The Role of Vigilance and Alarm Calling in the Red Junglefowl, *Gallus gallus*



A thesis presented in fulfilment for the DPhil in Zoology within the Department of Zoology

by Heather Catherine Kazara

Student Number: 851343

Supervised by Professor Tommaso Pizzari

St Anne's College, 56 Woodstock Rd, Oxford, OX2 6HS

ABSTRACT

Vigilance and alarm calling are considered exclusively anti-predator behaviours. However recent work suggests they may have other functions. My thesis explores the role of vigilance and alarm calling in red junglefowl, *Gallus gallus*. Male fowl are more vigilant than females and produce male-specific alarm calls for aerial predators. Chapter 2 tested hypotheses for male vigilance and aerial alarm calling by manipulating group size and sex ratio. Vigilance and alarm calling generally increased as groups became more female-biased, potentially reflecting compensation for reduced female vigilance. Chapter 3 explored how male vigilance and aerial alarm calling correlated with measures of male pre- and post-copulatory mating success; finding that more vigilant males and males that produced more alarm calls had higher mating success. One explanation for the link between male vigilance and alarm calling and mating success is that males invest more to protect their sexual partners to increase the survival of offspring that they may have sired. Chapter 4 tested this by experimentally manipulating male paternity confidence and mating success by allowing or preventing sexual access to a female in the presence or absence of male competitors. Males invested more in vigilance and alarm calling when they had sexual access to a female, regardless of the level of competition, which suggests that male investment is related to mating success *per se*. Finally, Chapter 5 considered the role of vigilance in females using two approaches. First, in mixed-sex groups females were three times less vigilant than males. Second, I examined whether female vigilance could play a role in foraging by allowing an individual to gather information on food location by watching a conspecific. I found no evidence that vigilance enables foraging females to gather information. Collectively, these results suggest that vigilance and alarm calling are linked to mating behaviour in male fowl.

Statement of Authorship

I declare that the work in this thesis is my own. Below I have outlined what I did within each chapter and stated instances where help was received from others.

For all four data chapters (2-5) my supervisor and I consulted on the ideas that we planned to test and designed the experiments together. I collected all the data used throughout my thesis; no data was provided by others. Although a second observer did provide information on any behaviours missed while conducting focal watches for the experiment used in Chapters 2 and 3. Furthermore I analysed the data alone, with some help through feedback from departmental statistics sessions and other students. Furthermore, I wrote all six thesis chapters with input from my supervisor.

In accordance with the Department of Zoology guidelines, this thesis does not exceed 50,000 words when excluding reference lists, table of contents, appendices, diagrams, figure/table legends and tables. The total word length when excluding these is 44,734.

Acknowledgements

I would like to thank my supervisor for his help with experimental design and his input on my thesis chapters. I want to thank all the members of the Pizzari lab, including Allison Roth for sharing pens and birds with me during the first two fieldwork seasons. I want to thank Liisa Veerus for her support and kind words these past six months. I want to thank Ellen Pasternack for giving me feedback on Chapter 5 despite being busy with her field season. I want to thank Rômulo Carleial for his help with my graphs in Chapter 3. I am grateful to Tom Mulder for checking the analysis in Chapter 3 and for giving me feedback on Chapters 2 and 3. I want to thank Sonya Clegg and Elizabeth Jeffers for their support and aid during my stressful final few months, you both made everything less stressful.

I especially must thank David Wilson, Lucy Larkman and Phil Smith for their assistance at the field site. David has gone out of his way to help me on multiple occasions despite always being busy with his own duties. I am eternally grateful for everything you all did to help me.

I need to thank all my friends and family for their support, unfortunately there are too many to list here. I especially want to thank Sue Leech for the advice and help you have given me, particularly this final year. I also want to thank James Jones for always trying to make me smile no matter how stressed I was. The person that I need to thank most of all is my wonderful partner, Richard Jones. Not only did you make my dream of doing a PhD a reality, but you have supported me every step of the way even when I doubted myself. I love you and cannot thank you enough for all you have done these past 4 years.

Dedication

I wish to dedicate my thesis to Tony Vater, a good friend that passed suddenly on the 2nd July 2019.

I remember you reading through my master's thesis and being impressed, so I was looking forward to showing you what these past few years had led to. Plus, you always poked fun at me for just studying chickens and I had hoped that while reading it you would have made one of your witty, sarcastic remarks, followed by your usual laugh.

I will miss you and I am sorry that it was not finished a month earlier so that we could have shared those moments.

Table of Contents

Chapter 1: Introduction 1
Strategies for Avoiding Predation 2
Confusion Effects 2
Cryptic Colouration 3
Oddity Effects 5
Selfish Herd Theory 6
Vigilance 7
Vigilance and Anti-predation 7
Social Uses of Vigilance 8
Vigilance and Sexual Selection 10
Alarm calls 13
Honest vs Dishonest Signals 14
Predator Vocalisations vs Alarm Calls 15
Adaptive Significance of Alarm Calling 17
Alarm Calling and Mate Investment 18
Alarm Calling and Sexual Selection 19
Vigilance, alarm calling and sexual selection in the Red junglefowl 20
Alarm Calling Behaviour in Fowl 21
Sexual Selection in Fowl 22
Vigilance, Alarm Calling and Mating Success 25
Thesis Aims 28
REFERENCES 31
Chapter 2: The Effect of Group Size and Sex Ratio on Vigilance and Alarm Calling 42
ABSTRACT 43
INTRODUCTION 44
METHODS 55
Study System 55
Analysis 57
Exploring Collinearity 57
Testing the effects of group size and sex ratio 61
Testing the effect of social status and sex ratio within each group size 61
RESULTS 65
Effect of group size and sex ratio 65
The effects of male social status and sex ratio within groups of the same size 73

DISCUSSION 81
Vigilance and Group Size 82
Vigilance and Sex Ratio 83
Alarm Calling and Sex Ratio 83
Vigilance and Alarm Calling 84
Dominance 85
Conclusions 87
REFERENCES 88
APPENDIX 96
Chapter 3: The Role of Vigilance and Alarm Calling in Mating Success 104
ABSTRACT 105
INTRODUCTION 106
METHODS 115
Study System 115
Mating Behaviours 115
Data Analysis 116
SCI 117
Building the Models 118
Alternative Analysis using Z-Score Standardized Variables 125
Analysis 126
RESULTS 127
Part 1: Correlates of Mating Success 127
Number of Matings 127
Number of females mated 129
Average remating rate 131
SCI 133
Part 2: Behaviours that Influence Mating Success 135
Solicitations 135
Courtship waltzes 137
Harassments 139
Copulation Interruptions 142
Interruptions Received 142
Interruptions Performed 144
DISCUSSION 148
Vigilance and Mating Success 150
Alarm Calling and Mating Success 152

Male Attractiveness vs Mate Investment 154
Dominance and Mating Success 155
Conclusions 156
REFERENCES 157
APPENDIX 165
Chapter 4: Testing Hypotheses of Male Vigilance and Alarm Calling Investment 179
ABSTRACT 180
INTRODUCTION 181
METHODS 192
Experimental Design 192
Behavioural Data 194
Anti-predation Behaviours 194
Male-Female Interactions 194
Data Analysis 195
RESULTS 199
Does treatment affect vigilance and alarm calling behaviour? 199
Does mating day affect vigilance and aerial alarm calling? 204
DISCUSSION 208
Paternity Confidence and Mate Investment 209
Mate Guarding 210
Male Compensation 211
Mating vs No-mating Days 211
Anti-predator Behaviours and Sexual Selection 212
Conclusions 213
REFERENCES 215
APPENDIX 223
Chapter 5: Exploring Vigilance Behaviour in Female Red Junglefowl, *Gallus gallus* 225
ABSTRACT 226
INTRODUCTION 227
METHODS 235
Study System 235
Part 1: Female vigilance and group sex ratio 235
Experimental Setup 235
Behavioural Data 235
Data Analysis 236
Part 2: Food knowledge and female vigilance 238

Experimental Set Up 238
Experimental Procedure 240
Training Phase 240
Experimental Phase 241
Data Analysis 241
RESULTS 244
Part 1: Exploring female vigilance in mixed sex groups 244
Part 2: Food knowledge and female vigilance 248
DISCUSSION 254
Part 1: Exploring female vigilance in mixed sex groups 256
Sex Differences in Vigilance 256
Sex Ratio and Female Vigilance 256
Dominance and Female Vigilance 257
Part 2: Food knowledge and female vigilance 258
Dominance and Exploration 259
Contrafreeloading 259
Foraging and Vigilance 260
Conclusions 261
REFERENCES 263
APPENDIX 269
Chapter 6: Discussion 270
Vigilance, Alarm Calling and Mating 271
Group Size and Sex Ratio Effects on Vigilance and Alarm Calling 277
Vigilance Behaviour in Female Red Junglefowl 279
Potential Effects of Personality on Vigilance and Alarm Calling 280
Potential Effects of Captivity on Vigilance and Alarm Calling 282
Redefining Vigilance 286
Future Work 289
Conclusions 290
REFERENCES 293

Chapter 1

Introduction

Strategies for Avoiding Predation

To maximise fitness an individual must avoid predation while performing fundamental functions such as foraging and reproduction. This often leads to trade-offs, e.g. between predator avoidance and foraging (Duriez *et al.*, 2005; Lind & Cresswell, 2005). One method of reducing predator avoidance is to aggregate. Predation risk in groups is reduced through the dilution effect, which predicts that increasing group size leads to the decreasing probability that an individual is attacked (Foster & Treherne, 1981; Beauchamp & Ruxton, 2008). For example ocean skater insects, *Halobates robustus*, gained a considerable reduction in the probability of being targeted when aggregated, demonstrating the benefit of grouping in predator avoidance (Foster & Treherne, 1981).

Confusion Effects

Larger groups of similar looking organisms can also confuse predators, making it more difficult for them to latch on and target a particular individual (confusion effect; Schradin, 2000; Jeschke & Tollrian, 2005). Disentangling confusion and dilution effects can be difficult. Leopard geckos, *Eublepharis macularius*, have been demonstrated to take longer to capture one prey item from a group of 20 compared to a solitary prey item (Schradin, 2000). In addition, three out of seven geckos failed to capture a single prey item from the groups of 20 mealworms in comparison to just one gecko failing when provided with one item, consistent with the confusion effect (Schradin, 2000). More recent work has suggested that dynamic colour changes in large groups, such as the flash mark colouration found in both birds and fish, could add to the confusion effect through hindering a predators ability to pinpoint prey location (Murali *et al.*, 2019). Murali et al. (2019) predicted that stimuli expressing colour change when in motion would be

harder to follow and tested this using human participants tracking objects within a group. This study has provided the first evidence that confusion effects can be enhanced through dynamic colour changes.

Cryptic Colouration

It is often presumed that bright, conspicuous colouration increases the likelihood of detection by predators, thus increasing risk. Brighter colours can be attractive to mates (e.g. Petrie *et al.*, 1991; Doucet & Montgomerie, 2003), so colouration variation is likely influenced by both natural and sexual selection (Endler, 1992). Despite the persistent belief that conspicuous individuals are more vulnerable to predators, few studies have explicitly tested this idea. A study on two prey species of Australian rock dragons, *Ctenophorus decresii* and *C. vadnappa*, using plaster models found that the more conspicuously coloured models received significantly more attacks than the duller models (Stuart-Fox *et al.*, 2003). A similar study was done in the sexually dimorphic collared lizard, *Crotaphytus collaris*, using three different populations with varying degrees of male conspicuousness (Husak *et al.*, 2006). The study found that as the contrast between the model and background increased, so did the number of attacks. In addition, the cryptic female models suffered no attacks across the three sites, suggesting that crypsis is advantageous in avoiding predation (Husak *et al.*, 2006). This has also been shown in birds where male chaffinch, *Fringilla coelebs*, models received more attacks from avian predators than the duller female models (Götmark, 1993). However, in another passerine, the pied flycatcher, *Ficedula hypoleuca*, avian predators in multiple studies have shown a preference for cryptic females rather than the more visible males (Götmark, 1992, 1993; Götmark & Hohlfält, 1995). This indicates that

predation risk as a function of conspicuousness may vary across species, and in some species conspicuousness may be advantageous.

Noxious species often display bright colours or conspicuous patterns to warn potential predators that individuals are unpalatable or unprofitable prey. This phenomenon is referred to as aposematism and is phylogenetically widespread, and particularly common in amphibians and reptiles (Wüster *et al.*, 2004; Wang & Shaffer, 2008; Crothers *et al.*, 2011; Valkonen *et al.*, 2011; Hegna *et al.*, 2013; Kikuchi *et al.*, 2014). Aposematism has been proposed to explain predator avoidance of conspicuous male pied flycatchers. Yet feeding deceased pied flycatchers to peregrine falcons, *Falco peregrinus*, did not support the idea that they were toxic or unpalatable (Götmark, 1992). Alternatively, conspicuous colouration in the pied flycatcher could warn predators that attacks would have lower success rates due to an increased ability to escape predation, making them unprofitable (Götmark, 1992). A similar pattern has been shown in skylarks, *Alauda arvensis*, with males singing during chases to deter merlins, *Falco columbarius*, from continuing the pursuit (Cresswell, 1994). Merlins were more likely to catch none or poorly singing skylarks and chased them for longer than full-singing individuals (Cresswell, 1994).

A recent study explored whether conspicuousness leads to greater predation risk by using 3D-printed models of fairy-wrens (Cain *et al.*, 2019). Cain et al. (2019) used three model types comprising of a conspicuous male and dull female purple-backed fairy-wrens, *Malurus assimilis*, and a conspicuous female lovely fairy-wren, *M. amabilis*, and placed them over a variety of habitats. There was no relationship between the rate of attacks and the degree of model-background contrast nor a relationship between model conspicuousness and number of attacks,

indicating that conspicuousness alone does not increase predation risk within these species. Conversely, the study showed that predation was higher for models placed at higher latitudes and in more open habitats, typically inhabited by fairy-wren species where females are mostly dull, which suggests that there is a cost to bright colouration in these higher risk habitats (Cain *et al.*, 2019). Therefore, despite the notion that conspicuousness increases predation risk being seemingly intuitive, the evidence is sparse and contradictory in birds, with brighter plumage either increasing or decreasing predation risk depending on the species. Irrespective of the influence of colouration on the risk of predation, being odd in colour or shape when compared to neighbours can increase predation risk through the oddity effect.

Oddity Effects

Predators may be able to overcome confusion when presented with groups of moving organisms if one or several within the group are phenotypically distinct from the others (Landeau & Terborgh, 1986; Peuhkuri, 1997; Rodgers *et al.*, 2011). Oddity was examined in black and white mollies, *Poecilia latipinna*, by presenting fish with various shoaling choices and assessing preference (Mcrobert & Bradner, 1998). When presented with a shoal of the same colour or no shoal, focal fish spent more time with the shoal. However, when presented with a matching and non-matching shoal, individuals preferred to spend time with conspecifics sharing their colouration (i.e. matching shoal). A similar approach was used in Trinidadian guppies, *Poecilia reticulata*, testing whether small or large fish differed in shoal associations. Only large fish had a strong preference for the size-matched group, indicating a greater importance of oddity effects in larger fish; potentially due to higher risk of predation associated with higher

calorific value of larger individuals (Rodgers *et al.*, 2011). The oddity effect could come into conflict with the dilution effect if individuals are forced to choose between smaller groups with similar individuals or larger groups with varied conspecifics. In Trinidadian guppies increasing group size in the unmatched shoal had no effect on the preference to group with matched individuals, suggesting the dilution effect was less important in this species (Rodgers *et al.*, 2011). Similarly, although both larger group sizes and size-matched groups were preferred initially in two swordtail species, *Xiphophorus birchmanni* and *X. malinche*, focal individuals showed no significant preference when presented with the choice of either grouping with one similar individual or with four dissimilar fish (Wong & Rosenthal, 2005).

Selfish Herd Theory

Relative predation risk in large groups is not necessarily equally distributed across individuals even in phenotypically homogeneous groups. Widely spaced individuals or those on the periphery of a group may be more at risk from attack (Hamilton, 1971). In his Selfish Herd Theory, Hamilton (1971) proposed that individuals in a group have "domains of danger" relating to how widely spaced they are from one another, and that individuals can reduce their domains of danger by moving closer to neighbours, with more widely spaced individuals incurring a higher predation risk. For example, when startled, sand fiddler crabs, *Uca pugilator*, ran towards the centre of the group rather than running away, suggesting that predation risk is lower when a prey is surrounded by conspecifics (Viscido & Wethey, 2002). However, predation risk associated with prey position within a group can vary depending on the attack strategy of the predator. In schools of Atlantic silversides, *Menidia menidia*, central fish were at the greatest

risk of predation by black seabass, *Centropristis striata*, due to seabass splitting the school into two groups through the middle, causing the central fish to become exposed (Parrish, 1989).

Thus, multiple strategies to avoid predation have evolved in group-living species, where predation risk is often not uniform across group members due to coloration, oddity or group position. Additionally, large groups have disadvantages such as increased mate competition (Beauchamp, 2001), increased risk of parasites (Mooring & Hart, 1992) and food competition (Grand & Dill, 1999; Sansom *et al.*, 2008; Beauchamp, 2014). For example, in juvenile coho salmon, *Oncorhynchus kisutch*, individuals engaged in riskier foraging behaviour in larger groups despite predator presence, suggesting an increased nutritional stress in large groups (Grand & Dill, 1999). An alternative, non-mutually exclusive anti-predation strategy is vigilance.

Vigilance

Vigilance and Anti-predation

Vigilance is the interruption of behaviours, usually foraging, to scan the environment for predators (Elgar, 1989; Bednekoff & Lima, 1998; Treves, 2000; Beauchamp, 2007). Classic vigilance studies examine the rate and time spent engaged in "head-ups" during foraging activity, with most work done in birds and mammals (Elgar, 1989).

Vigilance behaviour is thought to aid in predator detection and in the chaffinch, *Fringilla coelebs*, birds that had greater head-up rates responded to an avian predator model quicker than individuals with lower head-up rates (Cresswell *et al.*, 2003b). Despite vigilance behaviour being well studied (Abramson, 1979;

Bednekoff & Ritter, 1994; Bednekoff & Lima, 2002; Blanchard & Fritz, 2007; Beauchamp, 2009a) with multiple studies finding that aggregating in large groups (Lima, 1995; Blumstein *et al.*, 1999; Beauchamp, 2008) and reducing nearest neighbour distance (Rolando *et al.*, 2001; Quinn & Cresswell, 2006) can reduce vigilance due to decreased predation risk, there are few studies empirically testing whether vigilance itself reduces risk through quicker predator response.

A potential added benefit to being vigilant may be that predators avoid targeting vigilant individuals. In Thomson's gazelle, *Eudorcas thomsonii*, cheetahs preferentially targeted less vigilant individuals approximately 88% of the time by selecting individuals prior to chasing (FitzGibbon, 1989). Cheetahs and other cats are stalking predators, choosing prey before engaging in an attack; thus these predators likely have time to assess the vigilance status of potential prey before striking (Cresswell *et al.*, 2003a). Wild Eurasian sparrowhawks, *Accipiter nisus*, were presented with two model types; first, stuffed domestic fowl chicks, *Gallus gallus domesticus*, and second, resin casts of greenfinches, *Carduelis chloris*. For both model types, half the models were positioned with the head down to mimic foraging, and the remaining with the head up to mimic vigilance. Sparrowhawks showed no preference for the non-vigilant models (Cresswell *et al.*, 2003a). This study demonstrates that whether predators hunt by stalking or opportunistically can affect whether vigilance is an effective strategy to deter predators.

Social Uses of Vigilance

Most vigilance studies assume that individuals scan independently of one another; however, this is not necessarily the case in group-living animals. Black-headed gulls, *Chroicocephalus ridibundus*, with more alert neighbours spent 22% less time sleeping than those with sleeping neighbours; suggesting that gulls copied

the vigilance state of neighbours (Beauchamp, 2009b). Watching neighbours can have social benefits as well as coordinating vigilance. While vigilance is conventionally interpreted as an exclusively anti-predation behaviour, it can also serve social functions in groups. Monitoring neighbours provides information on mate searching (Gaynor & Cords, 2012), guarding juveniles (Favreau *et al.*, 2015), location of food and food stealing opportunities (Fernández-Juricic *et al.*, 2004, 2005), and avoiding aggression from other group members (Slotow & Rothstein, 1995; Gaynor & Cords, 2012).

Social functions may also explain sex differences in vigilance behaviour. For example, European rabbits, *Oryctolagus cuniculus*, exhibit two forms of vigilance; subtle (quadrupedal) and overt (upright position). Male subtle vigilance increased in the presence of potential male competitors. Female vigilance on the other hand, related to social context. Subtle vigilance in females lacking social mates increased with proximity to both sexes; suggesting that this female behaviour is influenced by both potential mates and competitors (Monclús & Rödel, 2008). Similarly, male wigeon, *Anas penelope*, are twice as vigilant as females, which is possibly due to mate guarding rather than sexual dimorphism (Mayhew, 1987). Male mate guarding allows females to decrease individual vigilance thus increase foraging time. Potentially leading to increased egg quality and better offspring. However vigilance could be used to find potential females to engage in extra-pair copulations; or to guard females from competitors (Mayhew, 1987). Finally, non-reproductive male elks, *Cervus canadensis*, were less vigilant than reproductive males; suggesting that vigilance might be partly used by reproductive males to monitor their harem females. Consistent with this idea,

males were more vigilant in the autumn, when competition for harem territories occurs (Childress & Lung, 2003).

Vigilance and Sexual Selection

Vigilance behaviour can therefore convey an advantage in reproductive competition over access to mates and reproductive opportunities, and sex differences in vigilance can reflect sex-specific patterns of sexual selection. Sexual selection is a form of natural selection with two components; competition within the same sex for access to mates (intrasexual selection), and mate choice (intersexual selection) (Darwin 1871; Andersson 1994; Ligon *et al.*, 1990; Møller & Petrie, 2002; Loyau *et al.*, 2005). Theory predicts that sexual selection is more intense in males because of the asymmetry in gamete size by males and females (anisogamy) (Bateman, 1948; Trivers, 1996). Male reproductive success is restricted by competition with other males to fertilise eggs because sperm outnumber ova, whereas females do not typically need to engage in intrasexual competition to fertilise their eggs (Pizzari & McDonald, 2019). Traditional sex roles have been documented across the animal kingdom (Janicke *et al.*, 2016).

Despite the plausible link between male vigilance and sexual selection, very few studies have examined this relationship. Grey partridges, *Perdix perdix*, are monogamous, forming long-lasting pairs, with both males and females partaking in offspring care (Dahlgren, 1990; Beani & Dessì-Fulgheri, 1995; Fusani *et al.*, 1997). The first study looking at vigilance in relation to female choice in this species hypothesised that males would be more vigilant than females, based on findings from other Tetraoninae species (Dahlgren, 1990). Vigilance was examined in two ways: a mate choice trial and monitoring vigilance in free-living pairs. During the mate choice trial females were placed into an experimental arena

and allowed to choose between three pens; one with a vigilant male, one with a less vigilant male and an empty pen containing grassland. Male vigilance was experimentally altered by restricting views towards the outside in one pen, yet females remained unaware of this alteration when inside the arena. To control for female preference towards certain pens females were first tested alone excluding males, finding that females significantly preferred the grassland pen. When tested with males however, females preferentially associated with the most vigilant male (Dahlgren, 1990). When observing free-formed pairs there was a clear difference in time allocated for vigilance between sexes during the pre-hatching period, with females allocating approximately 20% of time to vigilance compared to 65% in males. Females also engaged in greater foraging activity, likely due to increased male vigilance, spending nearly triple the amount of time foraging as males. After hatching, females increased vigilance post-hatching, suggesting high vigilance from both parents is essential to offspring survival. Males were still more vigilant than females however not significantly so (Dahlgren, 1990). Dahlgren (1990) concluded that females actively choose to pair with more vigilant males, and that pairing with vigilant males allows females to gain direct benefits through reduced personal vigilance and increased foraging, and greater protection of chicks (Dahlgren, 1990). In addition to being more likely to secure a mate, vigilant males may also benefit from the increased foraging of their mate since females that forage more produce more and better quality eggs (Ankney & Afton, 1988; Meijer *et al.*, 1989; Ankney *et al.*, 1991; Squires *et al.*, 2007), thus improving reproductive success. Some studies in socially monogamous birds suggest that male vigilance can be used to guard mates from rivals (Mayhew, 1987; Guillemain *et al.*, 2003; Squires *et al.*, 2007), therefore female grey partridges

could gain additional benefits from decreased sexual harassment from males and males could benefit by preventing cuckoldry. A second study in this species was the first study to test the relationship between vigilance and testosterone. Testosterone controls the expression of male-specific behaviours and traits in many vertebrates (Duckworth *et al.*, 2001; Hagelin & Ligon, 2001; Mougeot *et al.*, 2004; Pérez-Rodríguez *et al.*, 2006). Fusani et al. (1997) gave half of the males a testosterone implant to increase circulating androgen levels in the blood, and these treated males engaged in vigilance behaviours more frequently than the non-treated, control males (Fusani *et al.*, 1997). The handicap principle states that signals showing male quality should be costly to prevent poor quality individuals cheating (Zahavi, 1975). The immunocompetence handicap hypothesis is a case of the handicap principle referring to the expression of male sexually selected behaviours and traits being testosterone dependant, and testosterone depressing immunity (Folstad & Karter, 1992). Since vigilance is testosterone dependant in grey partridges, males performing high levels of vigilance must be able to survive with higher levels of testosterone reducing immunity; thus, male vigilance is a condition dependant trait that signals male quality. To the extent to which condition dependence captures additive genetic quality (Rowe & Houle, 1996), females reproducing with more vigilant males will on average produce offspring inheriting higher quality genes. In addition, due to the increased female foraging found in Dahlgren (1990) along with bi-parental care in this species females could gain direct benefits through increased foraging and increased offspring survival by choosing vigilant males. Regardless of the findings that link vigilance to female choice in the grey partridge, this topic remains underexplored.

Alarm calls

Vigilance in social species is often associated with forms of communication of predation risk among group members, e.g. through alarm calls. Alarm calling is therefore a part of anti-predation behaviour that fulfils a social role, by signalling a danger to a conspecific. A signal is a structure or action that changes an individual's behaviour by transmitting information from one organism (sender) to another organism (receiver), and which has evolved largely because of this function (Guilford & Dawkins, 1991; Hasson, 1994; Smith & Harper, 1995).

Signals vary widely, with examples including aposematism, Batesian mimicry, courtship displays, and alarm calls (Smith & Harper, 1995). Signals can be further divided into three types: cues, self-reporting and other-reporting. A cue is normally not considered a signal, but it provides information from the environment that may assist in decision-making. Sexual displays, dominance displays, and warning colouration are all self-reporting signals, providing information about the signaller. Other-reporting signals supply information on aspects other than the signaller, such as predation risk or food location (Smith & Harper, 1995). Early studies focussed on the signaller; however, Guilford and Dawkins (1991) examined signalling theory from the perspective of the receiver. This paper highlighted the importance of efficacy of signals by exploring the brain of the receiver, labelled the "psychological landscape", which comprises of three factors important in creating an efficient signal: memorability, discriminability and detectability (Guilford & Dawkins, 1991). Importantly, a signal can be either beneficial to the receiver by conveying useful information, or detrimental by conveying unreliable information, which the signaller uses to manipulate

receiver's behaviour in a way that is beneficial to the signaller but disadvantageous to the receiver (Smith & Harper, 1995).

Honest vs Dishonest Signals

A long-standing debate in animal signal literature regards whether signals should be honest. Literature suggests costly traits evolved to function as honest signals because only certain individuals will be able to express these signals (Dawkins & Guilford, 1991). Signaller costs are expressed either through efficacy or strategic costs. Efficacy costs refer to the costs needed to provide an effective signal; this is often relative to the environment. For example, calls in closed habitats should be longer in duration, to increase the likelihood of detection (Brown & Handford, 1996; Boncoraglio & Saino, 2007; Ey & Fischer, 2009). Strategic costs emerge from the Handicap Principle, asserting that further costs evolved to ensure honest signalling (Petrie *et al.*, 1991; Hasson, 1994; Smith & Harper, 1995). A classic example is the sexually selected train of peacocks, *Pavo cristatus,* which incurs costs from potential increased predation and parasite load, and can thus represent a reliable indicator of a male's genetic quality to females (Petrie, 1994; Petrie *et al.*, 1996; Møller & Petrie, 2002).

Yet some signals can be dishonest, as exemplified by the phenomenon of mimicry. While alarm calls offer positive information by alerting the receiver of a danger, although they offer potential for manipulation, mimicry gives false information to a receiver through deception, (Smith & Harper, 1995). Male peacocks produce a hoot call when copulating, acting as a signal of mating success to others. Females were shown to be attracted to this hoot call during playback experiments. Some males released the hoot call despite being alone, gaining new female visitors (Dakin *et al.*, 2014). Alarm calling can also be

manipulated to increase mating opportunities as shown in Topi antelopes, *Damaliscus korrigum*. In this species individuals warn conspecifics of danger from stalking predators through snorting, and one study investigated whether dishonest alarm snorts were the result of errors or were deliberately false (Bro-Jørgensen & Pangle, 2010). The study concluded that dishonest snorts were probably deliberately deceptive because they occurred more often when females were present, and males often attempted to copulate immediately after initiating the snort. Also snorts were often performed when females neared territory boundaries, causing females to move closer to the male after hearing the alarm (Bro-Jørgensen & Pangle, 2010). If cheating was more prevalent than honesty, then receivers would cease using that signal. Still cheating can arise providing they too suffer costs. Cheating is common in mourning cuttlefish, *Sepia plangon*, with approximately 40% of males mimicking females so that they can mate with females when in proximity to a solo rival male. Cheats do incur in costs if caught by the more dominant male (Brown *et al.*, 2012).

Predator Vocalisations vs Alarm Calls

Some literature has investigated whether individuals are more likely to respond to alarm calls than to predator vocalisations, postulating that many predator sounds are emitted while not hunting whereas alarm calls signify an immediate threat (Schmidt *et al.*, 2008; Magrath *et al.*, 2015; Makin *et al.*, 2019). This may be particularly true for stalking predators that rely on stealth because loud sounds could inform the prey of the predators presence (Makin *et al.*, 2019). Yellow-bellied marmots, *Marmota flaviventris*, for example increased vigilance and decreased foraging in response to a conspecific alarm call more than with predator vocalisations; reiterating the importance of alarm calls (Blumstein *et al.*, 2008). A

very recent study examined this using lions, *Panthera leo*, and four ungulate species: zebras (*Equus quagga*), wildebeest (*Connochaetes taurinus*), gemsbok (*Oryx gazelle*) and red hartebeest (*Alcelaphus buselaphus caama*); predicting that all four species would significantly increase vigilance more upon hearing conspecific/heterospecific zebra and wildebeest alarm calls than roaring (Makin *et al.*, 2019). Unlike some previous findings this study found that lion roars caused greater vigilance than alarm calls, but this varied depending on species and whether lions inhabited that area. For instance, both zebras and red hartebeest significantly increased vigilance to a greater extent when hearing roars instead of alarm calls in lion zones. Wildebeest also followed this pattern, though it was not significant, and gemsbok responded strongly to both roars and alarm calls. Yet in lion-free zones red hartebeest and wildebeest did not increase vigilance after hearing roars whereas zebras and gemsbok still increased vigilance to roars; suggesting that roars still indicate a potential danger despite these sections being lion-free for over 100 years (Makin *et al.*, 2019). The "information precision hypothesis", proposed by Rainey et al. (2004), states that alarm calls may not give accurate information regarding predator location because it depends on the signaller's location, whereas predator vocalisations can give accurate information on position. So, in some species, although alarm calling may still be important, predator vocalisations could provide more useful and accurate information needed to avoid an attack (Rainey *et al.*, 2004). Information on predator location is probably useful when avoiding ambush predators such as lions but may be less critical when facing attacks from non-stalking predators. Then again alarm calling rate in Black-casqued hornbills, *Ceratogymna atrata*, dramatically increased after hearing the shrieking sound of an eagle, even more so than after hearing primate

eagle-specific alarm calls (Rainey *et al.*, 2004). Another study tested the vigilance response from zenaida doves, *Zenaida aurita*, after hearing avian predator calls compared to conspecific alarm calls also finding that predator vocalisations caused higher vigilance than conspecific calls (Barrera *et al.*, 2011). As these doves spend time in solitude it is possible that responding to predator sounds is more helpful than alarm calls. Several studies have found that avian predators emit vocalisations prior or during hunts, potentially explaining why some species exhibit a behavioural response to these calls (Hendrie *et al.*, 1998; Gil-da-Costa *et al.*, 2003).

Adaptive Significance of Alarm Calling

Since alarm calling may increase predation risk at no apparent benefit for the signaller, the significance of this behaviour demands an adaptive explanation. Kin selection is often suggested as an explanation for the evolution of alarm calls. Despite increasing individual predation risk, an individual may benefit by protecting those that share their genes when the benefits of protecting relatives outweigh the direct costs of an increased personal predation risk (Wickler, 1985; Yasukawa *et al.*, 1992; Krams *et al.*, 2006). A classic example of this is shown in Richardson's Ground squirrels, *Spermophilus richardsonii*, where females produced alarm calls more frequently after their offspring emerged from the burrow, whereas females without young did not call at all during the experiment (Davis, 1984). Furthermore, when the young were nearby, adult males increased the number of alarm calls, consistent with the idea that alarm calling may function to protect descendants (Davis, 1984). Similarly, in thirteen-lined ground squirrels, *Spermophilus tridecemlineatus*, and yellow-bellied marmots alarm calling rates were highest for adult females with emerging litters compared to other age and

sex categories (Schwagmeyer, 1980; Blumstein *et al.*, 2008). Finally, a recent playback experiment study on superb fairy-wrens, *Malurus cyaneus*, found that individuals respond more urgently to alarm calls given by kin than non-kin (Colombelli-Négrel & Evans, 2017).

Alternative hypotheses have explained alarm calling in the absence of relatedness effects. For example, alarm calls could inform predators of detection, which may be advantageous to the signaller if the predator relies on being undetected for successful prey capture since the predator should then abandon the hunt (Seyfarth *et al.*, 1980; Zuberbühler, 2009). The signaller could also benefit according to the prey manipulation hypothesis. Alarm calls can cause panicked movements in flocks, confusing the predator and decreasing the probability of the caller being targeted (Charnov & Krebs, 1975). Additionally, alarm calls can enact mobbing behaviours onto predators, again providing an advantage to the caller (Woodland *et al.*, 1980). Alarm calls can also enable the signaller to manipulate others to its own advantage (Munn, 1986; Gouzoules *et al.*, 1996; Wheeler, 2009).

Alarm Calling and Mate Investment

Males may use alarm calling to further protect mates from predators, increasing their likelihood of survival and thus increasing their reproductive fitness (Krams *et al.*, 2006; Wilson & Evans, 2008, 2010; Zaccaroni *et al.*, 2013). In great tits, *Parus major*, solitary males do not alarm call but they do when accompanied by their mate, suggesting males increase efforts to protect mates (Krams *et al.*, 2006). This was further explored in monogamous red-legged partridges, *Alectoris rufa*, by looking at alarm calling in lone males compared to males paired with either their mate or an unfamiliar male or female (Zaccaroni *et al.*, 2013). This study manufactured alarm calls by presenting 18 males with an avian predator model.

