

**A STUDY OF THE ROLE ODOUR PLAYS IN  
RISK OF NEST PREDATION IN BIRDS**

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# Table of Contents

<b>LIST OF FIGURES .....</b>	<b>IV</b>
<b>LIST OF TABLES .....</b>	<b>VI</b>
<b>ABSTRACT.....</b>	<b>1</b>
<b>CHAPTER 1 .....</b>	<b>3</b>
<b>1.1 GENERAL INTRODUCTION</b>	
<i>Pathways of animal communication.....</i>	<i>3</i>
<i>Communication in birds.....</i>	<i>7</i>
<i>Island birds of New Zealand.....</i>	<i>13</i>
<i>Outline of thesis .....</i>	<i>15</i>
<b>1.2 REFERENCES .....</b>	<b>17</b>
<b>CHAPTER 2 .....</b>	<b>24</b>
<b>BIRDS USE ODOUR CUES TO RAT OUT PREDATORS</b>	
<b>2.1 ABSTRACT .....</b>	<b>24</b>
<b>2.2 INTRODUCTION .....</b>	<b>25</b>
<b>2.3 METHODS .....</b>	<b>28</b>
<i>Study site.....</i>	<i>28</i>
<i>Study species.....</i>	<i>28</i>
<i>Collection and presentation of predator scent at nests .....</i>	<i>31</i>
<i>Experimental procedure.....</i>	<i>31</i>
<i>Data analysis .....</i>	<i>34</i>
<b>2.4 RESULTS .....</b>	<b>35</b>

2.5 DISCUSSION.....	36
2.6 REFERENCES .....	42
<b>CHAPTER 3 .....</b>	<b>52</b>
<b>THE DETECTABLE SMELL OF NATIVE BIRDS</b>	
3.1 ABSTRACT .....	52
3.2 INTRODUCTION .....	53
3.3 METHODS .....	57
<i>Study site and species</i> .....	57
<i>Collection of preen waxes</i> .....	58
<i>Analyses of samples using rats in a Y shaped maze</i> .....	59
3.4 RESULTS .....	62
3.5 DISCUSSION.....	63
3.6 REFERENCES .....	67
<b>CHAPTER 4 .....</b>	<b>75</b>
<b>DO STARLINGS (<i>STURNUS VULGARIS</i>) REPEL NEST PREDATORS WITH ODOUR?</b>	
4.1 ABSTRACT .....	75
4.2 INTRODUCTION .....	76
4.3 METHODS .....	78
<i>Study site and species</i> .....	78
<i>Collection of starling nest samples</i> .....	79
<i>Analyses of samples using rats in a Y shaped maze</i> .....	80
4.4 RESULTS .....	82

4.5 DISCUSSION.....	84
4.6 REFERENCES .....	92
<b>CHAPTER 5 .....</b>	<b>101</b>
<b>5.1 GENERAL DISCUSSION</b>	
5.2 REFERENCES .....	105
<b>ACKNOWLEDGEMENTS .....</b>	<b>106</b>

## List of Figures

- Figure 2.1.** Evidence that starlings use odour to detect predators. Each figure shows the behaviour of parents returning to the nest before entering the nest box to feed 10 to 15 day old nestlings during the first hour of filming. The same nests were used in each treatment, so any difference between reactions to treatments shows a change in behaviour of the same parent birds between trials. The nest box contained a control sachet on one day, and rat scent sachet on a consecutive day (n=7 nests). (a) Box plot of the number of visits to nest box without entering; (b) Box plot of the amount of time in seconds spent visiting without entering; (c) Box plot of the number of times birds hesitated before entering; (d) Box plot of the amount of time in seconds birds spent hesitating outside nest box. Plots are mean, 25<sup>th</sup> and 75<sup>th</sup> percentiles, with vertical lines showing 90<sup>th</sup> percentiles. p.50-51
- Figure 3.1.** Y maze set up with a bellbird wax sample in the left arm, and a silvereye wax sample in the right. p.70
- Figure 3.2.** Box plots comparing the amount of time in seconds that rats investigated (handled) vials containing samples of New Zealand bird preen waxes compared to vials containing samples of introduced bird preen waxes (n=8). (a) Number of seconds rats investigated bellbird verses silvereye wax samples. (b) Number of seconds rats investigated brown creeper verses chaffinch wax samples. (c) Number of seconds rats investigated rifleman verses redpoll wax samples. Plots are mean, 25<sup>th</sup> and 75<sup>th</sup> percentiles, with vertical lines showing 90<sup>th</sup> percentiles. p.71-72
- Figure 3.3.** Line graphs showing change over time in the average number of seconds rats spent in each maze arm containing either New Zealand bird preen wax or continental introduced bird preen wax per trial. (a) Number of seconds on average rats spent in the bellbird and silvereye arms. (b) Number of seconds

on average rats spent in the brown creeper and chaffinch arms. (c) Number of seconds on average rats spent in the rifleman and redpoll arms. p.73-74

**Figure 4.1.** Example of set up of Y-shaped maze including the black sleeves and control nest material in the left arm of the maze. p.96

**Figure 4.2.** Box plots comparing the amount of time (seconds) that rats actively investigated control nest material and starling nest material (n = 7 trials). Plots are median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, with vertical lines showing 90<sup>th</sup> percentiles. p.97

**Figure 4.3.** Box plots comparing the amount of time (seconds) that rats spent in the arm of the maze containing control nest material compared to starling nest material. Plots are median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, with vertical lines showing 90<sup>th</sup> percentiles. p.98

**Figure 4.4.** Box plots comparing the number of times rats entered the maze arm containing control nest material and starling nest material (n = 7 trials). Plots are median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, with vertical lines showing 90<sup>th</sup> percentiles. p.99

**Figure 4.5.** Line graph showing change over time in the median number of second's rats spent in each maze arm containing nest material per trial. p.100

## List of Tables

- Table 2.1.** Table of results for paired t-tests showing where significant differences exist between treatments for each variable tested in each of the four species investigated. p.47
- Table 2.2.** Table of results for paired t-tests and repeated measures ANOVA showing if significant differences exist between treatments for each variable tested in each of the four species investigated. p.48

## Abstract

Nest predation is the most important source of reproductive failure in many bird species, and thus acts as a powerful selection pressure influencing the evolution of their life history traits. A number of studies have found that birds use a variety of visual and auditory cues to assess nest predation risk and alter their behaviour in ways that appear to minimise this risk. However, few studies have examined the relationship between odour cues and nest predation risk. In this thesis, I use several species of native and introduced bird species in New Zealand to examine the role that odour cues might play in mediating nest predation risk. The birds in New Zealand provide an ideal opportunity to study the evolution of odours and nest predation risk as they are comprised of both native and introduced continental species which differ in their evolutionary history with predatory mammals. The odour of a bird might be expected to affect nest predation because mammalian predators use a well developed sense of smell to locate prey items. Given this difference, I examined three ways in which birds may lower predation risk in regards to odour cues. First, I compared the ability of two native New Zealand, and two introduced bird species to respond to the presence of a rat (*Rattus norvegicus*) at the nest. I found some evidence to support my prediction that the native birds do not respond to a predator scent at their nest, perhaps due to their lack of co-evolutionary history with mammalian predators, while some introduced birds responded with anti-predator behaviours. I then looked at the differences in the detectability of preen waxes, a source of odour in both New Zealand and introduced birds, and found evidence to support that rats were more likely to detect the preen wax of bellbirds (*Anthornis melanura*), a native species, than at least one introduced bird species. Finally, I investigated the possibility that European starlings (*Sturnus vulgaris*) use the ammonia-like odour associated with active nests as a predator deterrent. I found that rats avoided nest material taken from active starling nests, but

did not avoid the raw materials similar to those used by starlings in nest building. Although future work involving field trials are needed, my results suggest that the odour associated with active starling nests may function as a predator deterrent. Overall, my findings suggest that at least some New Zealand native birds differ from introduced birds in both the way they “smell” and the way they use “smell.” However, there is now a need for field studies to test the generalities of this pattern in real world situations, and whether such information can be used to devise novel methods for reducing the risk of nest predation of native birds threatened by introduced predatory mammals.

# Chapter 1

## 1.1 General Introduction

### *Pathways of Animal Communication*

For humans, ‘communication’, is equated more or less with language. This is because language is the primary mode of communication between members of our species. Language is a complex amalgamation of sounds, the order and tone of which conveys additional meaning to the receiver. Yet, more than auditory signals are employed when speaking. Facial expression, posture, and hand gestures are used during conversation to put auditory sounds into context. Visual and auditory signals are the most obvious signals used in communication among humans, but smell and touch are also used (Ardiel and Rankin 2010, Wedekind et al. 1995). Thus, in general, communication can be defined as any attempt by one organism (the emitter) to manipulate the behaviour of another organism (the receiver) via the sending of a signal (Sebeok 1965). This can take a number of forms, including the sound, vision and touch signals used by humans. The emitter and receiver may be the same individual, for example a bat can use echolocation to gain knowledge of objects around it (Simmons et al. 1974), or to send signals between individuals of the same or a different species.

Almost any form of physical energy transmission can be used to send signals, and therefore can be used by animals in communication. One way to categorise the forms of signal transmission is by specifying the sense employed in receiving a signal. Thus, the pathways of communication can be visual, acoustic, vibrational, electroreceptive, olfactory and/or tactile. The evolution of these pathways depends on the organs possessed by the emitter to produce and send the signal, and the organs (receptors) that are able to receive and interpret the signal

by the intended receiver (Sebeok 1965). The form a signal takes is further selected by the environmental conditions and receptors which favour it (Endler 1993).

The evolution of communication signals often involves 'sensory drive', a term that describes the use of existing perceptual mechanisms which have already evolved in a species for reasons other than communication (Endler 1992). For example, male whistling moths (*Hecatesia exultans*) communicate with conspecifics using ultrasound (Alcock and Bailey 2009). The ability to detect ultrasound likely first evolved in whistling moths as part of an evolutionary arms race against predation from insectivorous bats which use ultrasound to detect their prey (Conner 1999). Thus, the use of ultrasound in intra-specific signals in moths developed through perceptual mechanisms which originally evolved to aid foraging or as a defence against predation. One of the major limitations on the development of communication signals is the danger that the message reaches unintended receivers (Sebeok 1965). There is always the possibility of an 'eavesdropper' taking advantage of the emitter, especially in the case of predators locating prey more easily when prey send out intra-specific signals.

Each communication pathway has its advantages and disadvantages. For example, vision is a very fast communication pathway that possesses many channels, including motion, speed, direction, posture, brightness, hue, chroma and polarization (Endler 1993). It has a high information transfer rate, but can only be received by individuals within line of sight and at a short distance from the emitter. It is also dependent on ambient light (except in the instance of bioluminescence) and the density or complexity of the habitat matrix may make long distance visual signals inefficient. Visual displays are often easy for unintended receivers to detect, and can increase the vulnerability of the emitter to predation. Some examples of visual signalling are colour displays (Stuart-Fox et al. 2003), movement (Fleishman 1992), facial expression (Weigel 1979) and posture (Fox 1969).

Acoustic communication is similarly a fast communication pathway with many channels (e.g., frequency and amplitude) and a medium to high information transfer rate. It can often be used over a longer distance than sight, but mixes easily with other noises present within the habitat, and again can make the emitter conspicuous to predators or parasites (Endler 1993). The emission of audible signals costs the emitter energy, and the message is only available to the receiver for the duration of the broadcast. The great advantage of auditory signals over visual communication, however, is that receivers do not have to be within line of sight of the emitter. Instead, receivers can be anywhere within the radius of ‘call’ distance to receive the signal. Of course this also means that predators can stalk an emitter by approaching the source of the sound without seeing the emitter.

In some animals, communication uses “vibrational” channels. Vibrations that are ‘felt’ rather than ‘heard’ are similar to hearing, but are not received by pressure changes in the ear drum. For example, one tactic used by the assassin bug (*Stenolemus bituberus*) to prey upon web building spiders is to mimic the vibrations of flies and other spider prey items caught in its web by drumming or pulling on the web of potential prey spiders (Wignall and Taylor 2009). Vibrations travel fast through the medium, and are similar to sound in advantages and disadvantages. Vibrational signals have a medium to high information transfer rate, but again the signal lasts only as long as the emitter broadcasts it, and like other signals will always cost energy. Background vibrations (e.g., a leaf caught in the net of a spider and vibrating in the wind) can also distract or ‘jam’ vibration signals from being received.

Electroreception is similar to hearing, but lies within a smaller frequency range. It attenuates faster than sound, and is used only in aquatic conditions where it is dependent on salinity and conductance of the water. Fish of the Mormyridae family use electric pulses emitted from a specialised organ, and received through lateral-line-derived electroreceptors in the skin

(Hopkins 1981). These pulses are used for both electrolocation and electrocommunication in much the same way as dolphins use echolocation. The main advantage of using electroreceptive signals is that it makes communication in low visibility habitats possible; it also possesses ‘channel privacy’ or noise immunity and is less vulnerable to jamming than other channels of communication (Hopkins 1981).

In contrast to visual, auditory or electroreceptive pathways, the use of olfaction is a slow communication pathway. This is because it takes time for chemical signals to diffuse through the environment or to otherwise reach a receiver. On the other hand, olfactory signals give the emitter the option of sending very specific signals which can relay a lot of information, and is a longer term form of communication compared to the other pathways, as it does not disappear immediately after the emitter stops producing the signal. The major advantage is that scent trails or markers can be laid in a way which may, for example, help members of an ant colony identify which colony is their own (Tranielli 1980) without the emitter being present. Similarly, wolves (*Canis lupus*) mark their territory with urine, leaving a long-term signal to warn intruders that the territory is ‘taken’ and to advertise their own fitness and ability to defend the territory, without having to constantly patrol the territory boundary (Sillero-Zubiri and Macdonald 2001). Olfaction signals can be good fitness indicators, but have low directional control in emission, and can have poor directionality for tracking (Endler 1993).

Finally, communication can involve pathways that involve tactile or contact between emitter and receiver, and can include both touch and taste. The major disadvantage of this pathway is that the emitter must be within a very close range to the receiver, and this can carry high predation risk if the emitter is a prey item that must be within range of the receiver predator to send a defensive signal. It is not as fast, nor does it have as high an information transfer rate as in vision or hearing, but it can be as direct and specific as olfaction (Endler 1993).

Examples of tactile communication include social grooming/preening in building bonds between mates, family or group members (Boccia 1983). Wrestling and biting behaviour are also examples of tactile communication and serve to establish social dominance in canines (Fox 1969).

No species uses just one communication pathway. Rather each species uses a number of pathways, each of which may play greater or lesser roles in different phases of an animal's life. The first step in understanding the complex interaction within and between species is to identify the pathways and channels of communication, the way these are used, and the context in which each is relevant to a given species.

### ***Communication in birds***

The two communication pathways most studied in birds are visual and acoustic communication. Researchers have focussed on these two pathways simply because of the obvious use of visual and auditory displays commonly exhibited in birds, and that such signals are also easily detected by humans. Bird displays have long fascinated humans and have been studied systematically by ornithologists since the 1800s, resulting in the large literature on the behavioural repertoires of birds from all around the world. Bird song in particular has received a large amount of attention from biologists for over a century (Scott 1901).

Bird vocalizations function in a wide range of situations, from alarm calls, individual identity, communicating food location, to aggressive displays, territory possession and indicating mate quality. For example, black-capped chickadees (*Parus atricapillus*) display differences in acoustic mobbing calls depending on the size of a predator that threatens them. This

information is received by other chickadees which then change the intensity of their mobbing behaviour (Templeton et al. 2005). Similarly, terns (family Sternidae) are able to identify their mates by their vocalizations, and distinguish these vocalizations from the cacophony of an active tern colony (Moseley 1979). House sparrows (*Passer domesticus*) give specific chirrup calls to attract conspecifics to share in divisible resources in an effort to reduce risk to self (Elgar 1986). Male song plays a large role in female mate choice (Christensen et al. 2006) and serves as a warning to competing males that the territory is occupied and will be defended (Hardouin et al. 2008). It is apparent from these few studies that acoustic communication in birds is well developed and complex.

As with song, visual signals are also used extensively as a communication pathway in birds. Depth perception, colour and hue detection are all well developed in birds, and are likely to have developed along with flight, as quick precise visual perception is necessary when flying through complex three dimensional matrixes (Martin and Katzir 2000). Birds often use vision as the main sense with which to locate food or prey items (Cuthill et al. 2000). Poisonous or unpalatable insects have even adapted to display warning colourations, in a bid to identify themselves to birds as ‘not for eating’ (Skelhorn and Rowe 2006). It is no surprise then, that visual cues play a major role in courtship displays. Female birds use a variety of visual cues to determine male fitness and mate choice, from hue (Delhey et al. 2003) and the amount of pigment on a male’s plumage (Hill 1991), to the rate of locomotion in a display (Husak and Fox 2008).

In contrast to auditory and visual signals, the use of other pathways for communication is either unknown or poorly studied in birds. At present, there are no known cases of electroreception in birds (even in aquatic birds), and the only use of vibrations in the literature is when foraging for invertebrate prey hidden in the leaf litter or soil. For example, the New Zealand robin (*Petroica australis*) forages among the leaf litter of the forest floor.

Foraging robins send short bursts of rapid vibrations through their legs onto the leaf litter in contact with its feet to stimulate invertebrates hidden within the substrate (Brindle 1999). The robins then pause and are able to detect any movement from the invertebrates. Kiwi (*Apteryx* sp.) and probing Scolopacidae waders possess pressure-sensitive mechanoreceptors found in specialised pits in the bill-tip, which allows them to feel for prey vibrations in the substrate (Susan et al. 2007). Tactile communication has also been described in birds, and is probably best known in courtship and aggressive displays. For example, some raptors use talon grappling as a test of strength and determination between two opponents (Craig et al. 1982). Mutual preening likewise involves tactile signals and has become a common element of courtship in some species (Sordahl 2001). Recent studies indicate that this behaviour may further serve to transmit signals based on odour (Bonadonna et al. 2007, Bonadonna and Nevitt 2004, Hagelin et al. 2003).

The use of odour or olfactory signals in birds has long been thought to be minimal. Although it is over 200 years since anatomists first began considering the importance of the olfactory sense in birds (Bang and Cobb 1968), it is only recently that this communication pathway has attracted the attention of field biologists. Bang and Cobb (1968) set the stage for this recent interest by their well known work comparing the size of the olfactory bulbs (compared to brain ratio) of over 100 species and 20 orders of birds. This work was based on the assumption that the size of the olfactory bulbs in relation to brain size was an indicator of how well developed the sense of smell is in an organism. A number of previous studies have shown birds possess a sense of smell (Bang and Cobb 1968), but the ecological contexts in which birds used olfaction was uncertain.

Large olfactory bulbs are found in a number of ground-nesting and colonial-nesting species, aquatic birds, and some carnivorous and piscivorous species. In contrast to the perceived view that birds have a poorly developed sense of smell, birds in these groups were considered

as ‘exceptions to the rule’ (Hagelin and Jones 2007), and olfactory cues in particular appear to play a key role in foraging. For example, kiwis (*Apteryx* sp.) are the only birds with nares positioned at the end of their long beaks. These birds possess the largest olfactory bulb to brain ratio of birds (Bang and Cobb 1968), and use olfactory cues to locate invertebrate prey items (Wenzel 1968). Piscivorous procellariiforms such as albatrosses, shearwaters, fulmars and petrels have also been found to detect the smell of fish oil floating on the water without visual stimuli, by approaching down wind and apparently following the odour (Hutchison and Wenzel 1980). Turkey vultures (*Cathartes aura*) display a similar ability, and can pin-point the location of a prey item even in the absence of visual cues (Houston 1986). Further studies of olfactory cues indicate that seabirds use smell to locate and distinguish burrows (Bonadonna and Bretagnolle 2002, Bonadonna et al. 2003), and may even identify each other by the unique volatiles that originate from uropygial gland excretions (Bonadonna et al. 2007, Bonadonna and Nevitt 2004). Roper (1999) provides a full review of the use of olfactory cues in birds, and their use in interspecific and intraspecific chemosignals in birds was summarised by Rajchard (2007).

