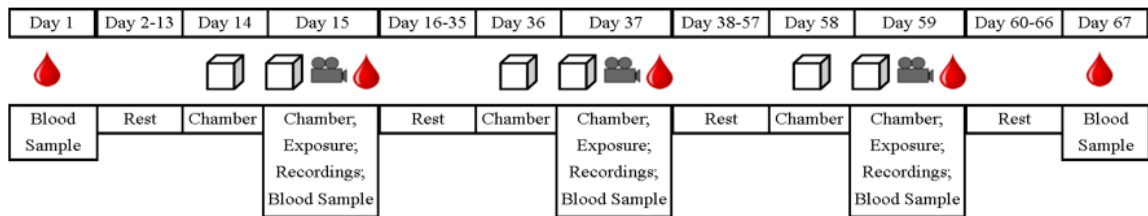


Figure 2.1. Timeline of exposure study; starting from the baseline blood sample as day 1 and ending at the final blood sample as day 67. Top row of the figure is the day number or the range of days. Middle row is icons that are a visual representation of the actions performed on the birds each day. Bottom row of the figure is a descriptive label for what was done on each day. Blood drop graphic indicates a blood sample taken, a white box graphic indicates the bird being in the chamber, and the video recording graphic indicates when there was a video recorded.

I randomly assigned each bird to one of the sensory modality treatments (visual, acoustic, olfactory), then further randomly assigned the birds to the stimulus presentation order within the modality treatment (predator, non-predator, or control). All birds were thus presented with each of the three types of stimuli in their respective modality, meaning all birds experienced a predator, a non-predator, and a control stimuli once in the experiment. The order of stimulus presentation was counterbalanced to achieve every possible order of presentation (Figure 2.2).

↙			↙			↙			Exposure 1	Exposure 2	Exposure 3
									Pred	Control	Non-Pred
									Pred	Non-Pred	Control
									Non-Pred	Control	Pred
									Non-Pred	Pred	Control
									Control	Non-Pred	Pred
									Control	Pred	Non-Pred

Figure 2.2. Visual representation of the stimulus randomization. Cartoon illustrations are used to represent all the bird species (house sparrows, black-capped chickadees, and zebra finches). The nose icon indicates birds that were put into the olfactory exposure group, the eye icon indicates birds that were put into the visual exposure group, and the ear icon indicates birds that were put into the acoustic exposure group. The exposure treatments are colour coded; red for predator exposure, yellow for non-predator exposure, and blue for control. Bird images were modified from Birdorable.com.

2.2.4 Stimuli

2.2.4.1 Visual

The birds in the visual experimental condition were exposed to taxidermized mounts from the University of Western Ontario's Zoological collection. The mounts were presented in the sound-attenuating chamber for 30 minutes, and the mount chosen for each subject was randomized. The taxidermized mounts included a variety of predators, non-predator, and controls (Table 2.1 and Figure 2.3).

Table 2.1. Acute visual exposure treatment conditions and species of taxidermized mounts used.

Treatment Exposure Types	Species	Dimensions
Visual Predator Exposure	northern saw-whet owl (<i>Aegolius acadicus</i>)	(25x17x48 cm)
	Cooper's hawk (<i>Accipiter cooperii</i>)	(18x32x48 cm)
	merlin (<i>Falco columbarius</i>)	(31x15x36 cm)
	eastern screech-owl (<i>Megascops asio</i>)	(20x17x28 cm)
Visual Non-Predator Exposure	American robin (<i>Turdus migratorius</i>)	(18x25x15 cm)
	rock pigeon (<i>Columba livia</i>)	(23x33x24 cm)
	northern flicker (<i>Colaptes auratus</i>)	(11x23x22 cm)
	pileated woodpecker (<i>Dryocopus pileatus</i>)	(20x25x37 cm)
Visual Control Exposure	Stand #1 made of cardboard and black cloth	(25x28x30 cm)
	Stand #2 made of cardboard and black cloth	(26x15x23 cm)



Figure 2.3. Species of taxidermized mounts used.

2.2.4.2 Acoustic

The birds in the acoustic experimental condition were exposed to a 30 minute playlist on an MP3 player (Hipstreet Prism, Cerritos, CA, U.S.A.) through speakers (Logitech, Newark, CA, U.S.A.), in the individual sound-attenuating acoustic chamber (50 cm × 70 cm × 50 cm, Industrial Acoustics Company, Inc., Bronx, NY). Calls were obtained from the Macaulay Library Database (Cornell University Lab of Ornithology, Ithaca, New York, USA). All sounds were edited in Audacity (Audacity 2.1.0 ®; Mazzoni 2015) to eliminate noise, to shorten calls to the proper length, and repeated. The playbacks included a variety of predators, non-predators, and a control sounds (Table 2.2). All audio files were assigned a number and run in a random number generator in R. Playbacks consisted of one species (e.g., mallard), but three different calls from three individuals of

that species (e.g., mallard a, mallard b, and mallard c). A typical call was 15 seconds in duration followed by 45 seconds of silence. This one minute playback was repeated 30 times, resulting in a period of 30 minutes with acoustic playbacks for both stimuli experimental categories (modified from Avey, Hoeschele, Moscicki, Bloomfield, & Sturdy, 2011; Hobbs, 2015). All sounds were played at 74 dBA SPL measured in the centre of the cage at the height of the perches using a sound level meter with slow response setting (Realistic, RadioShack).

Table 2.2. Acute acoustic exposure treatment condition and specific species used.

Treatment Exposure Types	Species
Acoustic Predator Exposure	northern saw-whet owl (<i>Aegolius acadicus</i>)
	Cooper's hawk (<i>Accipiter cooperii</i>)
	sharp-shinned hawk (<i>Accipiter striatus</i>)
	red-tailed hawk (<i>Buteo jamaicensis</i>)
Acoustic Non-Predator Exposure	hairy woodpecker (<i>Picoides villosus</i>)
	white-breasted nuthatch (<i>Sitta carolinensis</i>)
	mallard (<i>Anas platyrhynchos</i>)
Acoustic Control Exposure	MP3 in with a silent track playing

2.2.4.3 Olfactory

The birds in the olfactory experimental condition were exposed to an open Ziploc™ bag filled with samples specific to the exposure event. The samples were collected from colleagues and friends over the course of the study; the samples were frozen when not in use and thawed at least 24 hours before the exposure. The specific sample chosen for each subject was randomized. The samples used during an olfactory predator exposure event were collected from eight different indoor pet cats. The samples consisted of urine, feces, cat fur, and litter (if unavoidable); the average weight of the 15 total samples was 167 g. The samples used during an olfactory non-predator exposure event were collected from three different pet rabbits. The samples consisted of urine, feces, rabbit fur, and bedding; the average weight of the 14 rabbit samples was 176 g. The sample used during an olfactory control exposure event consisted of clay, cotton stuffing, damp paper towels; the weight of the sample was 134 g.

2.2.5 Behavioural Responses

For each stimulus exposure a 40 minute video comprised of behaviour prior to (10 minutes) and during the exposure treatment (30 minutes) was recorded. From the full 40 min video two five minute clips were selected, one during the period prior to the exposure and one during the exposure treatment. The videos were assigned randomly generated numbers to allow me to score them blind to subject, video type (baseline or treatment), and treatment (control, non-predator, and predator). I scored the videos using the event-logging software package BORIS (Behavioural Observation Research Interactive Software, Torino, Italy). Behaviour was scored using an ethogram developed to assess bird behaviour using the following categories of behaviour: grooming, food and water consumption, open beak, calling, beak wipe, sitting number and duration, as well as flight number and duration (Table 2.3).

Table 2.3. Behaviours quantified from videos captured before and during the exposure event.

Behaviour Types	Type	Definition
Grooming	Point	Contact between beak and body or wings
Eating	Point	Lowering beak into food dish
Drinking	Point	Using beak to drink from a vacuum-action inverted water bottle, or lowering beak into water dish
Open beak	Point	Beak was noticeably open with no sound
Beak wipe	Point	Beak making contact with the perching branch
Call	Point	Beak was noticeably open with sound
Sitting (freq)	Point	Number of times the bird started sitting
Sitting (dur)	State	Time spent sitting without moving for over 3 seconds
Flight (freq)	Point	Number of times the bird started flying
Flight (dur)	State	Time spent flying or continuously hopping in the cage

2.2.6 Corticosterone Assay

The blood was collected in under three minutes (mean 124.31 ± 31.71 SEM seconds) starting when researchers opened either the home-cage room door or the isolation chamber door. Blood samples were taken by puncturing the wing vein and drawing up blood into a microhematocrit capillary tube before being centrifuged and plasma was extracted and frozen at ~ 30 °C. Corticosterone levels were measured in plasma with a sensitive and specific corticosterone radioimmunoassay (RIA) (ImmuChem 07-120103; MP Biomedicals, Orangeburg, NY). Details on the validation (parallelism and precision) of our RIA protocol have been reported elsewhere (Newman, Pradhan, & Soma, 2008; Newman, MacDougall-Shackleton, An, Kriengwatana, & Soma, 2010; Newman & Soma, 2009). Two assays were run by the University of Western Ontario's Psychology Department Hormone Assay Lab, one for the zebra finch samples, and another for the chickadee and house sparrow samples. In brief, the assay protocol was to dilute avian plasma 1:50 with steroid diluent by combining 5 μ L of plasma with 245 μ L. Samples were mixed and allowed to equilibrate overnight at 4 °C. Afterward, we placed 50 μ L of the dilution in RIA tubes and added 100 μ L of CORT-I125 followed by 100 μ L of anti-corticosterone antibody (ANTI-CORT, Sigma C-8784). Samples were then assayed in duplicate along with blanks and six standards (0.0625–5 ng mL⁻¹ CORT). We measured the radioactivity using an automatic gamma counter. Intra-assay variation for the zebra finch assay (n=160 samples) was 9.8% (low control) and 1.05% (high control). Intra-assay variation for the black-capped chickadees and house sparrow assay (n=179 samples) was 6.2% (low control) and 5.6% (high control).

2.2.7 Data Analysis

2.2.7.1 Video

I conducted a linear mixed model (LMM) in IBM SPSS (Version 25; IBM Corporation, Armonk, NY, USA, 2017) comparing behaviours quantified from videos captured before and during the exposure event in all treatment conditions. To examine potential correlations among my behavioural measures (Table 2.3) I conducted a principal component analysis (PCA). After examining the scree plot (Appendix A), I retained three

PCs that together explained 51.88% of all variation in the measured behaviour (see Table 2.4 for variable loadings and descriptions). PC1 loaded strongly and positively with the duration and frequency of flight, additionally, PC1 loaded strongly negatively with the duration and frequency of sitting. Therefore I considered this to be a “movement” PC (i.e., higher values indicate that birds were in flight, or were active in the cage and lower values indicate the bird was sitting and inactive; Table 2.4). PC2 loaded strongly and positively with the frequency with of eating, drinking, and beak wiping, additionally, PC2 loaded strongly negatively with the frequency of beak opening. Therefore I considered this to be a “sustenance” PC (i.e., higher values indicate that birds were actively engaging in feeding or drinking behaviour and lower values indicate the bird was opening its beak with no intended purpose; Table 2.4). PC3 loaded strongly and positively with the frequency of grooming and beak wipe, additionally, PC3 loaded strongly negatively with the frequency of calling. Therefore I considered this to be a “maintenance” PC (i.e., higher values indicate that birds were using their beak for grooming behaviours and lower values indicate the bird was opening its beak to make vocalizations; Table 2.4). A difference score was calculated by subtracting the PC scores during exposure from the baseline PC score for each of the three PC outputs. This difference score reflects the behaviour change of the birds from the baseline/pre-exposure condition to the experimental/exposure condition.

Table 2.4. Factor loadings from principal components analysis on movement, sustenance, and maintenance behaviours (See Table 2.3). Duration refers to the length of total time spent doing a given behaviour, frequency refers to the number of times a behaviour occurred.

Variable	PC1	PC2	PC3	
Grooming	-0.07	0.163	0.64	
Eating	0.006	0.602	0.028	
Drinking	0.065	0.504	-0.022	
Open beak	0.345	-0.553	0.129	
Call	-0.008	0.357	-0.62	
Beak Wipe	0.152	0.508	0.419	
Sitting (freq)	-0.554	0.205	-0.146	
Sitting (dur)	-0.871	-0.302	0.162	
Flight (freq)	0.769	0.266	-0.199	
Flight (dur)	0.85	-0.195	0.086	
	% of Variance	25.30	15.75	10.83
	Cumulative %	25.30	41.05	51.88

Factors obtained with varimax with Kaiser Normalization and a rotation converged in 5 iterations.

Following the above principal components analysis I then carried out linear mixed models (LMM) in IBM SPSS (Version 25; IBM Corporation, Armonk, NY, USA, 2017), to test if the PC response variables were affected by the interaction between exposure conditions (control-exposed, non-predator-exposed, and predator-exposed) and/or the species (zebra finches, black-capped chickadees, and house sparrows).

2.2.7.2 Corticosterone

I conducted linear mixed models (LMM) in IBM SPSS (Version 25; IBM Corporation, Armonk, NY, USA, 2017), to test if CORT levels were affected by the interaction between exposure conditions (baseline, control-exposed, non-predator-exposed, and predator-exposed) and/or the species (zebra finches, black-capped chickadees, and house sparrows). Assay sensitivity was 3.13 ng/mL. One sample was above the standardized curve and was set at the maximum value of 250 ng/mL for statistical analysis. Eighteen of the samples were below the sensitivity of 3.13 ng/mL and were set at a 0.1 ng/mL for

statistical analysis, these were considered undetectable samples (falling below the lowest point on the standard curve).