While lone males produced hardly any calls, alarm calling was highest when the male's mate was present, indicating that males invest preferentially in their mates (Zaccaroni *et al.*, 2013). Additionally, alarm call structure significantly differed across audiences, with calls directed towards mates having lower peak frequencies than calls to unrelated males and females. This adds further support to the mate investment hypothesis because lower frequencies are harder to locate thus males warned mates whilst reducing detection from predators (Zaccaroni *et al.*, 2013). Investment in the survival of reproductive mates could be particularly important in this monogamous species (Zaccaroni *et al.*, 2013).

Alarm Calling and Sexual Selection

Alarm calling could also be under the influence of sexual selection. Firstly, females could prefer to associate with better alarm calling males due to males acting as a sentinel and warning females about potential predators. This may allow females to forage more efficiently and safely while reducing their own investment in anti-predator detection (Pizzari, 2003; Portugal & Guillemain, 2011); whilst males benefit through privileged access to reproductive females. Second, females could prefer to mate with males that perform more alarm calls. Alarm calling can increase predation risk, therefore only males that are more likely to escape an attack would be able to call regularly; acting as an honest signal of predator evasion (Wilson *et al*., 2008). To produce honest alarm calls an individual must be vigilant in detecting predators. High vigilance levels are also an honest signal of male quality since foraging time is reduced (Pizzari, 2003) and vigilant males are preferred in the grey partridge (Dahlgren, 1990; Fusani *et al.*, 1997). Only certain genotypes can afford costly behaviours like vigilance and alarm calling, therefore females may select alarm calling males to gain genetic

benefits by producing offspring that possess the genes associated with alarm calling. Finally, alarm calling could be used by males to manipulate others by interrupting extra-pair copulations. This idea was tested in male swallows, *Hirundo rustica*, by experimentally manipulating female presence on the nest by chasing females away (Møller, 1990). Males differed in their response to female absence depending on the breeding cycle, with males giving false alarm calls when mates were absent during the egg-laying period yet few alarm calls were given during the incubation period (Møller, 1990). This suggests that male swallows used alarm calling to disrupt extra-pair copulations. Furthermore birds hearing these deceptive calls responded the same way as when hearing honest calls, adding further evidence to the idea that copulations were disrupted (Møller, 1990).

Vigilance, alarm calling and sexual selection in the Red junglefowl

The above section has illustrated how the adaptive significance of vigilance and alarm calling behaviour can be complex, particularly in social species, and species where these behaviours are sex specific. The overarching goal of my DPhil is to investigate the function of divergent male and female vigilance and alarm calling behaviours in a population of the sexually dimorphic red junglefowl, *Gallus gallus.*

Vigilance in the related domestic fowl, *Gallus gallus domesticus*, was explored in one study by placing male pairs in visual contact with a female (Pizzari, 2003). The dominant male within the pair was significantly more vigilant than both the subordinate male and the female. Whereas there was no significant difference between the vigilance efforts of subordinates and females (Pizzari, 2003). Furthermore females were less vigilant providing at least one male was present,

and removing both males led to females increasing vigilance (Pizzari, 2003). Additionally in large flocks of domestic fowl females in female-only flocks spent approximately 4% of their time vigilant on average compared to 2% in mixed flocks, adding further support to the finding that females reduce vigilance in the presence of males (Odén *et al.*, 2005).

Alarm Calling Behaviour in Fowl

Red junglefowl have a diverse repertoire of vocalisations including crowing, food calls and alarm calls. Both red junglefowl, and the related domestic fowl, produce two distinct alarm calls, one to signal the presence of terrestrial predators and one for aerial predators (Gyger *et al.*, 1987; Wilson *et al.*, 2008; Kokolakis *et al.*, 2010). Interestingly female fowl do not perform aerial alarm calls; however, they do emit terrestrial alarm calls. An early study explored the potential stimuli for triggering aerial alarm calls in Golden Sebright bantam chickens, finding that males performed aerial calls due to airborne stimulus in 89% of cases. Although the study found that a large variety of stimuli triggered alarm calling including birds (74.6%), insects (5.2%), leaves (0.8%), airplanes (8.6%), humans (4.1%) and sounds (6.7%) (Gyger *et al.*, 1987). Of the 74.6% calls that were due to birds 20.4% were because of small passerines that present no harm to fowl nor their eggs; suggesting that alarm calling is not a good indicator of potential threats due to males calling for non-threatening stimuli. Exploration of the call structure in domestic fowl showed that aerial alarm calls have two variations; a scream-like call containing broad-band elements and a whistle-like call. Furthermore, calls could be broken into three or four distinct units with call structure differing depending on predation risk. This study found that the first two units within the call provide the most information regarding predation risk. When males called

because of a potential avian predator the first call unit was more likely to be the broad-band scream-like call with the second unit comprising of the whistle-like call (Gyger *et al.,* 1987). This indicates that male aerial alarm call structure differs depending on the threat; so when males are presented with an aerial predator they perform one call structure but when faced with a non-threatening stimuli such as small passerines they use a different structure (Gyger *et al.*, 1987). Following on from this both aerial and terrestrial alarm calls were tested in domestic fowl to see whether they are functionally referential. By definition a functional referential signal is one which is triggered by specific stimuli that causes conspecifics to modify behaviour in a way that is adaptive to avoiding the stimuli despite not detecting the potential threat themselves (Evans *et al.*, 1993; Evans & Evans, 1999; Scarantino, 2013). Again, using Golden Sebrights, females were placed into pens and male aerial and terrestrial alarm calls were played back. Hens responded to aerial calls by crouching, looking up, scanning and seeking cover (Evans *et al.*, 1993). Hearing terrestrial calls did not cause females to run for cover or to look up but females did adopt an erect conspicuous vigilant posture and increased horizontal scanning. Therefore this heavily implies that both aerial and terrestrial alarm calls are functionally referential (Evans *et al.*, 1993). More recent studies focussing on aerial alarm calling in fowl have sought to establish the role of male alarm calls in sexual selection and reproductive success (Wilson *et al.*, 2008; Wilson & Evans, 2008, 2010; Kokolakis *et al.*, 2010) .

Sexual Selection in Fowl

To increase mating success males often need to compete with one another for access to females and/or resources needed for reproductive success (intrasexual selection) (Morris *et al.*, 1992; Loyau *et al.,* 2005). For example larger swordtail

males, *Xiphophorus nigrensis*, are more likely to win male-male interactions, giving them privileged access to females (Morris *et al.*, 1992). In addition, males also undergo intersexual selection via female mate choice where females select males based on traits or behaviours (Petrie & Williams, 1993; Pryke *et al.*, 2002; Doucet & Montgomerie, 2003; Loyau *et al.*, 2005a, 2005b, 2008; Dakin & Montgomerie, 2014). The mechanisms of sexual selection have been extensively studied in red junglefowl and the related domestic chicken (Zuk *et al.*, 1990; Ligon & Zwartjes, 1995; Ligon *et al.*, 1998; Pizzari & Birkhead, 2000; Pizzari *et al.*, 2003), with the mating system of junglefowl being described as harem polygynandry (Birkhead & Pizzari, 2009; Pizzari & McDonald, 2019). Dominant males establish territories which are shared with subordinate males and females, and within these small groups dominant males monopolize females, outcompeting subordinates for the majority of matings (Duncan, 2009; Pizzari & McDonald, 2019). Males court females using behaviours like waltzing in order to synchronize mating between the pair (Duncan, 2009). Early studies on female mate choice found that females prefer larger combs over plumage colour when assessing males (Zuk *et al.*, 1990b; Ligon & Zwartjes, 1995). Males with smaller combs have a greater occurrence of nematode parasites, therefore females could gain direct benefits by assessing parasite resistance using comb length and avoiding infected males (Zuk *et al.*, 1990a). Furthermore females solicit matings from dominant males yet actively avoid subordinates, suggesting the importance of social status on female preference (Pizzari & Birkhead, 2000; Johnsen *et al.*, 2001; Pizzari & McDonald, 2019).

In species with polyandry, where females mate with multiple males, males must engage in postcopulatory intrasexual selection through sperm competition (Collet

et al., 2012; McDonald *et al.*, 2017; Pizzari & McDonald, 2019). Males can adopt various strategies to reduce sperm competition like mate guarding (Kokko & Morrell, 2005; Setchell *et al.*, 2005), increasing remating rates (Collet *et al.*, 2012; McDonald *et al.*, 2017) and allocating more sperm (Pizzari *et al.*, 2003; Dean *et al.*, 2011). Dominant male red junglefowl have been shown to have higher remating rates than subordinates (Collet *et al.*, 2012) and postcopulatory sexual selection favours males that remate frequently (McDonald *et al.*, 2017). Additionally male fowl can allocate sperm in response to the number of sperm competitors, with males allocating the minimal amount when provided with no competitors (Pizzari *et al.*, 2003). Pizzari et al. (2003) provided males with no competitors, one competitor and three competitors finding that dominant and subordinate males show differences in sperm allocation. Dominant males invested the most sperm when provided with three competitors whereas subordinates increased investment when provided with one competitor and decreased investment when facing three males (Pizzari *et al.*, 2003). Furthermore, male fowl face postcopulatory intersexual selection through female cryptic choice. Female fowl can alter insemination by contracting directly after copulation leading to the ejection of sperm. Although females can choose males pre-copulation through solicitation, less desirable males are still able to forcefully mate with females. Many studies describe female preference for more dominant males in fowl (Zuk *et al.*, 1990b; Pizzari & Birkhead, 2000; Pizzari *et al.*, 2002; Wilson *et al.*, 2008), and dominant males experience reduced sperm ejection compared to subordinates (Pizzari & Birkhead, 2000; Dean *et al.*, 2011).

Vigilance, Alarm Calling and Mating Success

There is evidence that vigilance effort and alarm calling may be modulated by sexual selection in red junglefowl and domestic fowl. First, males appear to be more vigilant than females. Female vigilance decreased when in the presence of males, regardless of male status; potentially allowing females to increase foraging efficiency (Pizzari, 2003). Additionally, junglefowl have two alarm calls with aerial calls being male-specific (Evans, 1997). In sexual selection males typically invest more into costly signals and aerial alarm calling could be an example of this.

Second, vigilance effort and honesty of aerial calls in males are related to male social status, with dominant males providing more honest calls and were overall more vigilant. (Pizzari, 2003). Pizzari (2003) found that vigilance did not differ between subordinate males and females, but dominant males were significantly more vigilant than both these groups. When the dominant males were removed the subordinates significantly increased aerial alarm call rate. When the subdominant males were removed vigilance and alarm calling in the dominant males did not change significantly. An individual male junglefowl has no competitors so becomes dominant by default; this would explain the significant increase in aerial call rate in the subordinates after the dominant males were removed (Pizzari, 2003). This shows the importance of male status on aerial alarm calling behaviour.

Third, aerial calls predict mating success (Wilson *et al*., 2008). A key study by Wilson et al. (2008) found that aerial alarm call rate was the best predictor of mating and reproductive success in a population of domestic fowl. Females may benefit from mating with alarm calling males due to increased protection from

predators from being alerted often. Only the best males may be able to call often because of the greater predation risk. This could indicate a male's ability to successfully escape predation (Wilson *et al.*, 2008). Conversely a more recent study tested whether aerial alarm calling in males adhered to the mate investment hypothesis (Wilson & Evans, 2008). Half the males used could mate with a female for a 2-week period during the 4-week experiment with the latter half being the control, leading to an increase in aerial alarm calling by approximately 30% in mated males. All males were provided with a female as an audience, however only treatment males were permitted to mate with the female. This experiment added support for the mate investment hypothesis driving the increase in alarm calling after mating (Wilson & Evans, 2008). This may imply that females do not necessarily choose better alarm calling males but that males increase their investment in alarm calling post-mating. Although mate investment and male attractiveness are not necessarily mutually exclusive hypotheses, and both could play a role in alarm calling and vigilance. For example, females could select better alarm calling males with the intention of gaining direct benefits from increased predator avoidance through mate investment. A third study followed up on the male attractiveness findings in Wilson et al. (2008) by manipulating aerial alarm calling across three experiments and assessing female choice in Sebright bantams (Wilson & Evans, 2010). Males had alarm calling rate either experimentally increased or decreased yet females showed no preference for the more frequent calling males. All three experiments examined female preference through associations yet they all differed in experimental design (Wilson & Evans, 2010). Experiment one placed females into a highly controlled lab environment and provided them with life size video footage of males performing

aerial alarm calls, with control footage replacing aerial alarm calls with ambient sound chamber noise. Additionally, when aerial alarm calls occurred, or were supposed to occur for the control, females were presented with raptor silhouettes on ceiling screens, simulating males responding or failing to respond to an attack. Yet females showed no preference in association with either video stimulus (Wilson & Evans, 2010). For the second experiment females were placed into large outdoor aviaries and could see and hear two live males, one whom was supplemented with his own pre-recorded calls. Females were only able to interact with males an hour before sunset and the final roosting preference was recorded. After females roosted a male was randomly selected, and the following morning females were able to access the selected male, giving her the opportunity to mate. However females did not preferentially mate with either male, nor did females have strong preferences regarding who to roost next to (Wilson & Evans, 2010). The final experiment again used video footage, but females were placed into the outdoor aviary, and video footage altered the frequency of producing aerial, terrestrial and food calls along with crowing. Similarly, to experiment one the control removed some of the calling sound and replaced it with ambient sound chamber noise. Females were able to interact with the videos until late at night, and the final roosting location was recorded. Again roosting location showed no preference nor did time spent associated with either video (Wilson & Evans, 2010). This study concluded that females showed no preference for better alarm calling males irrespective of the experimental set up. In addition, this study did not fully examine mating success because female preference was defined by time spent near the stimuli, so it is possible that these females may have been more

likely to solicit and mate with the better alarm calling males despite not preferentially associating or roosting with them (Wilson & Evans, 2010).

Therefore, the function of aerial alarm calling in fowl remains unclear. Current studies present various conclusions including male attractiveness, mate investment and one finding females show no preference towards better alarm callers. This thesis aims to further explore the role of aerial alarm calling in fowl, with focus on the role of anti-predator behaviours in mating success and mate investment. The potential relationship between vigilance and mating success has received no attention in fowl despite vigilance being used in female choice in another Galliforme, the grey partridge. Like aerial alarm calling, vigilance could increase after mating as a form of mate investment.

Thesis Aims

My thesis has four specific aims, structured into four chapters, which are outlined below. The first three aim to investigate the more pronounced vigilance and alarm calling behaviour in males and their potential role in male reproductive strategies, while the last explores the role of vigilance in females.

The Effect of Group Size and Sex Ratio on Vigilance and Alarm Calling:

This chapter aims to experimentally disentangle the effect of group size and sex ratio in modulating vigilance effort and aerial alarm calling in male fowl. If vigilance effort and alarm calling are naturally selected anti-predatory behaviours, individual males should reduce their investment as group size increases, as predation risk is diluted. However, if social effects play a role sex ratio could influence vigilance effort and/or alarm calling behaviour of individual males. Dominance status has been shown to effect vigilance and alarm calling in fowl,

with more dominant males being more vigilant and producing more calls. This chapter looks at the relationship between status and vigilance/alarm calling across varying group size and sex ratio combinations.

The Role of Vigilance and Alarm Calling in Mating Success:

This chapter takes a correlational approach by looking at the relationships between vigilance, aerial alarm calling and mating behaviours within groups of 12. If vigilance and/or aerial alarm calling are attractive traits then these behaviours may have a significant relationship with the number of females mated with along with remating rates, number of solicitations, number of matings and sperm competition intensity.

Testing Hypotheses of Male Vigilance and Alarm Calling Investment:

This chapter explores the mate investment hypothesis on vigilance and aerial alarm calling by following on from Wilson and Evans (2008). Wilson and Evans (2008) suggested that males may increase alarm calling investment to protect females with whom they have mated, increasing the chance of females surviving and siring his offspring. If males increase vigilance and alarm calling investment post-copulation as a form of mate investment, then investment should be highest when males are able to mate and have no sperm competitors. However, if males are presented with sperm competition then these males should decrease investment due to reduced paternity confidence. This was experimentally explored by manipulating male mating success using four treatments of mating with and without competition, and no mating with and without competition.

Vigilance Behaviour in Female Red Junglefowl:

Little is known about female vigilance behaviour in fowl, with this chapter aiming to explore female vigilance in two parts. Part one looks at female vigilance in groups of 4 across varying sex ratios to see whether this alters vigilance efforts as predicted in males. The effect of female status on vigilance is also explored along with comparing male and female vigilance efforts. Females have been found to be less vigilant than males (Pizzari, 2003), therefore it was predicted that males would be more vigilant than females and that female vigilance should increase when fewer males are present. Alternatively, the higher number of females in more female-dominated groups could lead to heightened female-female aggression, potentially leading to an increase in vigilance to avoid aggressive encounters. Furthermore female-female aggression has been linked to mating in fruit flies, *Drosophila melanogaster*, and more female-biased groups had higher numbers of matings (Bath *et al.*, 2017). Therefore, vigilance should be higher when groups become more female-biased, potentially because of fewer males or increased female-female aggression. Females are very food-driven, spending much of their time foraging. Vigilance can be used to monitor neighbours for scrounging opportunities so part two in this chapter examines whether female vigilance is used to gather information on food location.

REFERENCES

Abramson, M. (1979). Vigilance as a Factor Influencing Flock Formation Among Curlews Numenius Arquata. *Ibis*, **121**, 213–216.

Ankney, C.D. & Afton, A.D. (1988). Bioenergetics of Breeding Northern Shovelers: Diet, Nutrient Reserves, Clutch Size, and Incubation. *The Condor*, **90**, 459–472.

Ankney, C.D., Afton, A.D. & Alisauskas, R.T. (1991). The Role of Nutrient Reserves in Limiting Waterfowl Reproduction. *The Condor*, **93**, 1029–1032.

Barrera, J.P., Chong, L., Judy, K.N. & Blumstein, D.T. (2011). Reliability of public information: predators provide more information about risk than conspecifics. *Animal Behaviour*, **81**, 779–787.

Bateman, A.J. (1948). Intra-sexual selection in Drosophila. *Heredity*, **2**, 349.

Bath, E., Bowden, S., Peters, C., Reddy, A., Tobias, J.A., Easton-Calabria, E., Seddon, N., Goodwin, S.F. & Wigby, S. (2017). Sperm and sex peptide stimulate aggression in female *Drosophila*. *Nature Ecology & Evolution*, **1**, 0154.

Beani, L. & Dessì-Fulgheri, F. (1995). Mate choice in the grey partridge, *Perdix perdix*: role of physical and behavioural male traits. *Animal Behaviour*, **49**, 347–356.

Beauchamp, G. (2001). Should vigilance always decrease with group size? *Behavioural Ecology and Sociobiology*, **51**, 47–52.

Beauchamp, G. (2007). Exploring the role of vision in social foraging: What happens to group size, vigilance, spacing, aggression and habitat use in birds and mammals that forage at night? *Biological Reviews*, **82**, 511–525.

Beauchamp, G. (2008). What is the magnitude of the group-size effect on vigilance? *Behavioural Ecology*, **19**, 1361–1368.

Beauchamp, G. (2009a). How does food density influence vigilance in birds and mammals? *Animal Behaviour*, **78**, 223–231.

Beauchamp, G. (2009b). Sleeping gulls monitor the vigilance behaviour of their neighbours. *Biology Letters*, **5**, 9–11.

Beauchamp, G. (2014). A field investigation of scrounging in semipalmated sandpipers. *Behavioural Ecology and Sociobiology*, **68**, 1473–1479.

Beauchamp, G. & Ruxton, G.D. (2008). Disentangling risk dilution and collective detection in the antipredator vigilance of semipalmated sandpipers in flocks. *Animal Behaviour*, **75**, 1837–1842.

Bednekoff, P.A. & Lima, S.L. (1998). Randomness, chaos and confusion in the study of antipredator vigilance. *Trends in Ecology & Evolution,* **13**, 284–287.

Bednekoff, P.A. & Lima, S.L. (2002). Why are scanning patterns so variable? An overlooked question in the study of anti-predator vigilance. *Journal of Avian Biology,* **33**, 143–149.

Bednekoff, P.A. & Ritter, R. (1994). Vigilance in Nxai Pan Springbok, Antidorcas marsupialis. *Behaviour,* **129**, 1–11.

Birkhead, T.R. & Pizzari, T. (2009). Biology of Breeding Poultry. Chapter 9 Sperm Competition and Fertilization Success. In *Biology of Breeding Poultry*: 133–150. CABI, 2009.

Blanchard, P. & Fritz, H. (2007). Induced or routine vigilance while foraging. *Oikos,* **116**, 1603–1608.

Blumstein, D.T., Cooley, L., Winternitz, J. & Daniel, J.C. (2008). Do yellow-bellied marmots respond to predator vocalizations? *Behavioural Ecology and Sociobiology*, **62**, 457–468.

Blumstein, D.T., Evans, C.S. & Daniel, J.C. (1999). An experimental study of behavioural group size effects in tammar wallabies, *Macropus eugenii*. *Animal Behaviour,* **58**, 351–360.

Boncoraglio, G. & Saino, N. (2007). Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. *Functional Ecology,* **21**, 134–142.

Bro-Jørgensen, J. & Pangle, W.M. (2010). Male Topi Antelopes Alarm Snort Deceptively to Retain Females for Mating. *The American Naturalist,* **176**, E33–E39.

Brown, C., Garwood, M.P. & Williamson, J.E. (2012). It pays to cheat: tactical deception in a cephalopod social signalling system. *Biology Letters*, **8,** 729-732.

Brown, T.J. & Handford, P. (1996). Acoustic Signal Amplitude Patterns: A Computer Simulation Investigation of the Acoustic Adaptation Hypothesis. *The Condor,* **98**, 608–623.

Cain, K.E., Hall, M.L., Medina, I., Leitao, A.V., Delhey, K., Brouwer, L., Peters, A., Pruett-Jones, S., Webster, M.S., Langmore, N.E. & Mulder, R.A. (2019). Conspicuous Plumage Does Not Increase Predation Risk: A Continent-Wide Test Using Model Songbirds. *The American Naturalist,* **193**, 359–372.

Charnov, E.L. & Krebs, J.R. (1975). The Evolution of Alarm Calls: Altruism or Manipulation? *The American Naturalist,* **109**, 107–112.

Childress, M.J. & Lung, M.A. (2003). Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Animal Behaviour,* **66**, 389–398.

Collet, J., Richardson, D.S., Worley, K. & Pizzari, T. (2012). Sexual selection and the differential effect of polyandry. *Proceedings of the National Academy of Sciences of the United States of America,* **109**, 8641–8645.

Colombelli-Négrel, D. & Evans, C. (2017). Superb fairy-wrens respond more to alarm calls from mate and kin compared to unrelated individuals. *Behavioural Ecology,* **28**, 1101–1112.

Cresswell, W. (1994). Song as a pursuit-deterrent signal, and its occurrence relative to other anti-predation behaviours of skylark (*Alauda arvensis*) on attack by merlins (*Falco columbarius*). *Behavioural Ecology and Sociobiology*, **34**, 217–223.

Cresswell, W., Lind, J., Kaby, U., Quinn, J.L. & Jakobsson, S. (2003a). Does an opportunistic predator preferentially attack nonvigilant prey? *Animal Behaviour,* **66**, 643–648.

Cresswell, W., Quinn, J.L., Whittingham, M.J. & Butler, S. (2003b). Good foragers can also be good at detecting predators. *Proceedings of the Royal Society of London B: Biological Sciences,* **270**, 1069–1076.

Crothers, L., Gering, E. & Cummings, M. (2011). Aposematic Signal Variation Predicts Male–Male Interactions in a Polymorphic Poison Frog. *Evolution,* **65**, 599–605.

Dahlgren, J. (1990). Females choose vigilant males: an experiment with the monogamous grey partridge, *Perdix perdix*. *Animal Behaviour,* **39**, 646–651.

Dakin, R. & Montgomerie, R. (2014a). Deceptive Copulation Calls Attract Female Visitors to Peacock Leks. *The American Naturalist,* **183**, 558–564.

Dakin, R. & Montgomerie, R. (2014b). Condition-dependent mate assessment and choice by peahens: implications for sexual selection. *Behavioural Ecology,* **25**, 1097–1104.

Davis, L.S. (1984). Alarm Calling in Richardson's Ground Squirrels (*Spermophilus richardsonii*). *Zeitschrift für Tierpsychologie*, **66**, 152–164.

Dawkins, M.S. & Guilford, T. (1991). The corruption of honest signalling. *Animal Behaviour,* **41**, 865–873.

Dean, R., Nakagawa, S. & Pizzari, T. (2011). The Risk and Intensity of Sperm Ejection in Female Birds. *The American Naturalist,* **178**, 343–354.

Doucet, S.M. & Montgomerie, R. (2003). Multiple sexual ornaments in satin bowerbirds: ultraviolet plumage and bowers signal different aspects of male quality. *Behavioural Ecology,* **14**, 503–509.

Duckworth, R.A., Mendonça, M.T. & Hill, G.E. (2001). A Condition Dependent Link between Testosterone and Disease Resistance in the House Finch. *Proceedings of the Royal Society of London B: Biological Sciences,* **268**, 2467–2472.

Duncan, I.J.H. (2009). Biology of Breeding Poultry. Chapter 8 Mating Behaviour and Fertility. In *Biology of Breeding Poultry*: 111–132. CABI, 2009.

Duriez, O., Fritz, H., Binet, F., Tremblay, Y. & Ferrand, Y. (2005). Individual activity rates in wintering Eurasian woodcocks: starvation versus predation risk trade-off? *Animal Behaviour,* **69**, 39–49.

Elgar, M.A. (1989). Predator Vigilance and Group Size in Mammals and Birds: A Critical Review of the Empirical Evidence. *Biological Reviews,* **64**, 13–33.

Endler, J.A. (1992). Signals, Signal Conditions, and the Direction of Evolution. *The American Naturalist,* **139**, S125–S153.

Evans, C.S. (1997). Referential Signals. In *Communication*, Perspectives in Ethology: 99–143. Owings, D.H., Beecher, M.D. & Thompson, N.S. (Eds.). Springer US.

Evans, C.S. & Evans, L. (1999). Chicken food calls are functionally referential. *Animal Behaviour,* **58**, 307–319.

Evans, C.S., Evans, L. & Marler, P. (1993). On the meaning of alarm calls: functional reference in an avian vocal system. *Animal Behaviour,* **46**, 23–38.

Ey, E. & Fischer, J. (2009). The "Acoustic Adaptation Hypothesis"- A Review of the Evidence from Birds, Anurans and Mammals. *Bioacoustics,* **19**, 21–48.

Favreau, F-R., Pays, O., Fritz, H., Goulard, M., Best, E.C. & Goldizen, A.W. (2015). Predators, food and social context shape the types of vigilance exhibited by kangaroos. *Animal Behaviour,* **99**, 109–121.

Fernández-Juricic, E., Erichsen, J.T. & Kacelnik, A. (2004). Visual perception and social foraging in birds. *Trends in Ecology & Evolution,* **19**, 25–31.

Fernández-Juricic, E., Smith, R. & Kacelnik, A. (2005). Increasing the costs of conspecific scanning in socially foraging starlings affects vigilance and foraging behaviour. *Animal Behaviour,* **69**, 73–81.

FitzGibbon, C.D. (1989). A cost to individuals with reduced vigilance in groups of Thomson's Gazelles hunted by Cheetahs. *Animal Behaviour,* **37**, 508–510.

Folstad, I. & Karter, A.J. (1992). Parasites, Bright Males, and the Immunocompetence Handicap. *The American Naturalist,* **139**, 603–622.

Foster, W.A. & Treherne, J.E. (1981). Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature,* **293**, 466–467.

Fusani, L., Beani, L., Lupo, C. & Dessì-Fulgheri, F. (1997). Sexually selected vigilance behaviour of the grey partridge is affected by plasma androgen levels. *Animal Behaviour,* **54**, 1013–1018.

Gaynor, K.M. & Cords, M. (2012). Antipredator and social monitoring functions of vigilance behaviour in blue monkeys. *Animal Behaviour*, **84**, 531–537.

Gil-da-Costa R., Palleroni A., Hauser M.D., Touchton J. & Kelley J. (2003). Rapid acquisition of an alarm response by a neotropical primate to a newly introduced avian predator. *Proceedings of the Royal Society of London B: Biological Sciences,* **270**, 605–610.

Götmark. (1993). Conspicuous coloration in male birds is favoured by predation in some species and disfavoured in others. *Proceedings of the Royal Society of London B: Biological Sciences,* **253**, 143–146.

Götmark, F. (1992). Anti-predator effect of conspicuous plumage in a male bird. *Animal Behaviour,* **44**, 51–55.

Götmark, F. & Hohlfält, A. (1995). Bright Male Plumage and Predation Risk in Passerine Birds: Are Males Easier to Detect Than Females? *Oikos,* **74**, 475–484.

Gouzoules, H., Gouzoules, S. & Miller, K. (1996). Skeptical responding in rhesus monkeys (*Macaca mulatta*). *International Journal of Primatology,* **17**, 549–568.

Grand, T.C. & Dill, L.M. (1999). The effect of group size on the foraging behaviour of juvenile coho salmon: reduction of predation risk or increased competition? *Animal Behaviour,* **58**, 443–451.

Guilford, T. & Dawkins, M.S. (1991). Receiver psychology and the evolution of animal signals. *Animal Behaviour,* **42**, 1–14.

Guillemain, M., Caldow, R.W.G., Hodder, K.H. & Goss-Custard, J.D. (2003). Increased vigilance of paired males in sexually dimorphic species: distinguishing between alternative explanations in wintering Eurasian wigeon. *Behavioural Ecology,* **14**, 724–729.

Gyger, M., Marler, P. & Pickert, R. (1987). Semantics of an Avian Alarm Call System: The Male Domestic Fowl, *Gallus domesticus*. *Behaviour,* **102**, 15–40.

Hagelin, J.C. & Ligon, J.D. (2001). Female quail prefer testosterone-mediated traits, rather than the ornate plumage of males. *Animal Behaviour,* **61**, 465–476.

Hamilton, W.D. (1971). Geometry for the selfish herd. *Journal of Theoretical Biology,* **31**, 295–311.

Hasson, O. (1994). Cheating Signals. *Journal of Theoretical Biology*, **167**, 223–238.

Hegna, R.H., Saporito, R.A. & Donnelly, M.A. (2013). Not all colors are equal: predation and color polytypism in the aposematic poison frog *Oophaga pumilio*. *Evolutionary Ecology*, **27**, 831–845.

Hendrie, C.A., Weiss, S.M. & Eilam, D. (1998). Behavioural response of wild rodents to the calls of an owl: a comparative study. *Journal of Zoology*, **245**, 439–446.

Husak, J.F., Macedonia, J.M., Fox, S.F. & Sauceda, R.C. (2006). Predation Cost of Conspicuous Male Coloration in Collared Lizards (*Crotaphytus collaris*): An Experimental Test Using Clay-Covered Model Lizards. *Ethology*, **112**, 572–580.

Janicke, T., Häderer, I.K., Lajeunesse, M.J. & Anthes, N. (2016). Darwinian sex roles confirmed across the animal kingdom. *Science Advances*, **2**, e1500983.

Jeschke, J.M. & Tollrian, R. (2005). Effects of predator confusion on functional responses. *Oikos*, **111**, 547–555.

Johnsen, T.S., Zuk, M. & Fessler, E.A. (2001). Social Dominance, Male Behaviour and Mating in Mixed-Sex Flocks of Red Junglefowl. *Behaviour*, **138**, 1–18.

Kikuchi, D.W., Seymoure, B.M. & Pfennig, D.W. (2014). Mimicry's palette: widespread use of conserved pigments in the aposematic signals of snakes. *Evolution & Development*, **16**, 61–67.

Kokko, H. & Morrell, L.J. (2005). Mate guarding, male attractiveness, and paternity under social monogamy. *Behavioural Ecology*, **16**, 724–731.

Kokolakis, A., Smith, C.L. & Evans, C.S. (2010). Aerial alarm calling by male fowl (*Gallus gallus*) reveals subtle new mechanisms of risk management. *Animal Behaviour*, **79**, 1373–1380.

Krams, I., Krama, T. & Igaune, K. (2006). Alarm calls of wintering great tits *Parus major*: warning of mate, reciprocal altruism or a message to the predator? *Journal of Avian Biology*, **37**, 131–136.

Landeau, L. & Terborgh, J. (1986). Oddity and the 'confusion effect' in predation. *Animal Behaviour*, **34**, 1372–1380.

Ligon, J.D., Kimball, R. & Merola-Zwartjes, M. (1998). Mate choice by female red junglefowl: the issues of multiple ornaments and fluctuating asymmetry. *Animal Behaviour*, **55**, 41–50.

Ligon, J.D., Thornhill, R., Zuk, M. & Johnson, K. (1990). Male-male competition, ornamentation and the role of testosterone in sexual selection in red junglefowl. *Animal Behaviour*, **40**, 367–373.

Ligon, J.D. & Zwartjes, P.W. (1995). Ornate plumage of male red junglefowl does not influence mate choice by females. *Animal Behaviour*, **49**, 117–125.

Lima, S.L. (1995). Back to the basics of anti-predatory vigilance: the group-size effect. *Animal Behaviour*, **49**, 11–20.

Lind, J. & Cresswell, W. (2005). Determining the fitness consequences of antipredation behavior. *Behavioural Ecology*, **16**, 945–956.

Loyau, A., Jalme, M.S., Cagniant, C. & Sorci, G. (2005a). Multiple sexual advertisements honestly reflect health status in peacocks (*Pavo cristatus*). *Behavioural Ecology and Sociobiology*, **58**, 552–557.

Loyau, A., Jalme, M.S. & Sorci, G. (2005b). Intra- and Intersexual Selection for Multiple Traits in the Peacock (*Pavo cristatus*). *Ethology*, **111**, 810–820.

Loyau, A., Petrie, M., Jalme, M.S. & Sorci, G. (2008). Do peahens not prefer peacocks with more elaborate trains? *Animal Behaviour*, **76**, e5-e9.

Magrath, R.D., Haff, T.M., Fallow, P.M. & Radford, A.N. (2015). Eavesdropping on heterospecific alarm calls: from mechanisms to consequences. *Biological Reviews*, **90**, 560–586.

Makin, D.F., Chamaillé-Jammes, S. & Shrader, A.M. (2019). Alarm calls or predator calls: which elicit stronger responses in ungulate communities living with and without lions? *Oecologia*, **190**, 25-35.

Mayhew, P.W. (1987). Vigilance levels in European Wigeon - sexual differences. *Wildfowl*, **38**, 77–81.

McDonald, G.C., Spurgin, L.G., Fairfield, E.A., Richardson, D.S. & Pizzari, T. (2017). Pre- and postcopulatory sexual selection favor aggressive, young males in polyandrous groups of red junglefowl. *Evolution*, **71**, 1653–1669.