Despite the knowledge that at least some birds possess the necessary equipment for olfaction, the ridged structure of the nostrils and the lack of obvious sniffing behaviour, as well as the small size of the olfactory bulb in some species, has lulled scientists into thinking that odour does not play a large role in the biology of most birds (Hagelin and Jones 2007). However, this view is turning out to be incorrect. Recent studies on the number and form of olfactory receptor (OR) genes have shown birds from a number of orders other than those previously accepted as possessing a well developed sense of smell, have ‘surprisingly’ large numbers of potentially functional avian OR genes (Steiger et al. 2009). This indicates that olfaction may play a more important role than has previously been believed. Indeed, it has been suggested

that birds in general have a genetic predisposition to use olfactory cues in similar ways to mammals and reptiles (Steiger et al. 2008).

A growing number of studies investigating the use of olfaction in a wider range of bird species (including those with ‘smaller’ olfactory bulbs), have found that olfaction is a much more widely used communication pathway than previously thought (Balthazart and Taziaux 2009, Hagelin and Jones 2007, Kats and Dill 1998). Studies have now confirmed that odour cues are used in navigation (Wallraff 2004), mate selection (Hagelin et al. 2003, Zhang et al. 2009), nest material selection (Mennerat 2008), and prey palatability (Johnston and Burne 2008). For example, the dark-eyed junco (*Junco hyemalis*), a species not classically considered to have a well-developed sense of smell, have recently been found to differentiate between their own scent, the scent of a conspecific, and the scent of a hetero-specific at the nest (Whittaker et al. 2009).

Despite the recent interest in the role of odours in avian communication, few papers have investigated the use of olfactory cues in birds in relation to predation, or a bird’s ability to assess predation risk. One of the earliest papers investigated the response of young chickens (*Gallus gallus*) to domestic cat odour (Fluck et al. 1996). Fluck (1996) found that chickens of less than 4 days old did not respond to cat odour, but exhibited an avoidance response at 7 days of age. Most of the other research on the role of odour in predation risk has focussed on practical applications, such as the development of odours that could repel birds in an agricultural setting, such as the use of mustelid scent gland secretions to repel European starlings (*Sturnus vulgaris*) from crops (Mason et al. 1991). Crested auklets (*Aethia cristatella*) have also been found to avoid mammalian musk odours in a T shaped maze (Hagelin et al. 2003).

Recently, a handful of studies have revealed that birds possessing small olfactory bulb to brain ratios (Bang and Cobb 1968) use olfactory cues to assess predation risk, and display anti-predatory behaviour in the presence of predator odour. For example, Roth Li et al. (2008) found that house finches (*Carpodacus mexicanus*) reduce feeding bout length in the presence of predator faeces, but not in the presence of non-predator faeces. Amo et al. (2008) similarly found that blue tit (*Parus caeruleus*) parents refused to enter their nest boxes more often when predator odour was present. When one considers the importance of predation in determining reproductive fitness, including birds, and the multitude of studies investigating chemical cues in reference to predation in fish (Berejikian et al. 2003, Brown and Smith 1998), mammals (Jędrzejewski et al. 1993, Monclús et al. 2006, Russell and Banks 2007), reptiles (Amo et al. 2004, Downes 2002) amphibians (Ferrari et al. 2007, Flowers and Graves 1997), and invertebrates (Ferrari et al. 2008, McIntosh and Peckarsky 2004), it seems odd that this subject has been so neglected in birds. The growing number of studies revealing the role of olfaction in avian life history is slowly eroding the commonly held view that birds are anosmic or microsmatic (the olfactory system is unimportant or little used). This area of study is still very young, thus it is an exciting time for those involved in the development of this field. The study of odours and predation risk is especially poorly studied in the avifauna of New Zealand. This is unfortunate, as the unique history and diversity of New Zealand's birds provide some unique opportunities to study the evolution of olfaction in birds and the ways this communication pathway has developed.

## *Island birds of New Zealand*

New Zealand is an island nation where the flora and fauna has evolved over roughly 83 million years in isolation from any continent (Cooper and Millener 1993). For most of this time, no terrestrial predatory mammals, apart from a few insectivorous bats, were present in New Zealand leading to a spectacular radiation of bird species that filled the niches usually occupied by mammals. Island birds that evolved in the absence of mammalian predators display a number of different life history traits compared to their continental counterparts (Lima and Dill 1990). For example, a large number of island birds have become flightless or have a reduced ability to fly. They also tend to have low reproductive rates and behave 'naïve' (more tame) towards humans and the novel mammalian predators that often accompany them (Milberg and Tyrberg 1993). The usual anti-predator behaviours prevalent in continental bird species are often completely absent in island birds, which have been subject to predation by visually hunting birds during their evolutionary history. These factors combine to make many island birds particularly vulnerable to predation by mammalian predators that use odour cues to locate prey.

Two waves of introduction of mammalian predators have occurred in New Zealand (Holdaway 1989). The first was in 1200 AD with the arrival of Polynesian hunters, their dogs and kiore (*Rattus exulans*). The second wave accompanied the recent arrival of Europeans in the 1780's, who introduced mustelids (*Mustela furo*, *M. erminea*, and *M. nivalis*), rats (*Rattus rattus*, and *R. norvegicus*), possums, feral cats (*Felis domesticus*) and dogs (*Canis lupus*), all of which have become widespread, and are known to prey on birds (Holdaway 1989). Since the first of these introductions, over 40% of New Zealand birds have become extinct (Holdaway 1989), and a number of the existing species have been confined to predator-free islands, or small areas on the mainland with intensive predator control.

A number of exotic bird species have also been introduced to New Zealand in the last 200 years by ‘acclimatization societies’ (Oliver 1930), many of which have become widely established and are now common. Unlike native New Zealand birds, introduced birds from Europe, Australia and North America co-evolved with mammalian predators in their native range, and thus are likely to have adaptations to detect such predators and adopt behaviours to reduce the risk of predation to themselves or their nests. This is critical to reproductive success, as nest predation is the major cause of failure and mortality. New Zealand birds in particular, appear to suffer high rates of nest predation (Starling 2006).

Although the introduction of exotic mammalian predators has had disastrous consequences for native birds, it is important to understand why this has been the case in order to prevent further loss of species and to perhaps assist in the conservation of native birds still at risk from introduced mammals. Given the importance of odour in the food-searching behaviours of predatory mammals, it is critical to understand how native birds use odour (or fail to use odour) in their communication pathways and whether this puts them at risk from exotic mammals. The occurrence of introduced birds thus provides a unique opportunity to investigate the role of olfactory communication pathways in both native birds (which did not evolve with predatory mammals) and continental birds (which did evolve with mammals). Thus, the objective of my thesis is to examine the potential role of odour in communication in birds, and whether this differs between native and introduced species. Such information may become important in identifying the risks posed by introduced mammalian predators and to devise ways of reducing the vulnerability of native birds.

## *Outline of Thesis*

The objective of my thesis is to investigate the effect of nest predation on the evolution of odour cues in birds. It was expected that introduced European birds in New Zealand would exhibit greater responses to odour cues, and use their own odour cues for crypsis or discourage predator investigation. In contrast, due to their different evolutionary histories with mammalian predation pressures, it was expected that native New Zealand birds would lack such ability.

I first start by comparing the responses of introduced and native New Zealand birds to mammalian predator odours at the nest. This was done by comparing the behaviours of two introduced, and two native New Zealand bird species to rat urine at the nest. Previous studies have shown that European song birds have the ability to detect odours at the nest (Whittaker et al. 2009), and at least one species is able to differentiate between a foreign odour and predator odour, and responded by displaying anti-predator behaviours (Amo et al. 2008). It is expected that New Zealand native birds will lack this response due to the absence of mammalian predators during their evolutionary history, while anti-predator responses within the introduced species may help explain their success in establishing populations in New Zealand.

In the third chapter I investigate if the smell emitted by New Zealand endemic birds is more attractive to lab rats than the smell emitted by introduced birds. Much of this work has been stimulated by the research of Fluen (2008) and Reneerkens et al. (2005) on seasonal changes in 'preen wax' composition. Preen wax is a complex mixture of lipid-based compounds secreted from the uropygial gland (commonly called the preen gland), a sebaceous gland situated at the dorsal base of a bird's tail (Whittaker et al. 2010). Birds apply preen wax to their feathers with their beak during bouts of preening, hence the common name of 'preen

wax'. The main function of preen wax is to increase waterproofing and keep feathers flexible, protecting them from wear (Jacob and Ziswiler 1982). A number of studies have revealed that many bird species change the composition of preen wax from small molecule monoesters to larger diesters during breeding (Fluen 2008, Kolattukudy et al. 1985, Reneerkens et al. 2002, Soini et al. 2007). Reneerkens et al. (2005) proposed that as larger molecules are less volatile, such a shift may increase olfactory crypsis. The theory was tested with a single dog, which was less able to detect monoester preen waxes (Reneerkens et al. 2005). Fluen (2008) later found that New Zealand endemic birds did not change the composition of preen wax but continued to secrete monoester preen waxes while the continental introduced birds secreted less volatile diester waxes during breeding season. He explained this difference by the lack of nest predation pressure in the evolutionary history of New Zealand endemic birds compared to the introduced species. Fluen (2008) also proposed it might put native birds at greater risk from mammalian predators that use olfaction to locate their prey. As rats are a major introduced predator on the nests of native birds, the objective in this chapter is to determine if the preen waxes produced by native birds in the breeding season are indeed more likely to attract the attention of a rat.

Finally, in the last chapter I test if rats tend to avoid the strong ammonia-like odour I noticed coming from active starling nest cavities. If this is found to occur, it may indicate one of the few cases where a bird uses strong odour as a deterrent against predation, rather than as a form of crypsis.

There may be some repetition between data chapters where methodology, field site and study species are concerned, as the studies in chapter 2 to 4 were conducted at the same sites, and each study has been written as a separate paper.

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## Chapter 2

### Birds use odour cues to rat out predators

#### 2.1 Abstract

A number of studies have shown that birds assess predation risk through visual and auditory cues, and change their behaviours accordingly, but there has been little research into whether similar processes occur with olfactory cues. The objective of this chapter is to examine the possible role of odour cues in assessing and limiting the risk of nest predation in birds. I performed a comparative study on the ability of 2 introduced European species (starling, *Sturnus vulgaris*, and song thrush, *Turdus philomelos*) and 2 native New Zealand species (rifleman, *Acanthisitta chloris*, and South Island robin, *Petroica australis*) to respond to the scent of rat urine at the nest. I expected native birds, which did not co-evolve with mammalian predators, to lack behavioural adaptations to the scent of rats. This was indeed the case, but I also found that only the starling changed its behaviour in the presence of the rat urine. Neither song thrushes, nor rifleman and robins showed any change in their behaviour at their nest when rat urine was present compared to a control period in which no scent was present. In contrast, starlings with rat urine at the nest box were more likely to hesitate before entering. They also approached the nest, but refused to enter more in the presence of rat scent. Despite the small number of species, my preliminary survey suggests that responses to predator scent may be more common in European species than New Zealand species, and may be a factor contributing to the vulnerability of native birds to introduced mammalian predators.

## 2.2 Introduction

The use of chemosensory cues has been studied across a large number of taxa, but until recently, it has been a neglected area of study in birds (Roper et al. 1999). The overt use of visual and auditory signals, and the lack of obvious sniffing behaviour or possession of flexible nostrils, lead scientists to believe that smell played little role in birds, despite the presence of anatomical and neurological structures for detecting olfactory cues (Hagelin and Jones 2007, Kats and Dill 1998). Since the pioneering work of Bang and Cobb (1968) on the olfactory bulbs of 108 bird species, a growing literature has challenged early perceptions on the limited use of olfaction in birds. Papi et al. (1972) first proposed that pigeons used smell to build an odour map around their 'home loft', which is then used to pin-point their location (Wallraff 2004). While this suggestion has since been questioned (Jorge et al. 2010), the use of odours has been demonstrated as a means of nest location by Procellariiform birds (Bonadonna and Bretagnolle 2002) and food location in kiwis (Cunningham et al. 2009), turkey vultures (Wenzel and Sieck 1972), petrels and penguins (Cunningham et al. 2008, Nevitt et al. 1995). In the last decade, birds have also been shown to use odours in identifying their chicks (Cohen 1981), selecting mates (Hagelin et al. 2003, Zhang et al. 2009), and detecting predators (Amo et al. 2008, Mason et al. 1991, Roth Li et al. 2008).

Although it is now clear that olfaction can play an important role in bird behaviour, few studies have investigated olfaction in passerines, despite the fact that this order contains about half of all bird species. This may be because passerines have smaller olfactory bulbs (in comparison to brain and body size) than birds in other orders (Bang and Cobb 1963). While the olfactory sensitivity of passerines may not be as highly developed as in other birds (Nevitt 2008), recent studies have nonetheless confirmed fairly sophisticated discriminatory ability of odours in some species. For example, both starlings (*Sturnus vulgaris*) and blue tits (*Parus*

*caeruleus*) incorporate aromatic plant fragments into their nests, using the scent of the plants to differentiate and select the appropriate species (Gwinner and Berger 2008, Petit et al. 2002). Similarly, Mantyla et al. (2008) showed how insectivorous passerines may use volatile organic compounds released by mountain birches (*Betula pubescens*) subject to high insect herbivory, to locate insect-rich foraging sites. Detection of odours can also function in helping birds avoid dangers or unpalatable food. This ability has been used by agricultural scientists to create odorous repellents to protect crops from bird damage. For example, ortho-aminoacetophenone is an odorous compound present in the scent gland secretions of mustelid (Mason et al. 1991), which has been used as an avian repellent, reducing the amount of food eaten by starlings when contaminated with its smell (Mason et al. 1991, Shirley et al. 1996). Finally, odours can be as a mechanism for individual recognition. For example, dark-eyed Juncos (*Junco hyemalis*) have shown the ability to discriminate between their own odour, the odour of a conspecific, and the odour of a heterospecific at their nest (Whittaker et al. 2009).

Despite recent investigations into olfaction in birds, only a few studies to date deal with the role of olfaction in predator avoidance. One early study investigated the response of chicken chicks (*Gallus gallus*) to cat odour (Fluck et al. 1996). Fluck (1996) found that chicks less than 4 days old did not respond to cat odour, but exhibited an avoidance response at 7 days of age. Similarly, crested auklets (*Aethia cristatella*) have been found to avoid a mixture of mammalian musk odours, but not banana odour (a novel odour with no ecological significance for crested auklets) (Hagelin et al. 2003). Amo et al. (2008) presented mustelid scent inside the nests of blue tits (*Cyanistes caeruleus* L.) and found that the amount of time spent within the nest significantly decreased, as did the number of times birds approached but failed to enter the box. These results suggested that blue tits were able to detect the odours of a predator from outside the nest and modify their behaviour to reduce the risk to themselves (Amo et al. 2008). Recently house finches (*Carpodacus mexicanus*) were found to respond

more strongly to faeces from predatory cats than non-predatory rabbits (Roth Li et al. 2008). However, this ability is not ubiquitous, as presenting the scent of reptilian and mammalian predators to eastern bluebird (*Sialia sialis*) nest-boxes did not discourage birds from building nests (Godard et al. 2007).

The aim of this chapter is to test whether two New Zealand birds have the ability to detect the scent of a potential mammalian predator at their nest, similar to that found by Amo (2008) in blue tits. I examined this ability in both cavity and open-cup nesting species, as well as endemic and introduced continental species. Assessing predation risk using olfactory cues might be more advantageous for cavity-nesting birds, which are unlikely to be able to see the contents of their nests before entering, and might risk encountering predators such as mustelids or rodents. On the other hand, New Zealand birds might be expected to have a poorly developed ability to detect mammalian predators by odour cues alone. This is because the avifauna of New Zealand evolved in the absence of predatory mammals, and most native birds now exhibit quite different predator defence strategies compared to their continental counterparts (Maloney and McLean 1995). Only in the last couple of hundred years have mammalian predators been introduced (Atkinson 1973).

Previous studies have shown that continental species hesitate before entering their nest after the introduction of a foreign odour, suggesting they recognise the danger and alter their behaviour accordingly (Amo et al. 2008, Mennerat 2008, Whittaker et al. 2009). Given their different evolutionary histories, I would expect that native New Zealand species would lack a similar ability. Here I report on the experimental presentation of rat odours to the nests of native and introduced birds in New Zealand. Rats (*Rattus rattus*) were used in this study as they have been a major predator of passerines in New Zealand since their introduction in 1860 (Atkinson 1973). If native birds fail to respond to the scent of a mammalian predator at the nest, this might help explain why New Zealand's birds appear so vulnerable to exotic

predators. By conducting this study in New Zealand, I have the unique opportunity to compare responses to scent at the nest between two groups of birds living in the same locality, but possessing very different evolutionary histories.

## 2.3 Methods

### *Study site*

The study was carried out at two sites. The first site was Kowhai Bush, Kaikoura (173° 37'E, 42° 23'S); a 240 ha low-elevation native forest with an open interior. The canopy is 5-12 m high, averaging 7 m, and composed mainly of Kanuka (*Leptospermum ericoides*) with an understory of small shrubs. All exotic mammalian predators that have established in New Zealand are present at this site. For a more in-depth description of the physical characteristics and ecology of Kowhai Bush, see Hunt and Gill (1979). The second site, Waimangarara Bush (42°20' S, 173°40' E), is separated from Kowhai Bush by about 5 km of pasture. The two sites are at the same elevation, and are connected by continuous beech (*Nothofagus solandri*) forest at a higher elevation. Waimangarara Bush is similar in vegetation structure to Kowhai Bush, and the avifauna is similar between the two sites. However, removal of mammalian predators has been carried out at Waimangarara Bush since 2004 using tunnel traps and poison bait stations to control mustelids, rats, hedgehogs, cats and possums.

### *Study species*

The native species studied were rifleman (*Acanthisitta chloris*) and South Island robin (*Petroica australis*). The introduced species studied were European starling (*Sturnus vulgaris*) and song thrush (*Turdus philomelos*). Both rifleman and starlings are cavity nesters and readily use artificial boxes. A total of 25 starling boxes and over 50 rifleman boxes were

present in Kowhai bush during my study. Robins and song thrush both build tight woven open-cup nests. I located nests of robins and thrushes by following parents during building, incubation or nestling stages. For species nesting in nest boxes, the boxes were checked regularly to monitor occupancy. Experiments were run during the breeding season between November and December 2008 and from September 2009 to January 2010.

All rifleman and robin pairs used in this study were colour banded, so I could ensure re-sampling of parents did not occur. Not all starling or song thrushes were colour banded and thus there was a possibility I re-sampled the same birds twice. However, if I found a song thrush nest within 15 m of another nest that I had already used earlier in the season, I did not use it to avoid re-sampling. As the degree of breeding synchrony among starlings was high, and all nests were active within 3 weeks of each other, this eliminated the chance that I was re-sampling a second clutch from a pair of parents already sampled earlier in the season. All starling, song thrush and robin nests were filmed during the 2009 to 2010 breeding season. Only robin nests were filmed in Waimangarara Bush due to low numbers at Kowhai Bush. All other species were filmed in Kowhai Bush.

Bi-parental care is exhibited in all the species I studied, with both sexes caring for their young until fledging at 23 to 27 days of age, depending on the species. The European species tended to fledge earlier than the New Zealand species. Rifleman sometimes have helpers (non-breeding birds that help feed the young of other parents) at the nest, but for the purpose of this study, I used nests where only the parents raised their young, and thus only parental response was measured.