I did not statistically compare across the three modalities. The degree of exposure in each group cannot be assumed, for example the dBA level cannot be directly compared to the amount of odour present in the chamber as they are not measured on the same scale. For this experiment it was more important to determine how the three species of birds reacted in their given modality group than comparing between the degree of exposure types. A way to do this in the future would be to randomly assign to all nine conditions, this would entail exposing the birds to all control, non-predator, and predator exposures of the visual condition, the acoustic condition, and the olfactory condition. This method was not used in the current study due to time constraints and concerns of the sample sizes required to control for order effects, thus there was no statistical analysis done between the modalities.

2.3 Results

2.3.1 Acoustic Exposure on Behavioural Responses

The results of the linear mixed models (LMM) for the behaviour in response to acoustic exposure conditions showed no effects of experimental treatment. That is, there was no difference in scores between birds exposed to predator, non-predator, or control stimuli (Table 2.5). The behavioural responses of all birds in each condition of the acoustic exposure is demonstrated in Figure 2.4. There were species differences in sustenance behaviour (PC2) (Figure 2.5), there was also a significant interaction effect between condition and species on maintenance behaviour (PC3) (Figure 2.4). Overall, the birds did not respond to the predator condition as expected, movement did not significantly change, birds continued to eat and drink and groom. All species used in this study appeared to be largely unaffected by the acoustic presentation of predators, as their behaviour did not differ greatly between the pre-exposure recording, the control exposure, the predator exposure, and the non-predator exposure.

Table 2.5. Behaviour in the acoustic treatment conditions across all species.

Component	Factor	d.f.	<i>F</i>	<i>p</i>
PC1: Movement				
	Condition	2,41.53	0.91	0.41
	Species	2,21.09	2.08	0.15
	Condition*Species	4,41.58	0.70	0.60
PC2: Sustenance				
	Condition	2,62	0.54	0.58
	Species	2,62	3.196	0.048
	Condition*Species	4,62	0.24	0.913
PC3: Maintenance				
	Condition	2,62	2.67	0.08
	Species	2,62	2.25	0.11
	Condition*Species	4,62	2.67	0.04

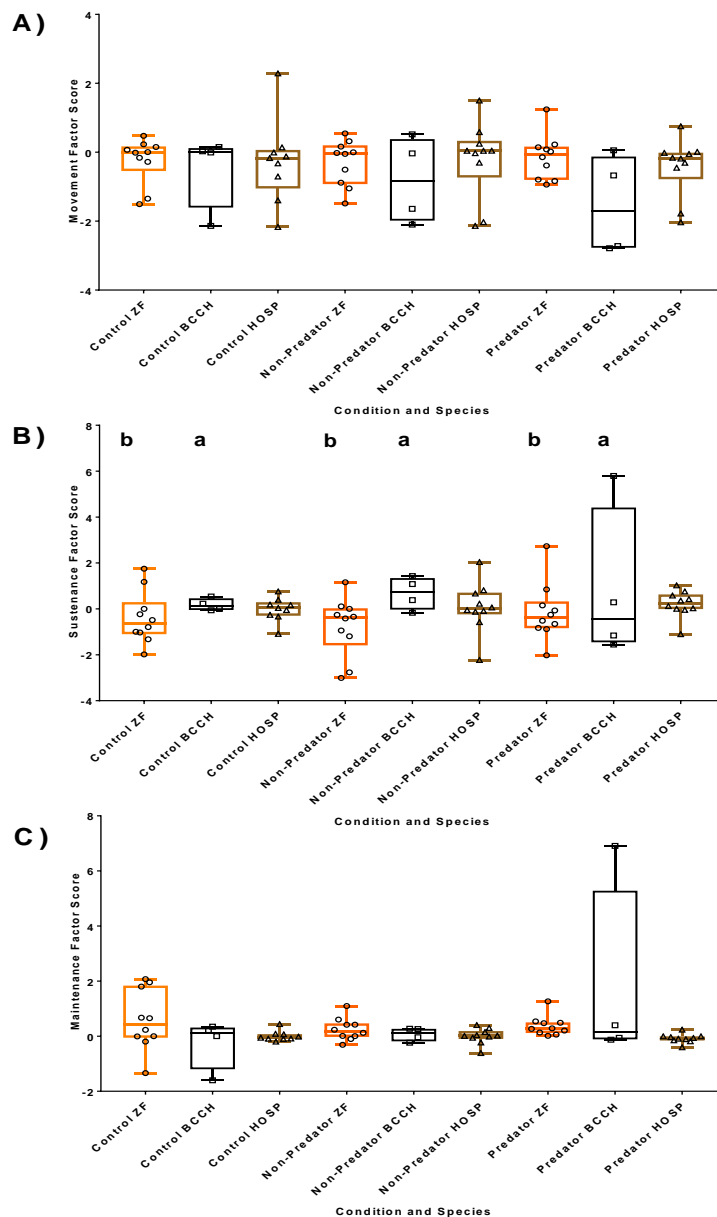


Figure 2.4. The behavioural responses of all bird species in each condition of the acoustic exposure, from each principle component (movement, sustenance, and maintenance). A) The movement factor scores, positive scores being duration and frequency of flight and more negative scores being duration and frequency of sitting. B) The sustenance factor scores, positive scores being frequency of eating, drinking, and beak wiping and more negative scores being frequency of beak opening. C) The maintenance factor scores, positive scores being frequency with of grooming and beak wipe and more negative scores being frequency of calling. Individual data overlaid over box and whiskers plots, Q2 is the median, and whiskers are the minimum and maximum of the data. Zebra finch represented by ZF, black-capped chickadee represented by BCCH, and house sparrow represented by HOSP.

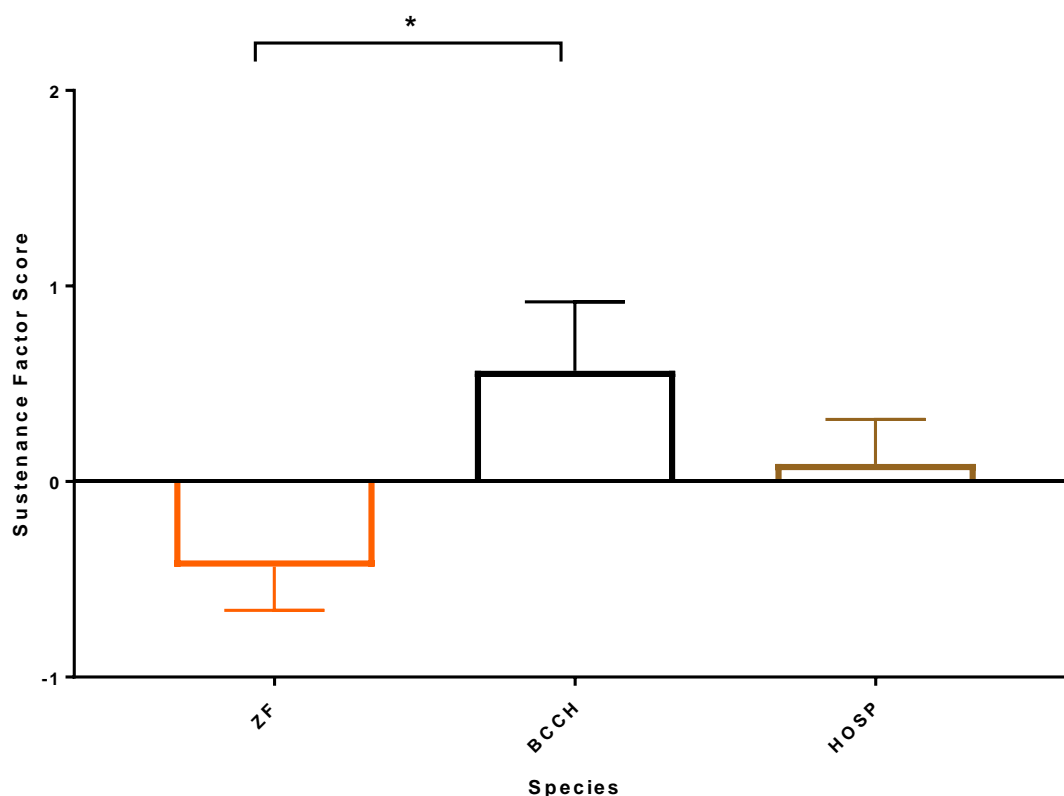


Figure 2.5. A significant species difference in sustenance behavioural responses (PC2) to the acoustic exposure, BCCH were significantly more likely to be eating, drinking, and/or beak wiping during all than zebra finches, who were likely to open their beaks during the exposure conditions. The sustenance factor scores, positive scores being frequency with of eating, drinking, and beak wiping and more negative scores being frequency of beak opening. Mean \pm SEM. Zebra finch represented by ZF, black-capped chickadee represented by BCCH, and house sparrow represented by HOSP.

2.3.2 Visual Exposure on Behavioural Responses

The results of the linear mixed models (LMM) for the behaviour in response to visual exposure conditions showed no significant difference in sustenance behaviour (PC2) (Figure 2.6), a significant species difference in movement (PC1) (Figure 2.7), and a significant condition effect and a significant interaction effect in maintenance behaviours (PC3) (Figure 2.8) (Table 2.6). House sparrows moved significantly less than zebra finches or black-capped chickadees in all exposure conditions. Pairwise comparisons established that there was significantly more grooming and beak wiping during the control condition than in the non-predator exposure condition or predator exposure condition, and that house sparrows were less active than the other two species.

Table 2.6. Behaviour in the visual treatment conditions across all species.

Component	Factor	d.f.	F	p
PC1: Movement	Condition	2,41.05	0.30	0.74
	Species	2,20.05	4.64	0.02
	Condition*Species	4,41.13	0.19	0.94
PC2: Sustenance	Condition	2,64	2.01	0.14
	Species	2,64	1.26	0.29
	Condition*Species	4,64	0.27	0.90
PC3: Maintenance	Condition	2,64	3.16	0.049
	Species	2,64	2.40	0.10
	Condition*Species	4,64	3.08	0.02

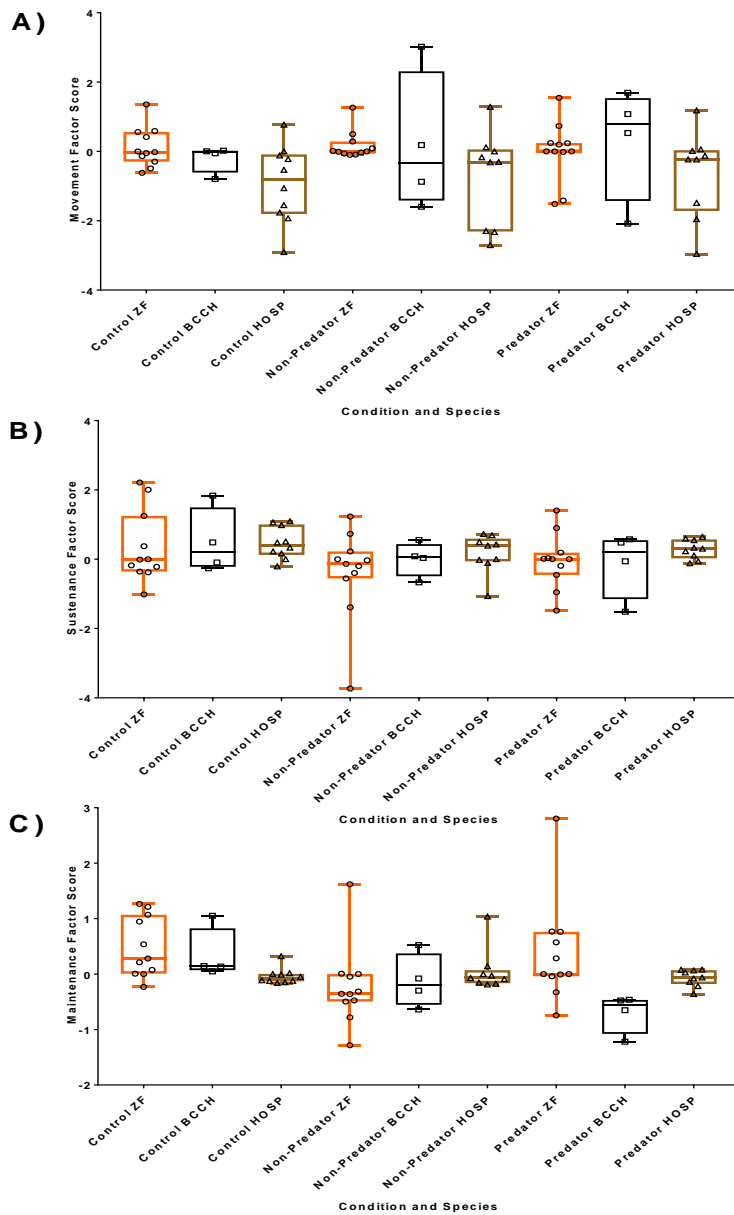


Figure 2.6. The behavioural responses of all bird species in each condition to the visual exposure, from each principle component (movement, sustenance, and maintenance). A) The movement factor scores, positive scores being duration and frequency of flight and more negative scores being duration and frequency of sitting. B) The sustenance factor scores, positive scores being frequency with of eating, drinking, and beak wiping and more negative scores being frequency of beak opening. C) The maintenance factor scores, positive scores being frequency with of grooming and beak wipe and more negative scores being frequency of calling. Individual data overlaid over box and whiskers plots, Q2 is the median, and whiskers are the minimum and maximum of the data. Zebra finch represented by ZF, black-capped chickadee represented by BCCH, and house sparrow represented by HOSP.