Mcrobert, S.P. & Bradner, J. (1998). The influence of body coloration on shoaling preferences in fish. *Animal Behaviour*, **56**, 611–615.

Meijer, T., Masman, D. & Daan, S. (1989). Energetics of Reproduction in Female Kestrels. *Auk*, **106**, 549–559.

Møller, A.P. (1990). Deceptive use of alarm calls by male swallows, *Hirundo rustica*: a new paternity guard. *Behavioural Ecology*, **1**, 1–6.

Møller, A.P. & Petrie, M. (2002). Condition dependence, multiple sexual signals, and immunocompetence in peacocks. *Behavioural Ecology*, **13**, 248–253.

Monclús, R. & Rödel, H.G. (2008). Different Forms of Vigilance in Response to the Presence of Predators and Conspecifics in a Group-Living Mammal, the European Rabbit. *Ethology*, **114**, 287–297.

Mooring, M.S. & Hart, B.L. (1992). Animal Grouping for Protection from Parasites: Selfish Herd and Encounter-Dilution Effects. *Behaviour,* **123**, 173–193.

Morris, M.R., Batra, P. & Ryan, M.J. (1992). Male-Male Competition and Access to Females in the Swordtail *Xiphophorus nigrensis*. *Copeia,* **1992**, 980–986.

Mougeot, F., Irvine, J.R., Seivwright, L., Redpath, S.M. & Piertney, S. (2004). Testosterone, immunocompetence, and honest sexual signaling in male red grouse. *Behavioural Ecology,* **15**, 930–937.

Munn, C.A. (1986). Birds that 'cry wolf.' *Nature*, **319**, 143.

Murali, G., Kumari, K. & Kodandaramaiah, U. (2019). Dynamic colour change and the confusion effect against predation. *Scientific Reports,* **9**, 274.

Odén, K., Gunnarsson, S., Berg, C. & Algers, B. (2005). Effects of sex composition on fear measured as tonic immobility and vigilance behaviour in large flocks of laying hens. *Applied Animal Behaviour Science,* **95**, 89–102.

Parrish, J.K. (1989). Re-examining the selfish herd: are central fish safer? *Animal Behaviour,* **38**, 1048–1053.

Pérez-Rodríguez, L., Blas, J., Viñuela, J., Marchant, T.A. & Bortolotti, G.R. (2006). Condition and androgen levels: are condition-dependent and testosterone-mediated traits two sides of the same coin? *Animal Behaviour,* **72**, 97–103.

Petrie, M. (1994). Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature,* **371**, 598–599.

Petrie, M., Cotgreave, P. & Stewart, I. (1996). Variation in the train morphology of peacocks (*Pavo cristatus*). *Journal of Zoology,* **238**, 365–371.

Petrie, M., Tim, H. & Carolyn, S. (1991). Peahens prefer peacocks with elaborate trains. *Animal Behaviour,* **41**, 323–331.

Petrie Marion & Williams Amanda. (1993). Peahens lay more eggs for peacocks with larger trains. *Proceedings of the Royal Society of London B: Biological Sciences*, **251**, 127–131.

Peuhkuri, N. (1997). Size-assortative shoaling in fish: the effect of oddity on foraging behaviour. *Animal Behaviour*, **54**, 271–278.

Pizzari, T. (2003). Food, vigilance, and sperm: the role of male direct benefits in the evolution of female preference in a polygamous bird. *Behavioural Ecology*, **14**, 593–601.

Pizzari, T. & Birkhead, T.R. (2000). Female feral fowl eject sperm of subdominant males. *Nature*, **405**, 787–789.

Pizzari, T., Cornwallis, C.K., Løvlie, H., Jakobsson, S. & Birkhead, T.R. (2003). Sophisticated sperm allocation in male fowl. *Nature*, **426**, 70–74.

Pizzari, T., Froman, D.P. & Birkhead, T.R. (2002). Pre- and post-insemination episodes of sexual selection in the fowl, *Gallus g. domesticus*. *Heredity*, **88**, 112–116.

Pizzari, T. & McDonald, G.C. (2019). Sexual selection in socially-structured, polyandrous populations: Some insights from the fowl. *Advances in the Study of Behavior*, **51**, 77-141.

Portugal, S.J. & Guillemain, M. (2011). Vigilance patterns of wintering Eurasian Wigeon: female benefits from male low-cost behaviour. *Journal of Ornithology*, **152**, 661–668.

Pryke, S.R., Andersson, S., Lawes, M.J. & Piper, S.E. (2002). Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color. *Behavioural Ecology*, **13**, 622–631.

Quinn, J.L. & Cresswell, W. (2006). Testing domains of danger in the selfish herd: sparrowhawks target widely spaced redshanks in flocks. *Proceedings of the Royal Society of London B: Biological Sciences*, **273**, 2521–2526.

Rainey, H.J., Zuberbühler, K. & Slater, P.J.B. (2004). The responses of black-casqued hornbills to predator vocalisations and primate alarm calls. *Behaviour*, **141**, 1263–1277.

Rodgers, G.M., Ward, J.R., Askwith, B. & Morrell, L.J. (2011). Balancing the Dilution and Oddity Effects: Decisions Depend on Body Size. *PLoS ONE*, **6**, e14819.

Rolando, A., Caldoni, R., De Sanctis, A. & Laiolo, P. (2001). Vigilance and neighbour distance in foraging flocks of red-billed choughs, *Pyrrhocorax pyrrhocorax*. *Journal of Zoology*, **253**, 225–232.

Rowe Locke & Houle David. (1996). The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society of London B: Biological Sciences*, **263**, 1415–1421.

Sansom, A., Cresswell, W., Minderman, J. & Lind, J. (2008). Vigilance benefits and competition costs in groups: do individual redshanks gain an overall foraging benefit? *Animal Behaviour*, **75**, 1869–1875.

Scarantino, A. (2013). Rethinking Functional Reference. *Philosophy of Science*, **80**, 1006–1018.

Schmidt, K.A., Lee, E., Ostfeld, R.S. & Sieving, K. (2008). Eastern chipmunks increase their perception of predation risk in response to titmouse alarm calls. *Behavioural Ecology*, **19**, 759–763.

Schradin, C. (2000). Confusion Effect in a Reptilian and a Primate Predator. *Ethology*, **106**, 691–700.

Schwagmeyer, P.L. (1980). Alarm calling behavior of the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*. *Behavioural Ecology and Sociobiology*, **7**, 195–200.

Setchell, J.M., Charpentier, M. & Wickings, E.J. (2005). Mate guarding and paternity in mandrills: factors influencing alpha male monopoly. *Animal Behaviour*, **70**, 1105–1120.

Seyfarth, R.M., Cheney, D.L. & Marler, P. (1980). Vervet monkey alarm calls: Semantic communication in a free-ranging primate. *Animal Behaviour*, **28**, 1070–1094.

Slotow, R. & Rothstein, S.I. (1995). Influence of Social Status, Distance from Cover, and Group Size on Feeding and Vigilance in White-Crowned Sparrows. *The Auk*, **112**, 1024–1031.

Smith, M.J. & Harper, D.G.C. (1995). Animal Signals: Models and Terminology. *Journal of Theoretical Biology*, **177**, 305–311.

Squires, K.A., Martin, K., Goudie, R.I. & Yasukawa, K. (2007). Vigilance Behavior in the Harlequin Duck (*Histrionicus histrionicus*) during the Preincubation Period in Labrador: Are Males Vigilant for Self or Social Partner? *The Auk*, **124**, 241–252.

Stuart-Fox, D.M., Moussalli, A., Marshall, N.J. & Owens, I.P.F. (2003). Conspicuous males suffer higher predation risk: visual modelling and experimental evidence from lizards. *Animal Behaviour*, **66**, 541–550.

Treves, A. (2000). Theory and method in studies of vigilance and aggregation. *Animal Behaviour*, **60**, 711–722.

Trivers, R.L. (1996). *Parental investment and sexual selection*. Foundations of animal behavior: Classic papers with commentaries. Chicago, IL, US: University of Chicago Press.

Valkonen, J., Niskanen, M., Björklund, M. & Mappes, J. (2011). Disruption or aposematism? Significance of dorsal zigzag pattern of European vipers. *Evolutionary Ecology*, **25**, 1047–1063.

Viscido, S.V. & Wethey, D.S. (2002). Quantitative analysis of fiddler crab flock movement: evidence for 'selfish herd' behaviour. *Animal Behaviour*, **63**, 735–741.

Wang, I.J. & Shaffer, H.B. (2008). Rapid Color Evolution in an Aposematic Species: A Phylogenetic Analysis of Color Variation in the Strikingly Polymorphic Strawberry Poison-Dart Frog. *Evolution*, **62**, 2742–2759.

Wheeler Brandon C. (2009). Monkeys crying wolf? Tufted capuchin monkeys use anti-predator calls to usurp resources from conspecifics. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 3013–3018.

Wickler, W. (1985). Coordination of Vigilance in Bird Groups. The "Watchman's Song" Hypothesis. *Zeitschrift für Tierpsychologie,* **69**, 250–253.

Wilson, D.R., Bayly, K.L., Nelson, X.J., Gillings, M. & Evans, C.S. (2008). Alarm calling best predicts mating and reproductive success in ornamented male fowl, *Gallus gallus*. *Animal Behaviour,* **76**, 543–554.

Wilson, D.R. & Evans, C.S. (2008). Mating success increases alarm-calling effort in male fowl, *Gallus gallus*. *Animal Behaviour,* **76**, 2029–2035.

Wilson, D.R. & Evans, C.S. (2010). Female fowl (*Gallus gallus*) do not prefer alarm-calling males. *Behaviour,* **147**, 525–552.

Wong, B.B.M. & Rosenthal, G.G. (2005). Shoal Choice in Swordtails when Preferences Conflict. *Ethology,* **111**, 179–186.

Woodland, D.J., Jaafar, Z. & Knight, M.-L. (1980). The "Pursuit Deterrent" Function of Alarm Signals. *The American Naturalist,* **115**, 748–753.

Wüster W., Allum C.S.E., Bjargardóttir I.B., Bailey K.L., Dawson K.J., Guenioui J., Lewis J., McGurk J., Moore A.G., Niskanen M. & Pollard C.P. (2004). Do aposematism and Batesian mimicry require bright colours? A test, using European viper markings. *Proceedings of the Royal Society of London B: Biological Sciences,* **271**, 2495–2499.

Yasukawa, K., Whittenberger, L.K. & Nielsen, T.A. (1992). Anti-predator vigilance in the red-winged blackbird, *Agelaius phoeniceus*: do males act as sentinels? *Animal Behaviour,* **43**, 961–969.

Zaccaroni, M., Binazzi, R., Massolo, A. & Dessì-Fulgheri, F. (2013). Audience effect on aerial alarm calls in the monogamous red-legged partridge. *Ethology Ecology & Evolution*, **25**, 366–376.

Zahavi, A. (1975). Mate selection- A selection for a handicap. *Journal of Theoretical Biology,* **53**, 205–214.

Zuberbühler, K. (2009). Chapter 8 Survivor Signals: The Biology and Psychology of Animal Alarm Calling. In *Advances in the Study of Behavior*, **40**: 277–322. Academic Press Inc 2009.

Zuk, M., Thornhill, R., Ligon, J.D. & Johnson, K. (1990a). Parasites and mate choice in red junglefowl. *American Zoologist,* **30**, 235–244.

Zuk, M., Thornhill, R., Ligon, J.D., Johnson, K., Austad, S., Ligon, S.H., Thornhill, N.W. & Costin, C. (1990b). The Role of Male Ornaments and Courtship Behavior in Female Mate Choice of Red Junglefowl. *The American Naturalist,* **136**, 459–473.

Chapter 2

The Effect of Group Size and Sex Ratio on Vigilance and Alarm Calling

Heather Kazara and Tommaso Pizzari

ABSTRACT

Individual investment in anti-predator behaviours such as vigilance and alarm calling are expected to decline with increasing group size. The role of the social composition of a group and its interaction with group size, however, are less understood. In sexually dimorphic species, the more conspicuous sex will suffer increased predation risk as it becomes scarcer, through the oddity effect. Alternatively, sex ratio can influence male investment in vigilance and alarm calling when these behaviours play a role in sexual selection. In red junglefowl, *Gallus gallus*, conspicuous males are more vigilant than females and only males produce alarm calls for aerial predators. Aerial alarm calls have been linked to male mating and reproductive success, and both vigilance and aerial alarm calling increase with male social status. This study explored the effects of group size, sex ratio and male dominance on both vigilance and aerial alarm calling in male fowl. We studied groups of 4 and 12 across four sex ratios (Female Biased, Even Ratio, Male Biased and Male Only). Vigilance effort was explained by an interaction between group size and sex ratio, peaking in Even Ratio groups of 12, and declining as groups became more male-biased. Alarm calling was also explained by an interaction between group size and sex ratio, progressively declining with the proportion of males in a group, and more so in groups of 4 than in groups of 12. Socially dominant males tended to invest more in vigilance and alarm calling. The patterns emerging from this work suggest that multiple mechanisms including oddity effect, social signalling and sexual selection may be at work to modulate male vigilance and alarm calling investment. Collectively, these results indicate that in addition to group size, the social composition of a group can be a critical driver of anti-predator behaviour.

INTRODUCTION

To maximise fitness individuals must avoid predation and there are multiple strategies that can be used to achieve this including aggregating (Wrona & Dixon, 1991; Rodgers *et al.*, 2011), cryptic colouration (Slagsvold *et al.*, 1995; Husak *et al.*, 2006) and vigilance (FitzGibbon, 1989; Cresswell *et al.*, 2003). Vigilance is defined as the stopping of behaviours, typically foraging, to scan the surrounding environment for potential predators. While it reduces predation risk (FitzGibbon, 1989), vigilance is costly; reducing time available for other fitness-related behaviours such as foraging or mating. For example, in four ungulate species increased vigilance led to a reduction in foraging (Creel *et al.*, 2014). Increasing vigilance can also lead to a reduction in other vital activities such as sleep. In Barbary doves, *Streptopelia risoria*, individuals exposed to a predator spent more time being vigilant and less sleeping than the control group, implying a trade-off between these two activities (Lendrem, 1984). Individuals should therefore aim to reduce vigilance where possible; with the simplest method being to aggregate (Pulliam, 1973). Increasing the number of individuals in a group can decrease predation risk through dilution effects; allowing individual vigilance to decrease (Wrona & Dixon, 1991; Beauchamp, 2008).

The group size effect refers to the decrease in individual vigilance observed as group size increases. Pulliam (1973) was the first to mathematically demonstrate this, showing that increased group size led to more eyes consistently scanning, increasing predator detection; causing a decrease in individual vigilance without compromising safety. For example, springboks, *Antidorcas marsupialis*, in herds with more than six individuals spent 4.6 seconds on scanning in comparison with 6.9 seconds in herds of five individuals or fewer (Burger *et al*., 2000). Similarly,

Tammar wallabies, *Macropus eugenii*, in groups of 10 spent 18% less time scanning and 16.5% more time foraging than solitary wallabies (Blumstein *et al.*, 1999). Group size also modulates the foraging-vigilance trade-off in the yellow-footed rock-wallaby, *Petrogale xanthopus,* where foraging increased while vigilance decreased with increasing group size (Blumstein *et al.*, 2001). In addition, by increasing proximity to conspecifics, an individual reduces their "domain of danger". This refers to a larger area surrounding an individual that is not filled by neighbours, where predation risk is higher. Reduction in domains of danger through grouping is known as the selfish herd effect (Hamilton, 1971; Parrish, 1989; Beauchamp, 2007; King *et al.*, 2012). Simulated predator attacks on sand fiddler crabs, *Uca pugilator*, caused individuals to move towards the centre of the group, rather than escape; suggesting an advantage to being central (Viscido & Wethey, 2002). In brown-headed cowbirds, *Molothrus ater*, vigilance increased with increasing neighbour distance (Fernández-Juricic *et al.*, 2007).

While increasing group size is expected to reduce individual vigilance effort, the composition of a group may also modulate vigilance behaviour through different social mechanisms. For example, several species exhibit marked sex differences in vigilance, with males typically investing more time in vigilance than females (Guillemain *et al.*, 2003; Squires *et al.*, 2007; Portugal & Guillemain, 2011). This may reflect the fact that males are often more conspicuous and thus more vulnerable to predation. Another explanation for this sex difference is that male vigilance may be favoured by sexual selection, which tends to be more intense in males in species with 'traditional sex roles' (i.e. in species where males must compete for mates) (Schärer *et al.*, 2012; Janicke *et al.*, 2016). Females may gain non-genetic benefits from copulating with vigilant males, as a more vigilant male

would offer a female and her offspring better protection from predators. In addition, females could gain genetic benefits when male vigilance effort reflects heritable condition dependence. Females may therefore evolve a preference to mate with more vigilant males, generating intersexual selection for male investment in vigilance. In addition, males could use vigilance to guard females from competitors, though whether mate guarding is an effective strategy to secure paternity likely varies depending on the mating system and could vary with male attractiveness (Kokko & Morrell, 2005). Increasing time in close proximity with mates may reduce the likelihood of extra-pair copulations but also likely decreases the male's extra-pair matings, causing a trade-off (Kokko & Morrell, 2005). In highly polygamous species mate guarding is less common, suggesting that within this system it is more beneficial for males to mate with multiple females than guard females (Kokko & Morrell, 2005). Using a game-theory approach Kokko & Morrell (2005) showed that more attractive males may spend less time engaged in mate guarding; potentially because their partners are less likely to mate with other males. Less attractive males on the other hand, may need to increase mate guarding effort to prevent cuckoldry. Therefore in certain mating systems, or for less attractive males, increasing vigilance effort to aid in mate guarding may be an adaptive strategy (Kokko & Morrell, 2005). Mate guarding has been suggested to explain sex differences in vigilance in Eurasian wigeon, *Mareca penelope* (Guillemain *et al.*, 2003). Male vigilance did not change when males adopted the more conspicuous breeding plumage, indicating that males do not suffer from increased predation risk. Furthermore, paired females did not reduce their vigilance compared to solitary females, indicating that male vigilance does not benefit paired females by allowing them to reduce their own vigilance

effort. Paired females did however engage in fewer interactions with other males, suggesting that male increased vigilance mainly to prevent their partners from engaging in extra-pair copulations (Guillemain *et al.*, 2003). On the other hand, there was no evidence that male white-tailed ptarmigans, *Lagopus leucura*, increase vigilance in the breeding period when an extra-pair copulation can result in fertilization; suggesting that male vigilance in this species does not aid in male mate guarding of their social partners (Artiss & Martin, 1995).

Intrasexual competition for reproductive opportunities is strongly influenced by the sex ratio of the population (Kvarnemo *et al.*, 1995; Kvarnemo & Ahnesjo, 1996); specifically, the operational sex ratio of a population which refers to the ratio of sexually receptive males to females (Tejedo, 1988; Kvarnemo & Ahnesjo, 1996; Mills & Reynolds, 2003). In some species, male-male competition and aggression are expected to increase in more male-biased populations, whereas in female-biased groups males could engage in more courting and less harassment (Kvarnemo & Ahnesjo, 1996; Jirotkul, 1999; Mills & Reynolds, 2003). In other species however, a male bias can offer females more choice and promote male investment in courtship displays. In guppies, *Poecilia reticulata*, females were more willing to copulate with courting males when the operational sex ratio was strongly male-biased (Jirotkul, 1999). Females were also more selective at these ratios, implying that female choosiness increases with increasing males available (Jirotkul, 1999). Male mate guarding behaviour post-mating is also predicted to increase in more male-biased populations to reduce sperm competition and increase paternity (Jirotkul, 1999). Biases in sex ratio in the marine sand goby, *Pomatoschistus minutus*, led to increased behavioural interactions among the

more common sex (Kvarnemo *et al.*, 1995). Sex ratio can therefore reflect aspects of the intensity of sexual selection on males (but see Kokko & Monaghan, 2001).

Group size and sex ratio may interact to determine the vigilance effort of individual males. Male white-fronted geese, *Anser albifrons flavirostris*, in pairs are significantly more vigilant than paired females. This sex difference disappears when geese join a flock (Fox & Madsen, 1981). When in pairs, sexual selection could favour male vigilance as a way for males to advertise their quality and investment in the current reproductive event to their social partner, prevent their social partners from copulating with extra-pair males, and/or to seek opportunities for extra-pair copulations. However, outside the breeding season, when birds flock in larger groups, vigilance is likely to be largely driven by natural selection and vigilance effort is shared more equally across all flock members.

Few studies have explored the relative roles of group size and sex ratio in modulating vigilance effort. Vigilance in natural mixed sex groups was studied in bighorn sheep, *Ovis canadensis*. Mixed sex groups were larger than single sex groups, causing overall vigilance to decrease in accordance with the group size effect. However, skewed sex ratios towards the opposite sex led to increased vigilance, particularly among older females (Meldrum & Ruckstuhl, 2009). This may reflect an oddity effect, which refers to predators preferentially targeting individuals that are phenotypically distinct from the rest of the group (Landeau & Terborgh, 1986; Peuhkuri, 1997; Rodgers *et al.*, 2011). The oddity effect raises the possibility that sex ratio bias could lead to the under-represented sex compensating for increased vulnerability by investing more in vigilance. This would only apply to sexually dimorphic species, and may be more pronounced in males, which are often more conspicuous than females because of stronger sexual

selection. Although this may be true for vigilance, it is unlikely to be true for aerial alarm calling.

Alarm calling is a form of signalling where the signaller relays information regarding a potential danger to receivers (Davis, 1984; Gyger *et al.*, 1988; Evans, 1997). Many species have distinct alarm calls to alert each other to the presence of different predators. A well-known example of this occurs in vervet monkeys, *Cercopithecus aethiops*, which emit three distinct alarm calls warning conspecifics of ground predators, aerial predators and snakes (Seyfarth *et al.*, 1980). In both natural situations and during playback experiments, alarm calls led to monkeys performing appropriate behavioural responses. For example, upon hearing terrestrial predator alarm calls whilst on the ground individuals immediately climbed up trees, whereas hearing aerial alarm calls led to monkeys seeking cover (Seyfarth *et al.*, 1980).

Individual alarm calling effort could decrease with increasing group size, like vigilance, because there are more animals available to alarm call. This has been shown in desert baboons, *Papio cynocephalus ursinus*, with female baboons increasing alarm calling rates with decreasing group size (Cowlishaw, 1997). Sex ratio could also have a strong effect on alarm calling, particularly if alarm calling differences between sexes exist. Sex differences in the alarm calling behaviour of Diana monkeys, *Cercopithecus diana,* was explored to test whether males or females were more likely to copy conspecific calls irrespective of predator type (Stephan & Zuberbühler, 2016). Playback experiments were conducted using either terrestrial or aerial predator sounds followed by either the correct or incorrect alarm call for that predator sound. Male monkeys were always played female alarm calls whereas females were always played male calls (Stephan &

Zuberbühler, 2016). Females always performed the correct predator alarm call for the predator sound even when they heard males performing a different call. Conversely males always copied female alarm calls even after hearing a different predator vocalisation. Additionally, female call rates decreased in treatments where male calls matched the predator sound heard but male call rates did not differ with treatment (Stephan & Zuberbühler, 2016). Males copying an incorrect alarm call could cause conspecifics to react inappropriately, leading to death of group members. However, the study suggested that it could be beneficial for males to rely on female calls because they may not see the predator due to foraging further from the group (Stephan & Zuberbühler, 2016). Alternatively, the study proposed that sexual selection could be acting upon male alarm calling, with copying female calls signalling a greater commitment to protect the group. Males that are more likely to copy female alarm calls may sire more offspring and be more likely to be accepted into the group (Stephan & Zuberbühler, 2016).

In addition to the sex ratio, the social structure of a group is also likely to modulate investment in vigilance and alarm calling. In groups structured into social hierarchies, social status can play an important role in individual strategies of anti-predator behaviours. For example, socially subordinate tufted titmice, *Baeolophus bicolor*, increased vigilance in the presence of more dominant individuals than when solitary (Waite, 1987). Higher vigilance in subordinates in the presence of more dominant individuals has also been shown in yellow-footed rock-wallabies (Blumstein *et al.*, 2001) and blue monkeys, *Cercopithecus mitis* (Gaynor & Cords, 2012). This could be explained by subordinates increasing vigilance to monitor higher ranking individuals to avoid aggressive encounters. In brown capuchin monkeys, *Cebus apella*, up to 80% of the scans by lower ranking

individuals were directed towards conspecifics whereas the highest ranking individuals devoted no more than 25% of scans towards neighbours (Pannozzo *et al.*, 2007). Additionally subordinate individuals directed scans towards individuals they frequently engaged in aggressive encounters with, suggesting in this species subordinates use social scanning to avoid conflict (Pannozzo *et al.*, 2007).

The aim of this chapter is to experimentally disentangle the effect of group size, group sex ratio and social dominance in modulating vigilance effort and alarm calling in male red junglefowl, *Gallus gallus*. Red junglefowl live in small social groups consisting of a dominant male, subordinate males and harem females (Johnsen *et al.*, 2001; Parker & Ligon, 2002; Wilson *et al.*, 2010). Fowl are sexually dimorphic, with males possessing bright conspicuous plumage whilst females adopt cryptic colouration (Zuk *et al.*, 1990b; Zuk *et al.*, 1995b; Ligon & Zwartjes, 1995). More conspicuous individuals could suffer high predation costs, and one study in domestic fowl found that male fowl are more vigilant than females (Pizzari, 2003), potentially reflecting these higher predation risks.

Like several non-human primates (Seyfarth *et al.*, 1980; Zuberbühler, 2000, 2001), red junglefowl also produce alarm calls for ground and aerial predators. Both calls have been experimentally demonstrated to be functionally referential in domestic fowl, with individuals responding to terrestrial calls by adopting alert postures and increasing scanning whilst aerial calls led to crouching and/or seeking cover (Evans, 1997). Furthermore, these alarm calls are sex-specific, as only male fowl perform aerial alarm calls (Gyger *et al.*, 1987; Evans, 1997; Wilson & Evans, 2008). Since aerial calls are male-specific their potential role in sexual selection and mating success has been explored in several studies using domestic fowl (Wilson *et al.*, 2008; Wilson & Evans, 2008, 2010). The mating

system of fowl is best described as harem polygynandry; and involves both male competition for females and female choice through females soliciting matings as well as sperm competition and cryptic female choice post-insemination (Zuk *et al.*, 1990b, 1990a; Pizzari & Birkhead, 2000; Johnsen *et al.*, 2001; Løvlie *et al.*, 2014; Pizzari & McDonald 2019). Early studies on sexual selection in red junglefowl focussed on plumage ornaments such as combs, finding males with larger combs were preferred during mate choice trials (Ligon *et al.*, 1990; Zuk *et al.*, 1990a, 1990b; Zuk *et al.*, 1995; Ligon *et al.*, 1998). Yet more recent work in both red junglefowl and the domestic fowl has found no preference for plumage ornaments and has shifted to focussing on dominance status and anti-predator behaviour (Ligon & Zwartjes, 1995; Pizzari & Birkhead, 2000; Wilson *et al.*, 2008; Wilson & Evans, 2010). Both male and female fowl form social hierarchies (Odén *et al.*, 1999; Pizzari & Birkhead, 2000; D'Eath & Keeling, 2003), and males status has been linked to greater mating and reproductive success in domestic fowl (Pizzari & Birkhead, 2000; Pizzari, 2001; Pizzari *et al.*, 2002; Pizzari *et al.*, 2003; Wilson *et al.*, 2008). Female fowl are more likely to solicit and retain the sperm of more dominant males whereas copulations from subordinates leads to increased risk of sperm ejection and resistance (Pizzari & Birkhead, 2000; Pizzari *et al.*, 2002; Løvlie *et al.*, 2014). In addition, dominance has been linked to a greater investment in both alarm calling and vigilance behaviour (Pizzari, 2003; Wilson *et al.*, 2008; Wilson & Evans, 2008); so females could potentially gain direct anti-predation benefits from copulating with these males, and possibly genetic benefits to the extent to which investment in status, vigilance and alarm calling is condition-dependent and heritable. One study found that the rate at which male fowl produced aerial alarm calls was the best predictor

of both mating and reproductive success (Wilson *et al.*, 2008), with more recent studies finding that mating causes an increase in aerial alarm calling effort in fowl (Wilson & Evans, 2008; Wilson *et al.*, 2010). Therefore, biasing sex ratios could lead to males increasing alarm calling efforts either in response to fewer males producing these calls, or because of increasing/decreasing competition for mates.

Although alarm calling could be thought of as a part of vigilance behaviour, in our data we found that vigilance and alarm calling were not strongly correlated, so these behaviours were explored separately. If vigilance effort and alarm calling are entirely naturally selected anti-predatory behaviours, we expect individual males to progressively reduce their investment as group size increases. However, if social effects play a role, we expect sex ratio to influence the vigilance effort and/or alarm calling behaviour of individual males. This can happen because these behaviours are sexually selected, and group sex ratio determines the intensity of male competition over females. In this case, we expect male investment to be minimal in the absence of females in a group, low when groups are strongly female-biased and male competition is relaxed, and high as the sex ratio becomes more male-biased. Alternatively, competition in the male-biased groups may be too great, reducing male investment in vigilance and promoting other strategies such as scramble competition and investment in postcopulatory sexual selection. We would also expect vigilance effort and alarm calling to co-vary with sexually selected male phenotypes, such as social status. Alternatively, male vigilance effort could be modulated by the oddity effect. In this case, one would expect males to maximise effort in female-biased groups and progressively reduce it as groups become more male-biased. A similar pattern is expected if males were simply increasing effort to compensate for the fact that females are less vigilant

than males. The oddity effect however, does not necessarily predict changes in alarm calling behaviour. If males were at a greater risk from predation because of the oddity effect, then a better approach when spotting an aerial predator would be to seek cover; not increase predation risk further. Yet studies show that male domestic fowl increase aerial alarm call effort in the presence of females (Gyger, 1990; Evans *et al.*, 1993; Wilson *et al.*, 2008; Wilson & Evans, 2008), suggesting the oddity effect is not driving aerial alarm calling, but sexual selection could be.

Based on previous findings, both vigilance and aerial alarm calling should be positively correlated with male dominance score. Altering group size and sex ratio can influence dominance by increasing or decreasing the number of males present. When there are few males in a group, dominance hierarchies should be very strong because the top male can more easily monitor and patrol subordinates. Therefore, dominance and sex ratio should have a significant two-way interaction on both vigilance and alarm calling in both group sizes, with the positive correlation between vigilance/alarm calling and dominance being stronger in groups with fewer males.

METHODS

Study System

We studied a captive population of red junglefowl at the John Krebs's Field Station in Wytham, across three seasons (May-September 2016, March-September 2017, July-August 2018). A total of 36 groups, 20 groups of 12 (five per sex ratio) and 16 groups of 4 (three Male Only, three Male Biased, five Even Ratio and five Female Biased) were created. Sixteen groups of 12 were studied in 2016, four in 2017 and none in 2018. Twelve groups of four were studied in 2017, and four more groups in 2018.

The trial to study each group took six days. Males were released on the first day, and females on the second. This was to give males enough time to build a dominance hierarchy in the absence of confounding effects of mating and intersexual interactions. Observations began the morning of day 3 and terminated on the evening of day 6. Due to constraints in bird availability, the experiment used a total of 48 males and 33 females across the three-year period, using the same birds across multiple groups. The most times a male was reused was 10 times, with the average number of times reused being 5 ± 0.16 (Standard Error). All individuals used were between 3-9 years of age, with the average male age being 4 ± 0.10 (SE).

The experimental pen was 11.5x7.2m with ballast substrate. Half the pen roof was covered in a translucent material, but the other half remained uncovered, allowing birds to see through the metal wire, increasing the likelihood of aerial alarm calls. Behavioural observations took place for 8 hours a day for a single group in the morning and evening. Observations started 30 minutes after sunrise for 4 hours. Evening observations ended 30 minutes before sunset.

Birds were defined as vigilant when they adopted an alert, upright posture with neck stretched upwards and both eyes open; often accompanied by moving the head from side-to-side (Inglis & Lazarus, 1981; Jacobsen & Ugelvik, 1994; Robinette & Ha, 2001; Pizzari, 2003; Randler, 2005; Squires *et al.*, 2007; Beauchamp, 2015). Birds with heads down exhibiting fixation on the ground, or birds with eyes closed were not considered vigilant. Two hours of the 4-hour observation period were dedicated to observing vigilance behaviour. This 2-hour period was spread across the first 2.5 hours in the morning, and the last 2.5 in the evening. This was done to coincide with the time a second observer would be in the pen. The observer conducting focal watches also recorded other behaviours *ad libitum* however monitoring interactions between all 12 individuals was difficult whilst simultaneously performing focal watches. Therefore, the second observer also recorded behavioural interactions that occurred during this 2.5-hour period, preventing data loss while recording vigilance. Vigilance was recorded via 10-minute focal watches using a stopwatch. The number of focal watches varied depending on the sex ratio. For example, in Male Only groups of 12, all males had one focal watch per session (120 mins/12 =10 mins). Whereas for Even Ratio groups of 12, each male had two focal watches per session. Female vigilance was also collected in the groups of four, therefore everyone in a group of four had three focal watches per 4-hour session. The order of focal watches was randomised for each session using a random number generator in R (version 1.1.423; R Core Team, 2017). For males with multiple focal watches in a single 4-hour session, the average time spent vigilant was taken for each 4-hour session, so that all males had one vigilance entry per 4-hour session.

Aerial and terrestrial alarm calls were recorded *ad libitum*. Both calls that were initiated by males and copied calls (i.e. when the focal male joined in with a call started by another male) were recorded separately. Male dominance behaviours were recorded *ad libitum* and included avoidances (bird A moved away from bird B within 1 minute), fight outcomes, chases and dominance pecks (aggressive peck to another male) (Rushen, 1982, 1984; Leonard & Weatherhead, 1996; Millman *et al.*, 2000). All dominance interactions between pairs of males across the 4-day period were totalled. A dominance matrix was then produced for each group using the total number of dominance behaviours performed between pairs within the group. These matrixes were entered into R and used to calculate normalized David scores using the "Steepness" package (version 0.2.2; Leiva & de Vries, 2014). Due to there only being one male Female Biased groups of 4 were excluded from this, and from the subsequent analysis.

Analysis

Exploring Collinearity

Correlations were explored to see whether the effect of dominance on vigilance and alarm calling varied across different group size and sex ratio combinations or remained constant across the groups. Correlations were explored between normalized David score, total time spent vigilant (seconds) and the total number of aerial alarm calls initiated for each group size and sex ratio combination using Kendall's tau-b correlations tests via the "cor.test" function within the "stats" package (version 3.4.3) in R (version 1.1.423; R Core Team, 2017). Kendall's was chosen because the variables were not normally distributed nor were they ordered variables. However, Pearson product-moment correlations and Spearman Rank correlations produced qualitatively similar estimates. The correlations show that

the strength of the relationship between dominance and both vigilance and alarm calling varies with differing sex ratios, being particularly strong in Female Biased groups of 12 and Male Biased groups of 4 (Tables 1 and 2). These correlations aided in model creation by suggesting the need for a two-way interaction between dominance and sex ratio on alarm calling within groups of 4 and 12. Furthermore the correlations between vigilance and alarm calling are similar in most groups but is strongest in Male Biased groups of 4; again, suggesting the need for a two-way interaction between vigilance and group size on alarm calling (Tables 1 and 2).