Both starling and song thrushes were introduced to New Zealand from Britain in the 19<sup>th</sup> century. Starlings were first introduced in 1862, when a shipment was imported to Nelson (Oliver 1930). They were subsequently released around New Zealand and are now widespread. Starling nestlings are fed by both parents for 23 days until fledging. Starlings in New Zealand breed between October and December and lay an average of 5 eggs per clutch (Bull and Flux 2006). Song thrushes were first introduced to New Zealand in 1862, in the same shipment of birds as Starlings that arrived in Nelson (Oliver 1930). Subsequent releases were made around New Zealand and thrushes are now abundant. Song thrushes forage for invertebrates such as worms and introduced snails among the leaf litter, and also eat fruit and berries. Song thrushes build large cup nests with a smooth lining of decayed wood and grass mixed with saliva. An average clutch of 4 eggs is laid between May and January.

The South Island robin is an endemic species. It is particularly 'tame', showing little fear of humans. Robins are typically found in broad-leaf forest where they forage among the leaf litter for invertebrates. They readily approach people who disturb the soil exposing tiny invertebrates for the gleaning. Robins declined after the arrival of stoats, rats and cats. Robins build cup nests out of grass, twigs and mosses, and line the inside with fine grass and sometimes feathers (Oliver 1930). Nesting occurs between mid-May to early January and they lay around 3 eggs per clutch. The rifleman is also an endemic species and the smallest bird in New Zealand, weighing 5 - 8 g (Sherley 1985). Rifleman usually live in the forest interior, where they make short flights from tree to tree and scale branches much the same way as a Northern Hemisphere creeper or nuthatch. Rifleman are vulnerable to predation from introduced mammalian predators. Their diet is composed of small caterpillars, moths and spiders, and they breed between August to January, building a round nest, typically within a cavity or dense ball of vegetation, with a short side entrance. Clutch size ranges between 4 to 5 eggs.

### ***Collection and presentation of predator scent at nests***

I used the same methods as in previous studies to collect and present the scent of a potential mammalian predator at a bird's nest (Amo et al. 2008, Godard et al. 2007). Predator scent was obtained by placing clean absorbent paper inside a cage containing four female rats. Rats are major nest predators of passerines in New Zealand (Empson and Miskelly 1999). Male and female rats release gland secretions along with urine, which is associated with scent-marking behaviour and intra-specific communication (Kannan and Archunan 2001). Papers were placed in cages at least three days before each experiment to ensure odour collection. Only papers wet with urine were used, and were removed from the cage within 6 hours of use in an experimental presentation. Papers were cut into 2x2 cm squares to control for surface area of odour. These small paper samples (hereafter referred to as "scent" papers) smelt strongly enough to be detected by the human nose. Control papers consisted of the same size squares of clean paper dampened with water. Two-by-two centimetre 'sachets' were made by stitching black nylon mesh together. Control and scent papers were enclosed in the sachets to reduce the glare of the paper but without obstructing diffusion of odours. The sachets also provided a more rigid frame which could be inserted easily into the side of nests.

### ***Experimental procedure***

Experiments were conducted when nestlings were between 10 to 15 days after hatching. Each experiment consisted of two treatments, a control trial and a scent trial. Only one trial was conducted per day but I ran the two treatments on consecutive days. The order of trials was alternated between each nest of each species (i.e., the first starling nest had the control sachet presented on day one and the scent sachet on day two, while the second starling nest had the

scent sachet presented on day one and the control on the second day). In cavity-nesting species, the sachet was hung on a thread from the entrance, or lid of the box so that it hung half way down the wall of the cavity. This position was used because nestlings often defecated upon sachets hidden around the edge of the nest. For cup-nesting species, the sachet was tucked into the material of the nest (where possible) or hidden under leaves in the nest wall (where leaves were incorporated in nest construction). In both types of nests, the sachet was hidden from view and did not provide a visual cue to the parents.

To assess the response of parents to the presence of predator scent at the nest, parental provisioning behaviour immediately after introduction of the sachet was recorded by video-camera. In each trial, a video-camera was set up 5 to 10 m from the nest an hour before the trial commenced, so that parents had time to habituate to the presence of the camera and tripod. The trial began once either the control or scent sachet was placed at the nest. Rifleman nests were filmed for 30 minutes as they feed frequently, and an hour of filming for all the other species. The two trials were always conducted 24 hours apart, so that filming occurred at the same time of day in each trial. Numbers of nestlings in the nest were always the same in both trials. To ensure that each treatment day had as similar weather conditions as possible, no trials were conducted in the rain. Rain can have a significant effect on incubation and brooding bout length in birds (Beintema and Visser 1989, Poisbleau et al. 2007). This study only examined short-term response to predator smell at the nest as previous work found anti-predator response to odours decreased quickly after the first 5 minutes (Amo et al. 2008, Mennerat 2008, Roth Li et al. 2008).

Video tapes were transcribed to record the number of visits to nests and time spent at the nests in each visit (measured in seconds). The number and amount of time birds “hesitated” before entering a nest and/or feeding chicks was compared between treatments. A hesitation was the amount of time parents paused within 20 cm of the nest before either entering a nest box, or perching on or directly beside nestlings within feeding distance (~5 cm). It was assumed that birds which spent time outside the nest before entering were “hesitating” (rather than going in directly without stopping), and that the longer the duration of this behaviour, the more hesitant the response. The number of times and the amount of time (in seconds) spent visiting but not touching the nest was also recorded. For nest-box species, this was the number and amount of time parents sat on the box, or just outside the hole of the box (within 20 cm) but did not enter the hole before leaving. For cup-nesting species, this was the number and amount of time parents spent within 20 cm of the nest, but did leave without approaching within ‘feeding distance’ (approximately 5 cm from nestlings) or attend to the nestlings in anyway, even though there were nestlings begging in the nest in each of these cases.

Each filming period for song thrush, starling and robin nests was divided into six segments of 10 minutes each, starting from the beginning of filming, to determine possible changes of parental response behaviour through the first hour after treatment in each species. Because rifleman nests were only filmed for 30 minutes, this footage was divided into six segments of 5 minutes each. Having six time segments allowed me to investigate any temporal patterns in behaviour changes occurred in the study species. Thus the variables transcribed from the video data were: time till first parent entered the nest or nest box, number of times parents visited but did not 'enter' the nest, time spent in or on the nest, and time spent within 20 cm of the nest, but not 'entering', and time spent hesitating before 'entering'. I defined entering the nest as putting the head right inside the hole of the nest box (as this can be sufficient to feed nestlings) or nest hole entrance in rifleman. For cup nesting species, 'entering' was any time the bird touched the nest, chicks, or the perch they usually fed the chicks from.

### ***Data analysis***

Data was log-transformed to ensure normality. I used a paired t-test to test for differences in time to enter the nest box for the first time, the average amount of time parents spent in the box over the hour, and the number of times parents visited without entering the nest between treatments. Paired t-tests were also used to test for differences in the number of times and the number of seconds birds hesitated within 20 cm of the nest before perching on cup nests, or entering nest boxes between treatments. Repeated measures ANOVA were used to test for differences in the time spent at the nest for each treatment between the six time sequences for each species. A critical value of 0.05 was used in all tests. Statistical analysis was performed using the computer program R, version 2.3.1

## 2.4 Results

There were no significant differences between treatments in time elapsed from the beginning of filming until the first parent entered the nest for the first time in any of the four species studied (paired t-test,  $P > 0.05$ , Table 2.1.). There was also no significant difference in the number of times parents entered the nest between treatments in any of the species (paired t-test,  $P > 0.05$ , Table 2.1.). The amount of time parents spent at the nest did not change significantly between treatments for any of the four species (paired t-test,  $P > 0.05$ , Table 2.1.). Nor was there a significant difference in the amount of time spent at the nest between any of the 6 time sequences for any of the species tested in either control or scent trials (ANOVA,  $P > 0.05$ , Table 2.2.).

Starling parents visited within 20 cm of the nest, but left without feeding or attending to the nestlings significantly more often in the scent treatments (paired t-test,  $df = 6$ ,  $t = -2.67$ ,  $P = 0.04$ , Figure 2.1.) compared to the control treatments. However, there was no significant difference between treatments in the number of times birds visited within 20 cm of the nest without attending to the chicks for any of the other species (paired t-test,  $P > 0.05$ , Table 2.1.). The amount of time parents spent visiting but leaving without entering the nest was again significantly higher in the scent treatments for starlings (paired t-test  $df = 6$ ,  $t = -4.67$   $P = 0.003$ , Figure 2.1.), but there was no significant difference between treatments for any of the other species tested (paired t-test,  $P > 0.05$ , Table 2.1.).

No robins or song thrushes ever hesitated when approaching their nests. All the birds in both species either flew right onto the nest when approaching the nest, or landed very close to the nest and hopped onto it within 1 second of landing. On the other hand, both starlings and rifleman parents often hesitated just before entering their nests. There was no significant difference in the number of times, or amount of time spent hesitating before entering the nest

for rifleman (paired t-test  $P > 0.05$ , Table 2.1.). Starling parents, however, hesitated a significantly greater number of times before entering the nest in the scent treatments (paired t-test,  $df = 6$ ,  $t = -3.68$ ,  $P = 0.01$ , Figure 2.1.) compared to the control treatments. Starling parents also spent significantly more time hesitating outside the nest during the scent treatments (paired t-test,  $df = 6$ ,  $t = -8.12$ ,  $P < 0.001$ , Figure 2.1.) compared to the control treatments.

## **2.5 Discussion**

Out of the four species tested, only starlings appeared to detect the odour of rats at the nest and exhibited behaviours that could potentially decrease predation risk to adults visiting the nest. Although none of the four species changed the number of times they entered the box, the amount of time they spent inside the nest box per 10 minute interval, or the time to first entry between treatments, starlings did approach the nest but left without feeding the chicks, and ‘hesitated’ more often when rat scent was present. As expected from their lack of evolutionary history with mammalian predators, neither the rifleman nor the robins changed any of the monitored behaviours between scent and control treatments. However, contrary to expectations, song thrushes also did not display any difference in behaviours at the nest between treatments.

Amo et al. (2008) interpreted the behaviour of parents approaching the nest-box but flying away without entering the box and feeding chicks as ‘refusing to enter the nest’. This could be interpreted as an anti-predatory behaviour because predation risk would decrease for such individuals in the event of returning to the nest while a predator is inside it. The amount of time hesitating outside the box, before finally going to the entrance and entering the nest box may reflect how long it takes before birds decide it is safe enough to enter the box. This

increases the amount of time birds spend listening and looking around the nest box for signs that a predator may still be around. Only upon hearing normal nestling begging sounds inside the box, and being unable to detect visual or auditory signs of a predator, does the bird enter the box.

No song thrush displayed hesitations during approaches to the nests, and only once did a robin 'hesitate' during the approach to the nest. This is probably because of the difference in the way a bird approaches an open-cup nest compared to a cavity nest. To approach a cavity nest, the bird usually lands outside the nest first, and then hops or flutters towards the entrance. The cup-nesting species I monitored tended to fly directly to the open nest, and land on, or directly beside it (within ~5 cm). This is possibly because they can see into the cup nest on approach, and assess the situation visually while flying, giving them enough time to change flight path if danger cues are visible at the nest. In contrast, cavity-nesting birds have fewer visual cues about the contents of their nest, and may risk entering a nest with a predator inside. Reliance on visual and auditory cues could be the primary means that cup-nesting birds use to detect predation risk, and once satisfied through these channels; birds may ignore scent at the nest. It is also possible that the strength of predator scent was greater within cavities than in open nests where urine can dry out and scent disperse faster.

My results suggest that anti-predator response to odour cues occurs in species other than blue tits, but is absent in other species. Both blue tits and starlings are known to use olfaction to select aromatic vegetation which is incorporated into the nest (Clark and Mason 1987, Petit et al. 2002), so further studies are needed to investigate how general the phenomenon of anti-predator response to predator odour cues is across other species. It is interesting to note that starlings were found to have an 'average' olfactory bulb to brain size ratio (for passerines) in Bang and Cobb (1963), with a ratio of 9.7 %. No tit species featured in the study on olfactory bulb size, but finches have been shown to respond to predator odour in previous studies (Roth

Li et al. 2008), and the finch brain data from Bang and Cobb indicates they have a small olfactory bulb to brain ratio of 4 %. This shows that even birds with small olfactory bulb to brain ratios can use their sense of smell in ecologically important ways. House sparrows (*Passer domesticus*) are another cavity-nesting species present in New Zealand and, like starlings, are one of the most widely distributed birds in the world (Anderson 2006, Flux and Flux 1981). If anti-predator response to odour cues at the nest is more common in cavity-nesting birds, house sparrows would provide another species which could be used to test the generality of these findings.

Native New Zealand birds (both adults and nestlings) have been found to suffer higher nest predation rates than introduced species within the same habitats (Starling 2006). Native birds display a number of different behavioural traits compared to their continental counterparts. For example, native New Zealand birds tend to visit their nest more often than continental species, which increases nest conspicuousness to predators (Starling 2006). Many native species appear unusually tame, and do not exhibit as many fear reactions towards humans, even at their nests (Maloney and McLean 1995). The results of my study indicate another behaviour in which New Zealand birds may differ from continental species that may contribute towards vulnerability to introduced mammalian predators. Despite the presence of rat scent at their nests, neither robins nor rifleman showed any indication they recognised a threat or altered their behaviour. As robins are open-cup nesters, this might simply be a result of this nest type (see above) but rifleman are a cavity nesting species and might be expected to show some change in their response. The fact that rifleman do not respond to the smell of a rat at the nest may increase their vulnerability to nest predation, and may explain why on one occasion in this study, a pair built their nest within an unoccupied rat burrow, which was subsequently predated. This lack of anti-predator behaviours in the presence of predator odour may increase the risk of predation, which could limit population size (Sherley 1985). It

would be valuable to test whether the hihi (*Notiomysis cincta*) or the saddleback (*Philesturnus carunculatus*), two other cavity-nesting native species, also lack the ability to detect rat scent. Both of these species can only survive in highly controlled predator free areas (Armstrong et al. 1999, Hooson and Jamieson 2003). The lack of a response is what might be expected given their lack of evolutionary history with mammalian predators.

Apart from testing additional species of native birds, future studies are also needed to investigate the effect of predator odour on nestlings. For example, great tit (*Parus major*) fledglings have been shown to be unable to recognise predators upon leaving the nest, and are reliant on parents to teach them anti-predator behaviour (Kullberg and Lind 2002). Long-term effects of responses of adults to continued predator odour cues at the nest are also needed, as well as the effects this may have on nestling growth rate. No effect was found on growth rate in Amo et al. (2008), although parents decreased the number of times they entered the nest box, as well as the amount of time spent inside the box, which could limit food deliveries. Starlings did not change the number of entries to the nest box, nor the amount of time spent inside the box, only the amount of time spent on behaviours outside the box. It is therefore unlikely that nestlings suffered adverse effects by reduced feeding in the presence of predator odour, but this would need further investigation. However, I was unable to determine if the behaviour of parents changed between treatments once inside nest boxes, and it is possible adults invest less in attending nestlings as a result of increased perceived risk. More time hesitating outside the nest, or returning with food but not feeding may reduce time for parents to forage for their own requirements. It would also be worth investigating if this behaviour can become maladaptive if continued for too long when a predator is not actually present (Amo et al. 2008).

It was outside the scope of this study to determine whether native New Zealand birds were able to detect the odour of predators at the nest, but failed to respond appropriately; or if they

lack the ability to detect such an odour cue at all. In other words, the lack of evolutionary history with mammalian predators may mean native birds do not have the “hardware” to detect such cues. On the other hand, if native New Zealand birds do have the ability to detect scent cues, it may be possible to train them to avoid such a scent using negative associations, much the same way that naïve New Zealand robins have been trained to visually recognise mammalian predators as a threat (Maloney and McLean 1995). If birds can be taught to associate a visual object with danger, there is no reason (given birds can detect odours) the same cannot be done with smell. In fact, when a specific odour is added to a visual cue to be associated with a negative stimulus, birds have been found to learn the negative association faster than when trained with the visual cue alone (Johnston and Burne 2008). Of course further studies would be needed to investigate if such behaviour is passed on from parent to young, or through social networking for such a learned behaviour to persist. If birds like the rifleman and saddleback can be taught to associate predator odour cues as a negative stimuli, this may be a useful conservation tool in the re-introductions of naïve individuals from predator free island populations back onto the mainland. At the very least, it may stop pairs nesting in rat burrows where predation is a certainty!

### ***Conclusion***

The use of odour cues in assessment of predation risk is common amongst fish (Berejikian et al. 2003, Brown and Smith 1998), mammals (Jędrzejewski et al. 1993, Monclús et al. 2006, Russell and Banks 2007), reptiles (Amo et al. 2004, Downes 2002), amphibians (Ferrari et al. 2007, Flowers and Graves 1997) and invertebrates (Ferrari et al. 2008, McIntosh and Peckarsky 2004). It is only in the last few years that scientists have begun to consider the use of odour cues in detection of predators influencing avian life histories. My results provide

evidence to support the theory that anti-predator behaviour triggered by odour cues may exist in at least some bird species, although it might be more developed in cavity nesting birds. My results also support the theory that 'naïve' island endemic birds lack this response, which may be a contributing factor in their rapid decline after the introduction of mammalian predators.

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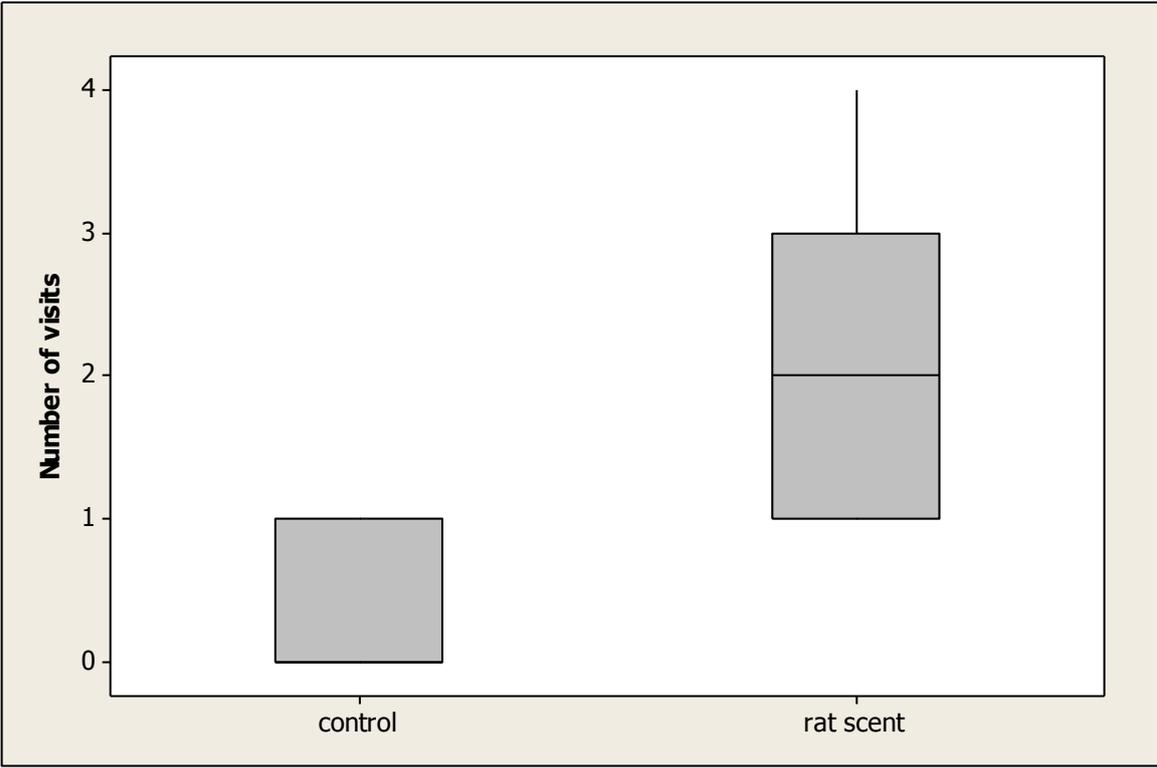
Table 2.1. Table of results for paired t-tests showing where significant differences exist between treatments for each variable tested in each of the four species investigated.