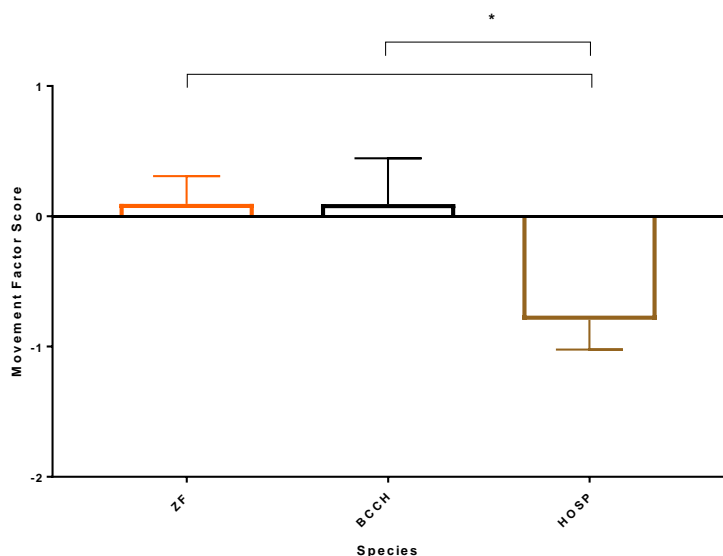


Figure 2.7. There was a significant difference between species movement behaviours (PC1) in response to the visual exposure conditions, HOSP's were sitting significantly more in all exposure conditions than both zebra finch and BCCH. The movement factor scores, positive scores being duration and frequency of flight and more negative scores being duration and frequency of sitting. Mean \pm SEM. Zebra finch represented by ZF, black-capped chickadee represented by BCCH, and house sparrow represented by HOSP.

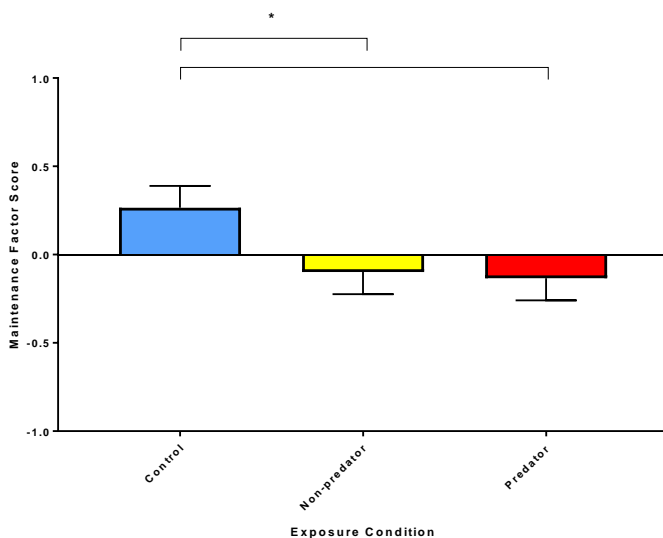


Figure 2.8. There was a significant difference between the birds in the visual exposure group in their maintenance behaviour (PC3) during the visual exposure conditions (control, non-predator, predator), there is significantly more grooming and beak wiping during the control than in the non-predator exposure or predator exposure. The maintenance factor scores, positive scores being frequency with of grooming and beak wipe and more negative scores being frequency of calling. Mean \pm SEM.

2.3.3 Olfactory Exposure on Behavioural Responses

The results of the linear mixed models (LMM) for the behaviour in response to olfactory treatment conditions did not specifically demonstrate a condition effect (Table 2.7, Figure 2.9). Though there was a significant difference between species in movement behaviour (PC1) (Figure 2.10) as well as a significant interaction effect of species and experimental condition on sustenance behaviour (PC2) (Figure 2.9). Overall, the zebra finches and house sparrows responded consistently with the predator stimuli by eating less, and moving less when the scent was present, it appears as if the chickadees either could not detect the scent or did not perceive it as a threat.

Table 2.7. Behaviour in the olfactory treatment conditions across all species.

Component	Factor	d.f.	<i>F</i>	<i>p</i>
PC1: Movement				
	Condition	2,43.94	0.34	0.72
	Species	2,21.83	3.68	0.042
	Condition*Species	4,44.01	1.16	0.34
PC2: Sustenance				
	Condition	2,44.78	0.20	0.82
	Species	2,22.81	0.26	0.78
	Condition*Species	4,44.86	2.68	0.044
PC3: Maintenance				
	Condition	2,67	0.48	0.62
	Species	2,67	1.62	0.21
	Condition*Species	4,67	0.39	0.82

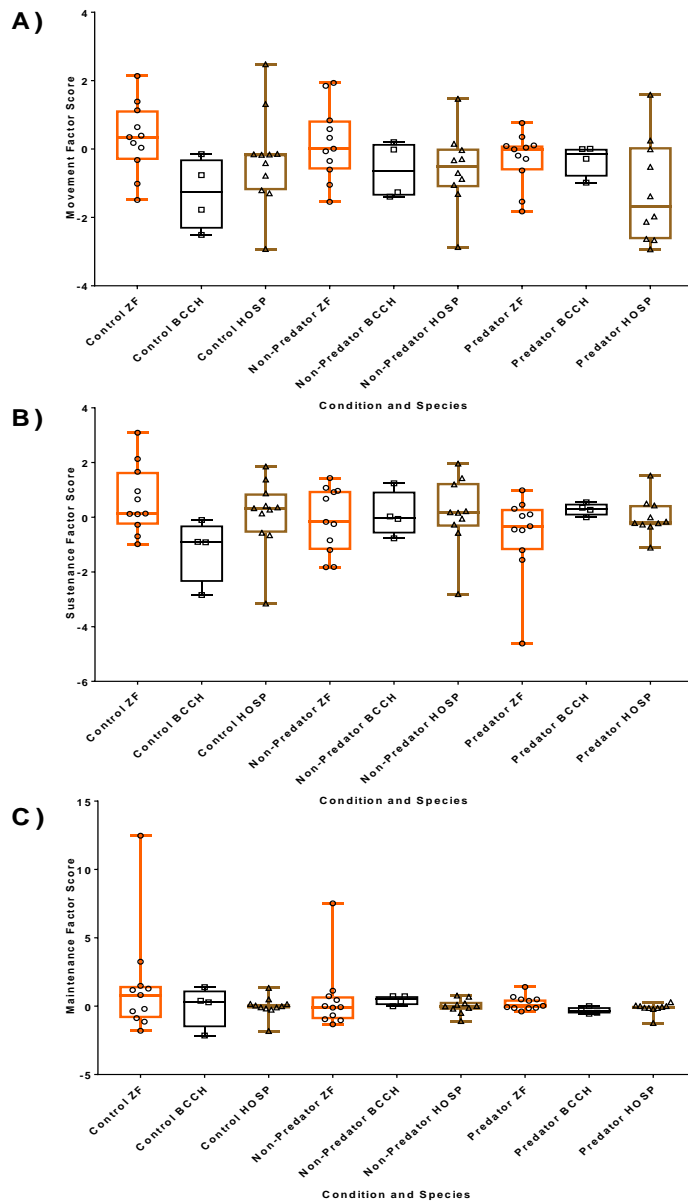


Figure 2.9. The behavioural responses of all bird species in each condition to the olfactory exposure, from each principle component (movement, sustenance, and maintenance). A) The movement factor scores, positive scores being duration and frequency of flight and more negative scores being duration and frequency of sitting. B) The sustenance factor scores, positive scores being frequency with of eating, drinking, and beak wiping and more negative scores being frequency of beak opening. C) The maintenance factor scores, positive scores being frequency with of grooming and beak wipe and more negative scores being frequency of calling. Individual data overlaid over box and whiskers plots, Q2 is the median, and whiskers are the minimum and maximum of the data. Zebra finch represented by ZF, black-capped chickadee represented by BCCH, and house sparrow represented by HOSP.

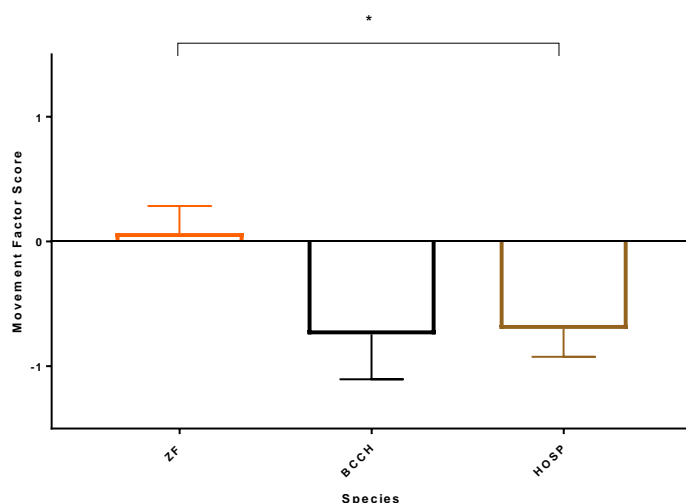


Figure 2.10. There is a significant difference between species for movement behaviour (PC1) in response to olfactory exposure conditions, HOSP flew significantly more than zebra finch. The movement factor scores, positive scores being duration and frequency of flight and more negative scores being duration and frequency of sitting. Mean \pm SEM. Zebra finch represented by ZF, black-capped chickadee represented by BCCH, and house sparrow represented by HOSP.

2.3.4 Corticosterone Assay Results

The results of the linear mixed model (LMM) for the corticosterone response to acoustic treatment conditions did not show any significant differences (Table 2.8), however, there was a significant difference between the baseline and the experimental conditions (Table 2.9). Birds in the acoustic exposure group did not differ in CORT levels (Figure 2.11, section A).

The results of the linear mixed model (LMM) for the corticosterone assay in response to visual exposure conditions did not show any significant differences in conditions or interactions, however, there was a significant species response difference (Table 2.8; Figure 2.11, section B). The species (zebra finch, chickadee, and house sparrow) had significantly different corticosterone levels. Results from the pairwise comparison reveal that chickadees and house sparrows on average had significantly higher corticosterone responses than the zebra finch in all visual exposure conditions. Furthermore, chickadees tended to have higher corticosterone responses than house sparrows (Figure 2.12). There was also a significant difference between the baseline and the experimental conditions corticosterone levels (Table 2.9, Figure 2.14, Figure 2.15).

The results of the linear mixed model (LMM) for the corticosterone assay in response to olfactory exposure conditions did not show any significant differences (Table 2.8), however, there was a trend towards a difference between species (Figure 2.11, section C). In pairwise comparisons the trend of species differences was between zebra finch and house sparrow, with house sparrow on average having a higher corticosterone response (Figure 2.13).

In summary, there was high variability and no strong CORT response to the conditions across the three groups. However, there was an increase in CORT between the baseline and the three experimental conditions. There was no significant CORT increase in any bird or any condition when exposed to acoustic stimuli. When exposed to visual stimuli chickadee and house sparrows had higher levels of CORT than zebra finches, there was also an increase in CORT between the baseline and the three experimental conditions. When exposed to olfactory stimuli house sparrows tended to produce higher levels of CORT than zebra finches in all conditions. Overall there appeared to be a trend for higher CORT response to predators in all three modalities, with acoustic and olfactory stimuli demonstrating the most difference.

Table 2.8. Corticosterone assay results across all modalities, conditions, species, and interactions.