Table 1. Correlation coefficients for Kendall's tau-b correlations between normalized David score, total time spent vigilant (seconds) and the total number of aerial alarm calls initiated. Correlations are shown for each sex ratio within the groups of 12. Correlation estimates are displayed below the black boxes, with *p* values shown above the black boxes. Statistical significance is shown by bold colouration. * indicates near significance. Sample sizes include 60 (Male Only), 45 (Male Biased), 30 (Even Ratio) and 15 (Female Biased).

	Female Biased			**Even Ratio**		
Variable	Dominance	Vigilance	Alarm Calls	Dominance	Vigilance	Alarm Calls
Dominance		0.050*	**<0.001**		0.200	**0.004**
Vigilance	0.39		0.124	0.16		**0.015**
Alarm Calls	**0.70**	0.30		**0.37**	**0.31**	
	Male Biased			**Male Only**		
Variable	Dominance	Vigilance	Alarm Calls	Dominance	Vigilance	Alarm Calls
Dominance		0.060*	**<0.001**		**0.008**	**<0.001**
Vigilance	0.19		**0.013**	**0.23**		**0.001**
Alarm Calls	**0.35**	**0.26**		**0.35**	**0.29**	

Table 2. Correlation coefficients for Kendall's tau-b correlations between normalized David score, total time spent vigilant (seconds) and the total number of aerial alarm calls initiated. Correlations are shown for each sex ratio within the groups of 4. Correlation estimates are displayed below the black boxes, with *p* values shown above the black boxes. Statistical significance is shown by bold colouration. * indicates near significance. Sample sizes include 12 (Male Only), 9 (Male Biased), 10 (Even Ratio) and 5 (Female Biased). Female Biased groups of 4 had no normalized David score, so correlations including this variable could not be calculated.

	Female Biased			**Even Ratio**		
Variable	Dominance	Vigilance	Alarm Calls	Dominance	Vigilance	Alarm Calls
Dominance		NA	NA		0.108	0.073*
Vigilance	NA		0.483	0.42		0.216
Alarm Calls	NA	-0.40		0.47	0.33	
	Male Biased			**Male Only**		
Variable	Dominance	Vigilance	Alarm Calls	Dominance	Vigilance	Alarm Calls
Dominance		0.075*	**0.016**		0.250	0.408
Vigilance	0.50		**0.016**	0.27		0.168
Alarm Calls	**0.65**	**0.65**		-0.18	0.31	

Testing the effects of group size and sex ratio

We explored the effects of sex ratio and group size on vigilance behaviour using a linear mixed model. The square-root transformed total number of seconds spent vigilant by each individual male in the trial was the response variable. Sex ratio was entered as a fixed effect (four-levels) with group size (two-levels), year (three-levels) and male age (five-levels). Male ID and group ID were added as random effects. A two-way interaction between sex ratio and group size was added into the model, which was significant and so was retained in the final model. Similarly, we explored the effects of sex ratio and group size on the number of aerial alarm calls initiated by a male using a generalised linear mixed model with a Poisson family error structure with square-root link. A Poisson model was chosen because this variable used count data. Once again sex ratio, group size, year and male age were entered as fixed effects. Since the number of alarm calls should be proportional to the time spent vigilant (Pizzari 2003), we also entered the total time spent vigilant (seconds) as a fixed effect. Male ID and group ID were entered as random effects. Male age was removed from the final model because it was not significant. Group ID was also removed as a random effect because it had a variance of zero so was not needed in the model. We tested two-way interactions between group size and sex ratio, vigilance and group size, and vigilance and sex ratio. All interactions were significant, so were retained in the final model.

Testing the effect of social status and sex ratio within each group size

We then explored the effect of male social status on vigilance and alarm calling efforts separately for groups of 4 and groups of 12. We did this because the role of male social status is likely to change with group size in this species (Pizzari &

McDonald, 2019). For groups of 12, we used a linear mixed model with a square-root transformed time spent vigilant as response variable, sex ratio, normalized David score, male age and year (two-levels) as fixed effects, with male ID and group ID as random effects. A two-way interaction between normalized David score and sex ratio was tested though this was not significant, so was removed from the final model. For aerial alarm calling, we used a Poisson generalized linear mixed model (square-root link) with the number of aerial alarm calls initiated as the response variable, sex ratio, normalized David score and male age were entered as fixed effects, male ID and group ID as random effects. Male age was removed from the final model due to lack of significance along with group ID as a random effect due to having a variance of zero. A two-way interaction between sex ratio and dominance was included and retained in the final model because it was significant.

For groups of four, we excluded Female Biased groups where the presence of a single male prevented the quantification of normalized David scores and the role of inter-male variation in social status. We used a linear mixed model with the square-root transformed total time spent vigilant (seconds) as the response variable, sex ratio (i.e. Even Ratio, Male Biased and Males Only), normalized David score and male age as fixed effects, male ID and group ID as random effects. Male age was removed because it was not significant. Similarly, we used a generalised linear mixed model with Poisson error structure (square-root link), the number of aerial alarm calls initiated as the response variable, sex ratio, normalized David score and male age as fixed effects, male ID and group ID as random effects. Male age was again removed because it was not significant along with group ID as a random effect because it explained no variance in the model. A

two-way interaction between normalized David score and sex ratio was significant in the alarm calling model and was retained in the final model.

Year was entered into the models as a fixed effect rather than a random effect because this variable had less than five levels, making it unsuitable as a random effect (Gelman & Hill, 2006; Clark & Linzer, 2015). Age did have five levels, so additional models were run with age as a random effect and these models were compared to the original models with age as a fixed effect using AIC. Models using age as a fixed effect had significantly lower AIC, so age was retained as a fixed effect rather than a random effect in all models used. To test that all models fitted the data they were graphically tested using qqnorm and residual vs fitted plots. All models showed normality and a lack of heteroscedacity. Additionally, all Poisson models were graphically explored using the "DHARMa" package (version 0.2.0; Hartig, 2018), which is ideal for testing normality and heteroscedacity assumptions of Poisson models (Dorning & Harris, 2017; Rodríguez *et al.*, 2017; Rodríguez *et al.*, 2018; Walker *et al.*, 2017; Bowerman *et al.*, 2018; Eveson *et al.*, 2018). All "DHARMa" outputs showed that no assumptions were violated.

The Poisson models for alarm calling looking at the effects of group size and sex ratio and the effect of dominance in groups of 12 were over dispersed so an obs_effect variable (observation-level random effect) with a unique value for each observation (1: nrow(data)) was created; allowing incorporation of overdispersion into the models by adding (1|obs_effect) as a random effect. This approach is commonly used in ecological studies to combat overdispersion in Poisson and binomial models (Richards, 2008; Harrison, 2014, 2015). Once the obs_effect variable was added, these two models no longer showed overdispersion. Finally,

specifying the family as negative binomial was also considered to deal with overdispersion (Thomas & Lello, 2017), but this was deemed less suitable as it resulted in models with higher AIC.

Individual effects in each model were analysed through Likelihood Ratio Tests (LRT) using the "anova" function in the "lmerTest" package (2.0.36; Kuznetsova *et al.*, 2017). To test the effect of independent variables outside of the significant interactions *p* values were obtained through Type 3 Wald Chi-square tests using the "Anova" function in the "car" package (version 3.0.2; Fox & Weisberg, 2011). All graphs were created using the "ggplot2" package (version 2.2.1; Wickham, 2009) and "sjPlot" package (version 2.6.3; Lüdecke *et al.*, 2019). For visually displaying both vigilance and aerial alarm calling both variables were standardized using z-scores to allow easier interpretation of the magnitude of the effects of group size, sex ratio and dominance on these behaviours.

RESULTS

Effect of group size and sex ratio

When analysing both group sizes together, there was a significant two-way interaction between group size and sex ratio on vigilance, whereby vigilance was highest in Even Ratio groups of 12 and lowest in Male Only groups of 4 (LRT: X^2 (3) =15.03, p=0.002; Table 3; Figure 1). We ran two additional analyses to explore this pattern further. First, we ran the same linear mixed model excluding Even Ratio groups from the data because vigilance was much higher in the Even Ratio groups of 12 and we wanted to ascertain the extent to which the group size and sex ratio interaction was driven by the Even Ratio groups of 12. This model found that when excluding Even Ratio groups, sex ratio had a strong significant independent effect on vigilance (LRT: X^2 (2) =32.32, p<0.001; See Appendix, Supplementary Material A), but neither the interaction between group size and sex ratio (LRT: X^2 (2) =1.94 p=0.380; See Appendix, Supplementary Material A) or group size significantly affected vigilance (LRT: X^2 (1) =2.58, p=0.108; See Appendix, Supplementary Material A). Second, a separate linear mixed model was conducted to confirm the effect of sex ratio on groups of 4. This showed that in these groups, sex ratio had a significant effect on vigilance, whereby a reduction in vigilance effort was observed as groups became more male-biased (LRT: X^2 (3) =17.73, p=0.001; See Appendix, Supplementary Material B; Figure 1).

Given that the groups were not run in a balanced design across years, we wanted to test whether year influenced vigilance in groups of 4 and 12 separately. We found that in the groups of 12 there was a strong two-way interaction between year and sex ratio, which was driven by the Even Ratio groups of 12 from 2016

being more vigilant than any other group size and sex ratio combination (LRT: X^2 (3) =23.77, p<0.001; Table 5; see Appendix, Supplementary Material C). There was no significant two-way interaction between year and sex ratio on vigilance in the groups of 4 (LRT: X^2 (3) =0.16, p=0.691; See Appendix, Supplementary Material D and E), nor did year have a significant independent effect (See Appendix, Supplementary Material D).

Table 3. Model output for the square-root transformed linear mixed model for the total time spent vigilant (seconds) testing group size and sex ratio effects. Year, age, group size, sex ratio and the two-way interaction between group size and sex ratio are fixed factors. Male ID and group ID are random effects. The model AIC is 1206.8. Model BIC is 1261.7, loglikelihood is -586.4 and deviance is 1172.8.

	Total Time Spent Vigilant(seconds)		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	14.57	5.80 – 23.35	**0.001**
2017	-7.63	-12.66 – -2.61	**0.003**
2018	-4.22	-13.20 – 4.76	0.357
Age 2010	5.69	-2.17 – 13.55	0.156
Age 2011	8.76	2.90 – 14.63	**0.003**
Age 2012	6.54	1.70 – 11.38	**0.008**
Age 2013	7.14	2.44 – 11.84	**0.003**
Female Biased	3.58	-3.85 – 11.00	0.345
Male Biased	-1.52	-9.53 – 6.49	0.710
Male Only	-8.10	-15.91 – -0.29	**0.042**
Group Size 12	10.71	2.72 – 18.69	**0.009**
Female Biased: Groups of 12	-15.66	-25.15 – -6.17	**0.001**
Male Biased: Groups of 12	-12.69	-22.43 – -2.95	**0.011**
Male Only:Groups of 12	-14.23	-23.78 – -4.69	**0.003**
Random Effects			
σ^2	21.65		
$\tau_{00\ ID}$	7.97		
$\tau_{00\ GROUP}$	16.89		
ICC	0.53		
N_{ID}	48		
N_{GROUP}	36		
Observations	186		
Marginal R^2 / Conditional R^2	0.600 / 0.814		

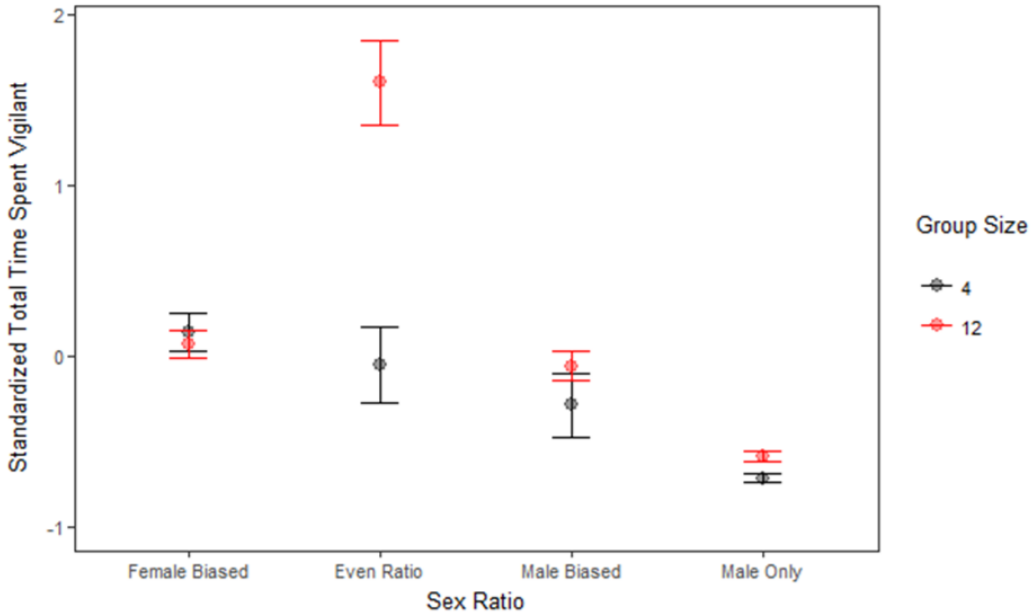


Figure 1. Average z-score standardized total time spent vigilant for each sex ratio. Error bars represent standard error.

There was a significant interaction between group size and sex ratio on alarm calling (LRT: X^2 (3) =8.74, p=0.033; Table 4; Figure 2), with alarm calling decreasing as groups became more male-biased, but especially so in groups of 4 (Table 4).

Overall, time spent vigilant significantly increased the number of alarm calls initiated (Table 4); however, this relationship was modulated by group size and sex ratio separately. There was a significant two-way interaction between time spent vigilant and group size, whereby the relationship between time spent vigilant and alarm calling was steeper in groups of 4 than in groups of 12 (LRT: X^2 (1) =8.63, p=0.003; Table 4; Figure 3). There was also a two-way interaction between sex ratio and vigilance on alarm calling, with a stronger correlation between vigilance and alarm calling in Female Biased groups (LRT: X^2 (3) =8.75, p=0.033; Table 4; Figure 3).

Like vigilance, individual models for groups of 12 and 4 were created to assess whether year had a strong effect on alarm calling. There was no significant two-way interaction between year and sex ratio in either the groups of 12 (LRT: X^2 (3) =1.54, p=0.673; See Appendix, Supplementary Material F and H) or the groups of 4 (LRT: X^2 (3) =0.79, p=0.375; See Appendix, Supplementary Material G and H). Nor did year have an independent effect on alarm calling in either group sizes (See Appendix, Supplementary Material F and G).

Table 4. Model output for the square-root linked Poisson generalized linear mixed model for the total number of aerial alarm calls initiated testing group size and sex ratio effects. Year, group size and sex ratio are fixed factors along with the two-way interactions between group size and sex ratio, sex ratio and vigilance, and group size and vigilance. Male ID is a random effect, and the (1|obs_level) random effect was added to combat overdispersion. Group ID was removed as a random effect due to having a variance of zero. The model AIC is 1487.1. Model BIC is 1542.0, loglikelihood is -726.6 and deviance is 1453.1.

	Total Number of Aerial Alarm Calls Initiated		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	5986.36	1246.08 – 28759.41	**<0.001**
Year 2017	2.37	1.10 – 5.10	**0.028**
Year 2018	0.02	0.00 – 0.12	**<0.001**
Group Size 12	0.00	0.00 – 0.02	**<0.001**
Female Biased	10.43	1.37 – 79.19	**0.023**
Male Biased	0.07	0.01 – 0.44	**0.004**
Male Only	0.19	0.02 – 1.82	0.151
Total Time Spent Vigilant (seconds)	71.01	18.39 – 274.27	**<0.001**
Female Biased: Group Size 12	2.00	0.19 – 20.57	0.562
Male Biased: Group Size 12	15.15	1.87 – 122.81	**0.011**
Male Only: Group Size 12	20.03	2.13 – 188.12	**0.009**
Female Biased: Total Time Spent Vigilant (seconds)	14.47	0.82 – 254.89	0.068
Male Biased: Total Time Spent Vigilant (seconds)	1.93	0.77 – 4.84	0.163
Male Only: Total Time Spent Vigilant (seconds)	8.76	1.28 – 59.94	**0.027**
Group Size 12: Total Time Spent Vigilant (seconds)	0.03	0.01 – 0.11	**<0.001**
Random Effects			
σ^2	2.69		
$\tau_{00\ obs_effect}$	2.44		
$\tau_{00\ ID}$	0.77		
ICC	0.22		
N_{ID}	48		
N_{obs_effect}	186		
Observations	186		
Marginal R^2 / Conditional R^2	0.573 / 0.668		

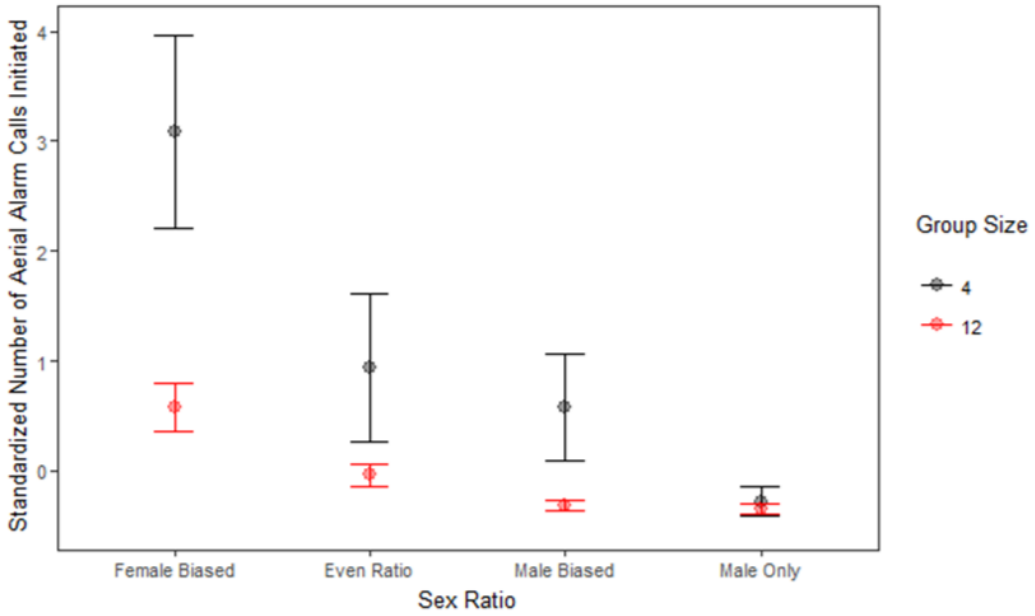


Figure 2. Average z-score standardized total number of aerial alarm calls initiated by a male across the 4-day observation period for each sex ratio. Error bars represent standard error.

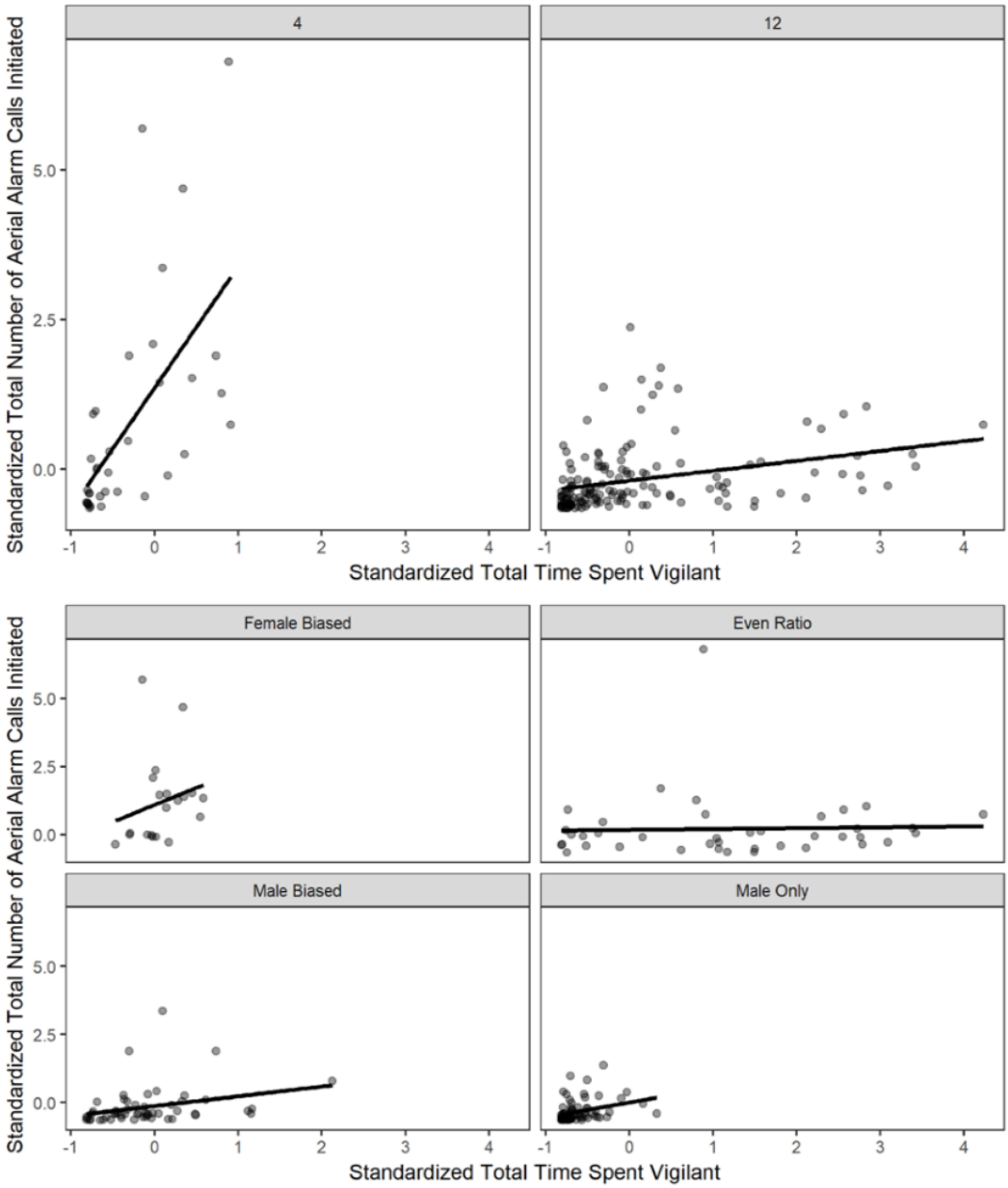


Figure 3. Each male's z-score standardized total time spent vigilant against the z-score standardized total number of aerial alarm calls initiated across the 4-day observation period for each a) group size and b) sex ratio.

The effects of male social status and sex ratio within groups of the same size

When considering the effect of male social status in groups of 12, we found that vigilance was positively correlated with normalized David score (LRT: X^2 (1) =21.45, p<0.001; Table 5; Figure 4a), and variation in aerial alarm calling was explained by a significant two-way interaction between normalized David score and sex ratio (LRT: X^2 (3) =18.16, p<0.001; Table 6; Figure 4b). More dominant males initiated more alarm calls, but this correlation was stronger in Female Biased groups (Figure 4b). Dominance score also had a significant independent effect on aerial alarm calling (Type 3 Wald Chi-square Test: X^2 (1) =24.48, p<0.001; Table 6).

Table 5. Model output for the square-root transformed linear mixed model for the total time spent vigilant (seconds) testing the effect of dominance in groups of 12 only. Year, age, sex ratio and normalized David score are fixed effects along with the two-way interaction between year and sex ratio. Male ID and group ID are random effects. The model AIC is 923.1. Model BIC is 971.3, loglikelihood is -445.6 and deviance is 891.1.

	Total Time Spent Vigilant (seconds)		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	24.98	19.75 – 30.21	**<0.001**
Year 2017	-24.07	-31.54 – -16.60	**<0.001**
Age 2010	5.44	-1.61 – 12.50	0.130
Age 2011	7.10	1.93 – 12.27	**0.007**
Age 2012	5.94	1.75 – 10.13	**0.005**
Age 2013	6.14	2.05 – 10.22	**0.003**
Normalized David Score	1.87	1.12 – 2.62	**<0.001**
Female Biased	-14.23	-19.33 – -9.12	**<0.001**
Male Biased	-20.38	-25.06 – -15.70	**<0.001**
Male Only	-32.40	-37.49 – -27.30	**<0.001**
Year 2017: Female Biased	26.60	15.42 – 37.77	**<0.001**
Year 2017: Male Biased	18.05	7.78 – 28.32	**0.001**
Year 2017: Male Only	21.65	11.50 – 31.80	**<0.001**
Random Effects			
σ^2	16.62		
$\tau_{00\ ID}$	5.38		
$\tau_{00\ GROUP}$	8.41		
ICC	0.45		
N_{ID}	48		
N_{GROUP}	20		
Observations	150		
Marginal R^2 / Conditional R^2	0.755 / 0.866		

Table 6. Model output for the square-root linked Poisson generalized linear mixed model for the total number of aerial alarm calls initiated testing the effect of dominance in groups of 12 only. Sex ratio, normalized David score and the two-way interaction between sex ratio and normalized David score are fixed factors. Male ID is a random effect, and the (1|obs_level) random effect was added to combat overdispersion. Group ID was removed as a random effect due to having a variance of zero. The model AIC is 1121.0. Model BIC is 1151.1, loglikelihood is -550.5 and deviance is 1101.0.

	Total Number of Aerial Alarm Calls Initiated		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	5.48	1.57 – 19.11	**0.008**
Normalized David Score	3.08	1.97 – 4.82	**<0.001**
Female Biased	7.80	1.38 – 44.00	**0.020**
Male Biased	0.56	0.09 – 3.66	0.544
Male Only	0.01	0.00 – 0.17	**0.002**
Female Biased: Normalized David Score	5.61	1.90 – 16.53	**0.002**
Male Biased: Normalized David Score	0.57	0.32 – 0.99	**0.045**
Male Only: Normalized David Score	0.95	0.50 – 1.81	0.886
Random Effects			
σ^2	1.90		
$\tau_{00\ obs_effect}$	1.65		
$\tau_{00\ ID}$	0.58		
ICC	0.23		
N_{ID}	48		
N_{obs_effect}	150		
Observations	150		
Marginal R^2 / Conditional R^2	0.504 / 0.620		

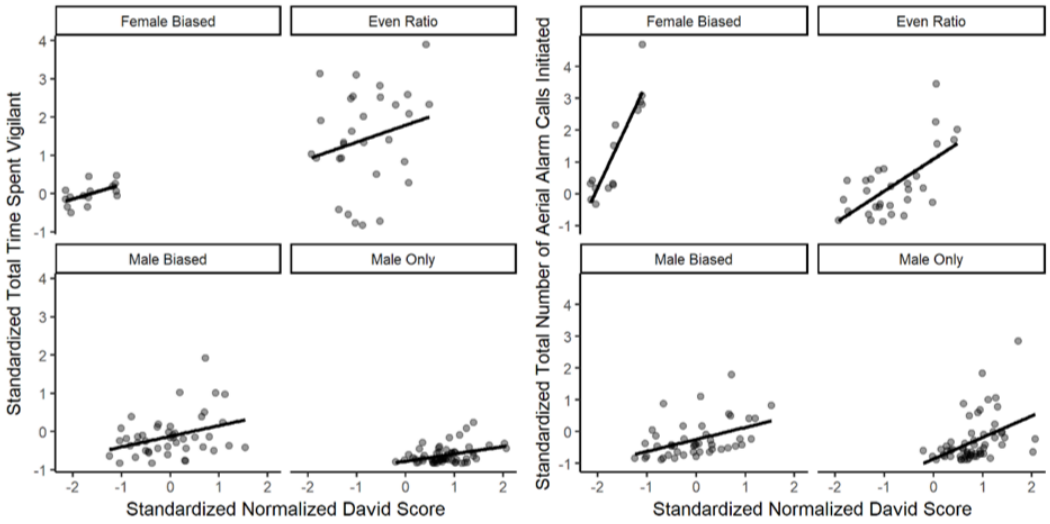


Figure 4. A scatter graph showing each male's z-standardized normalized David score against: a) the z-score standardized total time spent vigilant, and b) the z-score standardized total number of aerial alarm calls initiated in groups of 12 only across different sex ratios.

When looking at groups of 4, a male's normalized David score had a significant relationship with vigilance particularly so in groups with more females (LRT: X^2 (1) =5.22, p=0.022; Table 7; Figure 6a). There was a significant two-way interaction between sex ratio and dominance on aerial alarm calling, with Male Only groups showing a negative correlation whereas positive correlations are shown in the remaining sex ratios (LRT: X^2 (1) =15.79, p<0.001; Table 8; Figure 6b).

Table 7. Model output for the square-root transformed linear mixed model for the total time spent vigilant (seconds) testing the effect of dominance in groups of 4 only. Sex ratio and normalized David score are fixed factors with male ID and group ID as random effects. The model AIC is 215.4. Model BIC is 225.5, loglikelihood is -100.7 and deviance is 201.4.

	Total Time Spent Vigilant (seconds)		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	13.65	8.86 – 18.43	**<0.001**
Normalized David Score	4.37	1.08 – 7.67	**0.009**
Male Biased	-5.12	-12.11 – 1.88	0.151
Male Only	-14.91	-21.84 – -7.98	**<0.001**
Random Effects			
σ^2	21.54		
$\tau_{00\ ID}$	18.64		
$\tau_{00\ GROUP}$	7.67		
ICC	0.55		
N_{ID}	26		
N_{GROUP}	11		
Observations	31		
Marginal R^2 / Conditional R^2	0.386 / 0.724		

Table 8. Model output for the square-root linked Poisson generalized linear mixed model for the total number of aerial alarm calls initiated testing the effect of dominance in groups of 4 only. Sex ratio, normalized David score and the two-way interaction between sex ratio and normalized David score are fixed factors with male ID as a random effect. Group ID was removed as a random effect due to having a variance of zero. The model AIC is 275.8. Model BIC is 285.8, loglikelihood is -130.9 and deviance is 261.8.

	Total Number of Aerial Alarm Calls Initiated		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	1307.25	282.41 – 6051.09	**<0.001**
Normalized David Score	1.99	0.18 – 22.55	0.577
Male Biased	0.00	0.00 – 0.02	**<0.001**
Male Only	0.10	0.02 – 0.54	**0.007**
Male Biased: Normalized David Score	38.19	1.72 – 848.46	**0.021**
Male Only: Normalized David Score	0.20	0.03 – 1.22	0.081
Random Effects			
σ^2	0.25		
$\tau_{00\ ID}$	6.89		
ICC	0.96		
N_{ID}	26		
Observations	31		
Marginal R^2 / Conditional R^2	0.455 / 0.981		

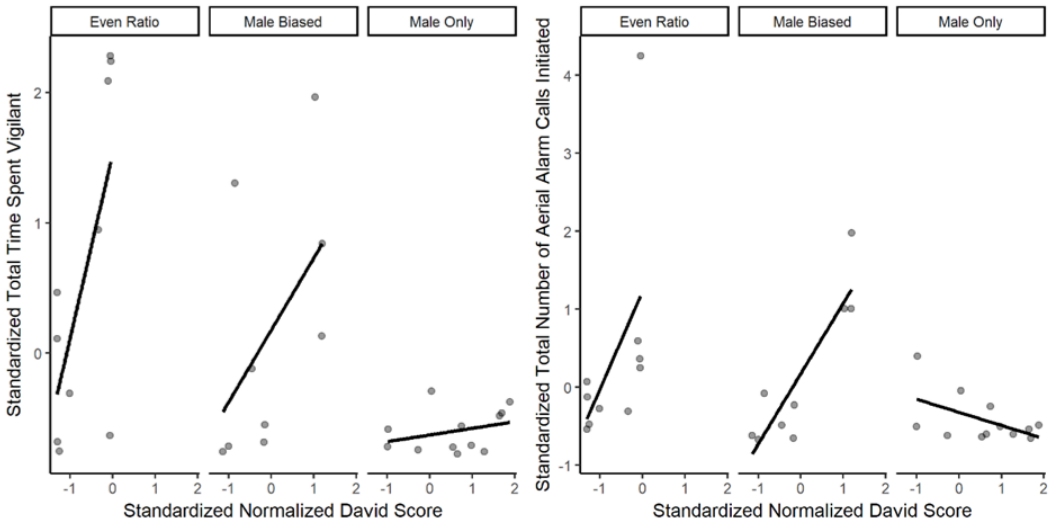


Figure 5. Each male's z-standardized normalized David score against a) the z-score standardized total time spent vigilant, and b) the z-score standardized total number of aerial alarm calls initiated in groups of 4 across different sex ratios. Female Biased groups are excluded because they contained only one male.

As Female Biased groups of 4, which contained a single male were excluded from the analysis, to compare the role of male status in Female Biased groups of 4 with the role of male status in other sex ratio and group size combinations, we plotted male social status measured as simple ranking (1=top-ranking). While less sensitive, this measure of social status allowed us to include the single males in Female Biased groups of 4 by assigning them the rank of 1 (i.e. lone males behave as socially dominant). Visual inspection of Figures 6 and 7 indicate that males in the Female Biased groups of 4 display a level of vigilance that is very similar to that of dominant males in Female Biased groups of 12 and a level of alarm calling that is higher than that displayed by dominant males Female Biased groups of 12 (or by male of any status in any other sex ratio/group size combination).

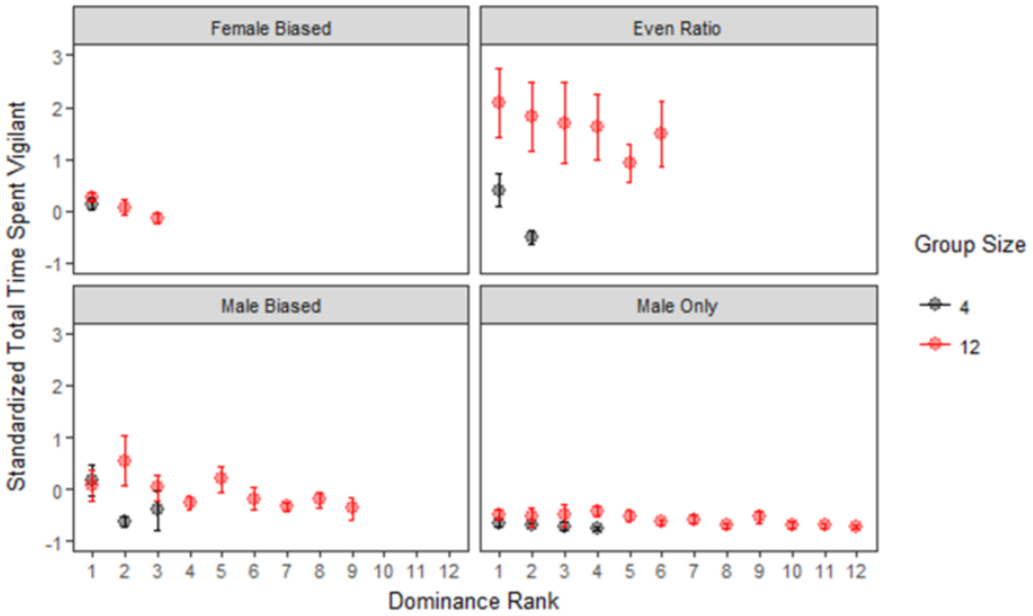


Figure 6. Average z-score standardized total time spent vigilant for each dominance rank. Error bars represent standard error.