Paired t test statistics	Starling n = 7 df = 6		Rifleman n = 14 df = 13		Song thrush n = 7 df = 6		Robin n = 6 df = 5	
	T value	P value	T value	P value	T value	P value	T value	P value
Time before first entry	-0.73	0.49	-0.38	0.71	0.43	0.68	0.19	0.86
Number of entries	1.38	0.21	-1.71	0.11	0.59	0.58	0.96	0.39
Total time spent at the nest	1.98	0.10	1.40	0.18	1.12	0.30	-0.95	0.39
Number of visits	-2.67	0.04	0.65	0.53	-1.00	0.36	-1.00	0.36
Time (seconds) spent visiting	-4.67	< 0.01	0.07	0.95	-1.35	0.23	0.68	0.53
Number of hesitations	-3.68	0.01	-0.34	0.74	NA	NA	NA	NA
Time (seconds) spent hesitating	-8.12	< 0.01	-0.83	0.42	NA	NA	NA	NA

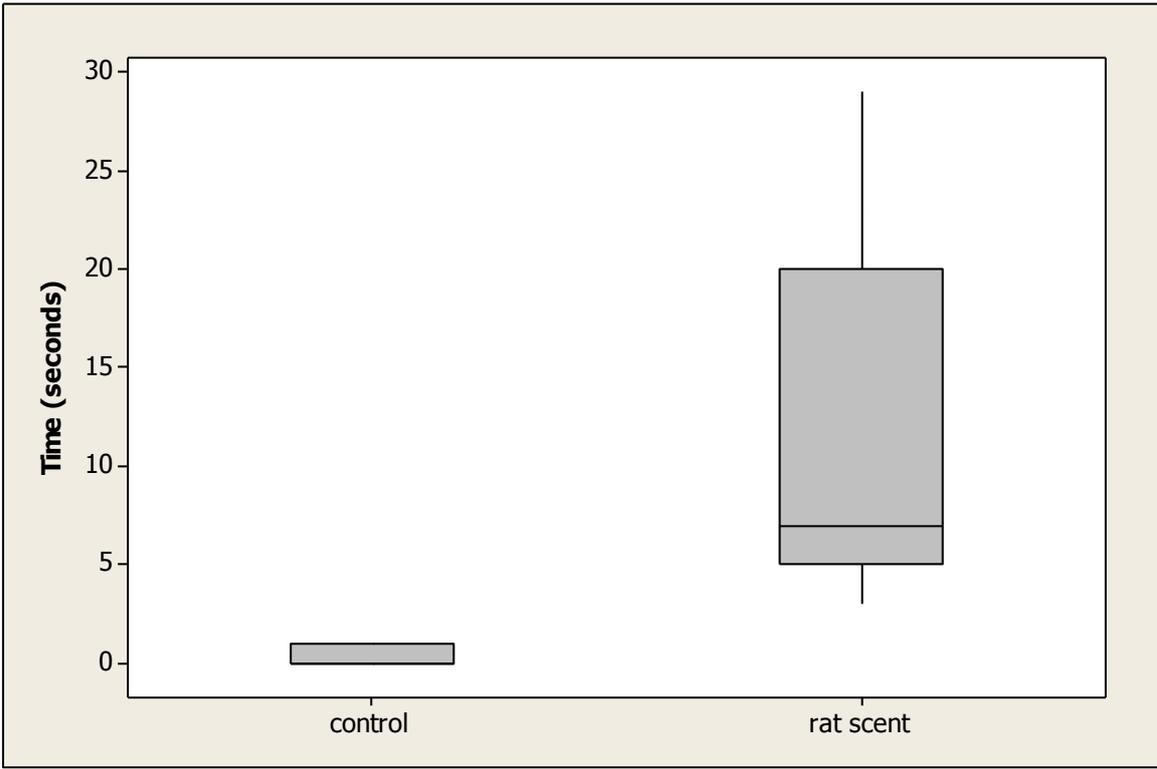
Table 2.2.

Table of results for paired t-tests and repeated measures ANOVA showing if significant differences exist between treatments for each variable tested in each of the four species investigated.

	Starling n = 7		Rifleman n = 14		Song thrush n = 7		Robin n = 6	
ANOVA F and P statistics	F value	P value	F value	P value	F value	P value	F value	P value
Time spent at nest between 6 time sequences for control df=1	F = 0.38	P = 0.25	F = 0.06	P = 0.81	F = 0.87	P = 0.36	F = 2.08	P = 0.16
Time spent at nest between 6 time sequences for scent df=1	F = 1.51	P = 0.22	F = 1.15	P = 0.29	F = 0.04	P = 0.83	F = 4.89	P = 0.34



(a)



(b)

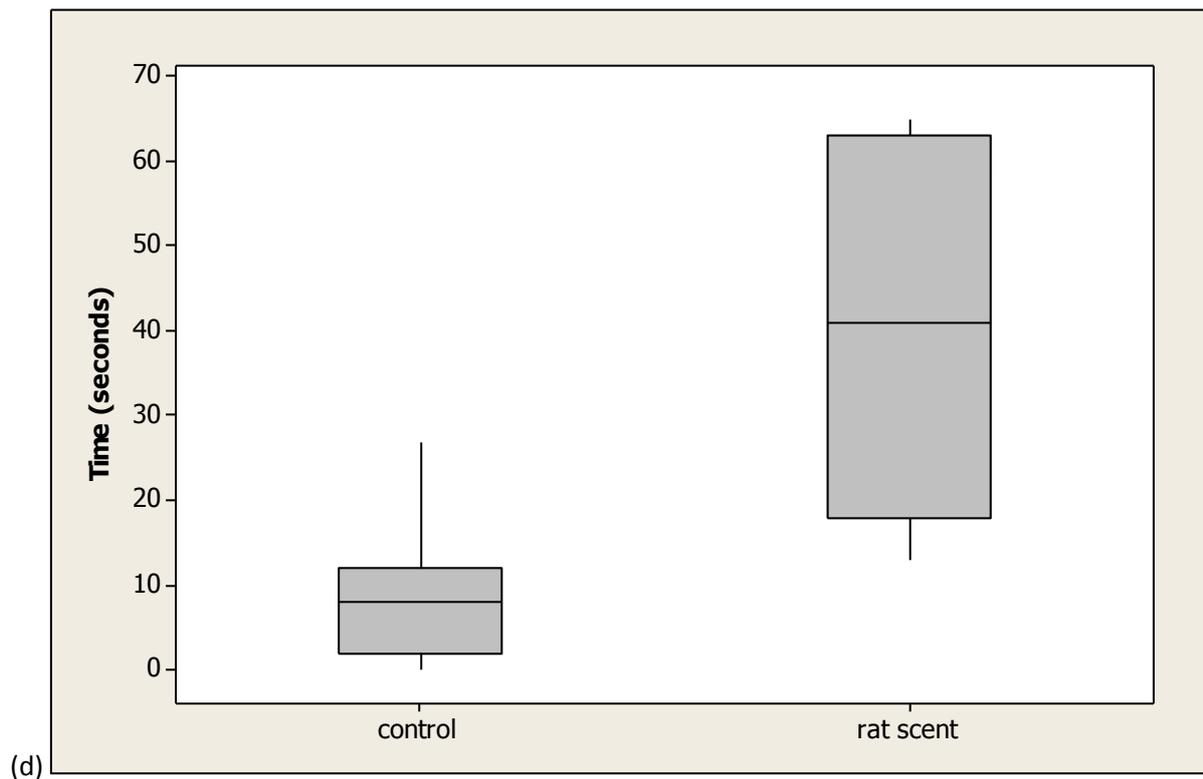
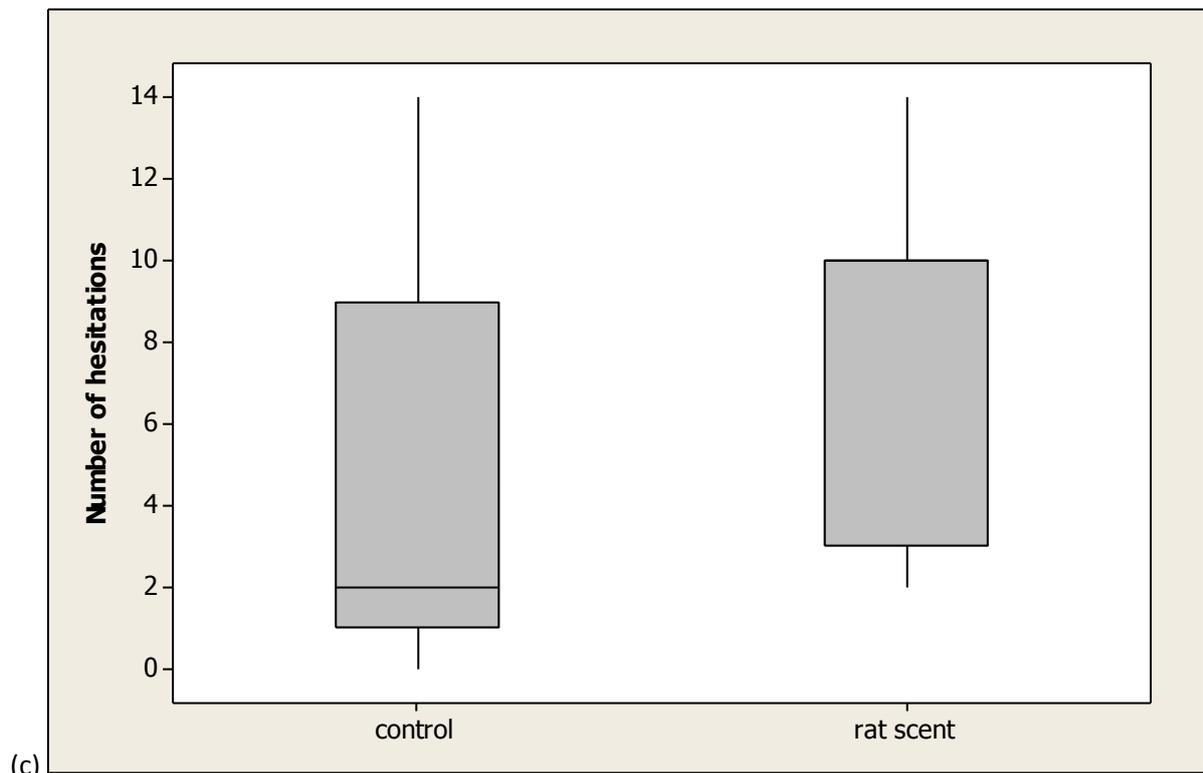


Fig. 2.1. Evidence that starlings use odour to detect predators. Each figure shows the behaviour of parents returning to the nest before entering the nest box to feed 10 to 15 day old nestlings during the first hour of filming. The same nests were used in each treatment, so

any difference between reactions to treatments shows a change in behaviour of the same parent birds between trials. The nest box contained a control sachet on one day, and rat scent sachet on a consecutive day (n=7 nests). (a) Box plot of the number of visits to nest box without entering; (b) Box plot of the amount of time in seconds spent visiting without entering; (c) Box plot of the number of times birds hesitated before entering; (d) Box plot of the amount of time in seconds birds spent hesitating outside nest box. Plots are mean, 25<sup>th</sup> and 75<sup>th</sup> percentiles, with vertical lines showing 90<sup>th</sup> percentiles.

# Chapter 3

## The detectable smell of native birds

### 3.1 Abstract

Previous studies have revealed European and American birds reduce the volatility of uropygial gland secretions during the breeding season, perhaps to enhance olfactory crypsis at the nest. Recent research has also shown that European birds introduced to New Zealand display seasonal change in wax composition, but endemic New Zealand birds that evolved in the absence of mammalian predators do not. I performed a comparative study on the detectability of preen waxes to rats (*Rattus norvegicus*) in three pairs of birds. Each pair consisted of one New Zealand endemic species, and one European introduced species. Rats were presented with each wax type in a Y-maze and the time spent in each arm used as an index of which species was more readily detected by olfaction. I found that rats detected the native species more often in only one species pair: rats spent more time handling uropygial secretion samples from the native bellbird (*Anthornis melanura*) than they did from the self-introduced silvereye (*Zosterops lateralis*), a continental species that co-evolved with rodents in its native Australian range. Rats showed no discrimination between the other samples taken from native New Zealand or introduced European species. These results indicate that some native birds may indeed produce preen waxes that are more detectable than that produced by European birds, but further research is needed to determine the general nature of this pattern.

## 3.2 Introduction

Birds need to maintain their plumage for a number of reasons. Waterproofing and keeping feathers properly positioned aids in thermoregulation, while the physical action of preening can limit ectoparasite numbers (Møller et al. 2009), aid in enhancing appearance, or be incorporated into courtship behaviours (Piault et al. 2008). One of the most important components of plumage maintenance is the application of an oily secretion to the feathers called preen wax. Preen wax is a complex mixture of lipid-based compounds secreted from the uropygial gland, which is situated at the base of a bird's tail (Whittaker et al. 2010). The composition of preen wax differs among species, between sexes in some species, and to a small degree between individuals (Haribal et al. 2005). Birds squeeze this nipple-like gland with their beak, which then causes the excretion of preen wax as a droplet. They then apply this oily drop to their outer feathers using their beak during preening sessions. A coating of preen wax on the feathers has been found to increase water resistance (Jacob and Ziswiler 1982) and inhibit the growth of feather-degrading bacteria and other microorganisms (Møller et al. 2009, Reneerkens et al. 2008).

The composition of preen wax is not constant in some species and shows seasonal changes associated with breeding. For example, in mallards (*Anas platyrhynchos*), the preen wax changes from containing short chain acyl groups to long chain acyl groups immediately after the moult into breeding plumage, and then back into short chain acyl groups after breeding, two months later (Kolattukudy et al. 1985). Seasonal changes in preen wax have been found in passerines such as dark-eyed juncos (*Junco hiemalis*) (Soini et al. 2007), house finches (*Carpodacus mexicanus*) (Haribal et al. 2005) and red vented bulbuls (*Pycnonotus cafer*) (Bhattacharyya and Chowdhury 1995). Recent studies have also identified seasonal changes in wax composition among 19 species of sandpipers, the European oystercatcher *Haematopus*

*ostralegus*, and six plover species (Reneerkens et al. 2002). In these wading birds, the composition of preen wax changes in an annual cycle, with lower molecular-mass monoester waxes during the non-breeding season, but a change to higher molecular-mass diester waxes at the time of migration to Arctic breeding grounds (Reneerkens et al. 2002). It was proposed that diesters are costlier to produce than monoesters, thus birds only produced diesters when most needed. It was originally suggested that diester waxes could act as substrates for pheromones (Kolattukudy et al. 1985). However, Piersma et al. (1999) found no traces of small volatile molecules that would result from hydrolysis of diesters. Piersma et al. (1999) also pointed out that the change in ester composition is opposite to that expected in terms of viscosity – in a colder climate, lipid compounds would become more viscous and harder to spread across feathers, therefore it seems counter-intuitive to produce more viscous waxes on the arctic breeding grounds. An opposite trend in seasonal preen wax composition has been observed in a few avian species, with volatility increasing (i.e., molecular size decreases) during breeding season. There is some evidence that in these cases, odour associated with the preen waxes may act as olfactory communication between birds or play a role in mate choice (Fluen 2008, Hirao et al. 2009, Zhang et al. 2010).

There are a number of hypotheses to explain seasonal changes in preen wax composition. One idea is that seasonal changes in preen wax are used to alter plumage coloration. Piersma et al. (1999) proposed the change in preen wax composition was a sexually-selected trait linked to brighter plumage needed for successful courtship. However, little evidence has been found to support this ‘make-up’ hypothesis. Neither plumage reflectance nor hue changes when covered with diester versus monoester waxes, and preen wax does not protect pigment or plumage colouration from sun bleaching (Reneerkens et al. 2007b, Reneerkens and Piersma 2004, Surmacki 2008). It has also been suggested that the change in preen wax composition could be related to changes in resistance to feather-degrading bacteria, which

may multiply quickly during the nesting period due to the added warmth and moisture trapped in a nest (Shawkey et al. 2003). However, no difference has been found in the amount of protection against bacterial degradation provided by diester compared to monoester waxes (Reneerkens et al. 2008).

For most birds, predation on nests is the single greatest limit on reproductive success, with many birds losing 50 to 90% of their nests to predation. Given the importance of predation as a selective pressure on the life history of birds, Reneerkens et al. (2002) proposed that the change from monoester to diester waxes could be an adaptation for olfactory crypsis at the nest. In this hypothesis, the larger molecule and lower volatility diesters produced in the breeding season would make it more difficult for predators using olfaction to locate prey. Indeed, a trained dog was less able to detect monoesters from red knots (*Calidris canutus*), than diester preen wax from the same species (Reneerkens et al. 2005). Wader nests are particularly vulnerable as they nest on the ground and the timing of the change in preen wax composition from small molecule to larger molecule esters is synchronised with the start of breeding and lasts until after incubation (Reneerkens et al. 2007b). In mallards the male plays no role in incubating the eggs or parental care. If the change in preen wax is an adaptation against nest predation, only the incubating sex in uni-parental care species would come under this pressure, where as both sexes should change preen waxes in bi-parental care species. Mallards fit this pattern, with only females changing preen wax composition. A recent study of wader species further confirms that both sexes in bi-parental care species changes wax composition, whereas only the incubating sex in uni-parental care species changes wax composition (Kolattukudy et al. 1987, Reneerkens et al. 2007a).

To further test the “cryptic olfactory hypothesis”, Fluen (2008) sampled a range of introduced and endemic passerines in New Zealand during breeding and non-breeding season. The eight species of continental birds introduced from Europe all exhibited a switch in preen wax

composition from small molecule monoesters during non-breeding season, to a high proportion of diester molecules during the 3 months of peak breeding condition. This is the same pattern seen in waders, mallards and other species examined elsewhere in the world. In contrast, among the 4 New Zealand endemic species tested, there was little or no change in preen wax composition between breeding and non-breeding seasons, with birds producing a high proportion of low molecular weight esters year-round. The exception to this rule was the male South Island robin, which produced more volatile preen wax compounds during breeding season, opposite to the pattern expected (Fluen 2008).

The New Zealand avifauna is particularly interesting for studying the evolution of preen wax composition, due to an evolutionary history with a complete lack of predatory terrestrial mammals. Birds on islands without predatory mammals, like New Zealand, evolved in the absence of the selective pressures associated with olfactory-searching predators. In contrast, birds in continental areas co-evolved with predatory mammals and would be expected to experience strong selective pressures to evolve ways of masking their odours and thus their detectability. One of these traits, may be the seasonal change in preen wax composition which may provide some degree of protection from predatory mammals using olfaction to locate nests. If the seasonal switching of preen waxes is an anti-predator defence strategy employed by birds, it is possible that New Zealand species either never developed this ability, or lost it after becoming isolated from mammalian predators. The fact that preen wax change to less volatile molecules does not occur in any of the New Zealand endemic species supports the theory of olfactory crypsis (Fluen 2008). However, it is not clear if the preen waxes of native birds are more readily detected by introduced mammalian predators.

In this chapter, I test whether a potential mammalian predator, the rat (*Rattus norvegicus*), is more likely to detect preen wax collected from a native New Zealand bird, than a continental

introduced species in breeding condition. Rats have been a major predator of passerines in New Zealand since their introduction in 1860 (Atkinson 1973). If rats are more likely to detect preen wax collected from a native New Zealand bird compared to a continental species, this could help explain why native birds appear so vulnerable to introduced mammalian predators.