Modality	Factor	d.f.	<i>F</i>	<i>p</i>
Acoustic	Condition	2,41.29	1.328	0.276
	Species	2,20.79	0.035	0.965
	Condition*Species	4,41.34	0.526	0.717
Visual	Condition	2,42.83	0.327	0.723
	Species	2,21.76	8.463	0.002
	Condition*Species	4,42.91	0.402	0.806
Olfactory	Condition	2,44.99	1.35	0.27
	Species	2,22.94	3.10	0.064
	Condition*Species	4,45.07	0.54	0.71

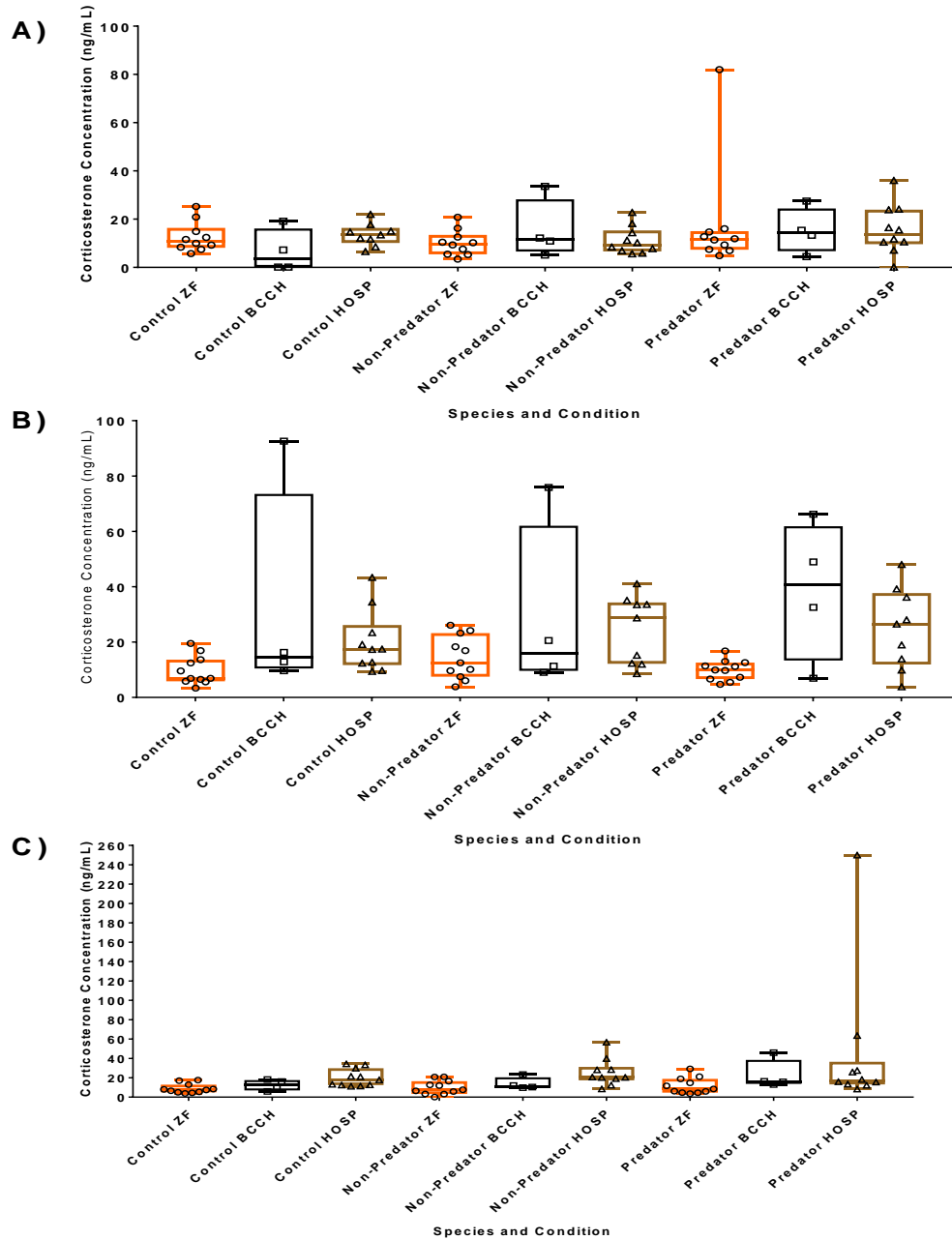


Figure 2.11. The corticosterone concentration responses of all bird species in each condition to the acoustic exposure, the visual exposure, and the olfactory exposure, demonstrating data range and means. A) The corticosterone concentration responses to acoustic exposure conditions. B) The corticosterone concentration responses to visual exposure conditions. C) The corticosterone concentration responses to olfactory exposure conditions. Individual data overlaid over box and whiskers plots, Q2 is the median, and whiskers are the minimum and maximum of the data. Zebra finch represented by ZF, black-capped chickadee represented by BCCH, and house sparrow represented by HOSP.

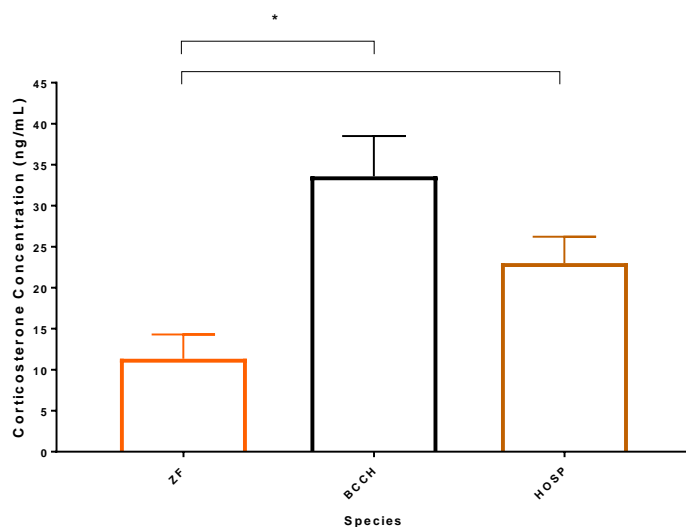


Figure 2.12. The species had significantly different corticosterone levels in response to visual exposure conditions, BCCH and HOSP on average had significantly higher corticosterone responses than the zebra finch in all visual exposure conditions. The corticosterone concentration responses to visual exposure conditions in each demonstrating differences between the means. Mean \pm SEM. Zebra finch represented by ZF, black-capped chickadee represented by BCCH, and house sparrow represented by HOSP.

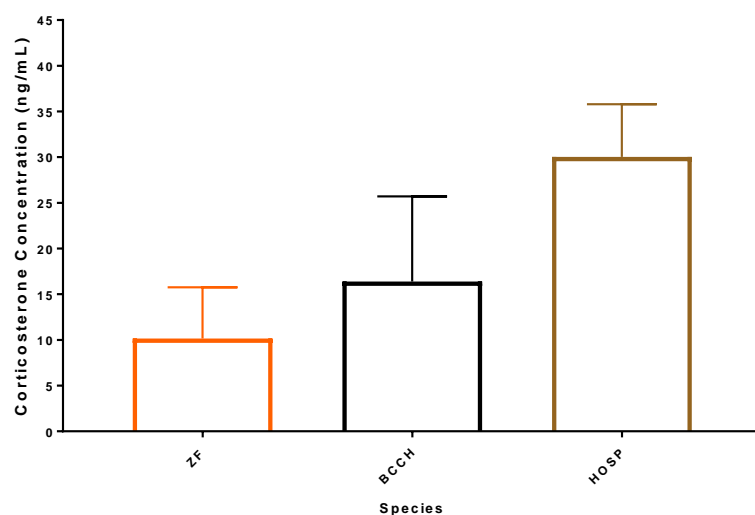


Figure 2.13. Trend of species differences between HOSP and zebra finch, HOSP on average having a higher corticosterone response than the zebra finch birds. Zebra finch were overall less reactive in all conditions than HOSP. The corticosterone concentration responses to olfactory exposure conditions in each demonstrating differences between the means. Mean \pm SEM. Zebra finch represented by ZF, black-capped chickadee represented by BCCH, and house sparrow represented by HOSP.

Table 2.9. Corticosterone concentrations in all modalities in all conditions across all species, including baseline condition.

Component	Factor	d.f.	<i>F</i>	<i>p</i>
Acoustic	Condition	3,63	5.802	0.001
	Species	2,21	0.145	0.866
	Condition*Species	6,63	0.503	0.804
Visual	Condition	2,86	11.861	<0.001
	Species	3,86	5.647	0.001
	Condition*Species	6,86	1.265	0.282
Olfactory	Condition	2,23.12	3.325	0.054
	Species	3,67.86	1.742	0.167
	Condition*Species	6,67.94	0.898	0.501

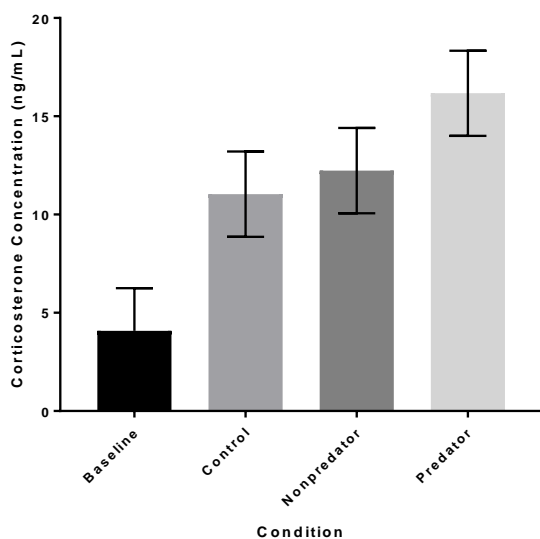


Figure 2.14. Corticosterone concentrations in the acoustic treatment conditions across all species, including baseline condition.

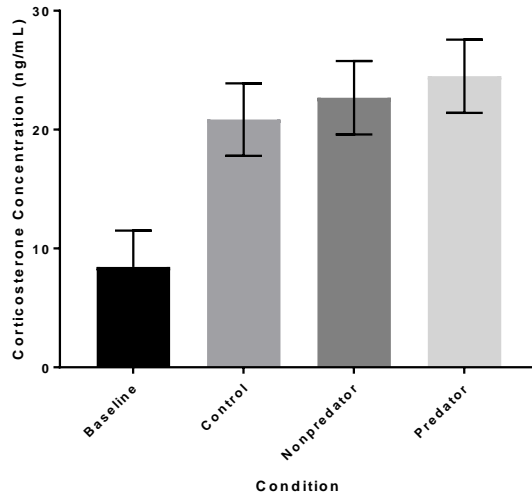


Figure 2.15. Corticosterone concentrations in the visual treatment conditions across all species, including baseline condition.

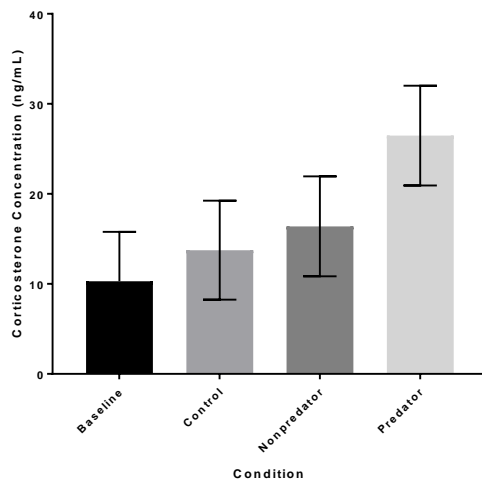


Figure 2.16. Corticosterone concentrations in the olfactory treatment conditions across all species, including baseline condition.

2.4 Discussion

I have identified behavioural and physiological responses to presented stimuli across three species of birds. During acoustic exposure presentations there was more maintenance behaviour during the acoustic predator exposure than in the control or non-predator exposure conditions. Baseline CORT levels were lower than all exposure conditions, with the predator condition tending to have higher CORT levels than all conditions. Acoustic exposure did not cause the predicted changes, as overall there was

not a strong decrease in behaviours during the predator exposure, and the CORT increase was not as prominent as expected. During the visual exposure presentations maintenance behaviour was significantly higher during the control exposure than in the non-predator or predator visual exposure condition. During the olfactory exposure there was a tendency overall for CORT levels to be higher during olfactory predator exposure. In all exposure modality types there was a variety of main effects of species.

For this study I had originally hypothesized that when birds perceived an increased predation risk they would show behavioural and physiological changes. Specifically, I predicted that the wild birds would respond to predator stimuli by reducing grooming, feeding, and movement behaviour as well as increased CORT in all modalities. My results did not follow this predicted pattern closely. Chickadees had high variability in their behaviour and CORT and in some instances responded as predicted but in the exact opposite way in others. House sparrows did not demonstrate strong reactions through behaviour or CORT levels in most of the conditions across the three modalities. I further predicted that the lab-bred predator-naïve zebra finch would not respond strongly to the predator exposure condition in any modality. Based on the results I would suggest that the zebra finch did support the predictions as they neither followed the predicted pattern of the wild birds nor reacted strongly to the predator exposure conditions in any modality.

2.4.1 Assumptions and Limitations

There were some initial assumptions made about what kind of stimuli should be used to be most effective and some limitations as to what stimuli were available. It was assumed that because the zebra finch were predator naïve and that if the response to predators were innate they would respond to a North American predator visual cues and acoustic calls as being similar to birds of prey native to Australia.

Synthetic sprays were not used in the olfactory condition as previous researchers have questioned their efficacy in eliciting a desired response (Apfelbach, Blanchard, Blanchard, Hayes, & McGregor, 2005). In the few studies that have been conducted with birds and olfactory capability of detection or avoidance to scent the studies have used cat odour and not synthetic repellents, that is why in this study the samples were collected

from real cats and synthetic repellents/sprays were not used (Jones & Roper, 1997). A limitation of the cat feces used may have been that they were indoor cats, however, none of the cats were on a vegetarian diet. It has been previously suggested that rodents react differently to cat feces resulting from a carnivorous diets as opposed to a vegetarian one (Berton, Vogel, & Belzung, 1998). There was also an assumption made that moving the bird to the isolation chamber from the homecage for 24 h prior to the exposure would habituate the birds to the new surroundings but there was a significant increase between the initial baseline and the exposure conditions in both the visual and acoustic group and a similar trend in the olfactory group.

Given the variance in my data and the current samples size in the chickadee groups, I would have only been able to detect very large effect sizes ($f > 0.4$). This would suggest that a larger sample size would be needed to detect condition effects, though the species effects were strong enough to be detected with the current sample size.