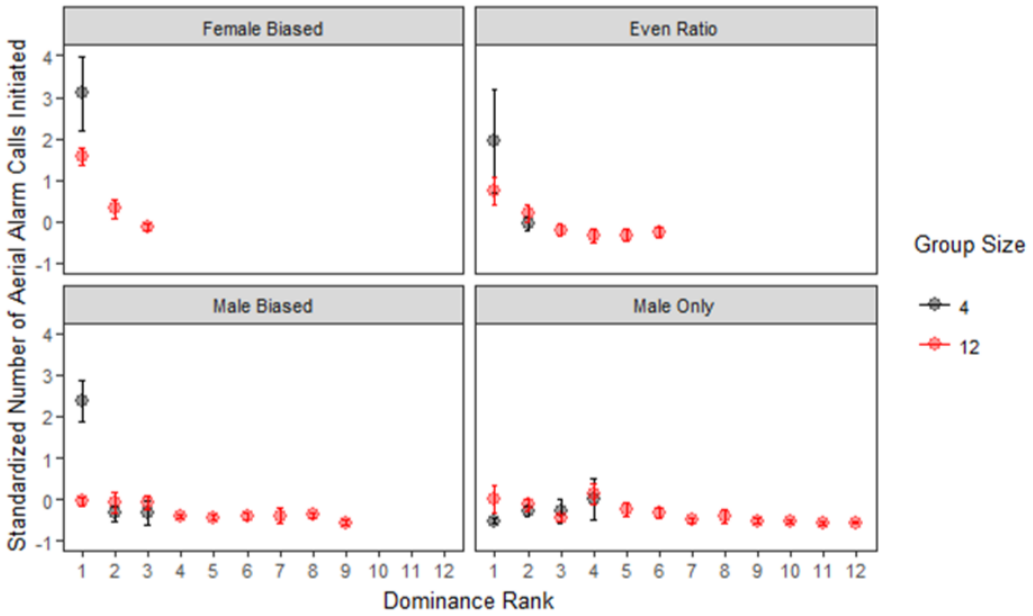


Figure 7. A point graph showing the average z-score standardized total number of aerial alarm calls initiated that a male gave across the 4-day observation period for each dominance rank. Error bars represent standard error.

DISCUSSION

The key aim of this study was to explore, through experimental manipulations, the way in which group size and sex ratio influence both male investment in vigilance and alarm calling. The main findings include sex ratio having a significant effect on vigilance, with an additional effect on individuals that were in Even Ratio groups of 12 (Table 9). Aerial alarm calling is significantly affected by both sex ratio and group size (Table 9). Furthermore, dominance was significantly positively correlated with both vigilance and alarm calling in both group sizes, except in Male Only groups of 4 (Table 9). Alarm calling was also significantly correlated with vigilance with an added effect of increased vigilance in groups of 4 and Female Biased groups.

Table 9. Summary of the predictions outlined in the introduction and whether the results support these predictions. Bold indicates that the prediction was supported.

Predictions	*Results*
Group Size Effect- vigilance/alarm calling should be lower in the groups of 12 compared to the groups of 4.	Vigilance was significantly higher in the groups of 12. Alarm calling was significantly higher in the groups of 4. **Prediction supported for alarm calling**.
Male Compensation- vigilance/alarm calling should be highest in Female Biased groups and decrease with increasing males.	Vigilance/alarm calling did generally decrease with increasing males but vigilance was highest in the Even Ratio groups. **Prediction supported.**
Sexual Selection- male investment should be low when females are absent and highest in Male-Biased groups due to more intense male-male competition.	Sex ratio had a significant effect but vigilance/alarm calling generally decreased with increasing males present. Prediction not supported.
Dominance- vigilance/alarm calling should be positively correlated with male social status.	Vigilance/alarm calling was significantly positively correlated with male social status in all group size and sex ratio combinations, except alarm calling in Male Only groups of 4. **Prediction supported**.
Dominance and Sex Ratio- there should be a significant interaction between dominance and sex ratio on vigilance/alarm calling, showing a stronger correlation between social status and vigilance/alarm calling in more female dominated groups.	There was no significant interaction between dominance and sex ratio for vigilance. There was a significant interaction between dominance and sex ratio in both group sizes. **Prediction supported for alarm calling**.

Vigilance and Group Size

This study predicted that if vigilance was primarily used for predator detection then vigilance should be higher in groups of 4 compared to 12. However, our findings oppose this prediction. Spatial positioning and proximity to conspecifics can greatly influence predation risk. In common redshanks, *Tringa tetanus,* birds more than five bird lengths away from conspecifics were significantly more likely to be targeted by Eurasian sparrowhawks, *Accipiter nisus* (Quinn & Cresswell, 2006). If the same is true in junglefowl, it is possible that individuals in groups of 4 remained in closer proximity to one another; explaining why vigilance is higher in groups of 12. Although redshanks, like many waders, inhabit open mudflats making them vulnerable to avian predation, so reducing neighbour distance may be an effective strategy for reducing predation risk in this habitat. Whereas junglefowl naturally inhabit forests, providing more cover from avian predators so flocking close together may be a less effective strategy in this species. Nevertheless, because nearest neighbour distance was not recorded during this experiment it is unknown as to whether this would have influenced vigilance, or whether neighbour distance would have differed depending on group size. Alternatively, groups of 12 could present males with challenges in relation to lost foraging opportunities, lost mating opportunities and increased aggression due to more individuals being present. In our experiment vigilance was unusually high in the Even Ratio groups of 12 and removing these groups from the analysis caused the previously significant interaction between group size and sex ratio to become insignificant. Therefore, the significantly higher vigilance in groups of 12 (regardless of sex ratio) as well as the significant interaction between group size and sex ratio, are predominantly caused by the Even Ratio groups of 12.

Vigilance and Sex Ratio

Sex ratio influenced time spent vigilant, with vigilance being highest in Even Ratio groups then decreasing with decreasing females present. Male junglefowl might have increased vigilance efforts in more female dominated groups because of mating opportunities. Alternatively, this may be explained by lower vigilance levels in female junglefowl compared to males (Pizzari, 2003), because of which a lower level of vigilance is required when more males are present. The high vigilance found on average in the Even Ratio groups is because of the unusually high vigilance observed in Even Ratio groups of 12 from 2016 compared to all other groups. There were five Even Ratio groups of 12, and four of these groups were completed in May 2016. So, it is possible that the greater amount of time spent vigilant is because of an abiotic factor such as temperature, rainfall, wind or sunlight differences, yet no unusual weather was noted during this month. Alternatively, a pair of birds of prey could have been nesting nearby however, there was no correlation between vigilance and alarm calling in Even Ratio groups, suggesting that aerial predation was not the cause. Regardless of these groups, the exclusion of Even Ratio groups entirely and re-running the models still shows sex ratio to have a significant effect on vigilance.

Alarm Calling and Sex Ratio

Sex ratio also had a strong significant effect on the number of aerial alarm calls initiated, with the number of calls decreasing with increasing number of males present. Aerial alarm calling is a male-specific behaviour (Johnsen & Zuk, 1995; Fusani *et al.*, 1997; Wilson & Evans, 2008); therefore, males in more female dominated groups may have increased their individual calling effort to compensate for females not producing these calls. Previous work in fowl has

shown that aerial alarm calling effort increases significantly when males mate (Wilson & Evans, 2008). Males obtained more matings and solicitations in Female Biased groups, as such aerial alarm calling may have been higher when more females were present due to increased mating activity. According to our findings, the interaction between group size and sex ratio was significant, with males in Female Biased groups of 4 producing more calls than any other group. If mating directly affected alarm calling effort then it should be highest in Female Biased groups of 12, not 4, because these groups had the most matings and the highest number of potential reproductive partners. Males in Female Biased groups of 4 had no male conspecifics, therefore the most probable explanation for alarm calling being highest in these groups is males compensating since they are the only member of the group that produces these calls. Males compensating for fewer males likely explains why aerial alarm calling was significantly higher in groups of 4 also.

Vigilance and Alarm Calling

As expected, the amount of time spent vigilant significantly increased the number of aerial alarm calls initiated. Males in groups of 4 initiated significantly more calls than those in groups of 12 but were less vigilant on average than males in groups of 12. Yet increased vigilance in the groups of 12 only led to a slight positive relationship with alarm calling, despite vigilance being higher in these groups; suggesting that the increased vigilance may not be used primarily for predator detection. However, time spent vigilant in the Even Ratio groups of 12 is much higher in comparison with the other groups, which might have affected the results. Nonetheless, after excluding all Even Ratio groups the same pattern emerges (See Appendix, Supplementary Material I). This adds more support to

the idea that males in groups of 12 directed more vigilance towards social uses such as mate guarding, food stealing, avoiding aggression etc. Whereas in the groups of 4, time spent vigilant had a very strong relationship with the number of calls initiated. Males in groups of 4 may have needed to increase individual investment in vigilance, leading to an increase in alarm calling, since they had fewer male conspecifics to rely on. Furthermore, vigilance and alarm calling had a stronger correlation in Female Biased groups, suggesting that fewer males leads to individuals spending more time vigilant to detect potential threats.

Dominance

Dominance score had a strong significant effect on time spent vigilant, with vigilance decreasing with decreasing score for all group size and sex ratio combinations. Increasing vigilance could allow dominant males to detect copulations quickly, increasing the chance of successful interruptions. In addition, dominant males could use vigilance to guard mates, better protecting against extra-pair copulations, thus further securing paternity (Artiss & Martin, 1995; Osorio-Beristain & Drummond, 1998). The correlation between vigilance and dominance was strongest in Even Ratio and Male Biased groups of 4. Potentially dominant males in groups of 4 could more easily monopolize and guard females, thus increasing vigilance.

Dominance was significantly correlated with the number of aerial alarm calls initiated in both group sizes, with more dominant males producing significantly more calls in all groups except Male Only groups of 4. Previous studies have demonstrated a link between aerial alarm calling and dominance in fowl (Pizzari, 2003; Wilson *et al.*, 2008; Kokolakis *et al.*, 2010), and our findings add support for this. Becoming the dominant male has been associated with physiological and

behavioural changes in fowl such as larger comb size and increased crowing (Ligon *et al.*, 1990; Johnsen & Zuk, 1995; Zuk *et al.*, 1995). Similarly aerial alarm calling has been linked to testosterone levels in fowl (Gyger *et al.*, 1988; Ligon *et al.*, 1990), therefore this endocrine change that occurs when becoming the top-ranking male could explain why aerial alarm calling is higher in the most dominant males. In Male Only groups the most dominant males initiated the most calls within the groups of 12, whereas groups of 4 did not follow this pattern; suggesting that lack of females is not the cause. It is presumably easier to monitor conspecifics in groups of 4 compared to groups of 12, therefore dominant males in Male Only groups of 12 could continue to call more often because they are unsure whether females may be present whereas dominant males in Male Only groups of 4 could be certain that females were not present; thus, reducing individual effort. The negative correlation between dominance and alarm calling in Male Only groups of 4 is unlikely to be caused by confounding factors associated with the dates these groups were examined, as these groups were spread out across the 2017 season and not confined to one observation block. However, this unusual result could be because of the individuals, or combination of individual used.

Increased vigilance and alarm calling in more dominant males could be a result of mating activity, explaining why the correlations are typically stronger in more female-biased groups. As discussed earlier fowl increase alarm calling efforts after mating (Wilson & Evans, 2008; Wilson *et al.*, 2010), and dominant males may be more able to gain mating opportunities through policing. Plus in fowl females have been demonstrated to eject the sperm of dominant males less than subordinates (Pizzari & Birkhead, 2000). So, the relationship between dominance, vigilance and alarm calling could be indirectly influenced by mating success.

Conclusions

To conclude, this study is unique by investigating the effects of group size, sex ratio and dominance on time spent vigilant and the number of aerial alarm calls initiated in male fowl. Despite the group size effect being demonstrated in many other species (Bertram, 1980; Lipetz & Bekoff, 1982; Roberts, 1996; Fernández-Juricic *et al.*, 2007) this study found that the group size effect was not acting upon vigilance behaviour, with vigilance being highest in the larger group size. Aerial alarm calling did follow the pattern expected for the group size effect, with more alarm calls initiated in groups of 4. This significant effect on aerial alarm calling is likely to be driven by groups of 4 having fewer males, thus individual males had to increase their own alarm calling effort to compensate. Very few studies explore the effect of sex ratio on anti-predation behaviours, yet this study found sex ratio to have an important role in determining both vigilance and aerial alarm calling behaviour. Future studies should consider manipulating sex ratio as well as group size to get a broader understanding of the factors influencing anti-predation behaviours. Sex ratio differences are particularly important when it comes to mating opportunities as more male-biased groups present increased competition. Finally, this study's findings on dominance support previous work showing that more dominant males are more vigilant and produce more aerial alarm calls. The strength of the correlations with dominance varied with sex ratio and group size, yet in most group size and sex ratio combinations the higher scoring males initiated more calls and spent more time vigilant.

REFERENCES

Artiss, T. & Martin, K. (1995). Male vigilance in white-tailed ptarmigan, *Lagopus leucurus*: mate guarding or predator detection? *Animal Behaviour*, **49**, 1249–1258.

Beauchamp, G. (2007). Vigilance in a selfish herd. *Animal Behaviour*, **73**, 445–451.

Beauchamp, G. (2008). What is the magnitude of the group-size effect on vigilance? *Behavioural Ecology*, **19**, 1361–1368.

Beauchamp, G. (2015). *Animal Vigilance: Monitoring Predators and Competitors*. Academic Press.

Bertram, B.C.R. (1980). Vigilance and group size in ostriches. *Animal Behaviour*, **28**, 278–286.

Blumstein, D.T., Daniel, J.C. & Evans, C.S. (2001). Yellow-Footed Rock-Wallaby Group Size Effects Reflect A Trade-Off. *Ethology*, **107**, 655–664.

Blumstein, D.T., Evans, C.S. & Daniel, J.C. (1999). An experimental study of behavioural group size effects in tammar wallabies, *Macropus eugenii*. *Animal Behaviour*, **58**, 351–360.

Bowerman, T., Roumasset, A., Keefer, M.L., Sharpe, C.S. & Caudill, C.C. (2018). Prespawn Mortality of Female Chinook Salmon Increases with Water Temperature and Percent Hatchery Origin. *Transactions of the American Fisheries Society*, **147**, 31–42.

Burger, J., Safina, C. & Gochfeld, M. (2000). Factors affecting vigilance in springbok: importance of vegetative cover, location in herd, and herd size. *acta ethologica*, **2**, 97–104.

Clark, T.S. & Linzer, D.A. (2015). Should I Use Fixed or Random Effects? *Political Science Research and Methods*, **3**, 399–408.

Cowlishaw, G. (1997). Alarm Calling and Implications for Risk Perception in a Desert Baboon Population. *Ethology*, **103**, 384–394.

Creel, S., Schuette, P. & Christianson, D. (2014). Effects of predation risk on group size, vigilance, and foraging behavior in an African ungulate community. *Behavioural Ecology*, **25**, 773–784.

Cresswell, W., Lind, J., Kaby, U., Quinn, J.L. & Jakobsson, S. (2003). Does an opportunistic predator preferentially attack nonvigilant prey? *Animal Behaviour*, **66**, 643–648.

David Leiva & Han de Vries. (2014). Steepness: testing steepeness of dominance hierarchies. R package version 0.2.2. Available at: http://CRAN.R-project.org/package=steepness.

Davis, L.S. (1984). Alarm Calling in Richardson's Ground Squirrels (*Spermophilus richardsonii*). *Zeitschrift für Tierpsychologie*, **66**, 152–164.

D'Eath, R.B. & Keeling, L.J. (2003). Social discrimination and aggression by laying hens in large groups: from peck orders to social tolerance. *Applied Animal Behaviour Science*, **84**, 197–212.

Dorning, J. & Harris, S. (2017). Dominance, gender, and season influence food patch use in a group-living, solitary foraging canid. *Behavioural Ecology*, **28**, 1302–1313.

Evans, C.S. (1997). Referential Signals. In *Communication*, Perspectives in Ethology: 99–143. Owings, D.H., Beecher, M.D. & Thompson, N.S. (Eds.). Springer US.

Evans, C.S., Evans, L. & Marler, P. (1993). On the meaning of alarm calls: functional reference in an avian vocal system. *Animal Behaviour*, **46**, 23–38.

Eveson, J.P., Bravington, M.V. & Farley, J.H. (2018). Accounting for environmental and observer effects in estimating abundance of southern bluefin tuna from aerial survey data. *PLoS ONE*, **13**, e0207790.

Fernández-Juricic, E., Beauchamp, G. & Bastain, B. (2007). Group-size and distance-to-neighbour effects on feeding and vigilance in brown-headed cowbirds. *Animal Behaviour*, **73**, 771–778.

FitzGibbon, C.D. (1989). A cost to individuals with reduced vigilance in groups of Thomson's Gazelles hunted by Cheetahs. *Animal Behaviour*, **37**, 508–510.

Fox, A.D. & Madsen, J. (1981). The pre-nesting behaviour of the Greenland White-fronted Goose. *Wildfowl*, **32**, 48–54.

Fox, J. & Weisberg, S. (2011). *An R Companion to Applied Regression*. SAGE.

Fusani, L., Beani, L., Lupo, C. & Dessì-Fulgheri, F. (1997). Sexually selected vigilance behaviour of the grey partridge is affected by plasma androgen levels. *Animal Behaviour*, **54**, 1013–1018.

Gaynor, K.M. & Cords, M. (2012). Antipredator and social monitoring functions of vigilance behaviour in blue monkeys. *Animal Behaviour*, **84**, 531–537.

Gelman, A. & Hill, J. (2006). *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge University Press.

Guillemain, M., Caldow, R.W.G., Hodder, K.H. & Goss-Custard, J.D. (2003). Increased vigilance of paired males in sexually dimorphic species: distinguishing between alternative explanations in wintering Eurasian wigeon. *Behavioural Ecology*, **14**, 724–729.

Gyger, M. (1990). Audience effects on alarm calling. *Ethology Ecology & Evolution,* **2**, 227–232.

Gyger, M., Karakashian, S.J., Dufty Jr., A.M. & Marler, P. (1988). Alarm signals in birds: The role of testosterone. *Hormones and Behavior,* **22**, 305–314.

Gyger, M., Marler, P. & Pickert, R. (1987). Semantics of an Avian Alarm Call System: The Male Domestic Fowl, *Gallus domesticus*. *Behaviour,* **102**, 15–40.

Hamilton, W.D. (1971). Geometry for the selfish herd. *Journal of Theoretical Biology*, **31**, 295–311.

Harrison, X.A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ,* **2**, e616.

Harrison, X.A. (2015). A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology & evolution. *PeerJ,* **3**, e1114.

Hartig, F. (2018). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.2.0. http://florianhartig.github.io/DHARMa/.

Husak, J.F., Macedonia, J.M., Fox, S.F. & Sauceda, R.C. (2006). Predation Cost of Conspicuous Male Coloration in Collared Lizards (*Crotaphytus collaris*): An Experimental Test Using Clay-Covered Model Lizards. *Ethology*, **112**, 572–580.

Inglis, I.R. & Lazarus, J. (1981). Vigilance and Flock Size in Brent Geese: The Edge Effect. *Zeitschrift für Tierpsychologie,* **57**, 193–200.

Jacobsen, O.W. & Ugelvik, M. (1994). Grazing and vigilance behaviour of breeding Eurasian Wigeon *Anas penelope* in relation to distance from water. *Wildfowl,* **45**, 119-123.

Janicke, T., Häderer, I.K., Lajeunesse, M.J. & Anthes, N. (2016). Darwinian sex roles confirmed across the animal kingdom. *Science Advances,* **2**, e1500983.

Jirotkul, M. (1999). Operational sex ratio influences female preference and male–male competition in guppies. *Animal Behaviour,* **58**, 287–294.

Johnsen, T.S. & Zuk, M. (1995). Testosterone and Aggression in Male Red Junglefowl. *Hormones and Behavior,* **29**, 593–598.

Johnsen, T.S., Zuk, M. & Fessler, E.A. (2001). Social Dominance, Male Behaviour and Mating in Mixed-Sex Flocks of Red Junglefowl. *Behaviour*, **138**, 1–18.

King, A.J., Wilson, A.M., Wilshin, S.D., Lowe, J., Haddadi, H., Hailes, S. & Morton, A.J. (2012). Selfish-herd behaviour of sheep under threat. *Current Biology,* **22**, R561–R562.

Kokko, H. & Morrell, L.J. (2005). Mate guarding, male attractiveness, and paternity under social monogamy. *Behavioural Ecology,* **16**, 724–731.

Kokko & Monaghan. (2001). Predicting the direction of sexual selection. *Ecology Letters,* **4**, 159–165.

Kokolakis, A., Smith, C.L. & Evans, C.S. (2010). Aerial alarm calling by male fowl (*Gallus gallus*) reveals subtle new mechanisms of risk management. *Animal Behaviour,* **79**, 1373–1380.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, **82**, 1–26.

Kvarnemo, C. & Ahnesjo, I. (1996). The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution,* **11**, 404–408.

Kvarnemo, C., Forsgren, E. & Magnhagen, C. (1995). Effects of sex ratio on intra- and inter-sexual behaviour in sand gobies. *Animal Behaviour,* **50**, 1455–1461.

Landeau, L. & Terborgh, J. (1986). Oddity and the 'confusion effect' in predation. *Animal Behaviour,* **34**, 1372–1380.

Lendrem, D.W. (1984). Sleeping and vigilance in birds, II. An experimental study of the Barbary dove (*Streptopelia risoria*). *Animal Behaviour,* **32**, 243–248.

Leonard, M.L. & Weatherhead, P.J. (1996). Dominance rank and offspring sex ratios in domestic fowl. *Animal Behaviour,* **51**, 725–731.

Ligon, J.D., Kimball, R. & Merola-Zwartjes, M. (1998). Mate choice by female red junglefowl: the issues of multiple ornaments and fluctuating asymmetry. *Animal Behaviour*, **55**, 41–50.

Ligon, J.D., Thornhill, R., Zuk, M. & Johnson, K. (1990). Male-male competition, ornamentation and the role of testosterone in sexual selection in red junglefowl. *Animal Behaviour,* **40**, 367–373.

Ligon, J.D. & Zwartjes, P.W. (1995). Ornate plumage of male red junglefowl does not influence mate choice by females. *Animal Behaviour,* **49**, 117–125.

Lipetz, V.E. & Bekoff, M. (1982). Group Size and Vigilance in Pronghorns. *Zeitschrift für Tierpsychologie,* **58**, 203–216.

Løvlie, H., Zidar, J. & Berneheim, C. (2014). A cry for help: female distress calling during copulation is context dependent. *Animal Behaviour,* **92**, 151–157.

Lüdecke, D., Bartel, A. & Schwemmer, C. (2019). *sjPlot: Data Visualization for Statistics in Social Science*. R package version 2.6.3, https://CRAN.R-project.org/package=sjPlot.

Meldrum, G.E. & Ruckstuhl, K.E. (2009). Mixed-sex group formation by bighorn sheep in winter: trading costs of synchrony for benefits of group living. *Animal Behaviour,* **77**, 919–929.

Millman, S.T., Duncan, I.J. & Widowski, T.M. (2000). Male broiler breeder fowl display high levels of aggression toward females. *Poultry Science,* **79**, 1233–1241.

Mills, S.C. & Reynolds, J.D. (2003). Operational sex ratio and alternative reproductive behaviours in the European bitterling, *Rhodeus sericeus*. *Behavioural Ecology and Sociobiology*, **54**, 98–104.

Odén, K., Vestergaard, K.S. & Algers, B. (1999). Agonistic behaviour and feather pecking in single-sexed and mixed groups of laying hens. *Applied Animal Behaviour Science*, **62**, 219–231.

Osorio-Beristain, M. & Drummond, H. (1998). Non-aggressive mate guarding by the blue-footed booby: a balance of female and male control. *Behavioural Ecology and Sociobiology*, **43**, 307–315.

Pannozzo, P.L., Phillips, K.A., Haas, M.E. & Mintz, E.M. (2007). Social Monitoring Reflects Dominance Relationships in a Small Captive Group of Brown Capuchin Monkeys (*Cebus apella*). *Ethology,* **113**, 881–888.

Parker, T.H. & Ligon, D.J. (2002). Dominant male red junglefowl (*Gallus gallus*) test the dominance status of other males. *Behavioural Ecology and Sociobiology*, **53**, 20–24.

Parrish, J.K. (1989). Re-examining the selfish herd: are central fish safer? *Animal Behaviour,* **38**, 1048–1053.

Peuhkuri, N. (1997). Size-assortative shoaling in fish: the effect of oddity on foraging behaviour. *Animal Behaviour,* **54**, 271–278.

Pizzari, T. (2001). Indirect partner choice through manipulation of male behaviour by female fowl, *Gallus gallus domesticus*. *Proceedings of the Royal Society of London B: Biological Sciences,* **268**, 181–186.

Pizzari, T. (2003). Food, vigilance, and sperm: the role of male direct benefits in the evolution of female preference in a polygamous bird. *Behavioural Ecology,* **14**, 593–601.

Pizzari, T. & Birkhead, T.R. (2000). Female feral fowl eject sperm of subdominant males. *Nature,* **405**, 787–789.

Pizzari, T., Cornwallis, C.K., Løvlie, H., Jakobsson, S. & Birkhead, T.R. (2003). Sophisticated sperm allocation in male fowl. *Nature*, **426**, 70–74.

Pizzari, T., Froman, D.P. & Birkhead, T.R. (2002). Pre- and post-insemination episodes of sexual selection in the fowl, *Gallus g. domesticus*. *Heredity*, **88**, 112–116.

Pizzari, T. & McDonald, G.C. (2019). Sexual selection in socially-structured, polyandrous populations: Some insights from the fowl. *Advances in the Study of Behavior*, **51**, 77–141.

Portugal, S.J. & Guillemain, M. (2011). Vigilance patterns of wintering Eurasian Wigeon: female benefits from male low-cost behaviour. *Journal of Ornithology*, **152**, 661–668.

Pulliam, H.R. (1973). On the advantages of flocking. *Journal of Theoretical Biology*, **38**, 419–422.

Quinn, J.L. & Cresswell, W. (2006). Testing domains of danger in the selfish herd: sparrowhawks target widely spaced redshanks in flocks. *Proceedings of the Royal Society of London B: Biological Sciences*, **273**, 2521–2526.

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Randler, C. (2005). Coots, *Fulica atra*, reduce their vigilance under increased competition. *Behavioural Processes*, **68**, 173–178.

Richards, S.A. (2008). Dealing with overdispersed count data in applied ecology. *Journal of Applied Ecology*, **45**, 218–227.

Roberts, G. (1996). Why individual vigilance declines as group size increases. *Animal Behaviour*, **51**, 1077–1086.

Robinette, R.L. & Ha, J.C. (2001). Social and ecological factors influencing vigilance by northwestern crows, *Corvus caurinus*. *Animal Behaviour*, **62**, 447–452.

Rodgers, G.M., Ward, J.R., Askwith, B. & Morrell, L.J. (2011). Balancing the Dilution and Oddity Effects: Decisions Depend on Body Size. *PLoS ONE*, **6**, e14819.

Rodríguez, A., Dann, P. & Chiaradia, A. (2017). Reducing light-induced mortality of seabirds: High pressure sodium lights decrease the fatal attraction of shearwaters. *Journal for Nature Conservation*, **39**, 68–72.

Rodríguez, A., Holmberg, R., Dann, P. & Chiaradia, A. (2018). Penguin colony attendance under artificial lights for ecotourism. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, **329**, 457–464.

Rushen, J. (1982). Development of social behaviour in chickens: A factor analysis. *Behavioural Processes,* **7**, 319–333.

Rushen, J. (1984). Frequencies of agonistic behaviours as measures of aggression in chickens: A factor analysis. *Applied Animal Behaviour Science,* **12**, 167–176.

Schärer, L., Rowe, L. & Arnqvist, G. (2012). Anisogamy, chance and the evolution of sex roles. *Trends in Ecology and Evolution,* **27**, 260–264.

Seyfarth, R.M., Cheney, D.L. & Marler, P. (1980). Vervet monkey alarm calls: Semantic communication in a free-ranging primate. *Animal Behaviour,* **28**, 1070–1094.

Slagsvold, T., Dale, S. & Kruszewicz, A. (1995). Predation favours cryptic coloration in breeding male pied flycatchers. *Animal Behaviour,* **50**, 1109–1121.

Squires, K.A., Martin, K., Goudie, R.I. & Yasukawa, K. (2007). Vigilance Behavior in the Harlequin Duck (*Histrionicus histrionicus*) during the Preincubation Period in Labrador: Are Males Vigilant for Self or Social Partner? *The Auk,* **124**, 241–252.

Stephan, C. & Zuberbühler, K. (2016). Persistent Females and Compliant Males Coordinate Alarm Calling in Diana Monkeys. *Current Biology,* **26**, 2907–2912.

Tejedo, M. (1988). Fighting for females in the toad *Bufo calamita* is affected by the operational sex ratio. *Animal Behaviour,* **36**, 1765–1769.

Thomas, R. & Lello, J. (2017). *Data Analysis with R Statistical Software: A Guidebook for Scientists*. Eco-explore.

Viscido, S.V. & Wethey, D.S. (2002). Quantitative analysis of fiddler crab flock movement: evidence for 'selfish herd' behaviour. *Animal Behaviour,* **63**, 735–741.

Waite, T.A. (1987). Dominance-Specific Vigilance in the Tufted Titmouse: Effects of Social Context. *The Condor,* **89**, 932–935.

Walker, R.H., King, A.J., McNutt, J.W. & Jordan, N.R. (2017). Sneeze to leave: African wild dogs (*Lycaon pictus*) use variable quorum thresholds facilitated by sneezes in collective decisions. *Proceedings of the Royal Society B: Biological Sciences,* **284**, 20170347.

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*, Use R! Springer-Verlag.

Wilson, D.R., Bayly, K.L., Nelson, X.J., Gillings, M. & Evans, C.S. (2008). Alarm calling best predicts mating and reproductive success in ornamented male fowl, *Gallus gallus*. *Animal Behaviour,* **76**, 543–554.

Wilson, D.R. & Evans, C.S. (2008). Mating success increases alarm-calling effort in male fowl, *Gallus gallus*. *Animal Behaviour,* **76**, 2029–2035.

Wilson, D.R. & Evans, C.S. (2010). Female fowl (*Gallus gallus*) do not prefer alarm-calling males. *Behaviour,* **147**, 525–552.

Wilson, D.R., McDonald, P.G. & Evans, C.S. (2010). Mechanisms of Mate Investment in the Polygamous Fowl, *Gallus gallus*. *Ethology,* **116**, 755–762.

Wrona, F.J. & Dixon, R.W.J. (1991). Group Size and Predation Risk: A Field Analysis of Encounter and Dilution Effects. *The American Naturalist,* **137**, 186–201.

Zuberbühler, K. (2000). Referential labelling in Diana monkeys. *Animal Behaviour,* **59**, 917–927.

Zuberbühler, K. (2001). Predator-specific alarm calls in Campbell's monkeys, *Cercopithecus campbelli*. *Behavioural Ecology and Sociobiology,* **50**, 414–422.

Zuk, M., Johnsen, T.S. & Maclarty, T. (1995a). Endocrine-Immune Interactions, Ornaments and Mate Choice in Red Junglefowl. *Proceedings of the Royal Society of London B: Biological Sciences,* **260**, 205–210.

Zuk, M., Popma, S.L. & Johnsen, T.S. (1995b). Male Courtship Displays, Ornaments and Female Mate Choice in Captive Red Junglefowl. *Behaviour,* **132**, 821–836.

Zuk, M., Thornhill, R., Ligon, J.D. & Johnson, K. (1990a). Parasites and mate choice in red junglefowl. *American Zoologist,* **30**, 235–244.

Zuk, M., Thornhill, R., Ligon, J.D., Johnson, K., Austad, S., Ligon, S.H., Thornhill, N.W. & Costin, C. (1990b). The Role of Male Ornaments and Courtship Behavior in Female Mate Choice of Red Junglefowl. *The American Naturalist,* **136**, 459–473.

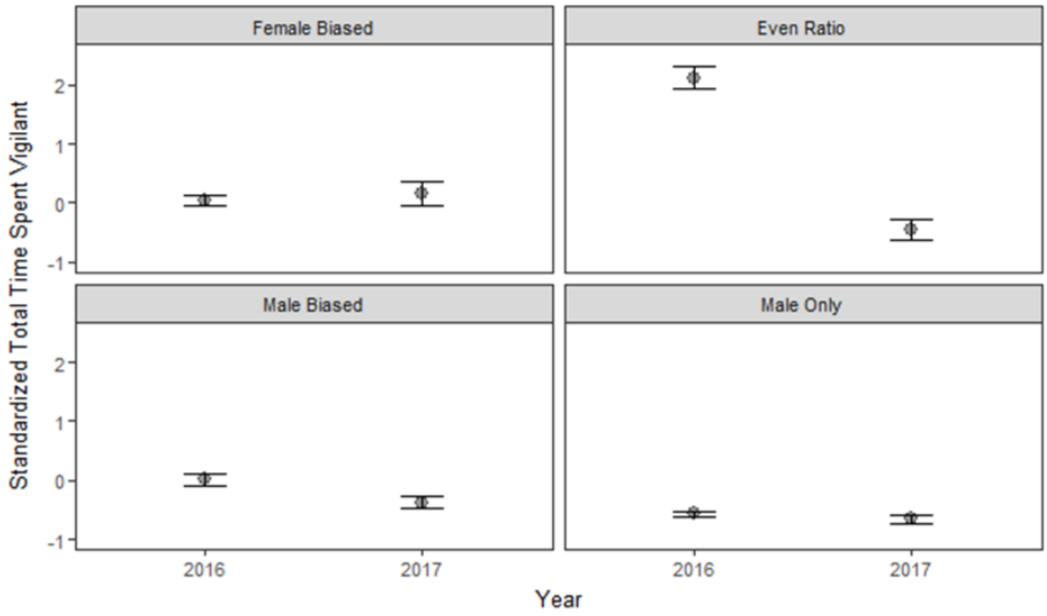
APPENDIX

	Total Time Spent Vigilant (seconds)		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	19.34	14.50 – 24.17	**<0.001**
Male Biased	-7.35	-13.61 – -1.08	**0.022**
Male Only	-13.50	-19.61 – -7.39	**<0.001**
Group Size 12	-0.75	-6.49 – 5.00	0.799
Male Biased: Group Size 12	5.03	-2.46 – 12.52	0.188
Male Only: Group Size 12	3.06	-4.23 – 10.35	0.410
Random Effects			
σ^2	21.08		
$\tau_{00\ ID}$	7.63		
$\tau_{00\ GROUP}$	5.54		
ICC	0.38		
N_{GROUP}	26		
N_{ID}	48		
Observations	146		
Marginal R^2 / Conditional R^2	0.383 / 0.620		

Supplementary Material A. Model output for the square-root transformed linear mixed model for the total time spent vigilant (seconds) testing group size and sex ratio effects after excluding Even Ratio groups. Group size, sex ratio and the two-way interaction between group size and sex ratio are fixed factors. Year and age were removed as fixed factors because they were not significant. Male ID and group ID are random effects. The model AIC is 923.6. Model BIC is 950.4, loglikelihood is -452.8 and deviance is 905.6.