### **3.3 Methods**

#### *Study site and species*

All preen wax samples were collected from birds nesting in Kowhai Bush, Kaikoura (173° 37'E, 42° 23'S) between October 2008 and January 2009. Kowhai Bush is a 240 ha low elevation forest with an open interior. The canopy is 5-12 m high, averaging 7 m, and composed mainly of Kanuka (*Leptospermum ericoides*) with an understory of small shrubs. For a more in-depth description of the physical characteristics and ecology of Kowhai Bush, see Hunt and Gill (1979).

Preen wax samples were taken from a variety of introduced and native New Zealand passerine species. Samples were collected from live adult birds caught with mist nets during the breeding season. The native species sampled were bellbird (*Anthornis melanura*), brown creeper (*Mohoua novaeseelandiae*) and rifleman (*Acanthisitta chloris*). The introduced species sampled were chaffinch (*Fringilla coelebs*), redpoll (*Carduelis flammea*) and silvereye (*Zosterops lateralis*). The silvereye (*Zosterops lateralis*) is often classed as a native species, but was self-introduced to New Zealand from Tasmania in the 19<sup>th</sup> century. For this reason it is classified as a continental species in this study as it evolved on a continent with mammalian predators, like the introduced species, and has only been in New Zealand for a short time. Chaffinch and redpoll first arrived in New Zealand around 1862, when a shipment

of English birds was imported to Nelson (Oliver 1930). They were subsequently released around several sites in New Zealand by acclimatization societies, and are now widely established.

### *Collection of preen waxes*

Small rounds (5 mm diameter) of filter paper were prepared before field collection, and stored in clean 0.6 ml eppendorf vials. These rounds were used to absorb preen wax samples, with each round being used to collect one sample. Rounds were cut using a whole punch to ensure the surface area over which wax was spread would be similar for all samples. The oils from the preen wax were found to spread over the entire area of the filter paper within a few hours. This ensured that the surface area covered in preen wax that was exposed to the air (which should affect how much preen wax will evaporate) was the same in all trials.

All preen wax samples were collected between October and January as this coincides with the breeding season for both the endemic and introduced continental species in the area. Breeding condition was confirmed in the hand by the presence of an engorged cloacal protuberance in the males (Wolfson 1952), and the presence of a brood patch in bi-parental species. I used only male samples in the trials, due to a lack of females. Birds were sexed according to plumage or angle/size of cloacal protuberance. All rifleman were caught near active nests of which they were parents and were known to be breeding at the time. Birds with undeveloped brood patches or small cloacal protuberances were not sampled as I could not be sure of their breeding status, and it is not known at what stage preen waxes change from monoesters to diesters in passerines.

Preen wax samples were collected by gently massaging the papilla of the uropygial gland with a pair of paraffin-tipped tweezers. This led to the excretion of a small amount of preen wax which was then absorbed onto a clean round of filter paper. Filter paper pieces were manipulated with a second pair of tweezers which was cleaned in ethanol between uses. Each filter paper containing a sample was placed in a coded airtight eppendorf vial and stored in a refrigerator at 4°C for up to 8 months before being used in tests. Each bird was marked with a metal band for individual identification before release to avoid re-sampling.

### *Analyses of samples using rats in a Y shaped maze*

Preen wax samples were presented to rats in a Y-shaped maze. To prepare samples for presentation, the outside of the eppendorf vials containing samples were first cleaned with alcohol to remove any contaminants, and handled only with latex gloves. Eppendorf vials were then placed in a water bath at 40°C for 3 minutes to simulate the normal temperature of a bird's body. The filter papers were positioned at the bottom of the vial. Because of the narrow end of the vials, this ensured that even when inverted, the filter paper did not fall out. After removal from the water bath, the lid was removed from the vial, and the sample was immediately used in a trial.

To test whether a rat was more likely to detect the preen wax of a native species compared to an introduced species, I paired preen wax samples from native and introduced bird's together for each Y-maze trial. Thus, I paired bellbird (native) with silvereye (introduced), brown creeper (native) with chaffinch (introduced) and rifleman (native) with redpoll (introduced). Bellbird and silvereye were paired together as they shared the greatest overlap in diet of any of the birds in the study. The other two pairs were arranged by weight, the larger introduced bird being paired with the larger native. A preen wax sample (contained in its original

ependorf vial) from one of each species per species pair was presented in each rat trial, one in each arm of a Y shaped maze (e.g., a bellbird sample in the left arm of the Y maze and a silvereeye sample in the right arm; figure 2.1.). Males were compared against males only, ensuring that differences between sexes was not a confounding factor. All the species used exhibit bi-parental care.

Presenting the preen wax sample at the bottom of an ependorf vial gave the rats an opportunity to handle each vial, but they were unable to extract it. Eight replicates of each trial was run, with a different rat used each time, and fresh preen wax samples collected from different individuals of the same species used in each replicate. Thus, I ran 8 replicates of bellbird vs. silvereeye trials, 8 replicates of brown creeper vs. chaffinch trials, and 8 replicates of rifleman vs. redpoll trials. I only had access to 8 rats however, and used the same 8 rats, one for each trial replicate, for all three ‘preen wax pair’ experiments. The order in which each rat received bird species was randomised to ensure that order of presentation did not influence the outcome. A video-camera was set up to record the movement of the rat in the maze, and the room was vacated for the duration of each trial to remove observer effects. Each arm of the maze was 46 cm long and the start box was 40 cm from the Y intersection. A trial began when the partition between the start box and the bottom of the Y maze was removed so that the rat was free to move anywhere within the maze. Each trial lasted 15 minutes. I alternated the arm (left or right) in which a native or introduced species sample was positioned in case one arm was favoured by the rats due to orientation in the room. All rats in the trials were female adult hooded rats, between 6 and 14 months old. Preen wax was a novel stimulus to all rats in these trials as none of the rats had ever come into contact with birds or preen waxes before these trials. Rats that participated in a trial were separated from rats that had not yet participated, to ensure no novel odours from preen waxes could be inadvertently introduced to rats before trials.

Video tapes were later transcribed. The number of visits to each arm was counted, and the amount of time spent in each arm during each visit recorded. The amount of time a rat spent in each arm of the maze in 5 minute blocks was recorded over 15 minutes and the total time spent in each arm during the 15 minutes was also calculated. The definition of a rat ‘visit’ to an arm of the maze was when the front half of its body was inside the edge of the black sleeve (Figure 2.1.). A rat sniffing in the entrance was not considered a visit, as the rat had to proceed two steps into the arm of the maze to be considered ‘in’ the arm of the maze. It is possible that a rat could have detected the smell of a preen wax sample without entering one arm of the maze by just sniffing, but I used the “two step” criteria as this was clearly visible on the video and indicated a stronger response to the stimuli. The number of seconds rats spent actively investigating each vial was recorded and compared between the two preen waxes. I defined ‘active investigation’ as any time a rat held a vial in its front paws, or the nose, teeth or whiskers were touching the vial.

Rats were chosen for this study as both ship rats (*Rattus rattus*) and Norwegian rats (*Rattus norvegicus*) are major predators of nests in the wild, and they use their acute sense of smell to locate prey items. Rats are also typically curious creatures and are likely to spend time investigating novel stimuli. Time data was used to assess the likelihood that a rat detected a particular vial, as the amount of time spent investigating novel stimuli is typically interpreted as indicative of how interested a rat is in the stimulus (Heth et al. 2000). Rats were deprived of food for 2 hours before trials to increase locomotion and investigation motivation (Heth et al. 1996).

All data was log transformed to ensure normality, and a paired t-test was used to examine any differences between the amounts of time rats spent in each arm of the maze arm. I used a critical value of 0.05.

### 3.4 Results

Rats spent significantly more time actively investigating (handling) bellbird preen wax than they did silvereye preen wax ( $t = 3.09$ ,  $df = 7$ ,  $P = 0.018$ ; Figure 2.2. a). Rats did not, however, spend a significantly different amount of time investigating brown creeper waxes compared to chaffinch waxes ( $t = 0.17$ ,  $df = 7$ ,  $P = 0.87$ ; Figure 2.2. b), nor was there any significant difference between the amount of time rats spent investigating rifleman compared to redpoll preen waxes ( $t = -1.55$ ,  $P = 0.17$ ; Figure 2.2. c).

There was no significant difference in the total amount of time per trial rats spent in the arm of the maze containing bellbird preen wax compared to silvereye preen wax ( $t = -0.13$ ,  $df = 7$ ,  $P = 0.90$ ). When separated into 5 minute blocks, the amount of time rats spent in each arm of the maze was not significantly different ( $P > 0.05$  for each 5 minute block; Figure 2.3.). Rats did not enter either arm of the maze significantly more times than the other in the bellbird and silvereye trials ( $t = -0.78$ ,  $df = 7$ ,  $P = 0.46$ ).

In the brown creeper and chaffinch trials, there was no significant difference between the total amount of time rats spent in either arm of the maze ( $t = 0.41$ ,  $df = 7$ ,  $P = 0.69$ ), nor did this change when time was separated into 5 minute blocks. The amount of time rats spent in each arm of the maze was not significantly different ( $P > 0.05$  for each 5 minute block; Figure 2.3.). There was no significant difference between the number of times rats entered the arm containing brown creeper preen wax compared to the arm containing chaffinch preen wax ( $t = 0.07$ ,  $df = 7$ ,  $P = 0.95$ ).

In the rifleman and redpoll trials, there was no significant difference between the total amount of time rats spent in either arm of the maze ( $t = -1.44$ ,  $df = 7$ ,  $P = 0.19$ ). There was also no significant difference between the amount of time rats spent in each five minute block

in each arm of the maze ( $P > 0.005$  for each 5 minute block; Figure 2.3.). No significant difference between the number of times rats entered the arm containing rifleman preen wax compared to the arm containing redpoll preen wax could be found ( $t = -0.28$ ,  $df = 7$ ,  $P = 0.79$ )

### **3.5 Discussion**

Contrary to what was expected, rats did not appear to discriminate between preen wax samples of native New Zealand and continental birds, except in the trial comparing bellbirds to silvereyes. Neither the samples of preen wax from rifleman or brown creepers (two native species) were handled more by rats, nor did rats spend more time in the arms of the maze containing the samples of these species over that of the introduced chaffinch and redpoll. Thus, the evidence that native birds might be more vulnerable to introduced mammalian predators than introduced species because of their more volatile preen waxes was only weakly supported.

An increased amount of time spent in investigating an object or scent mark is typically interpreted as the object or scent being more attractive to rodents (Heth et al. 2000). Although rats spent more time investigating bellbird preen wax than silvereye preen wax, this does not necessarily mean that this puts bellbirds at greater risk of predation than silvereyes. Likewise, I cannot completely rule out the lack of any effect of preen wax composition on the predation risk of brown creepers and rifleman relative to other introduced species. Putting my lab results into a field context is difficult as I have assumed that the propensity of a rat to handle or explore one arm of the maze equates to a greater ability to detect the nest of that species in the field, and in turn, lead to a greater rate of nest predation. Clearly, these assumptions need to be tested thoroughly before I can rule out no effect of preen wax composition on predation

risk and whether it varies in a systematic fashion between native and introduced species of birds.

One potential problem with my experimental design is whether or not the Y-maze actually gives rats two choices. For example, if the volatility of the preen waxes is particularly high, then it is possible that the entire air space of the maze was filled with the odour of both test species (native and introduced), and thus a rat would not detect any difference between the two arms. Alternatively, the volatility of the preen waxes may have been particularly low, such that neither odours was detected by the rat at the start of the maze (i.e. 46 cm from the vials) and could not be detected until the rat was at a very close distance. Determining the spread of odours through the airspace was beyond the scope of my study, but such work is needed to ensure the proper interpretation of any choice test in a Y-maze.

In all my trials, I used lab-bred rats that had no prior experience with either wild birds nor their nests or preen waxes. Thus, I made the assumption that rats have some form of natural attraction to the preen waxes or at least an inclination to explore novel odours than are more readily detected (i.e., explore the arm of the maze with the preen wax of the native bird if it is indeed more easily detected). On the other hand, if I had used rats captured from the wild, then I would be unsure if any preference for one type of preen wax over another was due to detection differences or differences in experience. For example, it is possible that wild rats may have learned to associate the odour of a particular preen wax with food items. If they are more successful at finding the nests of native birds through their odour (as proposed in this chapter), this could reinforce their preference for these species in a maze, and bias any lab test. It is interesting that only bellbird preen waxes were more attractive than the paired continental species in my study, not rifleman or brown creeper preen waxes. Perhaps this indicates some pre-existing bias in detection ability by rats or else maybe even a prior

association of the odour of bellbirds with a similar odour associated with food. As rats learn to associate and identify bird odours with food rewards however, the distance at which rats are able to detect nests becomes more important than how inherently attractive something smells.

While my results do not support the hypothesis that New Zealand birds are more easily detected by olfactory searching predators than continental birds during breeding season, this does not negate the hypothesis. These results show that rats have a preference for the smell of bellbirds, but not rifleman or brown creeper, which also suffer high nest predation rates.

This does not exclude the possibility that rats can smell New Zealand birds from further away than from continental species. Reneerkens et al. (2005) suggested that the smell of preen waxes and thus detection chance would decrease as the distance between the predator and the source increases. At a certain distance from the source, the smell of preen wax would have diminished to the point that it could no longer be detected by the predator. This critical distance was expected to be further away from birds producing monoester rather than diester preen waxes. Further trials are needed to investigate how far away a predator must be before the critical distance is reached for native and introduced continental birds, and whether this is different between native and introduced species of birds. New Zealand birds often suffer higher rates of nest predation than introduced species (Duncan and Blackburn 2004, Innes et al. 2010), and it is possible that lack of seasonal change in preen wax composition is one of the factors contributing to this fact.

Male budgerigars (*Melopsittacus undulatus*) have been found to secrete similar alkanol blends in their preen wax as found in female budgerigars, but these volatile components are four times more volatile and found in significantly greater amounts than in females (Zhang et al. 2010). Female budgerigars were able to distinguish between female and male body odour

using a Y maze choice test set up. Female birds exhibited a marked preference for the odour of male preen wax, as well as a synthetic 3-alkanol blend (the three most volatile compounds found in male budgerigar preen wax) over the odour of female or female and male mixed preen wax. The data obtained in this study isolated compounds in male preen wax secretions, and suggested that are likely to act as male pheromone or female attractant odour in budgerigars (Zhang et al. 2010).

Other studies examining smell preferences or scent differentiation in birds use similar maze choice test set ups to that used in this study. Like Zhang et al. (2010) these studies met with a reasonable level of success in terms of different behaviours performed by the subject when in close proximity to different preen wax samples, or other substances (Hagelin et al. 2003). This is the first study however, that presents different preen wax samples to rats within the confines of a Y maze set up, and it is apparent from this study that this procedure needs some further work.

The functions of seasonal fluctuations in preen wax secretions is a study that is still very much in its infancy. Future studies should focus on how general the change from monoester to diester preen wax is across a range of birds, especially in other island species that have evolved without mammalian predators. Field studies would also serve to reveal whether predation rates in the wild are higher at nests with monoesters present compared to those with diesters.

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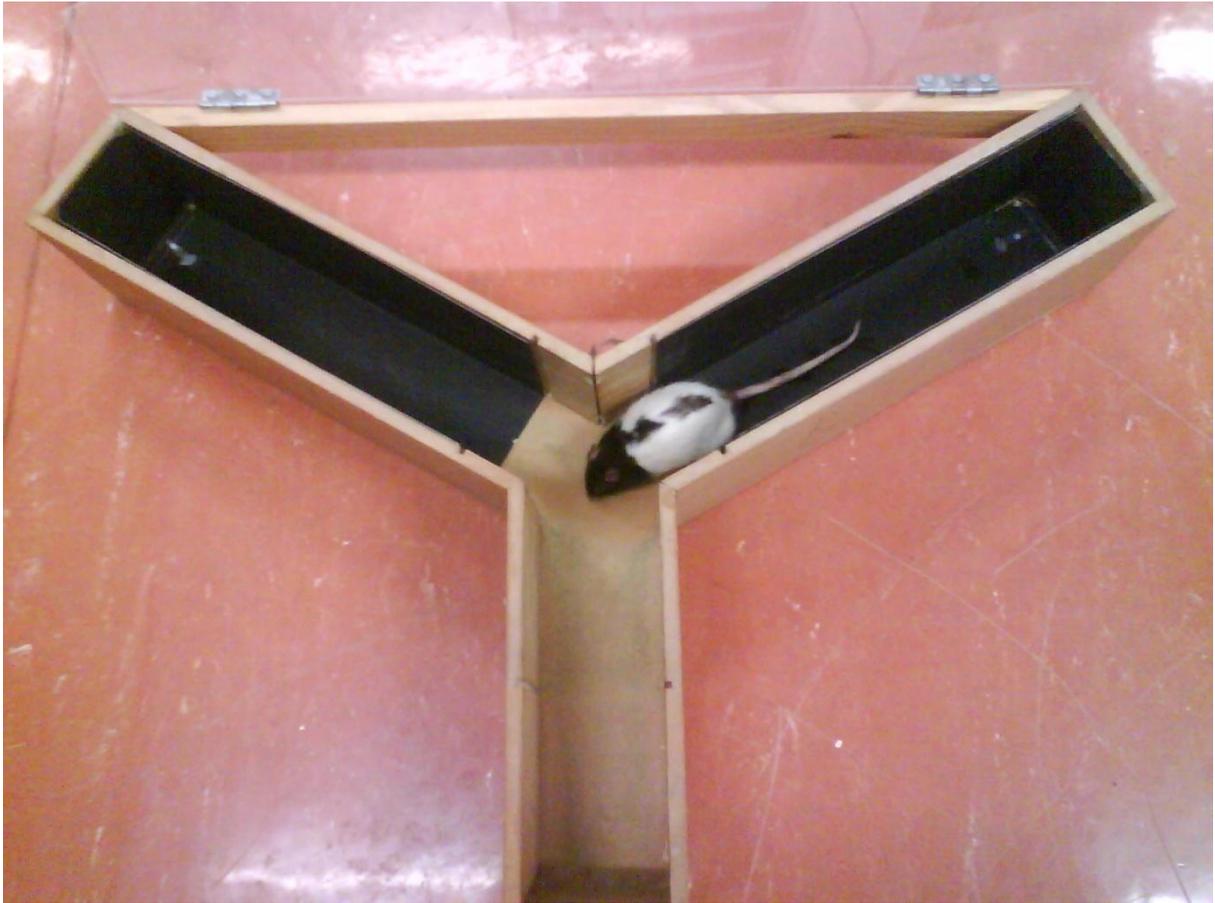
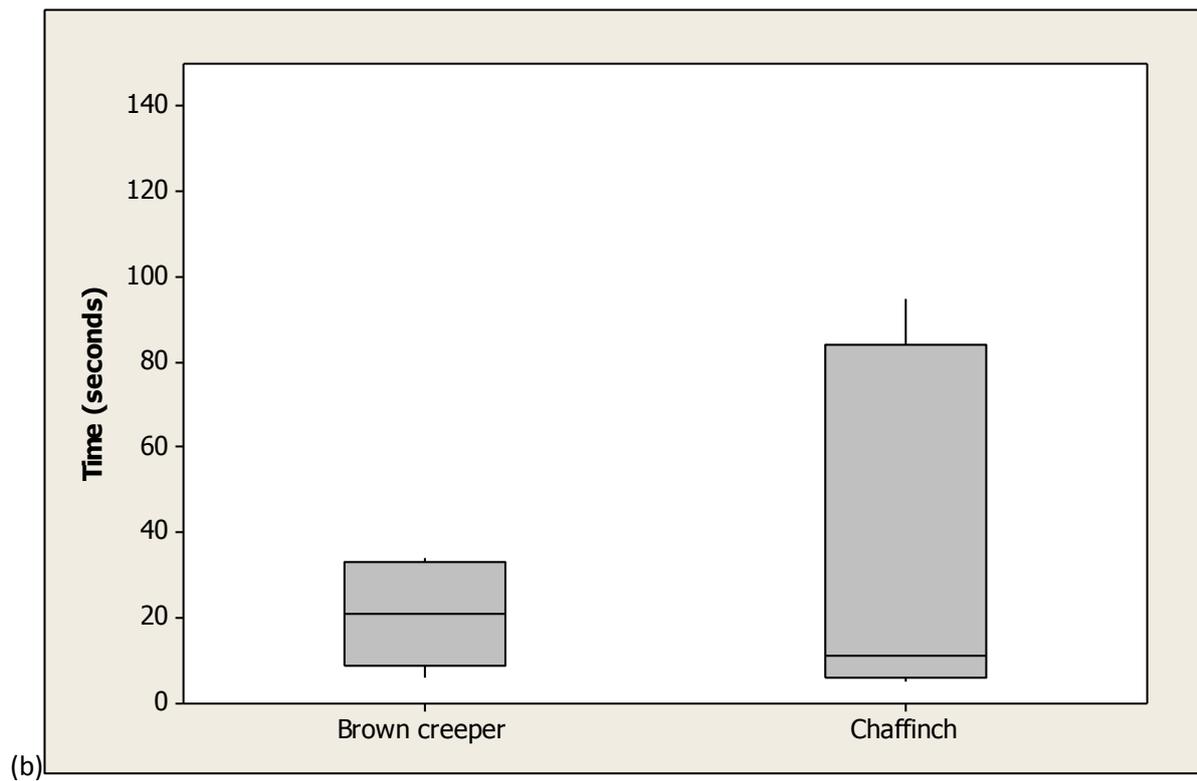
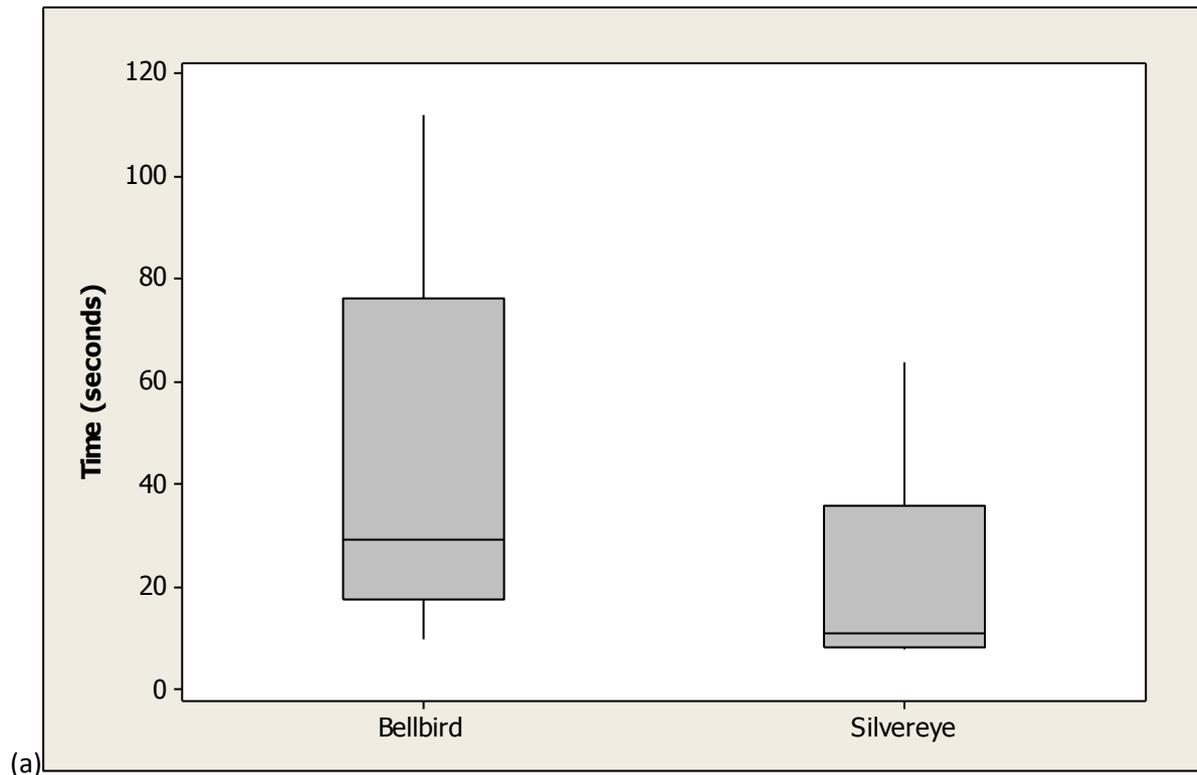