2.4.2 Future Studies

I would suggest three possible ways to improve and extend upon this study for future studies. Firstly, that future studies could aim to determine how the three senses examined in this study are recognized and how the discrimination could occur. This could be achieved by both by focusing on the specific trait differences in the stimuli used or by focusing on neural pathways and the activation caused by the stimuli. Secondly, future studies could recreate this experiment with multi-sensory stimuli. It has been suggested that multiple cues from a predator may have a compounding effect on the behavioural responses of prey species, for example a visual corroboration of a scent cue might strengthen the behavioural response to the visual cue (Roth, Cox, & Lima, 2008). Lastly, there are new opportunities to examine if and how urbanization impacts a bird's ability to respond to native predators. For example, increasingly urbanization has led to increased road use and construction where roads produce noise, pollution, and ambient light levels that might have an impact on adult and hatchling physiology or predator detection strategies. Studies that have been conducted exclusively on road noise found that body condition was lowered but did not examine the birds individual behavioural responses, the impacts on circulating CORT levels, or foraging vigilance (McClure, Ware, Carlisle, & Barber, 2017; Ware, McClure, Carlisle, & Barber, 2015).

2.4.3 Conclusion

This is the first study to examine how lab-bred and wild-caught birds respond to perceived predation threat through different sensory systems. Despite using a standardized methodology for all presentations across the visual, acoustic, and olfactory systems there were no strong patterns in the behaviours measured or the CORT responses between or within the sensory systems. In contrast to my predictions there was no significant predator condition effects on behaviour or CORT. There were significant species differences in at least one of each of the sensory system measures. The house sparrows did not differ between baseline and exposure behaviour responses except for decreased movement in all conditions in the visual and acoustic exposure groups as well as a generally higher CORT response than zebra finches. The zebra finches in the acoustic exposure group were not impacted by the predator condition as they did not change their behaviour in response to the predator condition but responded to all conditions with decreased feeding and drinking and increased grooming behaviour. However, the zebra finches in the visual group decreased grooming behaviour and in the olfactory group decreased feeding and drinking in the predator condition which was not predicted response for the lab-bred predator naïve birds. Black-capped chickadees were highly variable in their responses to the stimuli in all sensory stimuli groups, often increasing feeding, drinking, and grooming behaviours in response to the predator condition in contrast to my predictions. In conclusion, all species of birds used in each condition were highly variable in response to control, non-predator, and predator exposure cues and did not follow distinct patterns of behavioural responses or strong measurable CORT circulation when exposed to perceived predator threats of various modalities.

2.5 References

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Chapter 3

3 Effect of Chronic Perceived Predation Threat on Spatial Memory

3.1 Introduction

Predator-prey relationships provide a classic paradigm for studying stress and stress-related behavioural responses. Vertebrates cope with stress by using the hypothalamic–pituitary–adrenal (HPA) axis. The HPA axis, which when activated results in the secretion of glucocorticoids (primarily corticosterone (CORT) in birds), is essential for coping with unpredictable stressors and can influence behaviour directly and indirectly (Boonstra, 2004). The stress response can be activated by physical stressors such as actual attacks by a predator, or even a perceived predation threat (Clinchy, Sheriff, & Zanette, 2013). Predator cues are increasingly being used as a tool in acquiring information about how the brain and endocrine system respond during acute or chronic stress (Apfelbach, Blanchard, Blanchard, Hayes, & McGregor, 2005). While predation has obvious impacts on a population and on the individual through injury and mortality; there are also indirect impacts from a perceived predation threat which can change behaviour and reproductive output associated with changes in CORT levels (Bennett et al., 2016; Clinchy et al., 2013; Zanette, White, Allen, & Clinchy, 2011).

Rapid surges in CORT may increase the efficacy of an underlying memory process, however, the long-term changes may be damaging to these same processes (Boonstra, 2013; McEwen, 1998). Research has demonstrated that short term stress can be beneficial to memory performance. Evidence from mammals suggests that predator exposure does not impair the ability to solve four radial arm water maze with a centre entry after 1 day or 6 days of 30 min of exposure to a live cat (Diamond, Park, Heman, & Rose, 1999). Evidence from birds suggests that acute moderate elevations of CORT increases spatial memory retrieval efficacy; including consuming more food, recovering more previously cached seeds, a ratio of number of looks made by a bird per number of successful cache retrievals, and more cache-related site visits (Pravosudov, 2003; Saldanha, Schlinger, & Clayton, 2000). For example, when mountain chickadees (*Poecile gambeli*) ingested CORT prior to recovering previously cached seeds the birds recovered

more seeds and tended to visit more cache-related sites than controls during retrieval following a caching trial (Saldanha et al., 2000).

Taken together these findings suggest that glucocorticoid surges may enhance memory retrieval and therefore survival. However, when the stressor has been detected and stress response has been activated for a prolonged period of time it is suggested that the once beneficial and protective effects of CORT become detrimental. An organism is considered chronically stressed when there is long-term activation of the HPA axis caused by unpredictable or uncontrollable stimuli (stressors) in its environment. Long-term stress has been found to negatively impact learning and memory in rats, other mammals including humans. For example, Park et al. (2001) found that when rats were exposed to a predator for 5 weeks it impaired their spatial memory in the radial arm water maze task.

The majority of stress and memory research has been conducted with rats and other mammals. In general these studies found that glucocorticoids can impair spatial learning and memory task performance, and atrophy neurons critical to memory function (Hodgson et al., 2007; McEwen, 2000). However, there may be limitations to this approach and the generalizability of the results as rats are raised in artificial environments with unlimited food, no predators, no disease, and benign environmental conditions. To rats raised in these conditions, stressors are artificial and likely bear an unconvincing relationship to their wild counterparts (Boonstra, 2004). These experiments in captivity leave room for doubt that these results would generalize to natural conditions.

In the wild a functioning hippocampus is critical to spatial learning and memory particularly in regards to environmental demands and survival. Birds use the space around them to hide and locate food, as well as provision for their young (McEwen, 2000). The black-capped chickadee (*Poecile atricapillus*) is a species of bird that naturally caches its food, thus finding and retrieving the caches has important consequences for the life of an individual (Croston et al., 2016; Morand-Ferron, Cole, & Quinn, 2016). Chickadees are often used for spatial memory tasks as they have a natural predisposition for searching and have been used successfully in a variety of spatial memory tasks (Herz, Zanette, & Sherry, 1994; Pravosudov, 2003). The few studies that have investigated moderately elevated levels of CORT, using implants, found that there

was an enhanced cache-retrieval efficiency (Pravosudov, 2003; Pravosudov & Clayton, 2001). This suggests that chickadees may respond to elevated CORT levels differently than mammals. Additionally, when acoustic cues were presented to different species of chickadees the chickadees have been found to distinguish different predators by their vocalizations (Billings, Greene, & De La Lucia Jensen, 2015). Chickadees and other parids are an ideal group to investigate acoustically based discrimination among predator species because they are so vigilant and reliant on spatial memory abilities but it has yet to be tested if detection and vigilance over a long period of time impacts their spatial memory abilities.

The objective of this study was to test if chronic exposure to predator cues would impair spatial memory in a songbird. There is evidence that chronic stress in mammals impairs spatial memory, but this has yet to be investigated in a wild bird. Based on the assumption that predator cues will elicit both physiological and behavioural responses, I examined how chronic exposure to acoustic predator cues affected spatial memory retention in black-capped chickadees. I predicted that black-capped chickadees that are chronically exposed to acoustic predator cues would not perform as accurately on the spatial memory retention task as those exposed to acoustic non-predator cue control treatment because a predator-induced stress response would interfere with spatial memory retention.

3.2 Methods

3.2.1 Overview

In this study I investigated the effect of chronic exposure to acoustic predator cues on spatial memory retention in black-capped chickadees. Chickadees searched and retrieved hidden seeds in artificial trees. I trained birds to retrieve seeds from specific locations then exposed them daily to acoustic cues for two weeks; I then tested birds on their ability to retrieve the seeds from their initial trained locations.

3.2.2 Subjects

Between February 2017 and November 2017, I caught 15 black-capped chickadees using seed-baited Potter traps from several sites at the University of Western Ontario in

London, Ontario, Canada (43°00'37" N, 81°16'47" W). Ethics AUP # 2016-106, Environment Canada Scientific Collection permit # CA 0244. Birds were quarantined at the Advanced Facility for Avian Research, University of Western Ontario for a period of three weeks following the newest addition. Prior to the experiment the birds were housed indoors and individually in either a 60×40×45 cm cage or in an 80×40×45 cm cage supplied with enrichment materials. The chickadees were maintained at a temperature of 20–24 °C and a natural light cycle photoperiod. Chickadees were provided Mazuri small bird diet (catalog # 56A6; PMI Nutrition International, LLC, Brentwood, MO), black oil sunflower seeds, and water ad libitum prior to the experimental training and testing. Visual inspection throughout the experiment revealed that all birds had large bulging furcular fat deposits (fat scores of 4 on a 0 to 5 scale) and they were weighed monthly.

When the chickadees were moved to the testing holding room they were housed in smaller cages (31x39x31 cm) with a sliding back-door component (Figure 3.1). The birds were provided ad libitum water and when not being food restricted for the experiment they had full access to Mazuri small bird diet (PMI Nutrition International, LLC, Brentwood, MO), with crushed sunflower chips. The chickadees were maintained at a temperature of 20–24 °C and a quasi-natural light cycle photoperiod throughout the experiment. Food restriction was gradually worked up to 2 hours prior to testing and training, post-training/testing the food was returned.

3.2.3 Materials

3.2.3.1 Experimental Room

The experimental room (292x282 cm) had a wall of automated entry flaps, a one way mirror, and contained three artificial trees with height ranging from 150 cm to 195 cm (Figure 3.1). Each tree had a trunk cross-section of 3.7x3.7 cm, with a combination of long and short branches from 4.5 cm to 65 cm in length. Additionally, each tree had 8 holes drilled into it (0.6 cm in diameter), each placed above a branch, for a total of 24 holes in the room. When baited, these holes contained a small sunflower seed fragment. White yarn wrapped around the branches and tied in a knot was used to cover the holes.



Figure 3.1. Homeage and holding room for chickadees (left). Cages contained food cups, water cups and bottle, a perch, and a sliding door on the back. The sliding door and the metal flap allowed the birds to fly into the experimental room without being handled. There were switches to open the metal door in the attached room behind a one-way mirror (middle). The layout of experimental room (right); the room included three artificial trees with eight holes drilled in each tree.

3.2.3.2 Predator Cues

The sound attenuating chamber (50x70x50 cm, Industrial Acoustics Company Inc., Bronx, NY) held the birds homeage as well as speakers (Logitech, Newark, CA, U.S.A.) with a cord attaching an MP3 player (Hipstreet Prism, Cerritos, CA, U.S.A.), set at a volume of 39, on the outside of the chamber.

3.2.4 Experiment

For all birds there was habituation, training, and testing components to the experiment. Searching was observed in real-time by one or more observers behind a one-way mirror and coded using the data-logging software package BORIS (Behavioural Observation Research Interactive Software, Torino, Italy). Throughout the experiment the birds were deterred from foraging/caching on the ground, the ceiling, or the walls in the testing room by gently tapping on the glass when they performed those actions.

3.2.4.1 Habituation

The habituation consisted of all 24 of the holes in the artificial trees being baited, not covered, as well as food and water being provided in the room. A chickadee was released

and allowed to explore the experiment room daily and taught to fly back to its homecage. On average the birds habituated for 8 days.

3.2.4.2 Training

The first phase of training included all holes being baited and open (i.e., not covered by a yarn knot). Once the bird had collected seeds from a minimum of 23 of the holes over different training sessions the bird was randomly assigned its eight specific correct holes. The second phase of training allowed for 10 minutes of searching and all eight correct holes were baited and the sixteen incorrect holes were not baited, both were uncovered. Once the chickadee collected all the 8 baited seeds for 3-6 consecutive days, it was moved on to the third stage of training. The third and final training phase before testing involved alternating between correct covered and uncovered, with the incorrect consistently covered and a maximum search time of 10 minutes. Before moving onto the testing phase, the chickadee was again required to consistently collect all the eight baited seeds, as well as being accurate (measured as the number of baited holes searched divided by the total number of holes searched) greater than 70% for 5-6 consecutive training sessions.

3.2.4.3 Testing and Acoustic Exposure

For all tests the birds were released into the testing room and allowed to search for a maximum of 10 minutes, all correct holes were covered and baited whereas incorrect holes were covered but not baited. After completing a baseline test, the bird was randomly assigned to a predator, or a non-predator exposure condition. Each day of exposure procedure the birds were carried in their home cages to a sound-attenuating acoustic chamber. Once placed inside the chamber with the speakers, a specific auditory playback (predator calls or non-predator calls) was played for 30 minutes. All stimuli examples from the predator exposure condition, the non-predator condition are listed in Table 3.1. The calls were all obtained from the Macaulay Library Database (Cornell University Lab of Ornithology, Ithaca, New York, USA). The playback consisted of six calls of various lengths from species listed in Table 3.1; predator calls for the predator playback playlist and non-predator calls for the non-predator playback playlist. Within a

playback, there were approximately four minutes of silence between calls. To avoid habituation, birds never heard the same playback playlist twice. After exposure to the unique playback each day, the bird was carried back to the room where it was housed. The chickadees were not permitted either visual or physical access to the experimental room during their exposure treatment period. After completing the auditory exposure treatments, the chickadees were tested one time the day after the last exposure treatment.

Table 3.1. Chronic exposure treatment types and specific species used.