	Total Time Spent Vigilant (seconds)		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	14.06	9.62 – 18.50	**<0.001**
Year 2018	4.95	-1.07 – 10.97	0.107
Female Biased	3.51	-2.64 – 9.67	0.263
Male Biased	-0.24	-6.01 – 5.54	0.936
Male Only	-9.10	-13.99 – -4.20	**<0.001**
Random Effects			
σ^2	14.72		
$\tau_{00\ ID}$	34.53		
ICC	0.70		
N_{ID}	28		
Observations	36		
Marginal R^2 / Conditional R^2	0.402 / 0.821		

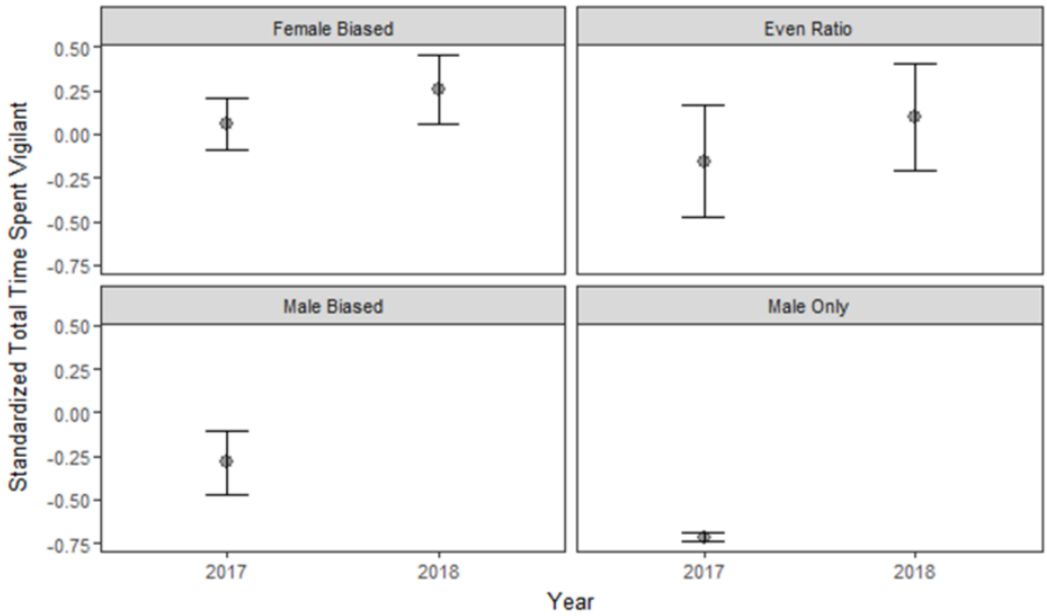
Supplementary Material B. Model output for the square-root transformed linear mixed model for the total time spent vigilant (seconds) testing sex ratio effects on the groups of 4 only. Year and sex ratio are fixed factors with male ID as a random effect. Group ID and was removed as a random effect because it had a variance of zero. The model AIC is 245.3. Model BIC is 256.3, loglikelihood is -115.6 and deviance is 231.3.



Supplementary Material C. Average z-score standardized total time spent vigilant for years 2016 and 2017 for each sex ratio for groups of 12 only. Error bars represent standard error.

	Total Time Spent Vigilant (seconds)		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	13.71	8.79 – 18.63	**<0.001**
Year 2018	5.85	-2.07 – 13.76	0.148
Female Biased	4.68	-3.86 – 13.22	0.283
Male Biased	-0.14	-6.14 – 5.87	0.965
Male Only	-8.72	-14.11 – -3.33	**0.002**
Year 2018: Female Biased	-2.55	-15.51 – 10.42	0.700
Random Effects			
σ^2	16.16		
$\tau_{00\ ID}$	33.96		
ICC	0.68		
N_{ID}	28		
Observations	36		
Marginal R^2 / Conditional R^2	0.399 / 0.806		

Supplementary Material D. Model output for the square-root transformed linear mixed model for the total time spent vigilant (seconds) testing year effects in groups of 4 only. Year, sex ratio and the two-way interaction between year and sex ratio are fixed factors. Normalized David score was excluded here so that we could test year effects using the Female Biased groups of 4. Male ID is a random effect. Group ID was removed as a random effect because it explained no variance in the model. The model AIC is 247.1. Model BIC is 259.8, loglikelihood is -115.6 and deviance is 231.1.



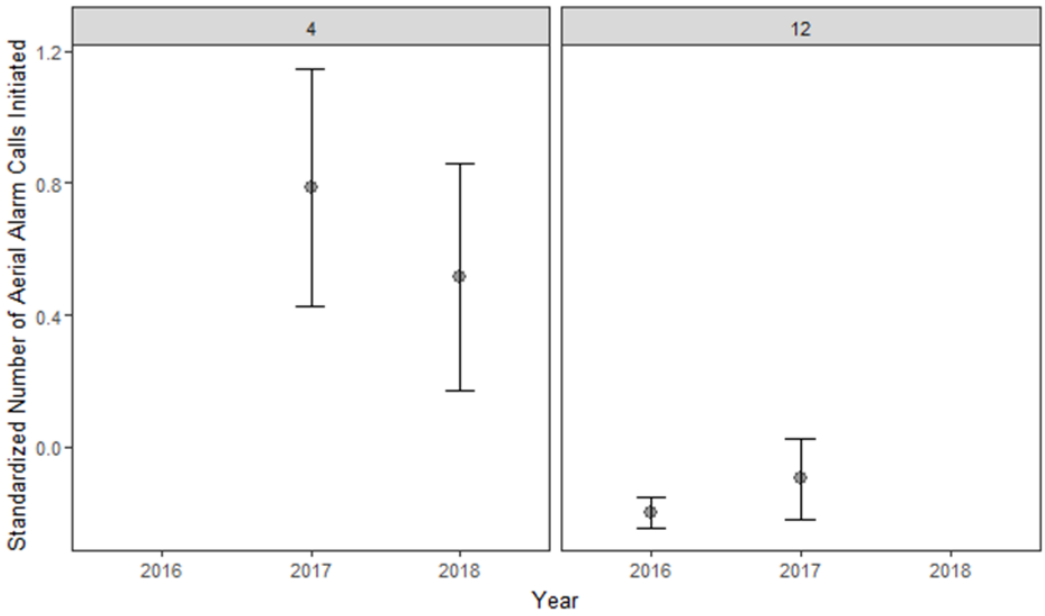
Supplementary Material E. Average z-score standardized total time spent vigilant for years 2017 and 2018 for each sex ratio for groups of 4 only. Error bars represent standard error. Male Biased and Male Only groups were not completed in 2018.

	Total Number of Aerial Alarm Calls Initiated		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	7.95	3.17 – 19.95	**<0.001**
Year 2017	1.11	0.27 – 4.67	0.883
Female Biased	27.52	8.62 – 87.91	**<0.001**
Male Biased	0.07	0.03 – 0.18	**<0.001**
Male Only	0.01	0.00 – 0.04	**<0.001**
Normalized David Score	2.62	2.01 – 3.41	**<0.001**
Year 2017: Female Biased	3.80	0.33 – 43.78	0.284
Year 2017: Male Biased	1.14	0.18 – 7.35	0.890
Year 2017: Male Only	0.95	0.16 – 5.53	0.958
Random Effects			
σ^2	2.35		
$\tau_{00\ obs_effect}$	2.10		
$\tau_{00\ ID}$	0.29		
ICC	0.11		
N_{ID}	48		
N_{obs_effect}	150		
Observations	150		
Marginal R^2 / Conditional R^2	0.465 / 0.524		

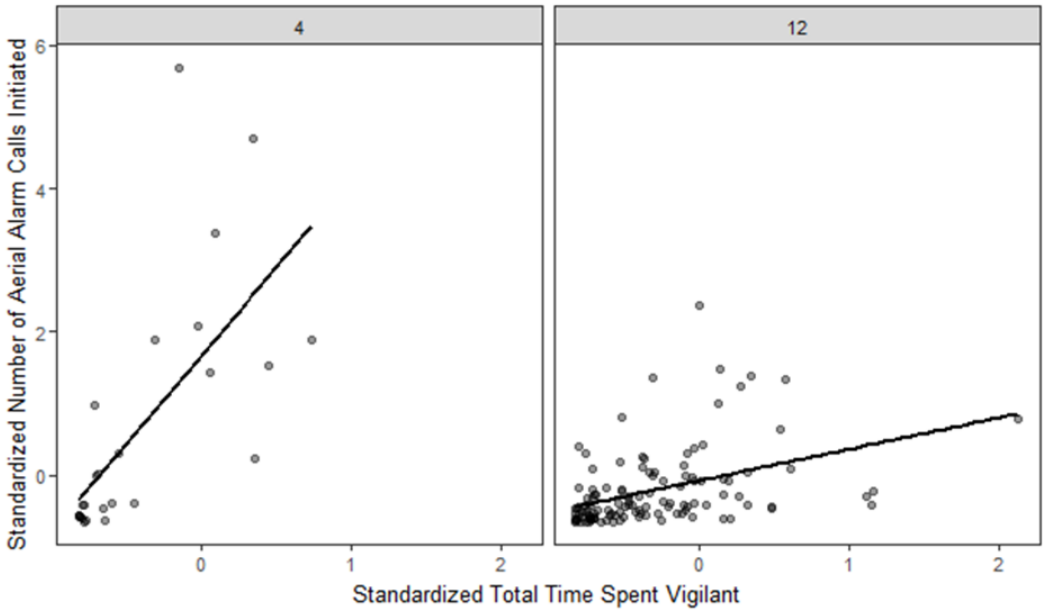
Supplementary Material F. Model output for the square-root linked Poisson generalized linear mixed model for the total number of aerial alarm calls initiated testing year effects in groups of 12 only. Year, sex ratio, normalized David score and the two-way interaction between year and sex ratio are fixed factors. Male ID is a random effect, and the (1|obs_level) random effect was added to combat overdispersion. Group ID was removed as a random effect due to having a variance of zero. The model AIC is 1139.0. Model BIC is 1172.1, loglikelihood is -558.5 and deviance is 1117.0.

	Total Number of Aerial Alarm Calls Initiated		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	78.52	35.54 – 173.48	**<0.001**
Year 2018	0.58	0.17 – 2.00	0.392
Female Biased	0.66	0.22 – 1.96	0.456
Male Biased	0.20	0.08 – 0.52	**0.001**
Male Only	0.11	0.05 – 0.24	**<0.001**
Year 2018: Female Biased	0.60	0.11 – 3.12	0.542
Random Effects			
σ^2	0.04		
$\tau_{00\ ID}$	1.73		
$\tau_{00\ GROUP}$	0.12		
ICC	0.98		
N_{ID}	28		
N_{GROUP}	16		
Observations	36		
Marginal R^2 / Conditional R^2	0.281 / 0.986		

Supplementary Material G. Model output for the log linked Poisson generalized linear mixed model for the total number of aerial alarm calls initiated testing year effects in groups of 4 only. Year, sex ratio and the two-way interaction between year and sex ratio are fixed factors. Normalized David score is excluded here to allow the exploration of year effects in Female Biased groups. Male ID and group ID are random effects. The model AIC is 352.3. Model BIC is 364.9, loglikelihood is -168.1 and deviance is 336.3.



Supplementary Material H. Average z-score standardized total number of aerial alarm calls initiated that a male gave across the 4-day observation period for years 2016, 2017 and 2018 for both groups of 4 and 12. Error bars represent standard error. No groups of 4 were completed in 2016, and no groups of 12 were observed in 2018.



Supplementary Material I. Each male's z-score standardized total time spent vigilant against the z-score standardized total number of aerial alarm calls initiated across the 4-day observation period for each group size. Even Ratio groups have been excluded.

Chapter 3

The Role of Vigilance and Alarm Calling in Mating Success

Heather Kazara and Tommaso Pizzari

ABSTRACT

Males and females face different constraints during reproduction. Males produce sperm which is inexpensive whereas eggs are costly; leading to females being more selective when choosing reproductive partners. As males benefit from multiple partners, and females exert pressure to possess certain qualities, sexual selection acts more strongly on males. To increase mating success males may need to simultaneously attract females, compete with rival males and protect paternity. Mating success in red junglefowl, *Gallus gallus*, and the related domestic fowl, *Gallus gallus domesticus*, has been thoroughly explored with more recent work finding a link between mating success and aerial alarm calling. Fowl produce two predator alarm calls, with aerial calls being a male-specific trait. Aerial alarm calling has been shown to be the best predictor of mating success, with studies showing an increase in alarm calling effort in males that mate. In addition, vigilance behaviour is a sexually selected trait in another Galliforme, the grey partridge, *Perdix perdix*, with females choosing to pair with more vigilant males. This study explored the relationship between vigilance and alarm calling with mating success in red junglefowl in groups of 12 using four measures of mating success: number of matings, number of females mated, remating rate and sperm competition intensity (SCI). Results found that vigilance was positively correlated with these behaviours, with vigilance also decreasing with increasing SCI; suggesting reduced sperm competition. Vigilance could be used to locate matings and/or guard females explaining these results. Alarm calling had significant positive correlations with the first three measures of mating success; with an additional positive relationship with female solicitations. Therefore, alarm calling may be an attractive trait to females, increasing mating success. Future studies should not ignore anti-predator behaviours when looking at predictors of mating success.

INTRODUCTION

Sexual selection was first theorised by Darwin (1871) to explain the development of elaborate features with no obvious advantages. Sexual selection is a form of natural selection where certain individuals within a population have greater reproductive success than others as a result of two factors: intra-sexual selection (male-male competition) and inter-sexual selection (mate choice) (Darwin, 1859, 1871; Arnold & Wade, 1984; Graves *et al.*, 1985; Kvarnemo *et al.*, 1995; Loyau *et al.*, 2005b). According to Bateman's Principle, females within species typically invest more into offspring production than males via eggs, leading to females benefitting from being more choosy when selecting mates (Bateman, 1948). Sperm is less costly to produce, therefore under traditional male and female roles, males typically benefit from mating with multiple females to increase reproductive success. Consequently, Bateman (1948) stated that male reproductive success relies on mate availability whereas female reproductive success is dependent upon egg production. Due to males competing for access to mates, and females being choosier than males during mate choice, sexual selection is often more pronounced in males. However, in species where males undergo pregnancy, like in pipefish and seahorses, it has been suggested that sex roles could be reversed with females competing and males being more selective with mate choice (Vincent *et al.*, 1992). Yet when this was experimentally tested in the Sri Lankan seahorse, *Hippocampus fuscus,* results indicated that males and females exhibited traditional sex roles despite higher male investment in offspring production (Vincent, 1994). Under traditional sex roles males must attract females, compete against rival males and defend/secure paternity.

Inter-sexual selection refers to female choice by female preference exerting selective pressures on males to possess certain qualities (Kvarnemo *et al.*, 1995; Loyau *et al.*, 2005b). By selecting certain traits females can gain direct (food, breeding territory, reduced harassment) or indirect (genetic) benefits (Loyau *et al.*, 2005a, 2005b). In many bird species males display bright plumage, which is often a result of female preference within sexual selection (Petrie *et al.*, 1991; Møller & Petrie, 2002; Pryke *et al.*, 2002; Doucet & Montgomerie, 2003a). In satin bower birds, *Ptilonorhynchus violaceus*, parasite resistance is predicted by the brightness of rump plumage colouration in males; allowing females to gain direct benefits by avoiding parasite riddled males (Doucet & Montgomerie, 2003a, 2003b). Although much of the work testing elaborate traits focusses on birds there is evidence of female choice for similar traits in other taxa. UV reflectance in blue throat patches of male European green lizards, *Lacerta viridis*, was assessed in respect to female preference (Bajer *et al.*, 2010). Male lizards were paired based on similar morphological features including snout length, head size and body weight to control for females selecting for other traits. One male in each pair had UV reflectance experimentally reduced using UV reducing agents. Females were then presented with both males, at which point female location was recorded to assess preference. Females significantly associated more with the unaltered male, suggesting UV reflectance could be used in mate choice (Bajer *et al.*, 2010). Peacocks, *Pavo cristatus*, are a classic example of sexual selection acting upon male plumage (Petrie & Williams, 1993; Møller & Petrie, 2002; Loyau *et al.*, 2005a; Loyau *et al.*, 2005b; Loyau *et al.*, 2007, 2008; Takahashi *et al.*, 2008; Dakin & Montgomerie, 2011). Male peacocks with larger eyespots have been demonstrated having larger offspring, which consequently led to increased

offspring survival (Petrie, 1994). Furthermore positive correlations between mating success and the number of eyespots along with eyespot brightness on the males train has been documented (Petrie *et al.*, 1991; Loyau *et al.*, 2007). Therefore, although it is unclear as to what aspect of train morphology females are using, it seems evident that trains are used in female selection.

Intra-sexual selection involves males competing over access to females, territories or reproduction-related resources. In sword tail fish, *Xiphophorus nigrensis*, larger males were more likely to win in male-male interactions over access to females (Morris *et al.*, 1992). A similar trend has been shown in red-sided garter snakes, *Thamnophis sirtalis parietalis*, where larger males have higher mating success. Male snakes of all sizes were able to court and gain female consent for mating however, larger males were more capable of displacing smaller males during copulations (Shine *et al.*, 2000). In addition to access to females, males may need to compete for access to reproduction-related resources like breeding territories or lekking grounds. In lekking species males must compete for a lekking area and defend their display territory. In peafowl, males that lek in more central positions received more matings, so male-male competition could indirectly influence male mating success (Loyau *et al.*, 2005b). One study found that peacocks that were more territorial and better able to defend their patch were heavier with larger trains. This study suggested that train length could be a handicap, so only the best males could produce longer trains; acting as an honest signal of male fighting ability (Loyau *et al.*, 2005b).

As well as gaining females, males need to secure paternity post-copulation and face sperm competition (Parker, 1970; Birkhead & Hunter, 1990; Price *et al.*, 1999; Birkhead & Pizzari, 2009). One strategy to reduce sperm competition is to

engage in mate guarding. In blue-footed boobies, *Sula nebouxii*, females were significantly less likely to engage in extra-pair copulations when mates were present (Osorio-Beristain & Drummond, 1998). Furthermore, in mandrills, *Mandrillus sphinx*, dominant males that engaged in mate guarding were more likely to sire offspring, with the percentage of time spent mate guarding significantly increasing the percentage of offspring sired (Setchell *et al.*, 2005). However, mate guarding can be costly as it reduces the amount of time that could be spent engaging in extra-pair copulations. Despite mate guarding, extra-pair copulations are common, even in socially monogamous species (Møller & Birkhead, 1993; Whittingham *et al.*, 1993; Sheldon *et al.*, 1997; Kokko & Morrell, 2005). As such, after mating has occurred males face post-copulatory sperm competition (Birkhead & Hunter, 1990; Kokko & Morrell, 2005). When males are faced with sperm competition, increasing sperm investment can lead to males fertilising a higher proportion of eggs. Some species can strategically allocate sperm (Marconato & Shapiro, 1996; Pizzari *et al.*, 2003). For example sand martins, *Riparia riparia*, increase sperm investment when competition risk is increased (Nicholls *et al.*, 2001).

Sexual selection on males therefore provides various challenges including males being attractive to females (Shutler & Weatherhead, 1990; Petrie, 1994), males being able to fight and compete for access to females/resources (Morris *et al.*, 1992; Loyau *et al.*, 2005b), and males needing to reduce sperm competition through mate guarding and/or increasing sperm investment (Birkhead & Hunter, 1990; Pizzari *et al.*, 2003; Kokko & Morrell, 2005). Mating behaviour in red junglefowl, *Gallus gallus*, and the related domestic fowl, *Gallus gallus domesticus*, has been extensively studied. Fowl have a harem polygynandrous

mating system with males competing for access to females while females exhibit female choice through soliciting matings and post-copulatory cryptic choice. Many of the classis studies examine mate choice in regards to morphological features like comb length (Zuk *et al.*, 1990a, 1990b), comb colouration (Zuk *et al.*, 1995), plumage colouration (Zuk *et al.*, 1990b; Ligon & Zwartjes, 1995; Zuk *et al.*, 1995a), or eye brightness (Zuk *et al.*, 1990c; Ligon *et al.*).

Both male and female fowl establish dominance hierarchies that can influence mating success (Kratzer & Craig, 1980; Pizzari & Birkhead, 2000; Zuk & Johnsen, 2000; Johnsen *et al.*, 2001; Pizzari, 2001; Parker & Ligon, 2002). Dominant males may be more capable of monopolising females, and thereby increase their reproductive success. Yet there is evidence that male dominance is selected for by females, so dominant males receive greater mating success through selection in addition to monopolisation (Kratzer & Craig, 1980; Pizzari & Birkhead, 2000; Johnsen *et al.*, 2001). Dominant males receive more solicitations and are less likely to have sperm ejected during post copulatory sexual selection (Pizzari & Birkhead, 2000). Females may benefit from mating with dominant males through direct benefits such as parasite avoidance (Zuk *et al.*, 1990a; Zuk & Johnsen, 2000), better courtship feeding (Zuk *et al.*, 1990b; Pizzari, 2003), protection from male harassment (Pizzari & Birkhead, 2000) and increased protection from predators (Pizzari, 2003; Wilson & Evans, 2008). Potential indirect benefits include offspring with increased immunity (Zuk *et al.*, 1995a; Zuk & Johnsen, 2000) and higher dominance status (dominance is heritable) (Pizzari & Birkhead, 2000).

Many studies aiming to assess which males are preferred by females adopt mate choice experiments. These trials are often short and may cause females to quickly

assess available cues while sacrificing more reliable indicators of quality (Sullivan, 1994). Mate choice experiments can furthermore be unreliable if they do not also assess more internal selection, such as sperm competition (Wilson & Evans, 2010). Past studies using mate choice trials have found that female fowl prefer males with larger ornaments (Zuk *et al.*, 1990b; Ligon *et al.*, 1998). However, in more natural conditions ornaments do not predict mating success, with dominance and alarm calling be the best indicators of success (Wilson *et al.*, 2008).

Fowl emit two alarm calls, one for aerial predators and one for terrestrial predators. Both calls have been experimentally demonstrated to be functionally referential, with aerial calls causing birds to seek cover and look upwards, whereas terrestrial calls caused individuals to increase horizontal scanning and adopt an alert posture (Evans *et al.*, 1993). However only males produce aerial alarm calls, leading to the suggestion that these calls could play a role in male mating success.

Alarm calling can reduce individual fitness by alerting a predator of the signaller's location (Davis, 1984; Cowlishaw, 1997; Collier *et al.*, 2010). Wilson et al. (2008) examined mating success via the number of copulations and offspring sired in domestic fowl. Numerous predictors were measured including dominance, weight and comb length. Aerial alarm call rate was the best predictor of mating and reproductive success, implying this behaviour may be a sexually selected trait (male attractiveness hypothesis). Another study explored the relationship between mating success and aerial alarm calling in fowl, finding that alarm calling effort substantially increased in males that were provided with the opportunity to mate; suggesting investment in reproductive partners and future offspring was

responsible (Wilson & Evans, 2008). The male attractiveness hypothesis was further tested in fowl by providing females with two sets of males that had calling increased and decreased respectively in three separate experiments. Females showed no preference for males with higher calling rates; indicating call rate is due to male investment in mates (Wilson & Evans, 2010). A potential criticism of this set up is that female preference was scored as the time spent in a specific section ($1/3^{rd}$) of the experimental pen, with females scored as showing preference for a specific male by spending more time in the section closest to him. As females were not able to mate or solicit males this cannot tell us whether females would have preferred to copulate with a better alarm caller.

Less work has been done exploring the role of vigilance in mating success. Vigilance is defined as the stopping of behaviours, typically foraging, to scan the environment for potential threats (Elgar, 1989; Beauchamp, 2015). The link between dominance rank and increased protection against predation was previously examined in fowl, finding that dominant males produced more alarm calls and spent more time vigilant than subordinates (Pizzari, 2003). Additionally, subordinate males performed alarm calls when in a non-vigilant state, showing dishonest signalling. This suggests that being vigilant is costly and only good quality males can afford to provide honest vigilance signals to females. In addition, increased male vigilance may allow females to relax individual vigilance levels, which may in turn increase time spent foraging in females, and so increase egg quality (Dahlgren, 1990). In another Galliforme the grey partridge, *Perdix perdix*, both vigilance and vocal performance are used in mate choice (Dahlgren, 1990; Beani & Dessí-Fulgheri, 1998). In this species parental care of offspring is shared, so direct benefits could be obtained by selecting for a vigilant male

(Fusani *et al.*, 1997). Furthermore, high vigilance could be used to guard females from competitors, which increases paternity confidence. Male Eurasian wigeon, *Mareca penelope*, are significantly more vigilant than paired females, with paired females spending significantly less time engaged in interactions with other males; implying that male vigilance increases to guard females from rivals (Guillemain *et al.*, 2003). Alternatively, vigilance may be used to detect potential mating opportunities, leading to greater reproductive success.

The link between mating success and vigilance/alarm calling in fowl is still unclear. Dominance is often highly correlated with alarm calling, making it difficult to distinguish whether females prefer alarm calling males or are selecting other traits (Wilson & Evans, 2010; Rodríguez-Muñoz *et al.*, 2011).

This chapter aims to explore the relationship between vigilance and aerial alarm calling and mating success in red junglefowl. The relationship is explored in two parts. Part one examines the correlations between vigilance and alarm calling with four measures of mating success including the number of matings, number of females mated, average remating rates per female and the sperm competition intensity (SCI). The number of matings and females mated allow the exploration of how these behaviours are correlated with pre-copulatory mating success, whereas remating rates and SCI represent post-copulatory mating success as higher remating rates, and lower SCI, represent reduced sperm competition. If vigilance and/or alarm calling are good predictors of mating success then they should increase with the number of matings, females mated and remating rates. Furthermore, if these behaviours aid in reducing sperm competition, then they should be negatively correlated with SCI. This is more probable for vigilance which could be used to either guard females and/or interrupt copulations.

Part two explores the correlations between vigilance and alarm calling on behaviours that could indirectly influence mating success. These behaviours include female solicitations, courtship behaviour (waltzing) and aggressive encounters including female harassment and the number of copulation interruptions received and performed. If vigilance and/or alarm calling are attractive traits to females, then males that invest in these behaviours should receive more female solicitations. As well as potentially being attractive to females, males could increase vigilance to either locate additional mating opportunities and/or guard mated females from rivals. If males do indeed invest in vigilance to guard females from competitors, then this should decrease the likelihood of rival males copulating with the mates of vigilant males; thus, reducing sperm competition. Like mate guarding, more vigilant males could further increase reproductive success by decreasing the number of times they are interrupted and increasing the number of copulation interruptions towards other males. Therefore, more vigilant males should significantly increase the number of interruptions performed while also decreasing the number of interruptions received. Lastly, waltzing in fowl is used as a form of courtship to help synchronize mating. So, it is possible that males that invest more heavily in vigilance and/or alarm calling also invest more into courtship.

METHODS

Study System

This chapter used a sub-set of groups discussed in Chapter 2. For more details on methods, group composition and behaviours recorded see the methods section from Chapter 2.

A total of 15 groups, five per sex ratio, were created. Each group took six days to run, with four days of observations. Males were placed into the experimental pen on the afternoon of day 1. Females were then released 24 hours later on day 2. This gave the males at least 24 hours alone to begin to establish a dominance hierarchy. Observations began the morning of day 3.

Observations took place for 8 hours per day in the morning and evening. Observations start 30 minutes after sunrise for 4 hours. Evening observations end 30 minutes before sunset.

Mating Behaviours

Only successful copulations are reported in this chapter, and these were defined by males mounting females followed by the lowering of the male's tail over the cloaca (Pizzari, 2003). Solicitations were recorded when females crouched down directly in front of males whereas all other matings were classified as forced (Pizzari, 2003). Waltzing in fowl is described as the lowering of one wing while circling the female (Zuk *et al.*, 1992; Zuk *et al.*, 1995b). Copulation interruptions were characterised by a male successfully preventing another male mating through either displacing and/or chasing away the copulating male (Rosher *et al.*, 2017). For each interruption event recorded both the actor and receiver was noted, allowing the exploration of both the number of interruptions received and

performed by males. Finally, harassing behaviours towards females were noted, including males chasing females, males pecking females aggressively and males lunging their necks towards females.

Data Analysis

To assess the relationship between both vigilance and alarm calling on mating success and mating behaviours nine main mixed models were created using R (version 1.1.423; R Core Team, 2017). Models looked at the total number of successful matings, number of females mated, average remating rates per female, sperm competition intensity (SCI), the number of solicitations, the number of courtship waltzes, number of harassments towards females, number of times a male's mating was interrupted, and the number of times a male interrupted copulation attempts by other males across the 4 days. Mixed models were chosen because our data was not independent due to reusing males. Adding male ID as a random effect controlled for this non-independence in the data. Mixed models also allowed us to control for any potential group-level effects by also adding group ID as a random effect.

The dependent variables (number of matings; females mated; solicitations; waltzes; harassments; interruptions received; interruptions performed) were created by totalling each male's mating behaviour for each of the 8 sessions into one weekly total. The standardized number of females mated was calculated by creating a standardised z-score by subtracting the mean and diving by the standard deviation. The mean and standard deviation was taken for the number of females mated with in the Male Biased groups and were used to create the z-score for males in groups adhering to this sex ratio. The same process was used to create z-scores for males in Female Biased and Even Ratio groups separately. As such, we

controlled for the different number of females available to mate within the varying sex ratios and allowed standardization of the number of females mated.

The vigilance variable was created by compiling the seconds spent vigilant from each of the 8 10-minute sessions into one combined total. The aerial alarm call variable was created by adding the number of aerial alarm calls initiated for each male across the 8 observation sessions; giving an overall total number of aerial calls initiated across the 4-day period.

All dominance interactions across the 4-day period were scored with a winner and a loser in each interaction. A dominance matrix was then produced for each group using the number of wins and losses for everyone within the group. These matrixes were entered into R and used to calculate normalised David scores using the "Steepness" package (version 0.2.2; Leiva & de Vries, 2014).

SCI

Female fowl are polyandrous causing males to suffer sperm competition (Pizzari & Birkhead, 2000; Birkhead & Pizzari, 2009; McDonald & Pizzari, 2016; McDonald *et al.*, 2017). To examine the effect of vigilance and alarm calling on the relative sperm competition faced by males the sperm competition intensity (SCI) was calculated. This is defined as the mean mating success of a male's reproductive partners (McDonald & Pizzari, 2016; McDonald *et al.*, 2017). This was calculated using the formula and methods outlined in McDonald and Pizzari (2016), with SCI representing the mean polyandry of mating partners. When males had no sperm competition then SCI equalled 1, with higher scores (maximum score is 6 in the data) indicating increased competition (McDonald & Pizzari, 2016).

Along with SCI, remating rates were examined. This was defined as a male's average number of successful matings per mated female, and was calculated by dividing the total number of matings a male obtained by the total number of females he mated with (McDonald *et al.*, 2017).

Building the Models

All the mixed models were completed using the "lme4" package (version 1.1.21; Bates *et al.*, 2015).

Normalized David scores and the number of aerial alarm calls initiated were highly correlated in Female Biased groups of 12 (see Chapter 2 for details). To avoid issues of multicollinearity two models were created for each mating dependant variable; one with alarm calling and one with dominance. Models contained a three-way interaction between vigilance, sex ratio (three-levels) and either alarm calling or dominance along with all subsequent lower order two-way interactions between these three variables. Models also contained year (two-levels) and age (four-levels) as fixed effects with Male ID and group ID added as random effects. For each mating dependant variable, the alarm calling, and dominance models were compared using AIC and AICc, with the model showing an AIC/AICc with a difference equal to or higher than two being kept and used in the results section of this chapter. AICc was obtained using the "MUMin" package (version 1.40.4; Bartón, 2018) and AIC using the "stats" package (version 3.4.3; R Core Team, 2017). Using AIC/AICc for model selection is commonly practiced in ecology (Anderson *et al.*, 1994; Dudley, 2006; Sullivan *et al.*, 2006; Ward, 2008; Aho *et al.*, 2014; James *et al.*, 2015; Prior *et al.*, 2018). Additionally dominance and aerial alarm calling models were compared using the "anova" function in the "lmerTest" package (version 2.0.36; Kuznetsova *et al.*,

2017), showing that models with the lower AIC were significantly better than models with larger AIC. The aerial alarm calling models were significantly better than the dominance models for all nine dependant variables, and so aerial alarm calling was used instead of dominance in the final analysis.

Due to issues in the models concerning scaling and convergence, the total time spent vigilant (seconds) variable was standardized using z-scores for all nine models. These issues were caused by the vigilance predictor being on a much larger scale than the aerial alarm calling continuous predictor. Standardization was achieved by subtracting each vigilance data point by the mean total time spent vigilant and dividing the resulting value by the standard deviation.

To explore the relationship between vigilance and aerial alarm calling with the nine mating variables nine mixed models were used. Poisson models were used for the number of matings, number of solicitations, number of courtship waltzes, number of harassments, number of copulation interruptions received, and the number of copulation interruptions performed because they use count data. The Poisson models for solicitations, the number of interruptions received and performed used the log link, whereas the remaining three Poisson models used the square-root link. Linear mixed models were used to explore the z-score standardized number of females mated, average remating rates per female and the SCI. The average remating dependant variable was log transformed to improve model fit.

The z-score standardized total time spent vigilant was entered as a fixed effect with the total number of aerial alarm calls initiated, sex ratio (three-levels), male age (four-levels) and year (two-levels) in all nine models. Male ID and group ID

were added as random effects in all nine models. However, group ID was removed as a random effect in the "interruptions performed" model and male ID was removed from the "average remating rate" model because they had variances of zero. A three-way interaction between vigilance, alarm calling, and sex ratio was tested in all the models in addition to all subsequent lower order two-way interactions between these three variables. The significance of these interactions was assessed using Likelihood Ratio Tests. When interactions were not significant, they were removed, allowing for the testing of significance from the main effects. Year and age were also removed when they were not significant. The fixed effects used in the final models are shown in Table 1.

Table 1. The fixed effects used in the final models for the nine mating dependant variables. GLMM refers to a generalized linear mixed model, whereas LMM refers to a linear mixed model. The colon signifies a significant interaction in the model.