Figure 3.1. Y maze set up with a bellbird wax sample in the left arm, and a silvereeye wax sample in the right.



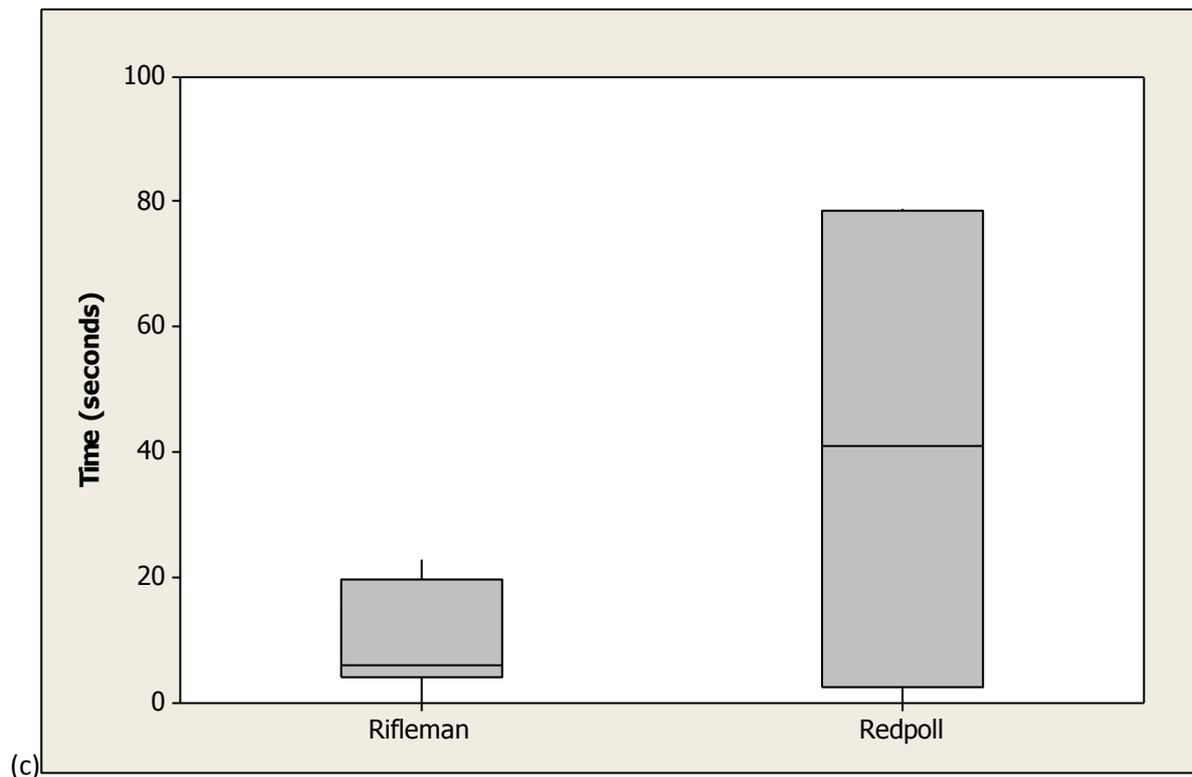
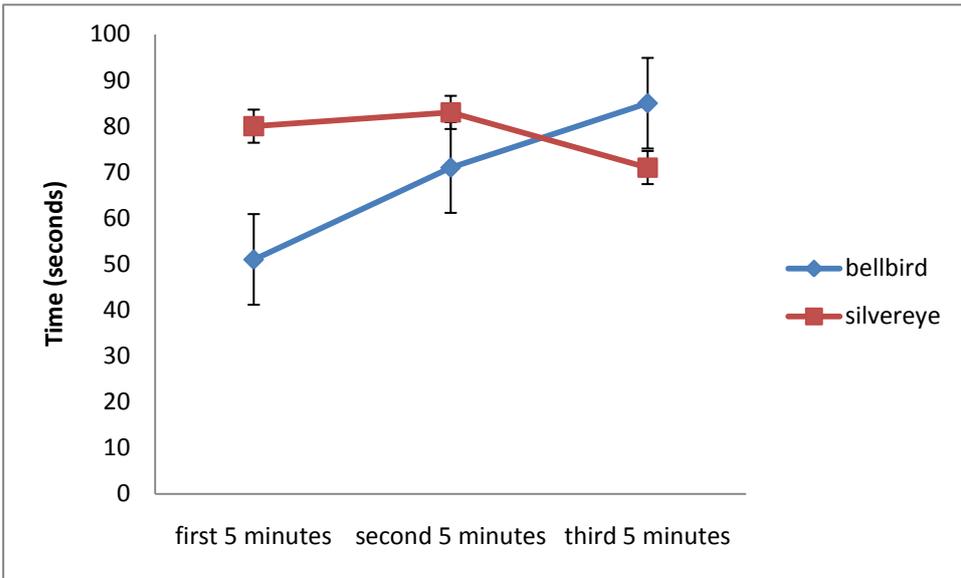
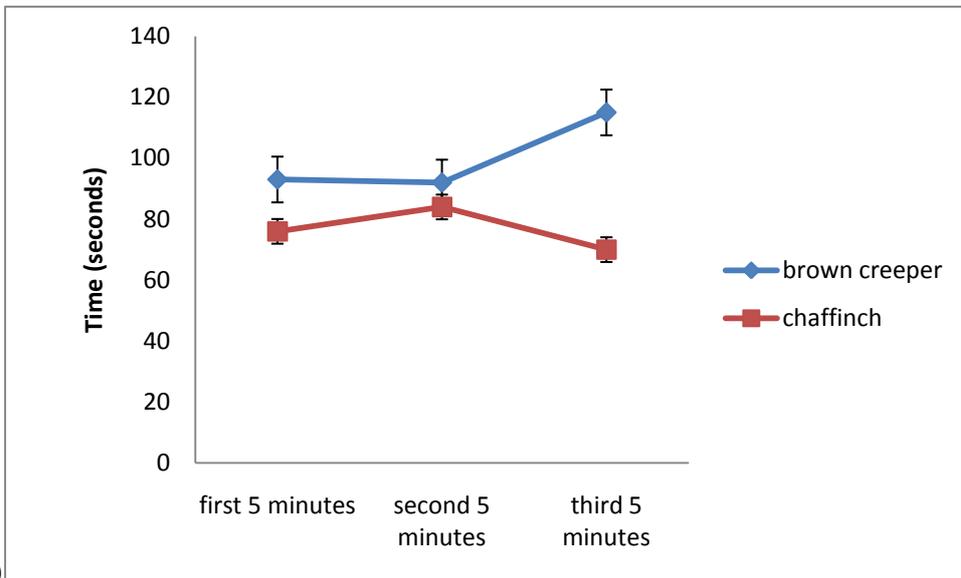


Figure 3.2. Box plots comparing the amount of time in seconds that rats investigated (handled) vials containing samples of New Zealand bird preen waxes compared to vials containing samples of introduced bird preen waxes (n=8). (a) Number of seconds rats investigated bellbird versus silvereye wax samples. (b) Number of seconds rats investigated brown creeper versus chaffinch wax samples. (c) Number of seconds rats investigated rifleman versus redpoll wax samples. Plots are mean, 25<sup>th</sup> and 75<sup>th</sup> percentiles, with vertical lines showing 90<sup>th</sup> percentiles.



(a)



(b)

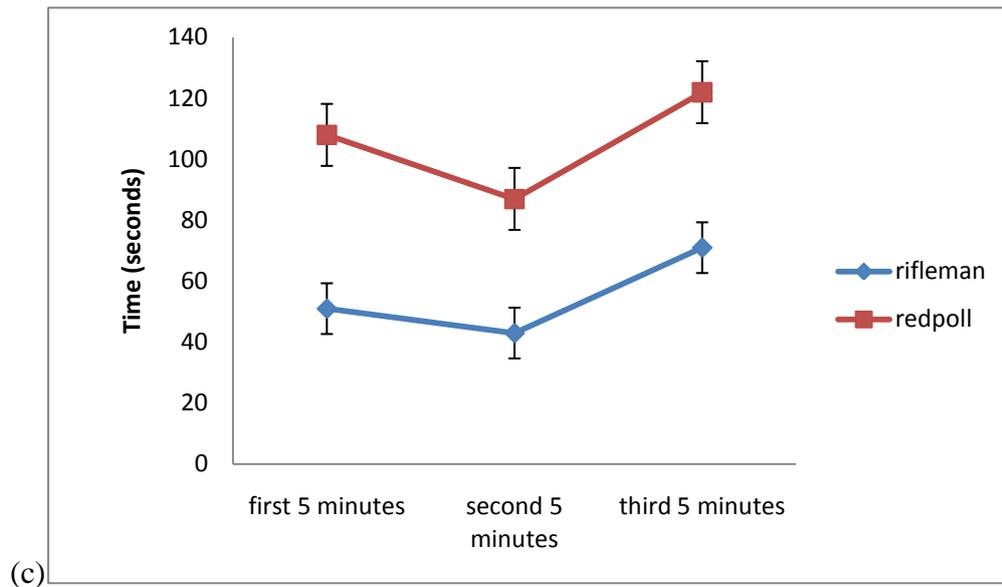


Figure 3.3. Line graphs showing change over time in the average number of seconds rats spent in each maze arm containing either New Zealand bird preen wax or continental introduced bird preen wax per trial. (a) Number of seconds on average rats spent in the bellbird and silvereye arms. (b) Number of seconds on average rats spent in the brown creeper and chaffinch arms. (c) Number of seconds on average rats spent in the rifleman and redpoll arms.

## Chapter 4

# Do starlings (*Sturnus vulgaris*) repel nest predators with odour?

### 4.1 Abstract

Nest predation is the largest contributing factor to bird mortality in most species. This puts strong selection pressures on birds to reduce the conspicuousness of nests, and a variety of behavioural and morphological traits have evolved which appear to reduce the chance of predators finding the nest. Most adaptations to nest predation function by lowering the visual, auditory and olfactory cues associated with the nest (i.e., crypsis) however it is also possible that some species may increase the conspicuousness of signals at the nest in order to directly deter predators. I investigated if the smell associated with active European starling (*Sturnus vulgaris*) nests, which is conspicuous to humans, could function as a deterrent to rats (*Rattus norvegicus*), a major predator on bird nests. Using a Y-maze test, I found that rats avoided nest material taken from active starling nests, but did not avoid the raw materials similar to those used by starlings in nest building. Although further work is needed to identify the chemical nature of the strong smell and to test whether it functions in limiting nest predation at real nests, my results suggest that the odour associated with active starling nests may function as a predator deterrent. Perhaps this form of predator deterrent contributes to their success in avoiding predation.

## 4.2 Introduction

Nest predation is the most important source of reproductive failure in many bird species, and thus acts as a powerful selection pressure influencing the evolution of their life history traits (Kleindorfer et al. 2005, Lima 2009, Thompson 2007). Nest predation has been shown to influence the evolution of traits such as the length of incubation periods (Remeš and Martin 2002), the structure of nestling vocalisations (Briskie et al. 1999), nest site selection (Kleindorfer et al. 2005), and the choice of nest materials (Schuetz 2005). For example, most birds build a nest to protect their eggs and nestlings. The materials selected by birds and incorporated into the nest may function not only to keep young warm but also to provide visual camouflage, making a nest less conspicuous and thus less likely to be detected by predators (Schuetz 2005). Similarly, the evolution of short incubation and nestling periods may reduce predation risk by minimising the time young are vulnerable to predation in the nest (Remeš and Martin 2002), while the evolution of inconspicuous begging vocalisations may function to reduce the risk of attracting a predator to the nest through auditory cues (Briskie et al. 1999).

In some species, camouflage of nests or young may not be possible, as birds may be constrained in nest placement or in the materials used for nest construction. Instead, a variety of deterrent adaptations appeared to have evolved to minimise the risk of nest predation. In other words, rather than selection favouring nest crypsis, in some species, adaptations have evolved to defend nests from the approach of predators. For example, white-breasted nuthatches (*Sitta carolinensis*) nest in tree cavities and are prone to predation by the red squirrel (*Tamiasciurus hudsonicus*) (Kilham 1968). To deter squirrels from approaching the nest, nuthatches employ a ‘sweeping’ behaviour, which entails smearing insects and sticky plant matter around the opening of the nest cavity, as well as on perches around and below

the nest entrance (Kilham 1968). Squirrels are reluctant to contact these sticky surfaces and thus approach nests. Similarly, the great crested flycatcher (*Myiarchus crinitus*) adds pieces of sloughed snake skin in and around its cavity nest. The presence of snake skins has been shown to decrease nest predation, especially by the southern flying squirrel (*Glaucomys volans*), presumably as snakes are potential predators of squirrels (Medlin and Risch 2006). Borgo et al. (2006) demonstrated that even snake scent alone was sufficient to deter southern flying squirrels from nest boxes. In some cases, birds may place their nests in situations where they are defended by other species. Some parrots nest in active termite mounds (Brightsmith 2000). While some termites may have a bite that is a direct deterrent against potential predators, the odour excreted by the insects can be enough to mask any odours of the bird, reducing the risk of detection from nocturnal predators relying on smell (Brightsmith 2000).

Removal of nestling faeces is a common parental behaviour, with parents either eating or removing faecal sacs from around the nest (Lang et al. 2002, Weatherhead 1984, Weitzel 2003, 2005). Removal of faeces is likely to be beneficial as it keeps the nest clean and dry, and discourages parasites and pathogens (Blair and Tucker 1941). In some species, parents go to significant lengths to move faecal sacs far away from nests in patterns that are consistent with the theory that nestling faeces attracts predators (Bulit et al. 2008, Lang et al. 2002, Weatherhead 1984, Weitzel 2005). However, in other species, faeces instead appear to be used to deter potential predators and act as a mechanism of nest defence. Both burrowing owls (*Athene cunicularia*) and common waxbills (*Estrilda astrild*) use mammalian faeces in nest construction. Burrowing owls scatter faeces from grazing animals around the burrow entrance, which may both, conceal the natural odours of an active owl nest, as well as attract arthropod prey (Smith and Conway 2007). Common waxbills build tightly woven grass nests with a side entrance but also incorporate carnivore faeces into and around the nest, to which

fresh faeces are added at intervals while the nest is active (Schuetz 2005). Schuetz (2005) found that artificial nests incorporating carnivore faeces in and around the nest structure enjoyed a lower rate of predation than those without carnivore faeces. It is likely that this is a case of either an olfactory deterrent or camouflage.

European Starlings (*Sturnus vulgaris*) are cavity nesting birds that construct “messy” cup-shaped nests of straw, grass and twigs within a small tree- or rock cavity. As with other small and medium-sized cavity nesting species, starlings are subject to nest predation by mammalian predators such as rats and mustelids (Bull and Flux 2006). However, starlings are unusual in that their nests accumulate large numbers of faecal sacs within 7 days of hatching, despite removal of some faeces by the adults. As a result, the nest begins to smell like ammonia, a feature that can be detected outside the nest entrance by humans (pers. obs.). As the nestlings continue to develop, the nest interior becomes increasingly smelly, and by the time starlings are fledging, the smell is so strong it can be detected by humans standing below the cavity opening. Such smells do not seem consistent with a strategy of reducing odours that might attract predators. Instead, it is possible that the ammonia-like odours produced by nestling starlings may function as a deterrent. Thus the aim of this study was to investigate if the strong smell associated with starling nestlings acts as a chemical deterrent to rats, a major predator of starlings in New Zealand (Bull and Flux 2006).