Treatment Exposure Types	Species
Acoustic Predator Exposure	Cooper’s hawk (<i>Accipiter cooperii</i>)
	American crow (<i>Corvus brachyrhynchos</i>)
	red-tailed hawk (<i>Buteo jamaicensis</i>)
	barred owl (<i>Strix varia</i>)
	sharp-shinned hawk (<i>Accipiter striatus</i>)
	northern saw-whet owl (<i>Aegolius acadicus</i>)
	merlin (<i>Falco columbarius</i>)
Acoustic Non-Predator Exposure	song sparrow (<i>Melospiza melodia</i>)
	mallard (<i>Anas platyrhynchos</i>)
	blue jay (<i>Cyanocitta cristata</i>)
	northern leopard frog (<i>Lithobates pipiens</i>)
	hairy woodpecker (<i>Picoides villosus</i>)
	wood frog (<i>Lithobates sylvaticus</i>)
	downy woodpecker (<i>Picoides pubescens</i>)

3.2.4.4 Experiment

There were twelve experimental birds in total. On average the birds habituated for 8 days and trained on average for 55 days. Subjects were habituated and trained in September 2017 and November 2017 to January 2018. Subjects were tested in January 2018 and February 2018, with fourteen days between the first and second test (Figure 3.2). Half of the subjects were randomly selected to be in the predator exposure group and the other half were selected to be in the non-predator exposure group. All the birds were taken to the isolation chamber daily for the fourteen days between the tests and exposed to its specific auditory stimulus.



Figure 3.2. Timeline for experimental birds.

3.2.5 Data Analysis

The data recorded for all birds included: the time in seconds it took to collect all 8 correct seeds with a maximum of 600 seconds (Latency), the number of correct seeds collected in a session (Number Correct), the number of incorrect holes checked in a session, revisiting was not counted as an error (Number of Errors), and the number of correct seeds collected divided by the total holes searched in a session multiplied by 100 (Collected Percent). For each of these dependent measures I conducted a two-way ANOVA in IBM SPSS (Version 25; IBM Corporation, Armonk, NY, USA, 2017) with treatment (predator-exposed and non-predator-exposed) as a between-subjects factor and session (pre-exposure test and the post-exposure test) as a within-subjects factor.

3.3 Results

The results of the two-way ANOVA showed that there were no significant interaction effect. Both predator-exposed and non-predator-exposed birds significantly changed performance between testing phases, however, the effect of exposure condition was not statistically significant in any area of performance measured (Table 3.2). Birds in both exposure conditions took significantly longer to collect all eight correct seeds after the exposure (Table 3.2, Figure 3.3). Birds in both exposure conditions collected less correct seeds after the exposure (Table 3.2, Figure 3.4); made significantly more errors after the exposure (Table 3.2, Figure 3.5); and were worse overall at the memory task after the exposure (Table 3.2, Figure 3.6). Overall, performance decreased after the chronic exposure, regardless of the playback stimuli.

Surprisingly condition did not significantly affect any measures of accuracy or latency used in the current experiment, which is not consistent with the prediction that chronic predator exposure would negatively impact spatial memory retrieval. I conducted a sensitivity power analysis using G*Power 3.1 software (Faul, Erdfelder, Lang, &

Buchner, 2007; Faul, Erdfelder, Buchner, & Lang, 2009), using Cohen's f effect size criteria (small = 0.1, medium = 0.25, large = 0.4) (Cohen, 1992). Given the variance in my data and the current samples size, I would have only been able to detect very large effect sizes ($f > 0.4$). Alternatively, if the sample sizes were increased by 12-20 subjects in both groups given the current correlations among repeated measures the test would have been strong enough to detect a large ($f = 0.4$) effect size. Thus, although I had sufficient statistical power to detect within-subject changes in performance, my statistical power to detect a treatment effect was low.

Table 3.2. Pre/Post-exposure effect on measures of performance during memory test. Measures of accuracy and time to task completion. Statistical results are main effects from Two-Way ANOVAs comparing predator-exposed and non-predator-exposed birds between the pre-exposure test and the post-exposure test.

Measure	Factor	d.f.	<i>F</i>	<i>p</i>
Latency	Condition	1,10	1.4	0.26
	Test	1,10	11.8	0.006
	Test*Condition	1,10	0.07	0.80
Number of Correct	Condition	1,10	0.07	0.80
	Test	1,10	11.0	0.008
	Test*Condition	1,10	0.07	0.80
Number of Errors	Condition	1,10	2.1	0.18
	Test	1,10	16.4	0.002
	Test*Condition	1,10	1.2	0.30
Collected Percent	Condition	1,10	1628.6	0.17
	Test	1,10	24.9	0.001
	Test*Condition	1,10	1.6	0.23

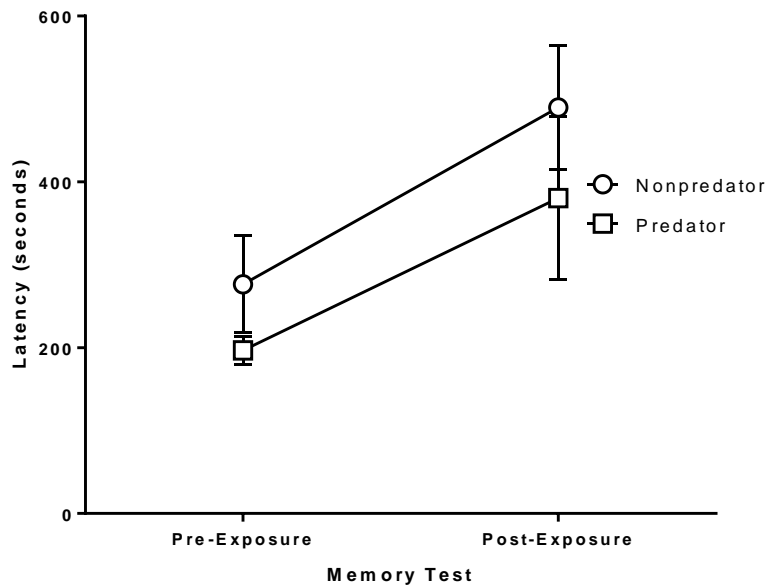


Figure 3.3. Mean seconds until collect all eight correct seeds in both exposure groups before and after the exposure. The non-predator-exposure condition before exposure ($n=6$) and predator-exposure before exposure ($n=6$), and the non-predator-exposure condition after exposure ($n=6$) and predator-exposure after exposure ($n=6$). The points are mean \pm SEM.

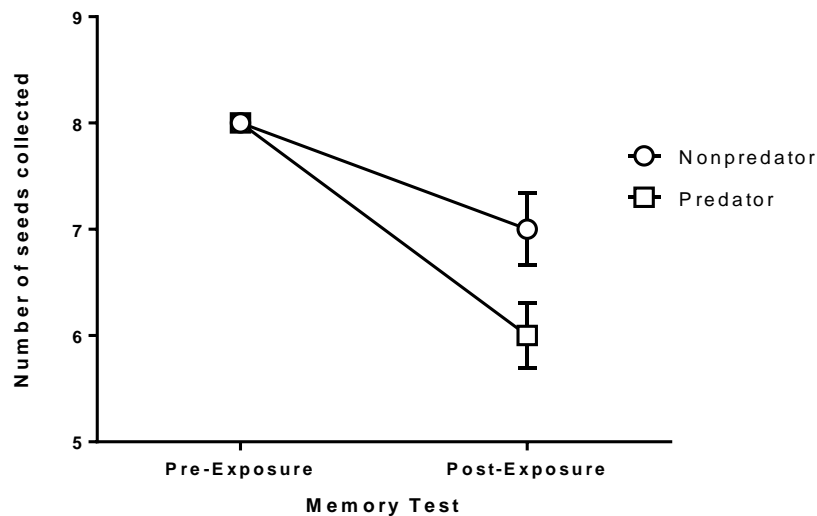


Figure 3.4. Mean number of correct seeds collected in the test session in both exposure groups before and after the exposure. The non-predator-exposure condition before exposure ($n=6$) and predator-exposure before exposure ($n=6$), and the non-predator-exposure condition after exposure ($n=6$) and predator-exposure after exposure ($n=6$). The points are mean \pm SEM.

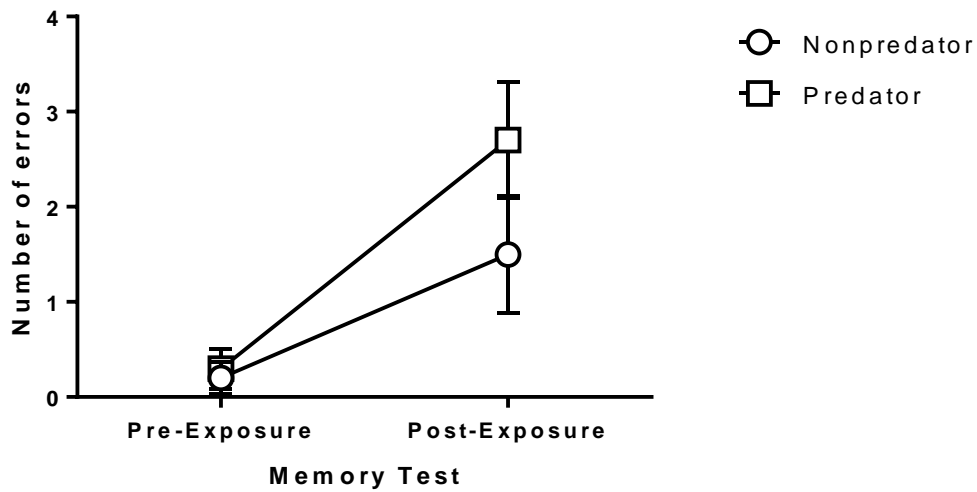


Figure 3.5. Mean number of incorrect seeds collected in the test session in both exposure groups before and after the exposure. The non-predator-exposure condition before exposure ($n=6$) and predator-exposure before exposure ($n=6$), and the non-predator-exposure condition after exposure ($n=6$) and predator-exposure after exposure ($n=6$). The points are mean \pm SEM.

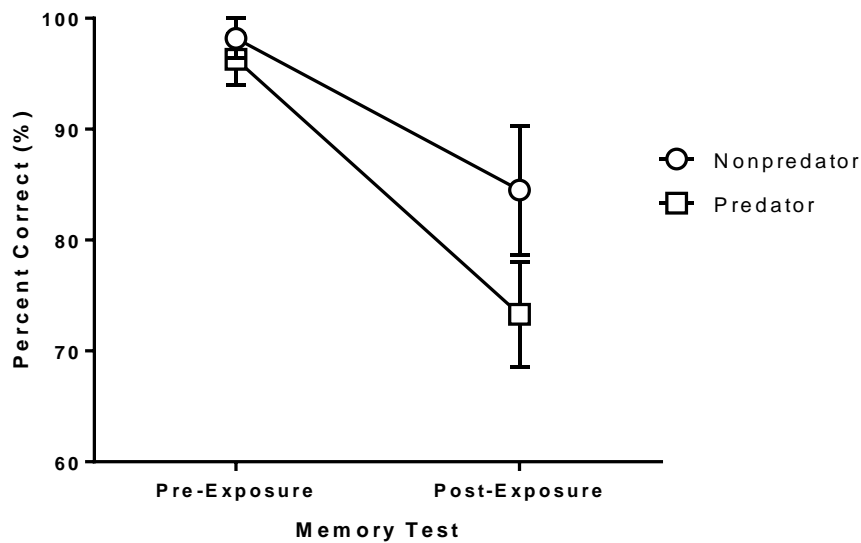


Figure 3.6. Mean number of correct seeds collected (divided by the total holes searched in a session multiplied by 100) in both exposure groups before and after the exposure. The non-predator-exposure condition before exposure ($n=6$) and predator-exposure before exposure ($n=6$), and the non-predator-exposure condition after exposure ($n=6$) and predator-exposure after exposure ($n=6$). The points are mean \pm SEM.

3.4 Discussion

This study is a novel investigation of chronic acoustic exposure on spatial memory retention in chickadees. I predicted that the chronic presentation of predator playbacks would make the birds perform less accurately than the test prior to the exposure and also perform worse than the non-predator chronic exposure control. The chickadees in the predator exposure group did perform worse after the exposure; they made more errors, took longer to finish, collected fewer correct seeds, and were overall less accurate in their searching. In contrast to the prediction the predator exposure group was not significantly less accurate than the non-predator exposed group, however, the predator exposed group tended to make more errors and collecting less correct seeds. There appeared to be a non-significant trend that the non-predator exposed group was slightly more accurate but took longer to collect all the correct seeds. This sample was not significant and did not have enough power to detect a small or medium effect, however, there was a trend in the sample which may be worth exploring in future studies. These results suggest that over time birds lose some accuracy but birds in an environment where they experience high levels of predation risk may slightly impact their spatial memory retrieval abilities.

3.4.1 Assumptions and Limitations

In this study there were some assumptions which were assessed in either an initial pilot test or in training the birds to perform the memory task. Pilot birds were used to verify timeline assumptions, that birds were able to perform the task after 1 or 2 weeks of being taken into the chamber and not having any access to the testing room. The pilot birds demonstrated that the birds were able to retain training for multiple weeks therefore it was assumed if the chronic predator playbacks impacted the retrieval ability it would be greater than any decrease over time. Though both groups were significantly less accurate after the exposure they were still above chance, which was calculated as if the birds were to randomly select the correct 8/24, chance was 33%.