Dependant Variable	**Fixed Effects**
Number of Matings GLMM	Sex ratio + standardized time spent vigilant + total number of alarm calls
Number of Females LMM	Sex ratio + standardized time spent vigilant + total number of alarm calls+ standardized time spent vigilant: total number of alarm calls
Remating Rate LMM	Sex ratio + standardized time spent vigilant + total number of alarm calls
SCI LMM	Year + sex ratio + standardized time spent vigilant + total number of alarm calls
Number of Solicitations GLMM	Sex ratio + standardized time spent vigilant+ total number of alarm calls
Number of Waltzes GLMM	Year + sex ratio + standardized time spent vigilant + total number of alarm calls
Number of Harassments GLMM	Sex ratio + standardized time spent vigilant + total number of alarm calls+ sex ratio: standardized time spent vigilant + sex ratio: total number of alarm calls
Number of Interruptions Received GLMM	Year + sex ratio + standardized time spent vigilant + total number of alarm calls+ sex ratio: standardized time spent vigilant: total number of alarm calls
Number of Interruptions Performed GLMM	Year + sex ratio + standardized time spent vigilant + total number of alarm calls+ sex ratio: standardized time spent vigilant: total number of alarm calls

Models exploring remating rates and SCI used 64 data points compared to 90 data points used in the other models. This is because SCI scores and remating rates can only be calculated in males that have mated at least once, and 26 males did not mate at all so were excluded.

There was a significant two-way interaction between sex ratio and vigilance, and between sex ratio and alarm calling, which was retained in the final harassment model (Table 1). To further explore this relationship between vigilance and alarm calling within different sex ratios, separate models were created for each sex ratio. The Male Biased model used the total number of harassments performed as the dependant variable in a Poisson (square-root link) generalized linear mixed model. The total time spent vigilant (seconds) and the total number of aerial alarm calls initiated were entered into the model along with year (two-levels) and male age (four-levels) as fixed effects; with male ID and group ID added as random effects (see Appendix, Supplementary Material A). Year and age were accordingly removed due to lack of significance. The Even Ratio model followed the same structure using a Poisson (log-link) generalized linear mixed model, removing year due to no significance (see Appendix, Supplementary Material A). Finally, the Female Biased model also used a Poisson (log-link) generalized linear mixed model with the total time spent vigilant (seconds) and the total number of aerial alarm calls initiated as fixed effects (see Appendix, Supplementary Material A).

The three-way interaction between sex ratio, vigilance and alarm calling had significant correlations with both the number of interruptions received and performed (Table 1). Following the same methods described for the harassment models, separate models were created for each sex ratio using the total number of

interruptions received and performed as the dependant variables.

For exploring the correlations between vigilance and alarm calling on the total number of interruptions received within each sex ratio, three Poisson generalized linear mixed models were created, with models for the Even Ratio and Male Biased groups using a square-root link. By contrast, the Female Biased model used the log-link. In all three models, male ID and group ID were random effects, and the total time spent vigilant (seconds) and the total number of aerial alarm calls initiated were fixed effects (See Appendix, Supplementary Material B). Year and age were removed from all three models as they did not have significant effects. The random effect of group ID had a variance of zero in the Even Ratio model, and was therefore removed from the final model.

For exploring the number of interruptions performed in Male Biased groups a Poisson (square-root link) generalized linear mixed model with the total number of interruptions performed as the dependant variable was created, with the total time spent vigilant (seconds), total number of aerial alarm calls initiated, year and male age as fixed factors. Male ID and group ID were added as random effects. Year and age were removed due to lack of significance (see Appendix, Supplementary Material C). The Even Ratio and Female Biased models were also Poisson (square-root link) generalized linear mixed models like the Male Biased model (see Appendix, Supplementary Material C). The random effect of ID for the Female Biased model had a variance of zero, so was removed from the final model.

The independent Male Biased models used 45 data points, whereas the separate Even Ratio models used 30 data points and the Female Biased models using 15.

The number of matings, waltzes and harassments Poisson models suffered from overdispersion. Additionally, the separate Even Ratio and Male Biased harassment and interruptions performed Poisson models along with the Male Biased interruptions received model also suffered from overdispersion. To combat this an observation-level effect was added as a random effect. This is a standard practice for dealing with overdispersion in ecological research (Harrison, 2014, 2015). An alternative approach to dealing with overdispersion is to use the negative binomial family (Thomas & Lello, 2017), but this was deemed less suitable as it resulted in models with higher AIC. Solicitations were the least recorded mating behaviour, with many zeros in this variable. This increased the risk of zero inflatedness in these models so this was checked using the "testZeroInflation" function in the "DHARMa" package (version 0.2.0; Hartig, 2018); finding no zero inflation.

Both year (two-levels) and age (four-levels) were entered as fixed effects instead of random effects in all models because both variables had fewer than five levels, making it statistically more appropriate to treat them as fixed effects (Gelman & Hill, 2006; Clark & Linzer, 2015). To test that all models adhered to the assumptions they were graphically tested using qqnorm and residual vs fitted plots. The Poisson models were also explored using the "DHARMa" package (version 0.2.0; Hartig, 2018), which better explores the fit of Poisson models. All the models adhered to normality and homoscedacity when tested with "DHARMa".

Alternative Analysis using Z-Score Standardized Variables

A secondary analysis was conducted using z-score standardized variables to check that sex ratio was not strongly influencing the correlations between vigilance and alarm calling in the original analysis.

All the z-scores were standardized within each sex ratio to control for sex ratio effects on behaviours. For example, the standardized number of matings was calculated by creating a z-score by subtracting the mean and dividing by the standard deviation. The mean and standard deviation was taken for the number of matings within the Male Biased groups, and these were used to create the z-score for males within this sex ratio. The same process was used to create z-scores for males in Female Biased and Even Ratio groups separately. This approach was used for all 9 mating dependant variables and the two predictor variables (vigilance and aerial alarm calls).

Linear mixed models were created to explore the relationship between vigilance and alarm calling on these nine standardized mating variables. The z-score standardized models for the number of matings, remating rate, solicitations, waltzes, harassments, interruptions received and performed were transformed using an adjusted log transformation, which is commonly used to transform data with negative values that adhere to Poisson-like distributions. This transformation improved the graphical outputs of all the models except the solicitation model. Therefore, a Gamma model (identity) was run for this dependant variable using the adjusted log transformed dependant variable. The results from the standardized models were like results from the original analyses without standardization, so only the original analysis is reported in the results. See the appendix for the standardized model outputs (Supplementary Material D-L).

Analysis

Results were analysed through Likelihood Ratio Tests (LRT) using the "anova" function from the "lmerTest" package version (2.0.36; Kuznetsova *et al.*, 2017). To explore the effect of independent variables outside of the significant interactions *p* values were obtained through Type 3 Wald Chi-square tests using the "Anova" function in the "car" package (version 3.0.2; Fox & Weisberg, 2011).

All graphs were made using the "ggplot2" package (version 2.2.1; Wickham, 2009) and "sjPlot" package (version 2.6.3; Lüdecke *et al.*, 2019). For visually displaying interactions between vigilance and aerial alarm calling both variables were standardized using z-scores to allow easier interpretation.

RESULTS

Part 1: Correlates of Mating Success

Number of Matings

When controlling for sex ratio, vigilance had a very strong significant correlation with the number of matings obtained (LRT: X^2 (1) =20.33, p<0.001; Figure 1a; Table 1). Aerial alarm calling also had a significant positive correlation with the number of matings (LRT: X^2 (1) =9.84, p=0.002; Figure 1b; Table 2).

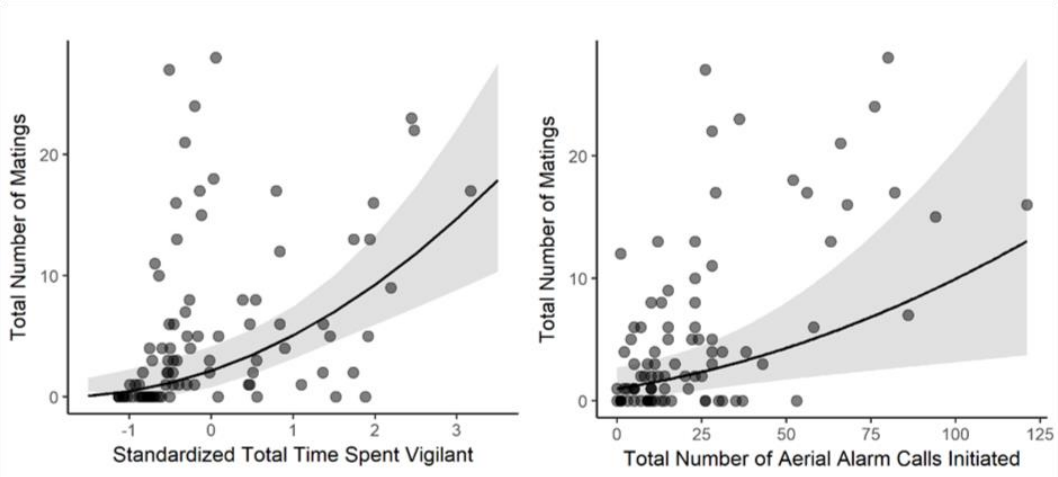


Figure 1. a) Z-score standardized total time spent vigilant and b) the total number of aerial alarm calls initiated against the total number of successful matings obtained. The line of best fit is the predicted line generated by the Poisson (square-root link) generalized linear mixed model. The grey area represents 95% confidence intervals.

Table 2. Model output for the Poisson generalized linear mixed model using the square-root link with the total number of matings as the dependant variable. Sex ratio, the z-score standardized total time spent vigilant and the total number of aerial alarm calls initiated ae fixed factors. Male ID and group ID are random effects. The (1|obs_level) random effect was added to combat overdispersion. The model AIC is 437.0. Model BIC is 457.0, loglikelihood is -210.5 and deviance is 421.0.

	Total Number of Matings		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	15.12	7.23 – 31.58	**<0.001**
Even Ratio	0.19	0.08 – 0.46	**<0.001**
Male Biased	0.22	0.11 – 0.44	**<0.001**
Standardized Total Time Spent Vigilant	2.20	1.55 – 3.13	**<0.001**
Total Number of Aerial Alarm Calls Initiated	1.02	1.01 – 1.03	**<0.001**
Random Effects			
σ^2	0.47		
$\tau_{00\ obs_effect}$	0.22		
$\tau_{00\ ID}$	0.31		
$\tau_{00\ GROUP}$	0.15		
ICC	0.50		
N_{ID}	31		
N_{GROUP}	15		
N_{obs_effect}	90		
Observations	90		
Marginal R^2 / Conditional R^2	0.587 / 0.792		

Number of females mated

Vigilance had a strong significant independent correlation with the standardized number of females mated (Type 3 Wald Chi-square Test: X^2 (1) =20.95, p<0.001; Figure 2a; Table 3) whereas alarm calling had a slight significant correlation (Type 3 Wald Chi-square Test: X^2 (1) =6.48, p=0.011; Figure 2b; Table 3). The visual distribution of datapoints in Figure 2b suggests that a quadratic term for aerial alarm calling may have been needed in the model, however, the addition of a quadratic term had no significant effect (LRT: X^2 (1) =0.16, p=0.685) and did not improve the model (determined per AIC comparisons) and was therefore not retained in the final model. There was a significant two-way interaction between vigilance and alarm calling, with vigilance having a stronger correlation with the number of females mated in males that initiated the fewest calls (LRT: X^2 (1) =5.59, p=0.018; Figure 3; Table 3).

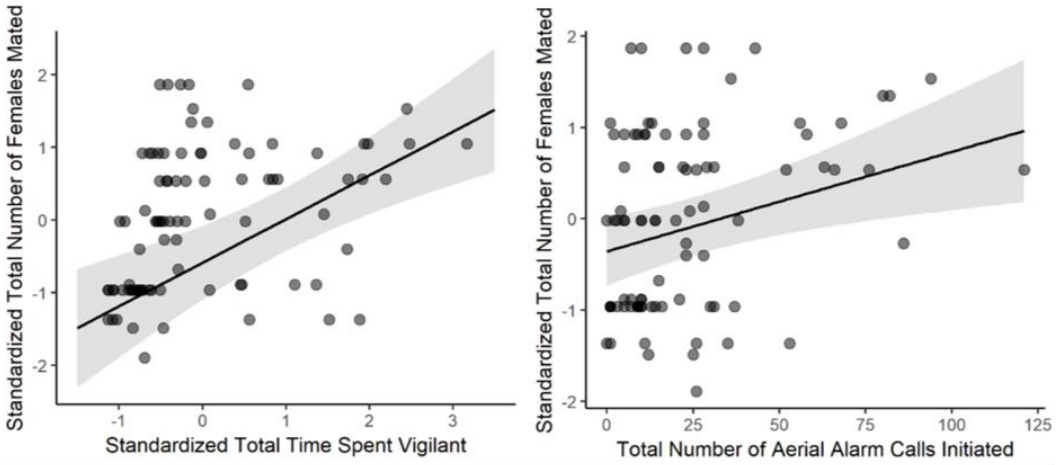


Figure 2. a) Z-score standardized total time spent vigilant and b) the total number of aerial alarm calls initiated against the z-score standardized total number of females mated. The line of best fit is the predicted line generated by the linear mixed model. The grey area represents 95% confidence intervals.

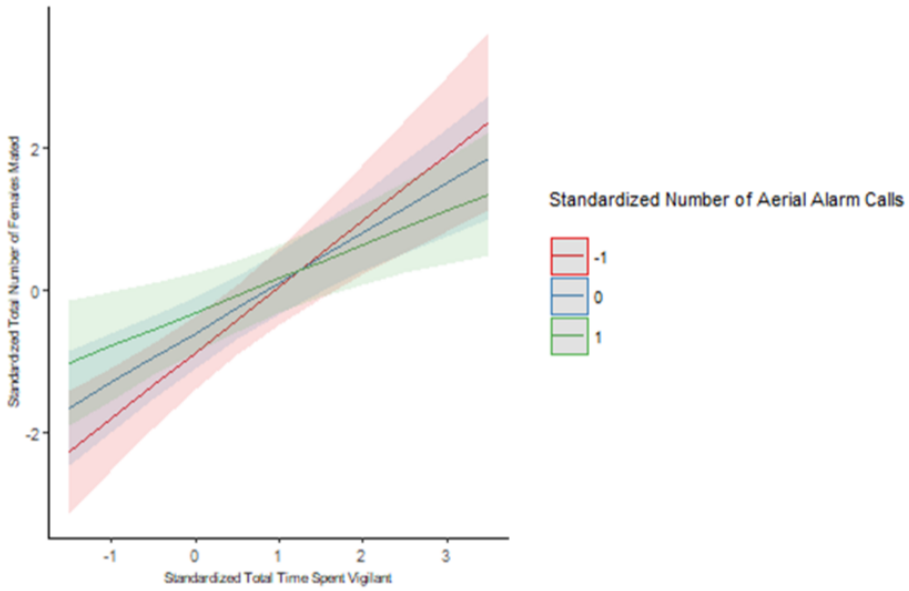


Figure 3. Z-score standardized total time spent vigilant against the z-score standardized total number of females mated. The graph is showing an interaction with the z-score standardized total number of aerial alarm calls initiated with males that initiated -1, 0 and 1 standard deviations of calls below and above the mean. The line of best fit is the predicted line generated by the linear mixed model. The shaded area represents 95% confidence intervals.

Table 3. Model output for the linear mixed model with the z-score standardized total number of females mated as the dependant variable. Sex ratio, the z-score standardized total time spent vigilant and the total number of aerial alarm calls initiated ae fixed factors along with a two-way interaction between vigilance and alarm calling. Male ID and group ID are random effects. The model AIC is 244.7. Model BIC is 267.2, loglikelihood is -113.4 and deviance is 226.7.

	Standardized Total Number of Females Mated		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	-0.45	-1.10 – 0.20	0.176
Even Ratio	-0.43	-1.20 – 0.34	0.273
Male Biased	0.67	0.01 – 1.33	**0.045**
Standardized Total Time Spent Vigilant	0.01	0.00 – 0.02	**0.003**
Total Number of Aerial Alarm Calls Initiated	0.92	0.55 – 1.30	**<0.001**
Standardized Total Time Spent Vigilant: Total Number of Aerial Alarm Calls Initiated	-0.01	-0.02 – -0.00	**0.012**
Random Effects			
σ^2	0.36		
$\tau_{00\ ID}$	0.22		
$\tau_{00\ GROUP}$	0.14		
ICC	0.49		
N_{ID}	31		
N_{GROUP}	15		
Observations	90		
Marginal R^2 / Conditional R^2	0.350 / 0.671		

Average remating rate

Controlling for sex ratio, both vigilance (LRT: X^2 (1) =6.12, *p*=0.013; Figure 4a; Table 4) and alarm calling had significant positive relationships with average remating rates per female (LRT: X^2 (1) =7.08, *p*=0.008; Figure 4b; Table 4).

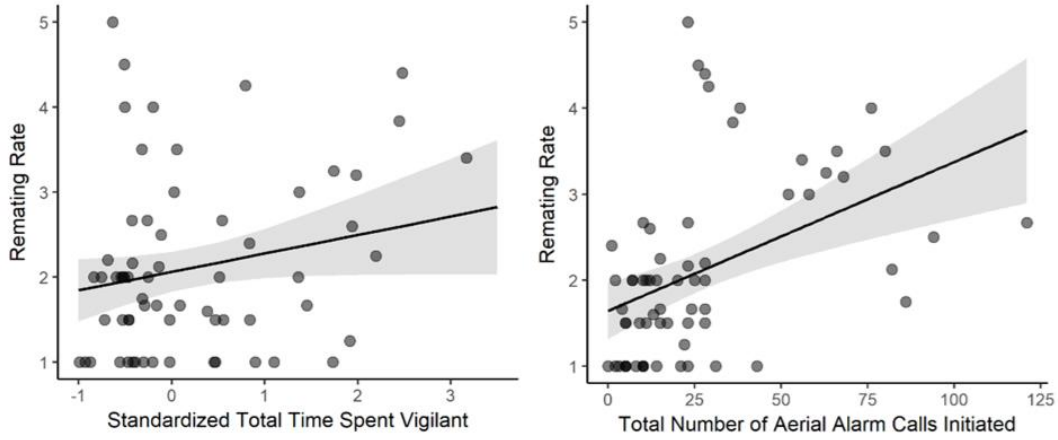


Figure 4. a) Z-score standardized total time spent vigilant and b) the total number of aerial alarm calls initiated against the average remating rate per female. The line of best fit is the predicted line generated by the log transformed linear mixed model. The grey area represents 95% confidence intervals.

.

Table 4. Model output for the log transformed linear mixed model using the average remating rate per female as the dependant variable. Sex ratio, the z-score standardized total time spent vigilant and the total number of aerial alarm calls initiated ae fixed factors. Group ID is a random effect. The model AIC is 76.58. Model BIC is 93.85, loglikelihood is -30.29 and deviance is 60.58

	Average Remating Rate Per Female		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	1.32	0.76 – 1.87	**<0.001**
Female Biased	0.75	-0.12 – 1.61	0.093
Male Biased	0.40	-0.29 – 1.10	0.253
Standardized Total Time Spent Vigilant	0.42	0.08 – 0.75	**0.015**
Total Number of Aerial Alarm Calls Initiated	0.01	0.00 – 0.02	**0.009**
Random Effects			
σ^2	0.81		
$\tau_{00\ GROUP}$	0.02		
ICC	0.02		
N_{GROUP}	15		
Observations	64		
Marginal R^2 / Conditional R^2	0.276 / 0.292		

SCI

Alarm calling did not significantly correlate with SCI (LRT: X^2 (1) =2.51, *p*=0.113; Figure 5b; Table 5) however vigilance did have a slight significant negative correlation with SCI (LRT: X^2 (1) =4.65, *p*=0.031; Figure 5a; Table 5) when controlling for sex ratio. More vigilant males appear to experience slightly less sperm competition (Figure 5). The visual distribution of datapoints in Figure 5a suggests that a quadratic term for vigilance may be needed in the model, however, this quadratic term had no significant effect (LRT: X^2 (1) =0.14, *p*=0.705) and did not improve the model (according to AIC comparisons) and was therefore removed from the final model. Additionally, there was no significant effect from a quadratic term using aerial alarm calling (LRT: X^2 (1) =0.02, *p*=0.876), nor did it improve model fit when comparing with AIC.

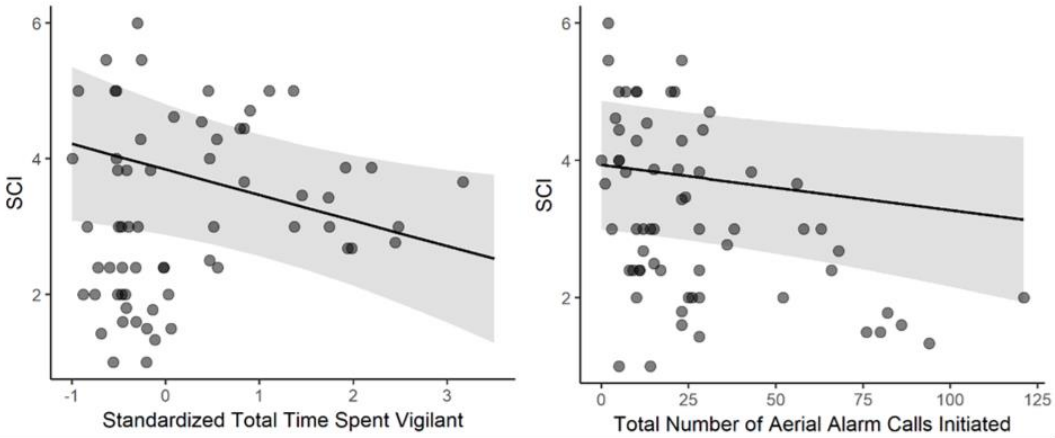


Figure 5. a) Z-score standardized total time spent vigilant and b) the total number of aerial alarm calls initiated against SCI score. The line of best fit is the predicted line generated by the linear mixed model. The grey area represents 95% confidence intervals.

Table 5. Model output for the linear mixed model with SCI as the dependant variable. Year (2016/2017), sex ratio, the z-score standardized total time spent vigilant and the total number of aerial alarm calls initiated ae fixed factors. Male ID and group ID are random effects. The model AIC is 163.5. Model BIC is 193.6, loglikelihood is -78.1 and deviance is 156.2.

	SCI		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	2.28	1.33 – 3.24	**<0.001**
Year 2017	-1.20	-2.42 – 0.02	0.054
Even Ratio	2.00	0.72 – 3.28	**0.002**
Male Biased	1.24	0.06 – 2.42	**0.039**
Standardized Total Time Spent Vigilant	-0.44	-0.78 – -0.09	**0.014**
Total Number of Aerial Alarm Calls Initiated	-0.01	-0.01 – 0.00	0.147
Random Effects			
σ^2	0.22		
$\tau_{00\ ID}$	0.23		
$\tau_{00\ GROUP}$	0.77		
ICC	0.82		
N_{ID}	27		
N_{GROUP}	15		
Observations	64		
Marginal R^2 / Conditional R^2	0.348 / 0.882		

Part 2: Behaviours that Influence Mating Success

Solicitations

Aerial alarm calling had a significant positive correlation with the number of solicitations received (LRT: X^2 (1) =20.36, p<0.001; Figure 6b; Table 6), with vigilance having a slight significant correlation (LRT: X^2 (1) =5.41, p=0.020; Figure 6a; Table 6) when controlling for sex ratio.

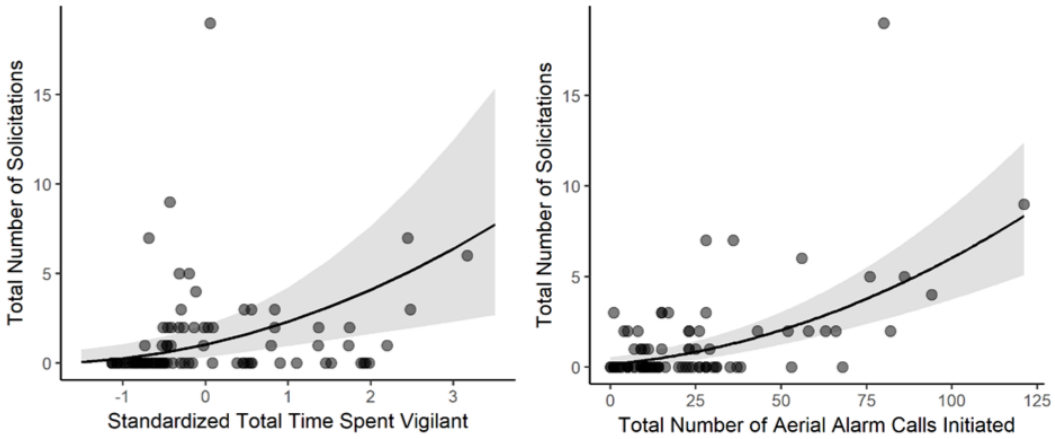


Figure 6. a) Z-score standardized total time spent vigilant and b) the total number of aerial alarm calls initiated against the total number of female solicitations received. The line of best fit is the predicted line generated by the Poisson (log link) generalized linear mixed model. The grey area represents 95% confidence intervals.

Table 6. Model output for the Poisson generalized linear mixed model using the log link with the total number of female solicitations received as the dependant variable. Sex ratio, the z-score standardized total time spent vigilant and the total number of aerial alarm calls initiated ae fixed factors. Male ID and group ID are random effects. The model AIC is 224.6. Model BIC is 242.1, loglikelihood is -105.3 and deviance is 210.6.

	Total Number of Solicitations		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	1.01	0.43 – 2.38	0.989
Even Ratio	0.24	0.09 – 0.62	**0.003**
Male Biased	0.19	0.08 – 0.43	**<0.001**
Standardized Total Time Spent Vigilant	1.65	1.12 – 2.45	**0.012**
Total Number of Aerial Alarm Calls Initiated	1.02	1.01 – 1.03	**<0.001**
Random Effects			
σ^2	1.04		
$\tau_{00\ ID}$	0.58		
$\tau_{00\ GROUP}$	0.08		
ICC	0.39		
N_{ID}	31		
N_{GROUP}	15		
Observations	90		
Marginal R^2 / Conditional R^2	0.447 / 0.663		

Courtship waltzes

When controlling for sex ratio, vigilance had a very slight significant correlation with the number of waltzes performed (LRT: X^2 (1) =3.95, p=0.047; Figure 7a; Table 7) whereas aerial alarm calling had a strong significant correlation with the number of courtship waltzes performed (LRT: X^2 (1) =31.65, p<0.001; Figure 7b; Table 7).

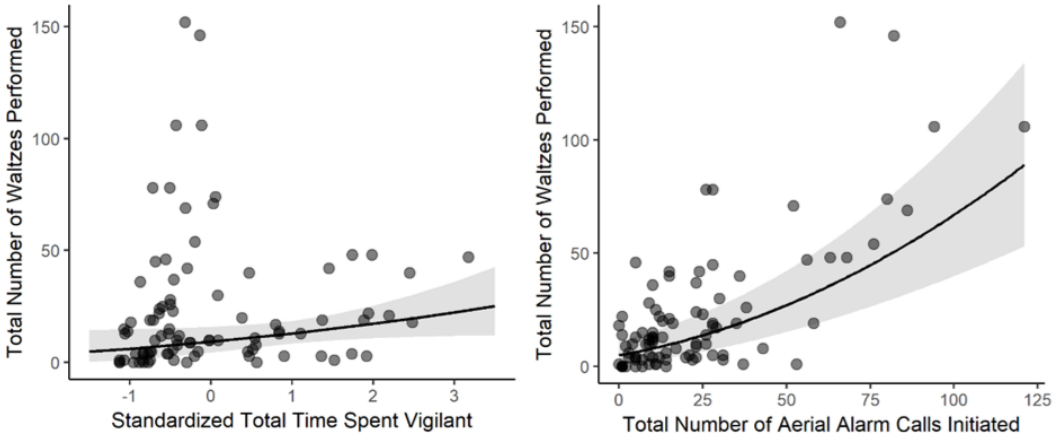


Figure 7. a) Z-score standardized total time spent vigilant and b) the total number of aerial alarm calls initiated against the total number of courtship waltzes performed. The line of best fit is the predicted line generated by the Poisson (square-root link) generalized linear mixed model. The grey area represents 95% confidence intervals.

Table 7. Model output for the Poisson generalized linear mixed model using the square-root link with the total number of courtship waltzes performed as the dependant variable. Year (2016/2017), sex ratio, the z-score standardized total time spent vigilant and the total number of aerial alarm calls initiated ae fixed factors. Male ID and group ID are random effects. The (1|obs_level) random effect was added to combat overdispersion. The model AIC is 678.5. Model BIC is 700.9, loglikelihood is -330.2 and deviance is 660.5.

	Total Number of Waltzes Performed		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	97.70	29.65 – 321.92	**<0.001**
Year 2017	6.41	2.33 – 17.66	**<0.001**
Even Ratio	0.06	0.02 – 0.23	**<0.001**
Male Biased	0.07	0.02 – 0.20	**<0.001**
Standardized Total Time Spent Vigilant	1.75	1.00 – 3.07	**0.050**
Total Number of Aerial Alarm Calls Initiated	1.06	1.04 – 1.07	**<0.001**
Random Effects			
σ^2	1.60		
$\tau_{00\ obs_effect}$	1.35		
$\tau_{00\ ID}$	1.11		
$\tau_{00\ GROUP}$	0.13		
ICC	0.44		
N_{ID}	31		
N_{GROUP}	15		
N_{obs_effect}	90		
Observations	90		
Marginal R^2 / Conditional R^2	0.634 / 0.794		

Harassments

Vigilance and sex ratio significantly interacted to correlate with the number of harassments performed by the focal male (LRT: X^2 (2) =22.62, p<0.001; Figure 8a; Table 8). The number of harassments increased with increasing vigilance in Male Biased groups whereas the opposite pattern was shown in Female Biased groups. Exploring the correlation between vigilance and the total number of harassments performed within each sex ratio separately found that vigilance had a significant correlation with the number of harassments in all three sex ratios (Female Biased LRT: X^2 (1) =6.32, p=0.012; Even Ratio LRT: X^2 (1) =4.84, p=0.028; Male Biased LRT: X^2 (1) =12.61, p<0.001; See Appendix, Supplementary Material A).

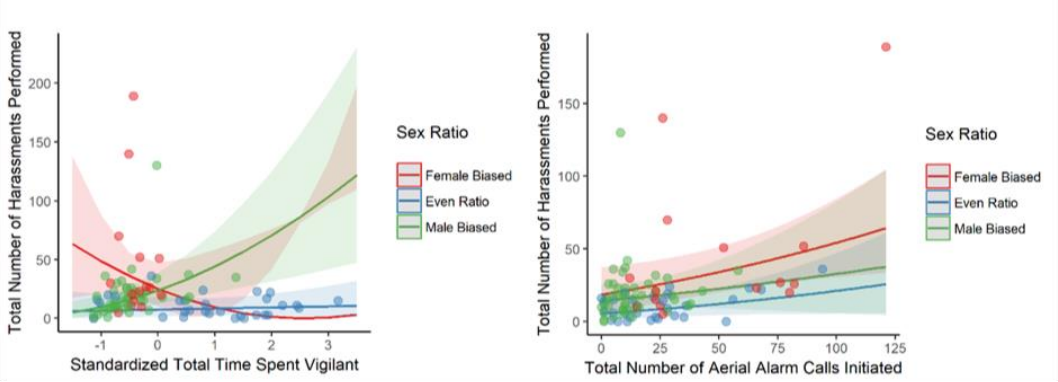


Figure 8. a) Z-score standardized total time spent vigilant and b) the total number of aerial alarm calls initiated against the total number of harassments performed for each sex ratio. The line of best fit is the predicted line generated by the Poisson (square-root link) generalized linear mixed model. The shaded area represents 95% confidence intervals.

Table 8. Model output for the Poisson generalized linear mixed model using the square-root link with the total number of harassments performed as the dependant variable. Sex ratio, the z-score standardized total time spent vigilant and the total number of aerial alarm calls initiated are fixed factors along with two-way interactions between vigilance and sex ratio, and alarm calling and sex ratio. Male ID and group ID are random effects. The (1|obs_level) random effect was added to combat overdispersion. The model AIC is 703.1 Model BIC is 733.1, loglikelihood is -339.6 and deviance is 679.1.

	Total Number of Harassments Performed		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	2.76	0.23 – 33.20	0.424
Even Ratio	4.52	0.27 – 75.08	0.293
Male Biased	57.04	3.61 – 901.71	**0.004**
Total Number of Aerial Alarm Calls Initiated	1.05	1.03 – 1.08	**<0.001**
Standardized Total Time Spent Vigilant	0.00	0.00 – 0.07	**<0.001**
Even Ratio:Total Number of Aerial Alarm Calls Initiated	0.97	0.94 – 1.01	0.103
Male Biased: Total Number of Aerial Alarm Calls Initiated	0.94	0.90 – 0.98	**0.002**
Even Ratio: Standardized Total Time Spent Vigilant	280.68	10.18 – 7738.13	**0.001**
Male Biased: Standardized Total Time Spent Vigilant	2175.71	79.28 – 59707.21	**<0.001**
Random Effects			
σ^2	1.20		
$\tau_{00\ obs_effect}$	0.95		
$\tau_{00\ ID}$	0.84		
$\tau_{00\ GROUP}$	1.54		
ICC	0.67		
N_{ID}	31		
N_{GROUP}	15		
N_{obs_effect}	90		
Observations	90		
Marginal R^2 / Conditional R^2	0.330 / 0.776		

Like vigilance, aerial alarm calling significantly interacted with sex ratio. This interaction significantly correlated with the number of harassments performed (LRT: X^2 (2) =9.09, p=0.011; Figure 8b; Table 8); such that males which called more frequently engaged in more harassments. Once again looking at the correlation between aerial alarm calling with the number of harassments

performed within each sex ratio group independently, we found that alarm calling had a significant correlation with the number of harassments in Even Ratio groups only, with a near significant correlation in Female Biased groups (Female Biased LRT: X^2 (1) =3.13, p=0.077; Even Ratio LRT: X^2 (1) =10.46, p=0.001; Male Biased LRT: X^2 (1) =1.12, p=0.290; See Appendix, Supplementary Material A).

Copulation Interruptions

Interruptions Received

There was a significant three-way interaction between vigilance, alarm calling and sex ratio on the number of interruptions received (LRT: X^2 (2) =7.14, p=0.028; Figure 9; Table 9). The correlation between vigilance and interruptions received was strongest in males that produced fewer calls in Even Ratio and Male Biased groups (Figure 9). In general, more vigilant males incurred more interruptions and initiated more calls. When testing each sex ratio independently vigilance had a significant correlation with the number of interruptions received in Even Ratio groups, with a near significant correlation in Male Biased groups (Female Biased LRT: X^2 (1) =0.94, p=0.333; Even Ratio LRT: X^2 (1) =4.42, p=0.035; Male Biased LRT: X^2 (1) =3.76, p=0.053; See Appendix, Supplementary Material B). Alarm calling had no significant correlation with the number of interruptions received when examining each sex ratio separately (Female Biased LRT: X^2 (1) =0.07, p=0.795; Even Ratio LRT: X^2 (1) =0.93, p=0.336; Male Biased LRT: X^2 (1) =0.02, p=0.892; See Appendix, Supplementary Material B).