## **4.3 Methods**

### ***Study site and species***

Starlings were introduced to New Zealand in 1862, when a shipment of English birds was imported to Nelson (Oliver 1930). They were subsequently released in several sites in New

Zealand by acclimatization societies, and have since benefited by the clearing of forests to create pasture. Starlings are widespread around New Zealand, living in both cities, where they nest in cavities in roof structures or walls of buildings, and in rural areas. Starling nestlings are altricial, with both parents feeding the nestlings for around 23 days until fledging. Starlings eat invertebrates and fruits and are regarded as a pest species due to the damage they can do to a crop. Starlings in New Zealand breed between October and December and lay a round average of 5 eggs per clutch (Bull and Flux 2006).

I studied starlings nesting along the edges of Kowhai Bush, Kaikoura (173° 37'E, 42° 23'S). Kowhai Bush is a 240 ha native forest with an open interior. The canopy is 5-12 m high, and composed mainly of Kanuka (*Leptospermum ericoides*), with an understory of small shrubs. For a more detailed description of the physical characteristics and ecology of Kowhai Bush, see Hunt and Gill (1979). As starlings are cavity nesters, twenty artificial nest boxes were put up around the edge of the forest facing onto the adjacent grazed pastures. The pastures are favoured by starlings for foraging. Starlings readily used the nest boxes as well as natural cavities around the forest edge.

### ***Collection of starling nest samples***

The nest materials used in this study were collected by myself, from birds nesting on the edge of Kowhai Bush, Kaikoura between November 2009 and January 2010. Four nests were in nest boxes, and three were in natural tree cavities. Natural nests were located by following the sound of begging nestlings. The nest boxes were checked regularly to monitor nest building. Nests were then checked every few days to monitor their progress. When the nestlings were 20 days old, about 18 g of nesting material was removed from the side of each nest. I collected only nest material from the top 4 cm of the nest. Nest material was removed

with the use of latex gloves to prevent the transfer of human scent, and samples were immediately transferred to individually labelled clean plastic bags. All nest samples were collected when nestlings were within 3 days of fledging. This ensured that nests were holding nestlings at the same stage of development. All trials with rats were conducted within 3 days of collection (see below). Samples not tested on the same day of collection were stored in a 4° C fridge until used. Stored samples were brought to room temperature before testing. For control trials, I collected an equivalent weight of dried grass, kanuka twigs and hay from near the nests (hereafter referred to as control nest material). These were similar to the materials used by starlings in their nests.

#### *Analyses of samples using rats in a Y shaped maze*

To test whether the odour of starling nests repelled a potential predator, I exposed rats to a choice test in which they had the opportunity to select between the starling nest material and a control. In each choice trial, a sample of starling nest material was placed in the end of one arm of a Y-shaped maze (figure 4.1). The other arm of the maze was left empty as a control. After the nest material was in place, I immediately introduced the test rat at the bottom of the Y-maze. A video-camera was set up above the maze to record the movements of the rat. The room containing the Y-maze and camera was vacated for the duration of each trial to reduce any effects of my presence. Each arm of the maze was 46 cm long, and the start box was 40 cm from the Y intersection. A trial began when the partition between the start box and the bottom of the Y maze was removed so that the rat was free to move anywhere within the maze. Each trial lasted 15 minutes. Seven test trials were conducted, with a different rat and a different starling nest sample in each trial. All rats were female adult hooded rats, and were approximately 14 months old. I alternated the arm in which nest material was placed in case

one arm was favoured by the rats due to orientation in the room. The maze was cleaned between trials by washing thoroughly with soap. None of the rats had ever come into contact with bird nests before, but they were familiar with the maze previous to the trials. Rats were deprived of food for two hours before trials to increase locomotion and investigation motivation.

To determine whether the presence of the nest material itself might have affected the choice of one arm over the other in the Y-maze, I conducted a second set of trials in which I gave rats a choice between control nest material in one arm and no material in the other arm. Seven control trials with seven different female hooded rats were then conducted in the same manner as above. I alternated arms with the control nest material and washed mazes between each trial.

After each trial was completed, I transcribed the videotapes to record the number of visits to each arm by the rat, and the amount of time each rat spent in each arm during each visit. The amount of time a rat spent in each arm of the maze over the 15 minutes was recorded, and I also calculated the total time spent in each arm in each successive 5 minute block to assess any temporal patterns in arm visitation. I defined a rat 'visit' to an arm of the maze as occurring when the back edge of the 'hood' of a rat crossed the edge of the black sleeve of the maze (figure 4.1). A rat sniffing in the entrance of an arm was not considered a visit, as the rat had to proceed at least two steps into the arm to be considered 'in' the arm of the maze. It is possible that a rat could have detected the smell of nesting material without entering one arm of the maze by just sniffing, but I used the "two step" criteria as this was clearly visible on the video and indicated a stronger response to the stimuli. The number of seconds rats spent investigating nesting material was recorded and compared between manipulation and control trials. I defined 'investigation' as occurring when a rat's front paws, nose or teeth touched the nest material.

Lab rats (*Rattus norvegicus*) were chosen for this study as ship rats (*R. rattus*) and Norwegian rats (*R. norvegicus*) are major predators of bird nests, including starlings, and are known to have an acute sense of smell which is used to locate prey items (Rajan et al. 2006). Rats are known to spend more time investigating novel stimuli, than a familiar empty chamber (Cowan 1977). Thus, I predicted the rats in my tests should spend more time in a chamber with nest material than in an empty chamber, unless such a chamber also contains a negative stimulus such as a deterrent odour. Any difference in time spent with starling nest material and control nest material should therefore reflect the deterrent properties of the former. The duration spent in each arm of the maze was used to measure interest by the rat, as the amount of time spent investigating novel stimuli is typically interpreted as indicative of the level of interest a rat has in the stimulus (Heth et al. 2000, Prud'homme et al. 2009).

As not all data was normally distributed, I used a Mann-Whitney test to examine differences between the amounts of time rats spent in the maze arm with control or starling nest material. All tests had a critical value of 0.05.

#### **4.4 Results**

Rats spent significantly more time investigating (handling) the control material than the starling nest material (Figure 4.2;  $W = 77.0$ ,  $P = 0.002$ ). The total amount of time rats spent within the maze arm containing control nest material was also significantly greater than the total amount of time spent in the arm containing starling nest material (Figure 4.3;  $W = 75.0$ ,  $P = 0.005$ ). The number of times rats entered the maze arm containing control material and the arm containing starling nest material was significantly different ( $W = 75.5$ ,  $P = 0.004$ ). Rats made significantly fewer visits to the arm containing the starling nest material (Figure 4.4).

Rats spent significantly more time in the empty arm of the maze than the one containing starling nest material ( $W = 33.0, P = 0.02$ ). The opposite was true of the control material trials where rats spent significantly more time in the arm of the maze containing control material than in the empty arm ( $W = 72.0, P = 0.02$ ). The avoidance of starling nest material differed over the 15 minute course of the trials (Figure 4.5). When I examined each 5 minute block separately, the mean time rats spent in the arm of the maze containing control nest material ( $100.6 \pm 9.4$  sec) was greater than that spent in the arm containing starling nest material ( $63.4 \pm 13.9$  sec), but this difference was not quite significant ( $W = 67.0, P = 0.074$ ). In the second 5 minute block there was no significant difference between the time rats spent in the arm containing control nest material ( $103.3 \pm 13.8$  sec) compared to the arm containing starling nest material ( $80.0 \pm 20.6$  sec;  $W = 63.0, P = 0.20$ ). However, in the third 5 minute block, rats spent significantly more time in the arm containing control nest material ( $124.0 \pm 22.1$  sec) than in the arm containing starling nest material ( $15.1 \pm 5.8$  sec;  $W = 73.0, P = 0.011$ ).

Six of 7 rats entered the maze arm containing control nest material the first time they reached the Y intersection. In contrast, only 2/7 rats entered the maze arm containing starling nest material the first time they reached the Y intersection. This difference was significant (Fisher exact test:  $P = 0.039$ ).

## 4.5 Discussion

I found that rats spent more time touching and sniffing the control nest material than they did the starling nest material. Rats also entered and explored the arm of the maze containing control nest material a greater number of times than the one holding the starling nest material. This meant the rats in the starling nest trials spent more time in the empty arm and start column of the maze, despite the lack of apparent stimuli. Exploratory behaviour is common in rats and should lead it to spend time investigating new stimuli (Ennaceur and Delacour 1988) (in this case nest material), yet rats avoided the starling nest material by making few visits to the arm in which it was held. An increased amount of time spent in investigating an object or scent mark is typically interpreted as the object or scent being more attractive to the subject (Heth et al. 2000). Rats showed clear discrimination between the two types of nest materials spending more time investigating control material than starling nest material, despite the fact that they were very similar in composition apart from the presence of faecal material in the starling nest material. These results can be interpreted as avoidance behaviour, and suggest that rats are repelled by starling nest material, and perhaps are less likely to investigate starling nests compared to clean dry nest material. If wild rats also use smell to avoid starling nests, this could reduce the predation risk to nests with accumulations of faecal material. This supports the hypothesis that the strong ammonia-like smell of an active starling nest at nestling stage may lower the risk to nestlings by predatory mammals.

The identity of the compound or compounds rats avoided in the starling nest trials was outside the scope of this experiment, but it seems likely to be contained within the nestling faeces. This is because the most obvious difference between the nesting materials used in the control and treatment trials was the presence of nestling faecal material in the starling nests, but further tests are needed to confirm this. Other possible sources of smell could be oils and

secretions from the skin, preen wax and other body fluids such as saliva, or food remains. Food remains were not visible in the nest material I collected and it is unlikely that preen wax or other skin secretions were present in any great amount on the material used in the Y-maze trials. This is because the samples were collected from the side of the nest where nestlings deposit faecal sacs, while the nestlings spend the majority of their time in the middle of the cup nest. A strong ammonia-like smell did emanate from the faecal matter around the edge of the nest (pers. obs.), but it is unclear whether fresh or damp decaying faecal matter (both present in quantity) was responsible, or whether the odour of the faeces alone was responsible for the avoidance of starling nest material. Faeces of other nestling birds (e.g. song thrush, rifleman) did not smell of ammonia (pers. obs.) and it is possible that this odour was produced specifically by nestling in their faeces in order to deter potential predators.

Nestling faeces in most passerine birds are produced within a mucus membrane, thereby allowing parents to handle 'faecal sacs' for removal. Faecal sac removal by parents is a widespread behaviour across passerine species (Dell'Omo et al. 1998, Lang et al. 2002, Weatherhead 1984). Parents may remove faecal sacs from the nest area by either picking up faecal sacs and swallowing them, or flying off with them to drop away from the nest. While no studies have tested as yet if predation rates rise when faecal sacs are not removed, the nest sanitation behaviour of birds is consistent with the predator cue reduction hypothesis. The predator cue reduction hypothesis is that parents remove nestling faeces in an attempt to reduce cues predators use to locate active nests when hunting (Weatherhead 1984).

The faecal sacs of starling nestlings appear visually similar to other birds. Like other birds, starlings are also known to remove faecal sacs from the nest cavity, but at a rate that decreases as nestling age increases (Wright and Cuthill 1989). No studies have yet compared rates of faecal sac removal between species, but it seems likely that starlings remove a lower proportion of faecal sacs from the nest than other species given the gradual accumulation of

faeces in their nests. For example, no faeces were observed in the nests of rifleman or song thrushes that I studied in chapters 2 and 3, yet more than a dozen (often more) faecal sacs were present in the nests of starlings. More study is needed on the pattern of faeces accumulation in starling nests, and when adults start reducing faecal sac removal.

If the accumulation of faeces in starling nests functions in predator deterrence, this behaviour can also have a number of costs. For example, increased faeces in the nest is associated with increased rates of parasites and pathogens which can affect growth or health (Blair and Tucker 1941, Herrick 1900). Recent studies demonstrate that fresh green volatile plants that are sometimes used by starlings in nest construction, function not to reduce ectoparasites, but instead increases a nestling's immune system. This could effectively counter the negative impacts of ectoparasites and bacteria on nestling growth due to increased faeces in the nest (Gwinner et al. 2000, Mennerat et al. 2009). If starling parents exhibit lower rates of faecal sac removal than other birds, the use of volatile herb plants may help reduce the negative effect of increased parasite and pathogen rates in nestlings.

The results of this study suggests that the ammonia-like odour of starling nest material, possibly resulting from the smell of nestling faeces, may be the mechanism that reduces nest predation.

Recent studies of preen wax (oils and fats secreted from a specialized 'uropygial' gland and applied by birds to waterproof feathers) composition have found that a range of continental species change their uropygial gland secretions to less volatile compounds during the breeding season in order to make them less detectable by predators using olfactory cue to locate their prey (Fluen 2008, Reneerkens et al. 2002). There is some evidence to suggest this is a form of 'olfactory crypsis' reducing the amount of smell present at a nest, thus reducing the rate of nest predation by olfactory searching predators (Reneerkens et al. 2005). The

strong smell of ammonia from starling nests would thus seem to counter this tendency, as it is difficult to understand how such a strong smell could ever act in a cryptic function. Instead, such a strong and distinctive smell likely evolved for some other function.

There are a few exceptions to the cryptic nature of preen wax odours, and some birds produce preen wax secretions which are highly volatile and may even increase in volatility during breeding season. One of these is the Eurasian Hoopoe (*Upupa epops*); preen wax secreted by females and their young has been reported as darker in colour, and to possess a strong smell during the breeding season, similar to the smell of rotting meat (Del Hoyo et al. 2006). A close relative, the Green Woodhoopoe (*Phoenicurus purpureus*), produces a dark drop of foul-smelling secretion which it presents towards a predator when threatened (Burger et al. 2004). Both species are obligate cavity roosters, and when roosting are particularly vulnerable to a range of predators including snakes and rats (Ligon and Ligon 1978). Some of the volatile compounds in this pungent preen wax have been found to be effective against feline predators and lizards, supporting the theory that the volatile components of these secretions function as a chemical deterrent to predators (Burger et al. 2004). If a strong distasteful odour can deter predators from predating cavities of roosting or nesting hoopoe, perhaps the strong ammonia-like smell of starling nests may function similarly.

Starling are not the only species in which the use of faeces has been suggested to deter nest predators. Incubating eiders (*Somateria mollissima*) and shovelers (*Anas clypeata*) have been observed to excrete watery faeces over their eggs when startled at the nest, before running away (Swennen 1968). This was described by early ornithologists as a possible nest defence measure, as the ducks took care to defecate elsewhere when leaving the nest spontaneously. Swennen (1968) used experimental trials to demonstrate that ferrets and rats, common nest predators, showed considerable reluctance to eat food after it had been contaminated by fresh

faeces collected from eiders during the breeding season. Yet ferrets and rats did not discriminate between uncontaminated food and that contaminated with faeces from non-breeding eiders or from other species such as pheasant (*Phasianus colchicus*), house sparrow (*Passer domesticus*) or black-headed gulls (*Larus ridibundus*).

Deliberate use of faecal pellets in the construction of a nest has been shown to reduce predation in one other bird species. Wild waxbills commonly use carnivore scat in nest construction, and continue to replace dry old faecal pellets with fresh new ones through out the period of activity at the nest. Schuetz (2005) found that false waxbill nests in which carnivore scat was used in the construction both outside and inside nests, suffered a significantly lower predation rate than those without. The carnivore scat used by waxbills came from cats, and may function to deter rodent predators (preyed upon by cats) from hanging around nests. It may also mask the natural smell of the birds and eggs in the nest presenting a novel form of olfactory crypsis.

Use of chemicals as a predator deterrent is particularly common in invertebrate species (Pasteels et al. 1983). This is often taste or toxin related, but there are also instances where species appear to invest more in repulsiveness of odours rather than toxicity (Idowu 1997). Mammals have also been documented using chemicals as a possible predator deterrent. Field rats (*Rattus Ratoides*) apply anal secretions from weasels to their fur as a form of olfactory crypsis (Xu et al. 1995). For a similar reason, squirrels and chipmunks apply rattlesnake scent to their fur (Kobayashi and Watanabe 1986, Xu et al. 1995). Use of odour as a predator deterrent is common among a number of classes of animals, but it is an area of study that has been neglected in the bird world.

Birds are not a class commonly thought of as using chemical defences, yet three passerine species in the genus *Pitohui* produce homobatrachotoxin, concentrated in the skin and

feathers of the birds rendering them poisonous and unpalatable to predators (Dumbacher et al. 1992). Seabirds of the order Procellariiformes store oil from fish in large glandular fore-guts primarily to feed their chicks, which some species are able to spit in defence or offense (Warham et al. 1976). The northern fulmar (*Fulmaris glacialis*) is particularly well known for spitting this oil over considerable distances (Swennen 1974), and has been observed to drench would be avian predators, destroying the insulating properties of the birds feathers, and causing death of the predator in a number of cases (Warham and Brooke 1996).

It is important to consider the evolutionary consequences of birds using chemical excretions to lower predation risk. Variation in the risk of nest predation has been linked to variation in avian life histories (Ghalambor and Martin 2002). Life history theory predicts that increased nest predation rates select for lower incubation length (Martin 2002), lower investment in single nesting attempts leading to multiple brood attempts (Cassey et al. 2009), faster nestling growth and early nestling development (Bosque and Bosque 1995, Remeš and Martin 2002) as well as conspicuousness of nestling begging behaviour (Briskie et al. 1999). These traits contribute to parental survival and reproduction strategies (Lack 1968). If the odour of a typically fouled starling nest releases nests from nestling predation compared to other passerine species breeding in the same area, selection pressure on traits such as number of broods, hatchling growth and development time and conspicuousness of nestling begging behaviour would be reduced.

The results of this study suggest that rats have an aversion to the smell of starling nests, but if an individual was ever to associate the smell with the reward of prey items, such a chemical cue has the potential to act as a beacon rather than a defence. Bull and Flux 2006 surveyed starling nesting times and success over 5 different sites in New Zealand each season between 1976 and 1979. At one site in 1976, the authors note predation increased suddenly when

‘stoats invaded the colony.’ It is interesting to note that this occurred only one season out of 4 at that site. Similarly at the site in Kowhai bush, two nest boxes only 10 m apart were predated both within 24 hours of each other. It is possible that both nest boxes were within the territory of a single mammalian predator, which after learning of the reward within one, proceeded to predate the other as well. Each box contained nestlings just before pin break. Yet none of the starling nests monitored during the 2009-2010 season was predated after the chicks reached pin break despite the conspicuous noise and smell of the nestlings inside. If the occurrence of an individual predator learning to associate the distasteful smell of a fouled starling nest with the prey inside is rare, and this behaviour is not passed on to other predators, perhaps predators keying into the scent of starling nests is not such a large problem. Certainly the reduced rate of nestling predation starlings enjoy indicates that this is not an issue for the species.

Applying the results of a lab experiment, such as this project, to the wider world is always difficult. This study presumes that where an experienced lab rats find the scent of starling fouled nests as distasteful, the same is likely true of wild rats. If the smell of an active starling nest causes rats to even hesitate before entering a nest cavity, this small change in behaviour would increase the chances a parent may arrive while the rat is in the vicinity, giving the parent a chance to perform mobbing behaviours in an effort to drive the would be predator away. It is possible that some other mechanism exists, by which starlings repel nest predators, and thus counteracts the conspicuousness of nests due to nestling smell. It is difficult however to think of any such mechanism.

Future work is needed to identify what compound it is that rats find so distasteful, and to check if this is found in the faeces of starling nestlings, or excreted some other way. Field experiments are also needed to investigate whether nests, where this compound is not present, suffer a greater predation rate than nests where the compound is present in the wild. Odour

camouflage may be more widespread in birds than previously thought, therefore future studies need to investigate if there are other bird species that excrete distastefully strong smelling substances to reduce nestling predation.