All birds were food deprived and thus assumed to be equally motivated. The experiment birds in both groups were less accurate and it was not because of a lack of motivation or a lack of searching. Birds in both groups were equally food deprived and the percent correct reveals that the birds were still searching just not in the correct places,

this is also supported by the number of correct seeds decreasing with an increase in incorrect holes searched. The birds were still motivated to search and capable of searching but were just less accurate. It could also be suggested that the chickadees in the predator exposure group were more motivated to retrieve the seeds quickly and forfeited accuracy compared to the non-predator exposed group.

Using a food-caching species in tests of spatial memory takes advantage of their natural behaviour and it was assumed that food-caching birds would be able to retrieve seeds from prepared sites after training which they were able to do, even when the seed was visually obstructed by a yarn knot (Hall, Delaney, & Sherry, 2014). Individual caching behaviour and retrieval of previously made caches was not used in this study as it was important for all birds initially to be retrieving the same number of seeds to accurately portray any deficits after exposure. There is difficulty in controlling for number of caches made and number of caches retrieved by any given subject (Bugnyar & Kotrschal, 2002) therefore the study methods were made to reflect the comparison between both groups on the overall spatial memory retrieval ability of collecting the eight prepared caches.

Limitations to training the birds to retrieve seeds correctly from the same holes over time meant that training was time-consuming and only permitted a small sample size given the length of the project and the availability of the testing room. As demonstrated in the binned data (Appendix B) birds learned the task at varying speeds and varying trial amount needed until they reached the criteria. This study was similar in size to other previous research but sample size should be increased in future studies for stronger ability in detecting effects between groups.

It was assumed that birds would be affected the same way as rats when exposed to chronic predator cues in that their spatial memory would be impaired. This study could suggest that chickadee would prioritize food retrieval over other behaviours because it is so vital, they would not forfeit memory of food placement. Chickadees may prioritize food caching memory over other cognitive processes and thus there was no strong difference after predator condition.

Lastly, as noted in chapter 2 there was a significant increase in CORT between the baseline blood sample and the blood samples taken from the isolation exposure

chamber. This is relevant to this study as the birds were carried down to the chamber daily for 30 minutes for two weeks, therefore it is possible that the act of moving the birds to the chambers was stressful. For future studies I would suggest a control group that remains in the homecage room and is not transported to the chambers, as this may have masked the effects of the condition effects. This could elucidate whether the effect is from the perceived predation threat or from the stress of being transported.

3.4.2 Future Directions

Future studies should further investigate if and how stress impacts memory capabilities of wild birds. An extension on the current study could involve the same training and testing/exposure protocol but only exposing the birds to acute predator or non-predator stimuli to determine if the consistent presence of predators or novel acoustic stimuli has a stronger impact on spatial memory than a brief exposure. Future studies could consider the neurobiological mechanisms that may be impacted by chronic stress and if the neural areas associated with learning and memory are specifically impacted. There also is a need for more field or large outdoor aviary experiments that assess the ecological and physiological costs of having major (or minor) detrimental effects on caching and retrieval in a more natural setting. Other future studies could investigate other behavioural measures and different kinds of cognitive tasks or memory tests that do not rely on caching to examine if chronic predator stress impairs other areas of behaviours while sparing food-storing memory abilities. Most of the current research on birds, stress and memory has focused on foraging habits and vigilance or the impacts of food shortages, however, there has been little research done on the effects of chronic or acute perceived predation threat and the impact on spatial memory retention or cache retrieval in birds.

3.4.3 Conclusion

Lots of research has focused on the differences in abilities and hippocampus size of food-caching versus non-food-caching birds but more research is required on what this ability means practically for the birds. Moreover, further research is needed to determine to what extent natural stressors impact necessary functions (e.g. food retrieval) which would be critical for a bird's survival in unfavourable conditions. As urbanization and habitat

fragmentation continues to occur there will be an increase in the presence of native predators and an increasing number of encounters with new predators (ex. stray cats), thus it is important to understand how chronic predator threat will impact spatial memory and food retrieval abilities of food-storing birds.

In conclusion, extended periods of time with acoustic stimuli as well as chronic threat of perceived predation does impact spatial memory, but it is not drastically detrimental to the critical spatial memory function of seed retrieval.

3.5 References

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Chapter 4

4 General Discussion

The goals of this thesis were to explore the effects of predation (i.e., the perceived threat of imminent harm or death resulting from a predator attack) on the behaviour and physiology of wild-caught and lab-bred birds, to investigate the immediate responses, to examine the effects of long-term exposure to increased predation risk, and to further our knowledge of predator-prey interactions.

In Chapter 1, I reviewed how perceived predation has been studied across sensory systems, and the short-term and long-lasting impacts on animals. I provided a specific focus on the physiological impacts of inducing a stress response. I introduced evidence of ecologically relevant behaviours that are likely to be mediated by perceived predator presence. Finally, I identified gaps in our knowledge surrounding the impacts of predator-induced stress on spatial memory abilities.

In Chapter 2, I explored the acute effect of perceived predation threat on movement, grooming, and maintenance behaviours as well as the activation of the HPA axis through the measure of CORT in wild-caught (black-capped chickadees, house sparrows) and lab-bred (zebra finches) birds. I manipulated perceived predation threat using taxidermized figures, acoustic playback calls, and olfactory cues. I then used video recordings and blood plasma to quantify the behaviours and circulating CORT. These studies used standardized methodologies that allowed me to note species differences between the wild-caught and lab-bred birds and to note that predator cue effects on behaviour or CORT were not as strong or predictable as expected.

In Chapter 3, I investigated whether chronic perceived predation threat would impact the spatial memory abilities of wild-caught black-capped chickadees. I trained and tested chickadees to retrieve food in a spatial memory task. I used acoustic playback calls to simulate predator or non-predator presence, and presented it chronically to the chickadees before testing and coding their retrieval efficiency and behaviours. The results of this study indicate that after exposure to either predator or non-predator cues birds made more errors, took longer, collected fewer correct seeds, and reduced precision and accuracy in seed retrieval overall. This study suggests that chronic acoustic predation

threat may not be detrimental overall to a critical memory function (i.e. food caching and subsequent retrieval of stored food).

In this final chapter, I summarize how my work addresses important issues relating to how predation threat impacts behaviour and physiology, and explore the broader significance of my findings in understanding the effects of perceived predation threat on prey species.

4.1 Perceived Predation Threat Effects on Behaviour, Physiology, and Spatial Memory

My results suggest that anti-predator behaviours can differ between lab-bred and wild-caught birds, as well as between wild species. Moreover, my results suggest that different environmental cues can trigger varying behavioural responses. There were a variety of behavioural and physiological changes between the species across all experimental exposure types. Movement behaviour was not affected by exposure conditions in any modality, this could be because the birds did not have room to escape the predator cues. It could be suggested that prey species respond to all sensory cue types, in various ways, and that previous experience with predation risk does not necessarily predict how a bird will respond behaviourally to predator cues. This suggests that birds retain some memory of traumatic situations, furthermore there may be innate cues present for birds that are predator naïve. Taken together it is possible that these different sensory cues can trigger alarm and behavioural responses in birds that would allow the birds to react quickly and adequately to threats. Contrary to my predictions I did not find a significant effect of perceived predation threat on behaviour, physiology, or spatial memory. Surprisingly, in the acoustic and visual sensory treatments all experimental exposures produced significantly higher levels of CORT than the baseline. Moreover, in the olfactory sensory treatment there was tendency for the baseline CORT to be lower on average than the experimental exposure conditions. This suggests that all of the exposures, even to non-predator stimuli, may have induced stress in the birds.

In regards to spatial memory my results suggest after the stress-inducing move to the isolation chamber and exposure to either predator or non-predator calls, experimental birds: made more errors, took longer, collected fewer correct seeds, and reduced

precision and accuracy in seed retrieval overall. Neither the pilot birds nor the experimental birds were performing at or below chance, and all birds tested appeared to decline over time. This suggests that chronic acoustic predation threat alone did not appear to change searching behaviour and accuracy, and was not detrimental overall to the critical memory function of food retrieval.

4.2 Implications for Perceived Predation Threat Study Design

My findings indicate that birds are sensitive to isolation testing procedures. Given that there was no main predator effect as well as no detectable effect of predator exposure in either study, the effects that were being investigated in this study could have been masked by the stress of social isolation and/or being moved to the exposure chamber. All three bird species used are social and were housed in homecage rooms with conspecifics until they were individually removed and placed in the isolation exposure chamber for 24 h prior to their exposure. This placement in the exposure chamber was necessary so as not to disrupt other studies in the facility, however, future studies could consider the impact this may have had on the birds and test two or more birds at a time within the chamber. As illustrated in Chapter 2 there were significant differences between the homecage baseline CORT levels and all the experimental exposure conditions; baseline blood samples were lower in the homecage room than when the birds were moved into the isolation exposure chambers. Overall, the CORT levels suggest that the birds were moderately stressed in all experimental condition. CORT in some conditions was increased to the same degree as capture-restraint protocol or a live attack on a conspecific (Baugh, van Oers, Naguib, & Hau, 2013; Jones, Smith, Bebus, & Schoech, 2016). This should be noted for future studies that 24 hour habituation to the isolation chamber was not enough to bring CORT levels down to an equivalent baseline level even for the control conditions. Therefore moving the birds to the chambers appears to be a stress-inducing process. This is also important to note for the experiment in Chapter 3, it is possible that both experimental groups performed worse after the exposure because they were being transported to the chamber for the playback exposures. I would suggest two

additional control groups in the future wherein the birds are not moved to the chamber, and a second control group that are moved but receive no playbacks.

As previously stated the way in which birds are exposed and experimented on across the modalities vary widely, this is also true for the amount and types of controls used in past studies. The strongest example of disparity between study designs is within the visual investigations of perceived predation threat. For example, Jones, Smith, Bebus, and Schoech (2016) used a variety of live raptor attacks on conspecifics, used simulated human attacks on conspecifics, capture and restraint, a control of pulling a conspecific into a camouflaged blind, and a baseline whereas Vitousek, Jenkins, and Safran (2014) only compared the pre- and post-exposure to a stuffed cat stimulus. Some studies of visual predator cues examined a variety of predators and a non-predator control (Grabarczyk & Ritchison, 2015; Welbergen & Davies, 2008), others had an additional empty or lack of stimulus control (Soard & Ritchison, 2009). Further still, some studies compared all these groups as well as a non-threatening control such as a box (Cockrem & Silverin, 2002). For studies investigating acoustic playbacks the most common method is to do a simple comparison between predators and a control: either a control of no sound (Ibáñez-Álamo, Chastel, & Soler, 2011) or a non-predator sound (Grabarczyk & Ritchison, 2015; Hobbs, 2015; Lamanna & Martin, 2016; Soard & Ritchison, 2009; Witterick, 2017; Zannette, White, Allen, & Clinchy, 2011). A few studies examined predator calls, non-predators, and a third group such as: reverse mob calls (Avey, Hoeschele, Moscicki, Bloomfield, & Sturdy, 2011), comparing to a baseline prior to an exposure (Billings, Greene, & De La Lucia Jensen, 2015), or birds receiving no treatment (Eggers, Griesser, Nystrand, & Ekman, 2006). In most of the studies investigating olfactory cues the researchers used predator scents, odourless controls (water), and an odourous control such as: cologne (Amo, Visser, & Oers, 2011), quail feces (Amo, Galván, Tomás, & Sanz, 2008) or rabbit feces (Roth, Cox, & Lima, 2008). Other studies implemented a simple comparison between a predator cue and a control (hay (Griggio, Fracasso, Mahr, & Hoi, 2016) or unscented paper (Godard, Bowers, & Morgan Wilson, 2007)). Very rarely did researchers use experimental controls to determine if the testing procedure itself was stressful. All factors that require controls should be considered and

that implementing those controls is an important process in determining what condition effects are from the stimulus and what effects are from the method of examination.

4.3 Consequences of Different Sensory Detection

Previous research has demonstrated strong evidence that birds are able to detect and respond to visual, acoustic and olfactory information as well as having effects on physiology (e.g., CORT) and behaviour (e.g., calling, provisioning, and avoiding). These studies individually postulate that each modality is an important sensory modality for all avian species (Billings, Greene, & De La Lucia Jensen, 2015; Corfield et al., 2015).

While it is accurate that birds gather information about predators through a variety of sensory cues, I would suggest that more evidence is required to determine exactly how birds respond to individual stimuli. My studies suggest that when testing in the lab these reaction effects are nuanced and easily influenced by testing procedures. There was not a consistent response between the species used or overall trends between the sensory systems tested. All birds responded differently when presented with the variety of sensory stimuli, and behaviour often did not differ from the initial baseline recording. It has been suggested that the failure of some studies to obtain a repellent effect or aversive reaction may relate to a mismatch between the predator cue and prey species used, an individual sensitivity difference to the present cue, and/or the use of low threat predator cues (Apfelbach, Blanchard, Blanchard, Hayes, & McGregor, 2005). I extensively evaluated the likeliness of previous exposure and threat each predator or non-predator posed to the three species, but there are other methods of exposure that were not explored in these studies. Perhaps it is the case that birds living in larger or more naturalistic conditions are able to respond differently, by evading predators or making larger scale behaviour changes that cannot be identified in laboratory settings.