### ***Conclusion***

Common starlings, originally native to Europe and Asia, are one of the most abundant and widespread birds in the world (Flux and Flux 1981). I found that rats (a potential natural predator) display an aversion to starling nest material that was not exhibited towards clean nest material. This supports the hypothesis that predation risk may be reduced by the peculiarly strong ammonia-like smell associated with nestling excretions. Faecal matter has been shown to be distasteful to mammalian predators in other systems, which may in turn avoid such a smell in the wild. This is a novel example of chemical production to aid in olfactory deterrence in a bird species. Future work is needed to determine how efficiently this mechanism functions in the wild, and how the evolutionary consequences of this phenomenon might have contributed to the specific life history traits exhibited by the common starling.

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Figure 4.1. Example of set up of Y-shaped maze including the black sleeves and control nest material in the left arm of the maze.

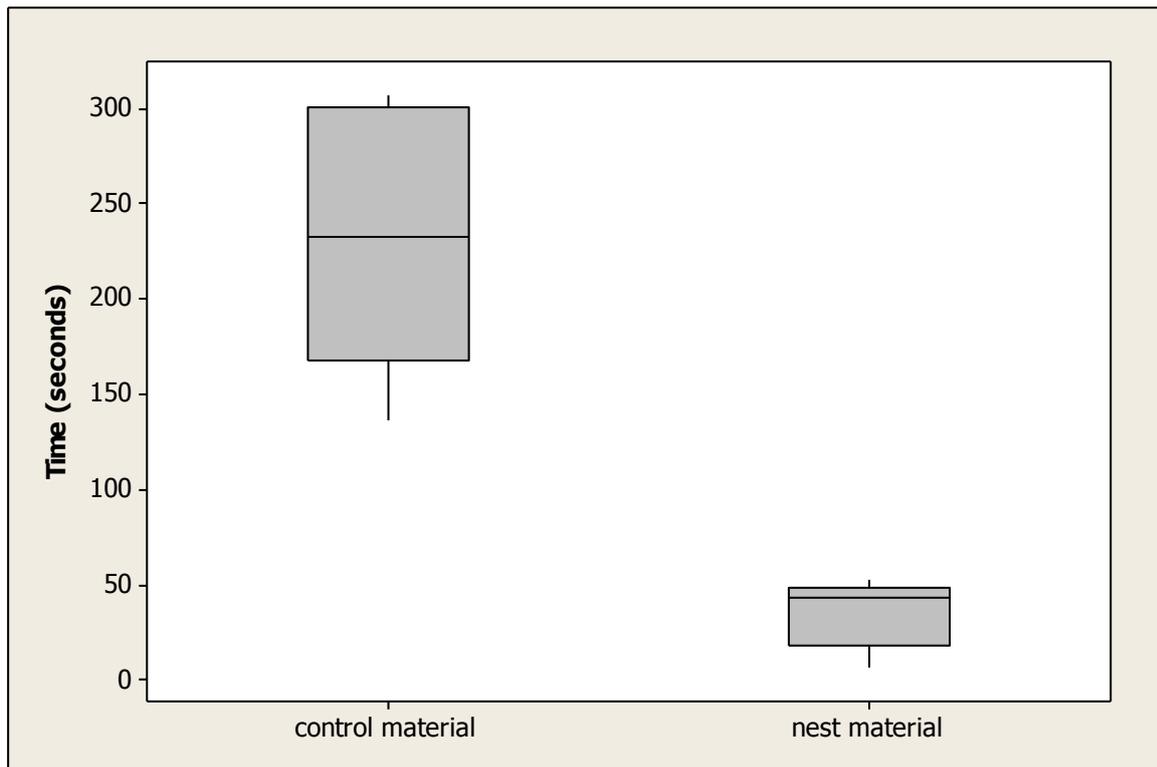


Figure 4.2. Box plots comparing the amount of time (seconds) that rats actively investigated control nest material and starling nest material ( $n = 7$  trials). Plots are median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, with vertical lines showing 90<sup>th</sup> percentiles.

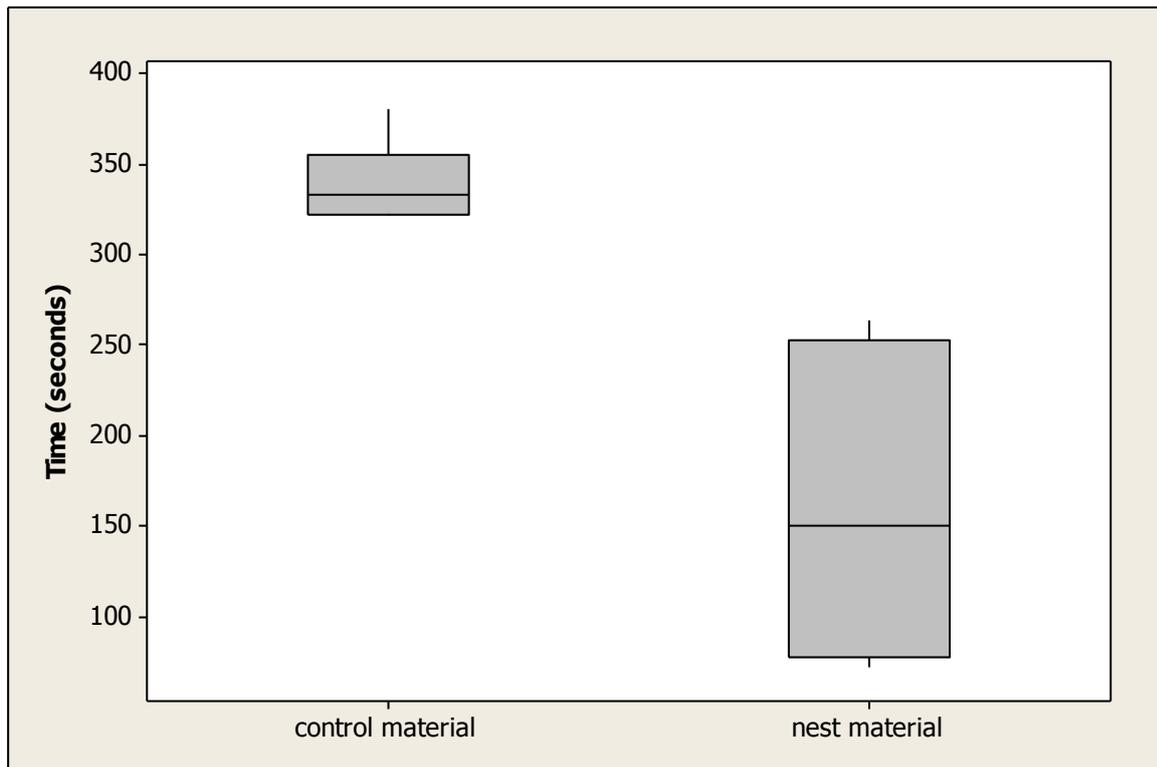


Figure 4.3. Box plots comparing the amount of time (seconds) that rats spent in the arm of the maze containing control nest material compared to starling nest material. Plots are median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, with vertical lines showing 90<sup>th</sup> percentiles.

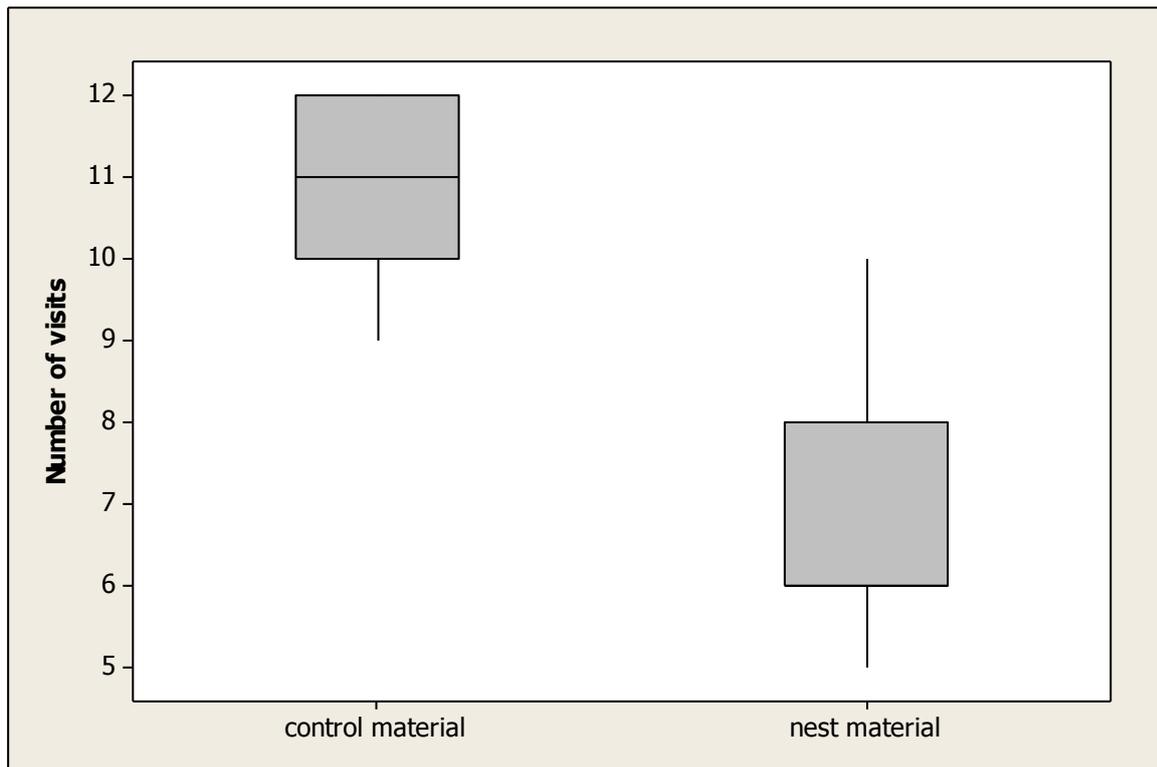


Figure 4.4. Box plots comparing the number of times rats entered the maze arm containing control nest material and starling nest material ( $n = 7$  trials). Plots are median, 25<sup>th</sup> and 75<sup>th</sup> percentiles, with vertical lines showing 90<sup>th</sup> percentiles.

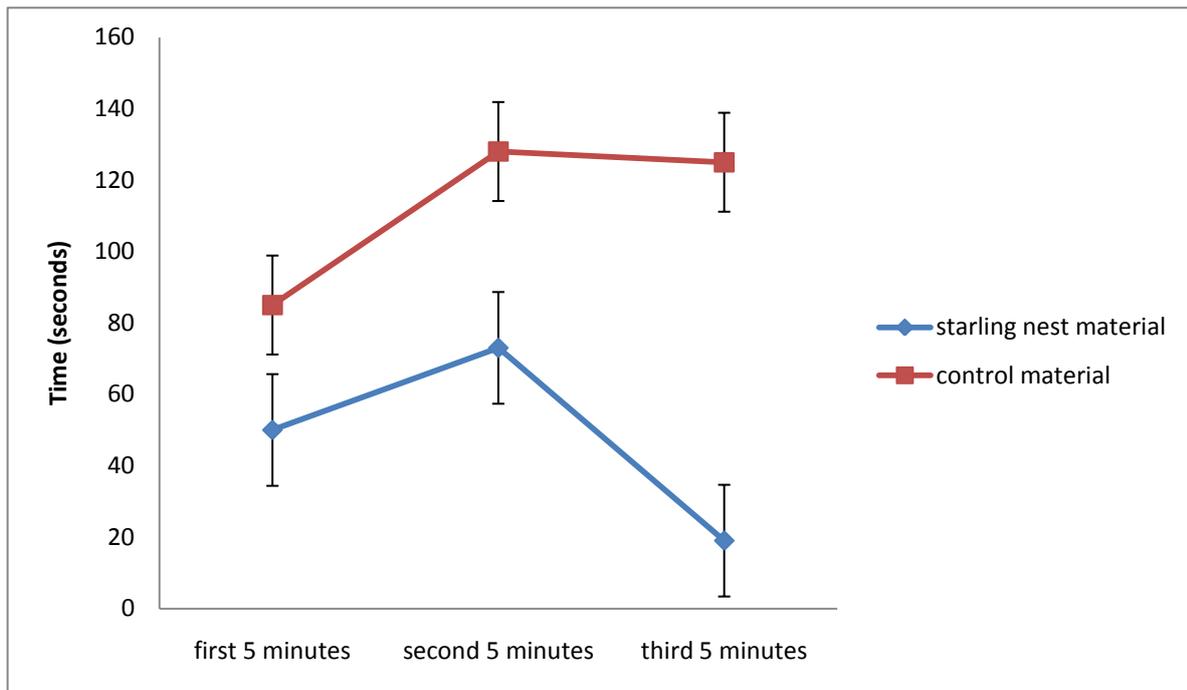


Figure 4.5. Line graph showing change over time in the median number of seconds rats spent in each maze arm containing nest material per trial.

# Chapter 5

## 5.1 General Discussion

Communication is an important component of animal life, and the selective forces that have acted on the evolution of different pathways and channels are particularly interesting. Probably one of the most important limiting factors on the development of a particular communication channel is the risk a predator will ‘eaves drop’, using the signal an organism sends to target an individual or its young as prey (Endler 1993). Olfaction is a widely used communication pathway that has been well documented in every class of animal except for birds (Hagelin and Jones 2007). Until recently, birds have been considered anosmic or microsmatic (with a few exceptions made for some carnivorous or piscivorous species), and for this reason few studies have investigated ways in which birds may use odours to avoid predation (Kats and Dill 1998).

The purpose of this thesis is to examine some of the ways in which predation risk has affected the evolution of olfactory cues in the behaviour of birds. New Zealand provides a unique opportunity to study the role of olfaction in communication in birds as the avifauna now contains a variety of native species and introduced continental species. These the two groups of birds share very different evolutionary histories with predatory mammals that are known to use olfaction to locate their prey. Introduced species from Europe and Australian evolved in the presence of such olfactory searching mammalian predators, and have become well established in New Zealand within the last couple of hundred years (Oliver 1930). As these introduced species now co-exist with the endemic New Zealand birds that evolved in the absence of mammalian predators, it is possible to compare the two groups in the same environment (the classic “common garden experiment”).

I began by comparing the ability of introduced and native birds to respond to predator odour at the nest. I focused on whether parent birds from four different species (two New Zealand endemics, two introduced European birds) could detect a potential increase in predation risk due to the presence of mammalian predator odour at the nest, and change their behaviour accordingly. Such anti-predator

behaviour to odour cues at the nest has been documented in a European bird, and is likely to exist in other species as well (Amo et al. 2008). New Zealand birds are thought to be more vulnerable to predation than introduced species, because they evolved without the constraint of high nest predation pressure on their life history strategies (Starling 2006). My results suggest that only one species responded to the presence of predator odour at the nest.

The results from my experiments with rat urine at the nest show that New Zealand birds lacked any of the anti-predator behaviours exhibited by European species. Of the two New Zealand bird species that I tested (one cup nesting species and one cavity nester), neither exhibited any change in visitation rates, hesitation rates, entries to the nest, or any other behaviour that I could assess during the presence and absence of scent treatments. However, contrary to expectations, there was also no change in the behaviour between treatments for one introduced species that builds a cup nest; only the introduced cavity nesting species exhibited behavioural changes in the presence of predator scent that are likely to reduce its chance of predation on either itself or its nest. Although the number of species I tested is small, my results do suggest that the use of odour cues to detect changes in predator risk may be present in continental cavity nesting species only, but not in all species. Clearly, more species need to be tested in both groups of birds to confirm this suggestion. Future research should also investigate on how such anti-predator behaviours actually increase reproductive success. If it is found to provide substantial benefits, then it may also be useful to investigate whether “training” native birds to avoid predator scent would be a viable tool for long term management of endangered native species.

My third chapter tested whether a morphological trait (preen wax composition) that is known to differ between native New Zealand and introduced European birds, could lead to native New Zealand birds being more readily detected by introduced predators, such as a rat. Reneerkens et al. (2002) first proposed that nest predation was the selective pressure causing the change from monoester uropygial secretions during non-breeding season, to less volatile diester secretions throughout the breeding season. This theory of olfactory crypsis was further supported by the work of Fluen (2008) who found this switch in preen wax composition existed in introduced birds in New Zealand, but not in the

endemic native species that evolved without predation pressure from olfactory searching mammals. Nevertheless, when I compared whether rats were more attracted to the smell of preen wax from native birds compared to the preen wax from introduced species, the results were equivocal. Only one species of native bird (bellbird) appeared to attract the attention of the rats more often, while no difference was found in two other trials between native and introduced species. While these results do not provide general support the theory of olfactory crypsis, they also do not preclude it, as it is possible that such crypsis might be present in only some species, and/or that the use of lab rats may have missed some of the processes that occur in a wild situation. Given the preliminary nature of my study, my results also invite future research in this area, including the testing of more species, and confirming the link between a preference in the lab and a greater risk to nest predation in the wild. If it was confirmed that the odour of preen wax from native birds can significantly increase the risk of nest predation, due to such waxes being more readily detected by introduced mammalian predators, it might even be possible to use this to devise novel ways of protecting native birds through masking the smell.

Finally, in Chapter 4 I looked at the possibility that a very successful continental species (European starling) has developed an 'odour repellent' to lower the risk of nest predation. I specifically tested if rats introduced to a Y-shaped maze would avoid starling nest material that had been smeared with nestling faeces. The reason I chose to test the odour of faeces as a potential repellent was quite straightforward: during the routine monitoring of starling nests I found myself repelled by the strong smell coming from the nests (and specifically the faeces that remained in the nest) and wondered if real predators might likewise be affected in the same way, thereby providing protection from nest predators. I found that lab rats did indeed avoid starling nest material, yet showed a strong interest in clean nest materials similar to those used by starlings in building their nest. It is likely this result was attributed to the strong ammonia-like smell emanating from the faeces in the nest, although I was not able to confirm this through a chemical analysis. Starlings are one of the most abundant and wide spread birds on the planet (Flux and Flux 1981), and a novel use of odour deterrent in the faeces of nestlings may be a contributing factor to their success.

The results of my study necessarily only touch on the role of olfaction in nest predation, but indicate an exciting area for future research, and perhaps even the possible development of a practical tool for nest protection of endangered species that could be used in management of bird species vulnerable to predation. For example, if the smell of starling faeces is found to reduce predation rates of nests in field trials, it might be worth identifying the compound or compounds responsible. It may then be possible to produce a similar predator-deterring odorant which could be used to reduce the risk of predation of nests belonging to threatened species, and perhaps even counteract the lack of olfactory crypsis in some native New Zealand birds. Of course, there is a lot of ground to cover in research before we can know if such possibilities are feasible, and whether the application of such odour deterrents would affect the normal behaviour of native birds.

Together, my three studies reveal the depth and complexity odour cues may play in how birds cope with predation risk, and indicate that current research is only just beginning to scratch the surface of an area which has long been neglected. The general findings show that some birds are able to use odours to assess predation risk in the absence of visual cues. The type and the strength of odours emitted from birds and their secretions may also affect rates of predation. Future work is clearly needed however, to discover if the presence of such behavioural and morphological anti-predator traits significantly affect life expectancy and reproductive success in such species, compared to those species lacking these traits. The results from my work suggests that there are at least some differences between the behavioural and morphological traits expressed in birds that evolved with high nest predation pressures (continental species), and those that evolved without (island natives like those in New Zealand). The generalities of this theory have yet to be tested. Island nations such as New Zealand have suffered a large numbers of extinctions and reductions in bird populations since the arrival of mammalian predators (Blackburn et al. 2004, Holdaway 1989). Understanding the differences between continental birds and island birds is essential to understanding why island species are so vulnerable to predation. Only when we fully appreciate the factors causing the vulnerability of these birds, can we design the most efficient ways to manage species.

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