4.4 Future Directions

My results have expanded our knowledge on the acute and chronic effects of perceived predation risk on prey behaviour, CORT response, and memory capacities. My results emphasize the importance of examining basic questions about how birds perceive predation threat, and show that each type of sensory modality selected can have different

impacts on behaviour and physiology. These results also lay the groundwork for future studies on the impacts of perceived predation risk on prey behaviour and neurobiology. I would suggest three areas for directions and improvements to be made in future research on the topic of perceived predation threat effects, the first being ecologically meaningful impacts, the second being elucidating single cue information and expanding to compounded cues, and lastly the methodological approaches and controls to use.

The first area that could be researched further is ecologically meaningful impacts of perceived predation threat across the different sensory modalities. For example, the stimuli used in the first study could be used to assess impacts on foraging amounts, provisioning, or cognitive abilities. This is particularly important in regards to olfactory stimuli, as previous studies primarily focus on nest box choice and avoidance. In the second study presented in this thesis chronic acoustic playbacks were used to assess whether predator calls could disrupt food retrieval. Food retrieval is a vital and necessary cognitive ability for food-caching birds, but other cognitive behaviours not explicitly related to food retrieval should be investigated to explore the possibility that there are effects of perceived predation threat on a variety of natural behaviours. A disconnect exists between research done on an individual level and on a larger ecological level, this disconnect is pronounced in the stimuli used in naturalistic environments as olfactory presentations are lacking.

Secondly, I suggest that there is a need for further evaluation and investigation into the single cues (e.g. calls, scents, and/or visual models) and how they are registered and interpreted in the neural sensory system and along the sensory organs (i.e. eyes, ear, and nostrils). The breakdown of the single cues would provide information about what is necessary for the birds to perceive and interpret threatening and non-threatening cues. For example, using chromatography to determine the volatile compounds in odour cues. From this proposed study, research could then start combining single cues to examine compounding effects, to determine if the layering of cues produces a stronger response than any single cue.

My third and final recommended direction is for future researchers to carefully examine the controls and testing protocols used. Both wild and lab-bred birds responded differently than expected and did not increase CORT or systematically change behaviour

in response to the predator condition in any of the three experimental modalities, however, it was determined that the baseline blood sample was significantly lower than the control condition. The stress produced by being moved (despite the 24 hour habituation period) and/or isolated may have masked both the behavioural and CORT responses to the stimuli presentations. This may have also been the case in the second study as both predator and non-predator groups decreased performance over time, future studies should consider controls that are not moved from homecages as this may have been a source of stress for both groups and thus masked any impacts of the chronic acoustic predator playbacks. Thus I would suggest implementing more controls to the testing conditions, such as animals that are not transported or isolated before or during the experiment.

This study attempts to fill gaps in the current literature, specifically in regards to how individuals perceive and respond to threats as well as what long-lasting effects of predator's threats on cognitive abilities. There is still much to be determined and explored in the field of perceived predation threat, hopefully this project will encourage future researchers to consider which type of stimuli they are using and why, and possibly inspire future research into underrepresented sensory systems.

4.5 References

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Appendices

Appendix A

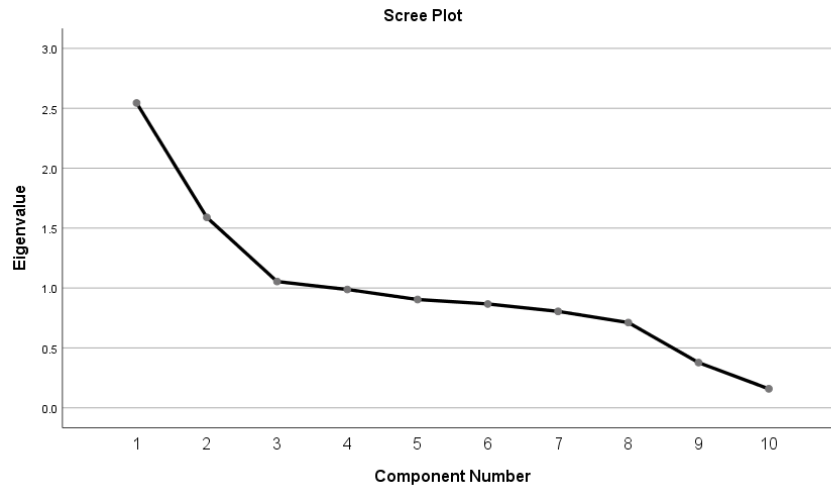


Figure A.1. Scree plot for principle component analysis for behaviour responses.

Appendix B

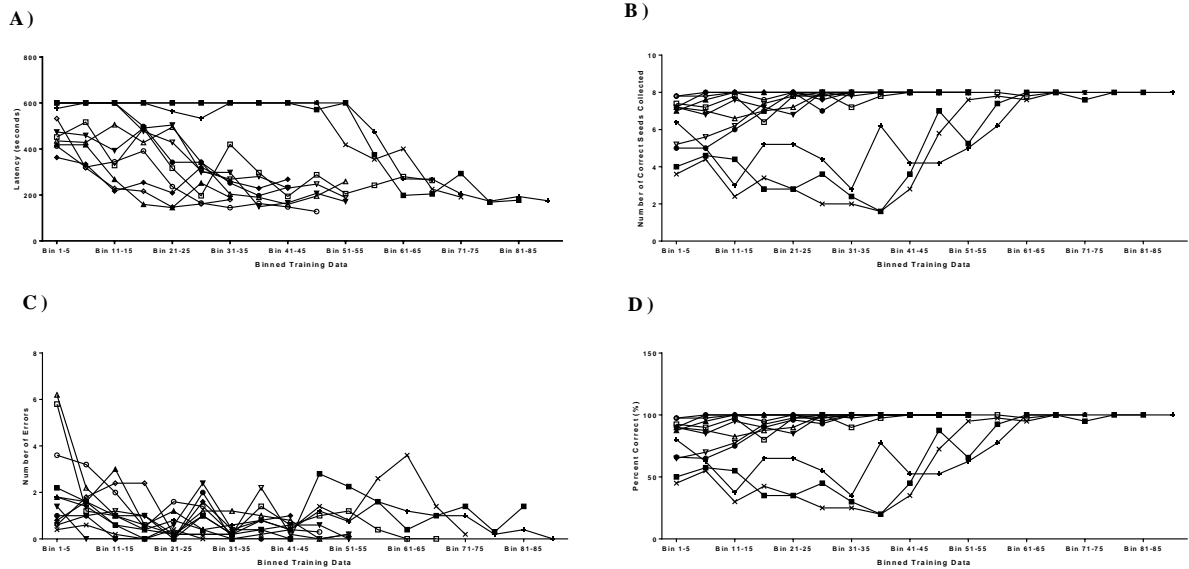


Figure B.1. Over the course of training all birds became faster, collected correct seeds more consistently, made less errors, and became more accurate in their searching by the end. A) The latency over time for the birds to collect all eight correct seeds. B) The number of correct seeds collected during the sessions with a maximum of eight. C) The number of errors, wrong holes searched, during the sessions with a maximum of 16 possible errors. D) The collected percent which is a measure of the number of correct seeds divided by the total holes searched in a session multiplied by 100. All points are an average over 5 training sessions, and each line represents an individual from the start of training to the end of training.

Appendix C



AUP Number: 2016-106

PI Name: Macdougallshackleton, Scott A

AUP Title: Effects of Predator Cues on Stress Response and Memory.

Official Notification of ACC Approval: A MODIFICATION to Animal Use Protocol 2016-106 has been approved.

Please at this time review your AUP with your research team to ensure full understanding by everyone listed within this AUP.

As per your declaration within this approved AUP, you are obligated to ensure that:

- 1) Animals used in this research project will be cared for in alignment with:
 - a. Western's Senate MAPPs 7.12, 7.10, and 7.15
http://www.uwo.ca/univsec/policies_procedures/research.html
 - b. Council on Animal Care Policies and related Animal Care Committee procedures
 - c. http://uwo.ca/research/services/animalethics/animal_care_and_use_policies.htm
- 2) As per UCAC's Animal Use Protocols Policy,
 - a. this AUP accurately represents intended animal use;
 - b. external approvals associated with this AUP, including permits and scientific/departmental peer approvals, are complete and accurate;
 - c. any divergence from this AUP will not be undertaken until the related Protocol Modification is approved by the ACC; and
 - d. AUP form submissions - Annual Protocol Renewals and Full AUP Renewals -will be submitted and attended to within timeframes outlined by the ACC.
http://uwo.ca/research/services/animalethics/animal_use_protocols.html
- 3) As per MAPP 7.10 all individuals listed within this AUP as having any hands-on animal contact will
 - a. be made familiar with and have direct access to this AUP;
 - b. complete all required CCAC mandatory training (training@uwo.ca);
 and
 - c. be overseen by me to ensure appropriate care and use of animals.
- 4) As per MAPP 7.15,
 - a. Practice will align with approved AUP elements;

- b. Unrestricted access to all animal areas will be given to ACVS Veterinarians and ACC Leaders;
 - c. UCAC policies and related ACC procedures will be followed, including but not limited to:
 - i) Research Animal Procurement
 - ii) Animal Care and Use Records
 - iii) Sick Animal Response
 - iv) Continuing Care Visits
- 5) As per institutional OH&S policies, all individuals listed within this AUP who will be using or potentially exposed to hazardous materials will have completed in advance the appropriate institutional OH&S training, facility-level training, and reviewed related (M)SDS Sheets,
<http://www.uwo.ca/hr/learning/required/index.html>

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

Curriculum Vitae

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Education

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B.Sc. (Honours) (Psychology) 2012-2016
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Research Experience

Graduate Student and Research Assistant 2016-2018
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Teaching and Supervisory Experience

Volunteer and Work-study Supervisor 2017-2018
Supervised seven students, including one work-study student
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Graduate Teaching Assistant 2016-2018
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PSYC 1000F: Introduction to Psychology Course 2016
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Tutor 2015-2016
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PSYC 3015Y Advance Statistics and Research Design
PSYC 3210H Principles of the Nervous System

Honours, Awards, & Scholarships

Bird Studies Canada/Society of Canadian Ornithologists' Student Travel Award	2018
Western Graduate Research Scholarship	2016-2018
Trent University J.P. Scott Memorial Prize \$1000	2015-2016
Trent University Undergraduate Student Research Award (USRA-NSERC)	2015
Trent University National Renewable Scholarship	2013-2016
Trent University Entrance Scholarship	2012-2013
Trent University Dean's Honour Roll	2012-2016

Academic Volunteer & Outreach Experience

Founder and Secretary, Society of Neuroscience Graduate Students	2017-2018
Neuroscience Council Member, Society of Graduate Students, University of Western Ontario	2016-2018
GradCast Interview, "Gradcast #145: What Does Stress Smell Like For a Bird?"	2017
Thames Valley Science and Engineering Fair Judge, London, Ontario	2017
Graduate Student Representative on ad-hoc SCAPA subcommittee Senate Committee on Academic Policy and Awards; implementing a fall reading break at University of Western Ontario	2016
Trent Student Psychology Society Treasurer, Trent University	2015-2016
Volunteer Notetaker, Trent University	2012-2016
Volunteer, Northern Saw-whet Owl banding station Trent University's James McLean Oliver Ecological Centre	2015
Trent Student Psychology Society Secretary, Trent University	2014-2015

Presentations and Conferences

Carter C., MacDougall-Shackleton S. (August 2018) The Effects of Perceived Predation Threat on Stress Response and Memory in Birds. Poster. International Ornithological Congress (IOC) Vancouver, British Columbia
Carter C., MacDougall-Shackleton S. (August 2018) The Effects of Perceived Predation Threat on Stress Response and Memory in Birds. Talk. Animal Behavior Society (ABS) Annual Meeting, Wisconsin, United States
Carter C., MacDougall-Shackleton S. (May 2018) The Effects of Perceived Predation Threat on Stress Response and Memory in Birds. Talk. Ontario Ecology, Ethology and Evolution Colloquium (OE3C) London, Ontario
Staples W., Carter C., MacDougall-Shackleton S. (May 2018) Songbird Neurological Response to Olfactory Predator Cues. Poster. Southern Ontario Neuroscience Association (SONA) Guelph, Ontario
Carter C., MacDougall-Shackleton S. (April 2018) The Effects of Chronic Perceived Predation Threat on Spatial Memory Retention in Black-capped Chickadee's (<i>Poecile atricapillus</i>). Talk. Conference on Comparative Cognition (CO3) Melbourne Beach, Florida, United States
Carter C., MacDougall-Shackleton S. (November 2017) The Effects of Perceived

- Predation Threat on Stress Response and Memory in Birds. Talk. **Neuroscience Seminar Series**, London, Ontario
- Carter C., MacDougall-Shackleton S. (**March 2017**) The Impact of Various Predator Perceptions on Stress Response and Spatial Memory in Birds. Talk. **Western Research Forum (WRF)**, London, Ontario
- Carter C., MacDougall-Shackleton S. (**February 2017**) The Effects of Perceived Predation Threat on Stress Response and Memory in Birds. Talk. **Neuroscience Seminar Series**, London, Ontario
- Carter C., MacDougall-Shackleton S. (**October 2017**) The Effects of Perceived Predation Threat on Stress Response and Memory in Birds. Talk. **Biology Graduate Research Forum (BGRF)** London, Ontario
- Shepherd E., Carter C., Lehmann H. (**October 2015**) Non-Hippocampal memory systems contributing to overtrained context fear memory. Poster. **Society for Neuroscience (SfN)**, Chicago, United States,

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