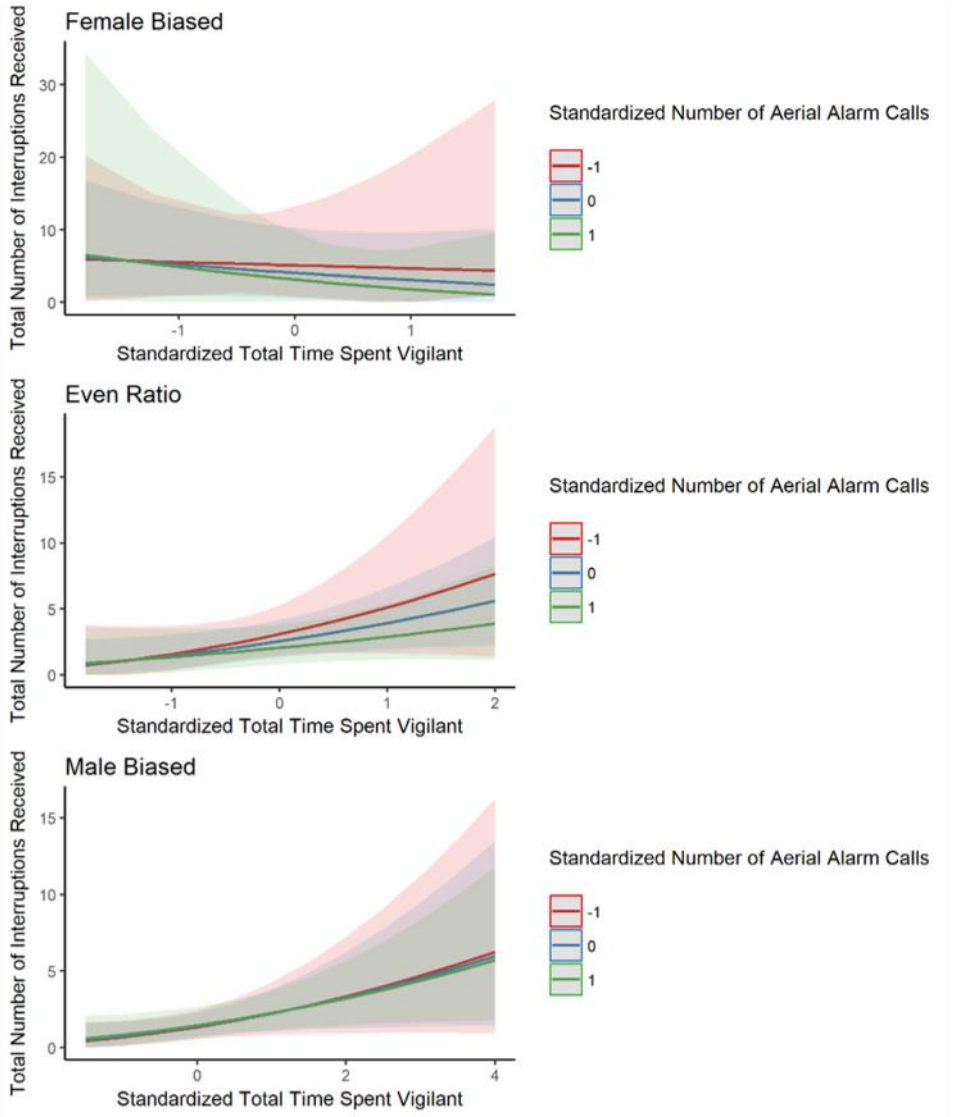


Figure 9. Z-score standardized total time spent vigilant against the number of copulation interruptions received for all sex ratios. The graphs are showing an interaction with the z-score standardized total number of aerial alarm calls initiated with males that initiated -1, 0 and 1 standard deviations of calls below and above the mean. The line of best fit is the predicted line generated by the Poisson (log link) generalized linear mixed model. The shaded area represents 95% confidence intervals.

Table 9. Model output for the Poisson generalized linear mixed model using the log link with the total number of copulation interruptions received as the dependant variable. Year (2016/2017), sex ratio, the z-score standardized total time spent vigilant and the total number of aerial alarm calls initiated are fixed factors along with the three-way interaction between vigilance, alarm calling and sex ratio. All lower order two-way interactions between these three variables are also fixed factors. Male ID and group ID are random effects. The model AIC is 373.5 Model BIC is 413.5, loglikelihood is -170.8 and deviance is 341.5.

	Total Number of Interruptions Received		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	11.04	1.57 – 77.76	**0.016**
Year 2017	2.61	1.75 – 3.90	**<0.001**
Even Ratio	0.10	0.01 – 0.71	**0.021**
Male Biased	0.12	0.02 – 0.84	**0.033**
Total Number of Aerial Alarm Calls Initiated	0.96	0.93 – 0.99	**0.021**
Standardized Total Time Spent Vigilant	73.47	1.70 – 3166.78	**0.025**
Even Ratio:Total Number of Aerial Alarm Calls Initiated	1.03	0.99 – 1.07	0.098
Male Biased: Total Number of Aerial Alarm Calls Initiated	1.03	0.99 – 1.07	0.169
Even Ratio: Standardized Total Time Spent Vigilant	0.04	0.00 – 1.46	0.078
Male Biased: Standardized Total Time Spent Vigilant	0.03	0.00 – 1.47	0.077
Total Number of Aerial Alarm Calls Initiated: Standardized Total Time Spent Vigilant	0.89	0.82 – 0.96	**0.003**
Even Ratio: Total Number of Aerial Alarm Calls Initiated: Standardized Total Time Spent Vigilant	1.11	1.03 – 1.20	**0.008**
Male Biased: Total Number of Aerial Alarm Calls Initiated: Standardized Total Time Spent Vigilant	1.12	1.04 – 1.22	**0.005**
Random Effects			
σ^2	0.47		
$\tau_{00\ ID}$	1.22		
$\tau_{00\ GROUP}$	0.01		
ICC	0.72		
N_{ID}	31		
N_{GROUP}	15		
Observations	90		
Marginal R^2 / Conditional R^2	0.208 / 0.780		

Interruptions Performed

Like the number of interruptions received, there was a significant three-way interaction between vigilance, alarm calling, and sex ratio on the number of interruptions performed (LRT: X^2 (2) =15.32, p<0.001; Figure 10; Table 10). Both Male Biased and Even Ratio groups show a positive correlation between vigilance and the number of interruptions performed; however, within Female Biased groups the number of interruptions performed strongly declined with increasing vigilance, particularly in individuals that initiated the most calls (Figure 10).

When testing the correlation between the total number of aerial alarm calls initiated and the total number of interruptions performed within each sex ratio separately alarm calling had a significant correlation with the number of interruptions performed in Female Biased and Even Ratio groups (Female Biased LRT: X^2 (1) =22.74 p<0.001; Even Ratio LRT: X^2 (1) =21.87, p<0.001; Male Biased LRT: X^2 (1) =1.80, p=0.180; See Appendix, Supplementary Material C). When exploring the correlation between vigilance and the number of interruptions performed within each sex ratio independently vigilance did not have a significant correlation in any sex ratio (Female Biased LRT: X^2 (1) =1.92, p=0.166; Even Ratio LRT: X^2 (1) =1.77, p=0.184; Male Biased LRT: X^2 (1) =1.84, p=0.176; See Appendix, Supplementary Material C).

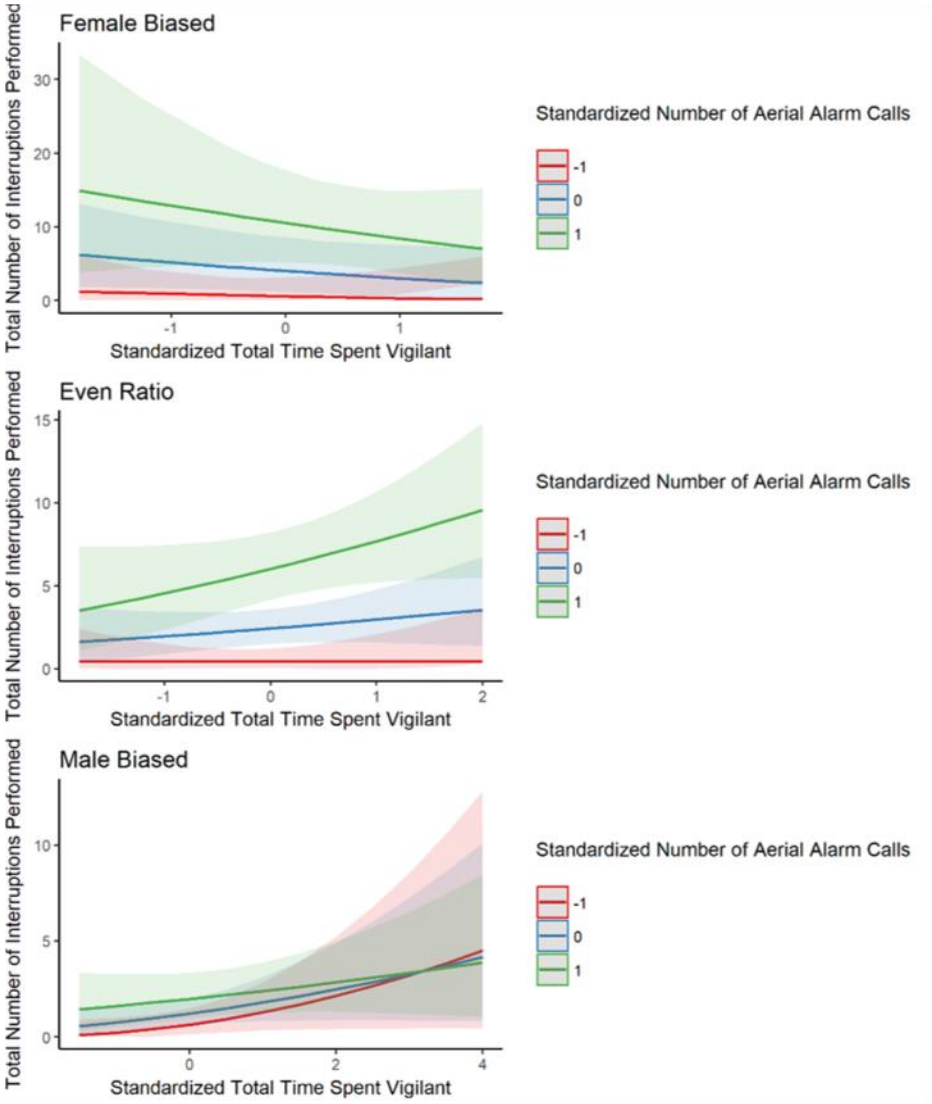


Figure 10. Z-score standardized total time spent vigilant against the number of copulation interruptions performed for all sex ratios. The graphs are showing an interaction with the z-score standardized total number of aerial alarm calls initiated with males that initiated -1, 0 and 1 standard deviations of calls below and above the mean. The line of best fit is the predicted line generated by the Poisson (log link) generalized linear mixed model. The shaded area represents 95% confidence intervals.

Table 10. Model output for the Poisson generalized linear mixed model using the log link with the total number of copulation interruptions performed as the dependant variable. Year (2016/2017), sex ratio, the z-score standardized total time spent vigilant and the total number of aerial alarm calls initiated are fixed factors along with the three-way interaction between vigilance, alarm calling and sex ratio. All lower order two-way interactions between these three variables are also fixed factors. Male ID and group ID are random effects. The model AIC is 364.7. Model BIC is 409.7, loglikelihood is -164.3 and deviance is 328.7.

	Total Number of Interruptions Performed		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	3.16	0.54 – 18.40	0.200
Year 2017	1.60	1.04 – 2.46	**0.031**
Even Ratio	0.29	0.05 – 1.58	0.152
Male Biased	0.57	0.10 – 3.22	0.525
Total Number of Aerial Alarm Calls Initiated	0.60	0.24 – 1.51	0.279
Standardized Total Time Spent Vigilant	10.25	0.41 – 254.40	0.155
Even Ratio:Total Number of Aerial Alarm Calls Initiated	2.48	0.89 – 6.95	0.084
Male Biased: Total Number of Aerial Alarm Calls Initiated	3.09	1.09 – 8.76	**0.033**
Even Ratio: Standardized Total Time Spent Vigilant	0.14	0.01 – 3.54	0.235
Male Biased: Standardized Total Time Spent Vigilant	0.15	0.01 – 4.04	0.260
Total Number of Aerial Alarm Calls Initiated: Standardized Total Time Spent Vigilant	0.03	0.00 – 0.29	**0.002**
Even Ratio: Total Number of Aerial Alarm Calls Initiated: Standardized Total Time Spent Vigilant	32.27	4.07 – 255.71	**0.001**
Male Biased: Total Number of Aerial Alarm Calls Initiated: Standardized Total Time Spent Vigilant	16.30	1.68 – 158.59	**0.016**
Random Effects			
σ^2	0.52		
$\tau_{00\ ID}$	0.84		
ICC	0.62		
N_{ID}	31		
Observations	90		
Marginal R^2 / Conditional R^2	0.335 / 0.745		

DISCUSSION

The key findings from this study include positive correlations between both vigilance and aerial alarm calling on remating rates, along with the number of matings and females obtained (Table 11). Additionally, vigilance was significantly negatively correlated with SCI, indicating that higher vigilance could aid in reducing female promiscuity. Furthermore, both vigilance and alarm calling were positively correlated with the number of solicitations received, suggesting that females may prefer mating with males that invest in anti-predator behaviours (Table 12).

Table 11. Summary of the predictions outlined in the introduction and whether the results support these predictions for the correlates of mating success (part 1). Bold indicates that the prediction was supported.

Predictions	*Results*
Pre-copulatory- if vigilance and alarm calling are good measures of pre-copulatory mating success then they should be positively correlated with the number of matings obtained and the number of females mated.	Both vigilance and alarm calling were significantly positively correlated with number of matings and number of females mated. **Prediction supported**.
Post-copulatory- if vigilance and alarm calling are good measures of post-copulatory mating success then they should be positively correlated with remating rate. Furthermore if these behaviours reduce sperm competition then they should be negatively correlated with SCI.	Both vigilance and alarm calling were significantly correlated with remating rate. **Prediction supported**. However only vigilance was significantly negatively correlated with SCI. **Prediction supported for vigilance only**.

Table 12. Summary of the predictions outlined in the introduction and whether the results support these predictions for behaviours that indirectly influence mating success (part 2). Bold indicates that the prediction was supported.

Predictions	*Results*
Solicitations- if vigilance and alarm calling are attractive traits to females then these behaviours should be positively correlated with the number of solicitations received.	Both vigilance and alarm calling were significantly positively correlated with number of female solicitations received. **Prediction supported**.
Waltzing- males that invest in costly behaviours may invest more into courtship, therefore vigilance/alarm calling should be positively correlated with the number of waltzes performed.	Both vigilance and alarm calling were significantly positively correlated with number of waltzes. **Prediction supported**.
Harassments- if more vigilant/alarm calling males are preferred by females then they should not need to harass females into mating. Therefore vigilance/alarm calling should be negatively correlated with harassment.	Alarm calling was significantly positively correlated with the number of harassments in all sex ratios. Vigilance was positively correlated in Male Biased groups only. Prediction not supported.
Interruptions- if vigilance is used to monitor rivals to increase mating success then vigilance should be negatively correlated with the number of copulation interruptions received.	Typically more vigilant males received more interruptions. Prediction not supported.

Vigilance and Mating Success

Vigilance was a good predictor of mating success, with more vigilant males receiving significantly more matings and mating with more females. Vigilance could be a desirable trait to females, which is supported by vigilance having a significant positive effect on the number of solicitations received. Furthermore, in the grey partridge vigilance is a sexually selected trait, with females preferentially pairing and mating with more vigilant males (Dahlgren, 1990). In this species females benefited from pairing with vigilant males prior to mating by decreasing their vigilance efforts and increasing foraging intake; leading to higher reproductive success. Post-hatching males spent more time vigilant than females, increasing chick survival (Dahlgren, 1990). Notably, however, in fowl males do not contribute to chick raising nor do they pair with females, suggesting alternative reasons for selecting vigilant males (Wilson *et al.*, 2008; Wilson & Evans, 2008). Still it is possible for female fowl to benefit from copulating with more vigilant males by reducing their personal vigilance and increasing time spent foraging. Higher vigilance could furthermore lead to increased number of matings if males were using vigilance to locate potential mating opportunities. In male elk, *Cervus elaphus*, reproductive males were twice as vigilant as non-reproductive males during the breeding season; implying that reproductive male elk use vigilance to guard harem females and monitor rival males (Lung & Childress, 2007). Therefore, it is probable that male fowl also use vigilance to monitor social situations, opportunistically locating females to mate with. More vigilant males could have gained more matings/females through increased harassment. However, increasing vigilance did not lead to more harassments within Female Biased and Even Ratio groups. Competition for mates was strongest in Male Biased groups so

perhaps males in these groups benefitted from using increased vigilance to search for mates, leading to more vigilant males finding unguarded females and attempting to harass them into copulating.

Vigilant males experienced significantly higher average remating rates per female and significantly lower SCI, revealing vigilance to be a good predictor for post-copulatory mating success. The higher remating rates and lower SCI experienced by more vigilant males may be explained by reduced interactions between male competitors and females, as more vigilant males can prevent such competitively undesirable interactions. However, in contrast to our predictions, vigilance had a strong significant positive correlation on the number of interruptions received in Even Ratio and Male Biased groups. Potentially males in these groups used vigilance primarily to locate females rather than avoid rivals. The relationship within Female Biased groups is interesting as we found a negative relationship between vigilance and interruptions performed for males that initiated the most calls. Perhaps the most vigilant males that also alarm called most frequently within this sex ratio group were able to monopolize a subset of females. If these males have their own harem of the most preferred females then they might not be invested in the less desirable females, thus engaging in fewer interruptions when rival males copulate with those females. Within these groups we did observe the most dominant male associating with a greater number of females than the subordinates, with the two subordinate males often foraging away from the dominant male and his subset of females. However, it is important to note that the sample size used (90) was possibly not large enough to support this three-way interaction between vigilance, alarm calling and the number of interruptions received/performed. Furthermore, interpretation of three-way interactions is

tricky, so it is also possible that this interaction has been misinterpreted here. Overall increasing vigilance did not appear to aid in preventing interruptions by other males, but may be used to locate mating opportunities, and to detect and prevent rival copulations, reducing SCI.

If increasing vigilance is an effective strategy for mating success, then more males should adopt this strategy. However, vigilance behaviour is often costly, reducing time that could be spent foraging or resting (Pöysä, 1987; Sansom *et al.*, 2009); so only the fittest males may be able to invest heavily in vigilance. Males that are unable to face the costs associated with high vigilance could use alternative tactics such as sneaky matings or presenting females with resources like food. Conversely the correlation between vigilance and mating success could be due to increased investment post-copulation to increase the odds of maternal survival and consequential offspring survival.

Alarm Calling and Mating Success

Aerial alarm calling was also a good predictor of mating success, as males that initiated more calls significantly obtained more successful matings, had higher remating rates and received more solicitations. Additionally, males that initiated fewer calls had a stronger positive correlation between vigilance and the number of females mated; potentially suggesting that the top callers may be more desirable to females and do not need to rely on vigilance to find mating opportunities. Notably, vigilance has been shown to be heritable, albeit with low heritability estimates, in yellow-bellied marmots, *Marmota flaviventris* (Blumstein *et al.*, 2010). Whether alarm calling or vigilance are heritable traits in fowl is unknown, but if these traits are heritable and sexually selected then females would benefit from siring males possessing those genes. Conversely,

males that initiated more calls may have gained more matings and mated with more females because they were more likely to harass females. Alarm calling had a positive correlation with the number of harassments in all sex ratios, with the strongest correlation in Female Biased groups; which could be a by-product of dominance (fewer males results in stronger dominance hierarchies). However more dominant males did not perform more harassments (See Appendix, Supplementary Material M). The act of mating in birds can cause elevations in testosterone level (Gyger *et al.*, 1988), and aerial alarm calling has been linked to testosterone production in fowl (Gyger *et al.*, 1988; Ligon *et al.*, 1990; Wilson & Evans, 2008). Therefore, a rise in testosterone post-copulation could lead to heightened aggression, causing this rise in harassments.

With regards to post-copulatory mating success; aerial alarm calling had a strong positive effect on remating rates and a non-significant negative correlation with SCI. Alarm calling had significant correlations with both the number of solicitations received and remating rates, so it is possible that females prefer mating with these males; thus, reduce their promiscuity. Alternatively, to gain maximum protection from predators females may stay near better alarm callers, decreasing the likelihood of copulations by competing males. The higher remating rates and lower SCI could also be explained by males that initiated more calls interrupting more copulations, though this was not significant (See Appendix, Supplementary Material N).

Alarm calling in a fowl could be an honest signal since it is a risky behaviour because predators are now informed of the signallers location (Charnov & Krebs, 1975; Makin *et al.*, 2019). Therefore, males that engage in this behaviour more frequently may be advertising their ability to successfully evade an attack.

Alternatively, males may not increase alarm calling efforts because of policing from other males. Crowing in fowl is a vocalisation associated with dominance, with more dominant males crowing more often (Gyger *et al.*, 1987; Leonard & Weatherhead, 1996; Parker & Ligon, 2002). Dominant males can attack subordinate males that crow, reducing crowing efforts. Therefore, it is possible that males that initiate the most calls police this behaviour in other males through bullying, leading to fewer males increasing alarm calling efforts.

Male Attractiveness vs Mate Investment

Although both vigilance and alarm calling are good measures of mating success, these behaviours may have increased post-copulation as a form of mate investment. In fowl, males that were given the opportunity to mate significantly increased aerial alarm calling effort by approximately 30% (Wilson & Evans, 2008). Wilson and Evans (2008) suggested that males could increase alarm calling investment in reproductive partners to increase the chance that they will survive and sire his offspring. Therefore, the increased vigilance and alarm calling behaviours observed in our study could be a result of mate investment rather than these behaviours being preferred by females. Importantly, these two hypotheses are not necessarily mutually exclusive. Females could prefer vigilant/alarm calling males (male attractiveness) while males simultaneously increase efforts post-mating (mate investment). Our study is unable to test whether anti-predator behaviours are acting under the male attraction or the mate investment hypothesis because there is no way to tell post-hoc whether males increased efforts pre-mating or post-mating. However, the aim of this chapter was to explore these behaviours and their relationship with correlates of mating success, not to disentangle between these two hypotheses.

Dominance and Mating Success

Dominance may have indirectly influenced the relationship between vigilance and alarm calling and mating success. However, during model selection, the dominance models were significantly less well suited to the data than alarm calling models. Nonetheless dominance has been linked to vigilance and alarm calling effort in previous studies (Pizzari, 2003; Wilson *et al.*, 2008; Chapter 2); with numerous studies citing the importance of dominance status on mating and reproductive success (Pizzari & Birkhead, 2000; Johnsen *et al.*, 2001; Pizzari, 2001, 2003). Although dominance had no strong relationship with the four correlates of mating success (See Appendix, Supplementary Material O). Since more dominant males have been shown to be more vigilant (Pizzari, 2003; Chapter 2) and produce more aerial calls (Pizzari, 2003; Wilson *et al.*, 2008; Chapter 2) than subordinates, the correlations often attributed to status might actually be caused by anti-predator behaviours.

Unlike dominance, other potential correlates of mating success like comb length and plumage brightness were not recorded. A classic study in red junglefowl by Zuk et al. (1990a) infected males with an intestinal parasite, finding that infected males had smaller combs than control males and entered eclipse plumage sooner; implying these may be costly signals of male quality. A further study in fowl discovered that testosterone implanted males developed larger and brighter combs and were preferred in mate choice trials (Zuk *et al.*, 1995a). High testosterone levels can cause suppressed immune systems and males in the testosterone group had both larger combs and fewer lymphocytes; suggesting ornamentation is a handicap (Zuk *et al.*, 1995a). Consequently, the results found in our study may be caused by an unrecorded measure of mating success like comb length.

Conclusions

To conclude, the main findings indicate vigilance and aerial alarm calling as suitable indicators of pre- and post-copulatory mating success. This has implications for future studies since anti-predator behaviours are often overlooked when exploring mating success. However, our research does not conclusively determine whether vigilance and alarm calling directly affect mating success, nor whether they increase prior to copulation in order to attract females, and/or are sexually selected traits. Future work could encompass cryptic female choice to test whether females are more likely to retain the sperm of more vigilant males and/or better alarm callers. Positive results in such an experiment would indicate they are sexually selected traits. By contrast, if females do not preferentially retain the sperm of these males, but mating still causes an increase in these behaviours, then mate investment is more probable.

REFERENCES

Aho, K., Derryberry, D. & Peterson, T. (2014). Model selection for ecologists: the worldviews of AIC and BIC. *Ecology*, **95**, 631–636.

Anderson, D.R., Burnham, K.P. & White, G.C. (1994). AIC Model Selection in Overdispersed Capture-Recapture Data. *Ecology*, **75**, 1780–1793.

Arnold, S.J. & Wade, M.J. (1984). On the Measurement of Natural and Sexual Selection: Theory. *Evolution*, **38**, 709–719.

Bajer, K., Molnár, O., Török, J. & Herczeg, G. (2010). Female European green lizards (*Lacerta viridis*) prefer males with high ultraviolet throat reflectance. *Behavioural Ecology and Sociobiology*, **64**, 2007–2014.

Bartón, K. (2018). MuMIn: Multi-modal inference. R package version 1.40.4. http://cran.r-project.org/web/packages/MuMIn/index.html.

Bateman, A.J. (1948). Intra-sexual selection in Drosophila. *Heredity*, **2**, 349.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1–48.

Beani, L. & Dessí-Fulgheri, F. (1998). Anti-predator behaviour of captive Grey partridges (*Perdix perdix*). *Ethology Ecology & Evolution*, **10**, 185–196.

Beauchamp, G. (2015). *Animal Vigilance: Monitoring Predators and Competitors*. Academic Press.

Birkhead, T.R. & Hunter, F.M. (1990). Mechanisms of sperm competition. *Trends in Ecology & Evolution*, **5**, 48–52.

Birkhead, T.R. & Pizzari, T. (2009). Biology of Breeding Poultry. Chapter 9 Sperm Competition and Fertilization Success. In *Biology of Breeding Poultry*: 133–150. CABI, 2009.

Blumstein, D.T., Lea, A.J., Olson, L.E. & Martin, J.G.A. (2010). Heritability of anti-predatory traits: vigilance and locomotor performance in marmots. *Journal of Evolutionary Biology*, **23**, 879–887.

Charnov, E.L. & Krebs, J.R. (1975). The Evolution of Alarm Calls: Altruism or Manipulation? *The American Naturalist*, **109**, 107–112.

Clark, T.S. & Linzer, D.A. (2015). Should I Use Fixed or Random Effects? *Political Science Research and Methods*, **3**, 399–408.

Collier, T.C., Blumstein, D.T., Girod, L. & Taylor, C.E. (2010). Is Alarm Calling Risky? Marmots Avoid Calling from Risky Places. *Ethology*, **116**, 1171–1178.

Cowlishaw, G. (1997). Alarm Calling and Implications for Risk Perception in a Desert Baboon Population. *Ethology*, **103**, 384–394.

Dahlgren, J. (1990). Females choose vigilant males: an experiment with the monogamous grey partridge, *Perdix perdix*. *Animal Behaviour,* **39**, 646–651.

Dakin, R. & Montgomerie, R. (2011). Peahens prefer peacocks displaying more eyespots, but rarely. *Animal Behaviour,* **82**, 21–28.

Darwin, C. (1859). *On the Origin of Species by Means of Natural Selection, Or, The Preservation of Favoured Races in the Struggle for Life*. London: John Murray.

Darwin, C. (1871). *The Descent of Man, and Selection in Relation to Sex*. D. Appleton.

David Leiva & Han de Vries. (2014). Steepness: testing steepeness of dominance hierarchies. R package version 0.2.2. Available at: http://CRAN.R-project.org/package=steepness.

Davis, L.S. (1984). Alarm Calling in Richardson's Ground Squirrels (*Spermophilus richardsonii*). *Zeitschrift für Tierpsychologie*, **66**, 152–164.

Doucet, S.M. & Montgomerie, R. (2003a). Multiple sexual ornaments in satin bowerbirds: ultraviolet plumage and bowers signal different aspects of male quality. *Behavioural Ecology,* **14**, 503–509.

Doucet, S.M. & Montgomerie, R. (2003b). Structural plumage colour and parasites in satin bowerbirds *Ptilonorhynchus violaceus*: implications for sexual selection. *Journal of Avian Biology,* **34**, 237–242.

Dudley, L.S. (2006). Ecological correlates of secondary sexual dimorphism in *Salix glauca* (Salicaceae). *American Journal of Botany,* **93**, 1775–1783.

Elgar, M.A. (1989). Predator Vigilance and Group Size in Mammals and Birds: A Critical Review of the Empirical Evidence. *Biological Reviews,* **64**, 13–33.

Evans, C.S., Evans, L. & Marler, P. (1993). On the meaning of alarm calls: functional reference in an avian vocal system. *Animal Behaviour,* **46**, 23–38.

Fox, J. & Weisberg, S. (2011). *An R Companion to Applied Regression*. SAGE.

Fusani, L., Beani, L., Lupo, C. & Dessì-Fulgheri, F. (1997). Sexually selected vigilance behaviour of the grey partridge is affected by plasma androgen levels. *Animal Behaviour,* **54**, 1013–1018.

Gelman, A. & Hill, J. (2006). *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge University Press.

Graves, H.B., Hable, C.P. & Jenkins, T.H. (1985). Sexual selection in gallus: Effects of morphology and dominance on female spatial behavior. *Behavioural Processes,* **11**, 189–197.

Guillemain, M., Caldow, R.W.G., Hodder, K.H. & Goss-Custard, J.D. (2003). Increased vigilance of paired males in sexually dimorphic species: distinguishing between alternative explanations in wintering Eurasian wigeon. *Behavioural Ecology*, **14**, 724–729.

Gyger, M., Karakashian, S.J., Dufty Jr., A.M. & Marler, P. (1988). Alarm signals in birds: The role of testosterone. *Hormones and Behavior*, **22**, 305–314.

Gyger, M., Marler, P. & Pickert, R. (1987). Semantics of an Avian Alarm Call System: The Male Domestic Fowl, *Gallus domesticus*. *Behaviour*, **102**, 15–40.

Harrison, X.A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, **2**, e616.

Harrison, X.A. (2015). A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology & evolution. *PeerJ*, **3**, e1114.

Hartig, F. (2018). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.2.0. http://florianhartig.github.io/DHARMa/.

James, A.M., Burdett, C., Mccool, M.J., Fox, A. & Riggs, P. (2015). The geographic distribution and ecological preferences of the American dog tick, *Dermacentor variabilis* (Say), in the U.S.A. *Medical and Veterinary Entomology*, **29**, 178–188.

Johnsen, T.S., Zuk, M. & Fessler, E.A. (2001). Social Dominance, Male Behaviour and Mating in Mixed-Sex Flocks of Red Junglefowl. *Behaviour*, **138**, 1–18.

Kokko, H. & Morrell, L.J. (2005). Mate guarding, male attractiveness, and paternity under social monogamy. *Behavioural Ecology*, **16**, 724–731.

Kratzer, D.D. & Craig, J.V. (1980). Mating behavior of cockerels: Effects of social status, group size and group density. *Applied Animal Ethology*, **6**, 49–62.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, **82**, 1–26.

Kvarnemo, C., Forsgren, E. & Magnhagen, C. (1995). Effects of sex ratio on intra- and inter-sexual behaviour in sand gobies. *Animal Behaviour*, **50**, 1455–1461.

Leonard, M.L. & Weatherhead, P.J. (1996). Dominance rank and offspring sex ratios in domestic fowl. *Animal Behaviour*, **51**, 725–731.

Ligon, J.D., Kimball, R. & Merola-Zwartjes, M. (1998). Mate choice by female red junglefowl: the issues of multiple ornaments and fluctuating asymmetry. *Animal Behaviour,* **55**, 41–50.

Ligon, J.D., Thornhill, R., Zuk, M. & Johnson, K. (1990). Male-male competition, ornamentation and the role of testosterone in sexual selection in red junglefowl. *Animal Behaviour,* **40**, 367–373.

Ligon, J.D. & Zwartjes, P.W. (1995). Ornate plumage of male red junglefowl does not influence mate choice by females. *Animal Behaviour,* **49**, 117–125.

Loyau, A., Gomez, D., Moureau, B., Théry, M., Hart, N.S., Jalme, M.S., Bennett, A.T.D. & Sorci, G. (2007). Iridescent structurally based coloration of eyespots correlates with mating success in the peacock. *Behavioural Ecology,* **18**, 1123–1131.

Loyau, A., Jalme, M.S., Cagniant, C. & Sorci, G. (2005a). Multiple sexual advertisements honestly reflect health status in peacocks (*Pavo cristatus*). *Behavioural Ecology and Sociobiology,* **58**, 552–557.

Loyau, A., Jalme, M.S. & Sorci, G. (2005b). Intra- and Intersexual Selection for Multiple Traits in the Peacock (*Pavo cristatus*). *Ethology,* **111**, 810–820.

Loyau, A., Petrie, M., Jalme, M.S. & Sorci, G. (2008). Do peahens not prefer peacocks with more elaborate trains? *Animal Behaviour,* **76**, e5–e9.

Lüdecke, D., Bartel, A. & Schwemmer, C. (2019). *sjPlot: Data Visualization for Statistics in Social Science*. R package version 2.6.3, https://CRAN.R-project.org/package=sjPlot.

Lung, M.A. & Childress, M.J. (2007). The influence of conspecifics and predation risk on the vigilance of elk (*Cervus elaphus*) in Yellowstone National Park. *Behavioural Ecology,* **18**, 12–20.

Makin, D.F., Chamaillé-Jammes, S. & Shrader, A.M. (2019). Alarm calls or predator calls: which elicit stronger responses in ungulate communities living with and without lions? *Oecologia,* **190**, 25–35.

Marconato, A. & Shapiro, D.Y. (1996). Sperm allocation, sperm production and fertilization rates in the bucktooth parrotfish. *Animal Behaviour,* **52**, 971–980.

McDonald, G.C. & Pizzari, T. (2016). Why patterns of assortative mating are key to study sexual selection and how to measure them. *Behavioural Ecology and Sociobiology,* **70**, 209–220.

McDonald, G.C., Spurgin, L.G., Fairfield, E.A., Richardson, D.S. & Pizzari, T. (2017). Pre- and postcopulatory sexual selection favor aggressive, young males in polyandrous groups of red junglefowl. *Evolution,* **71**, 1653–1669.

Møller, A.P. & Birkhead, T.R. (1993). Certainty of paternity covaries with paternal care in birds. *Behavioural Ecology and Sociobiology*, **33**, 261–268.

Møller, A.P. & Petrie, M. (2002). Condition dependence, multiple sexual signals, and immunocompetence in peacocks. *Behavioural Ecology*, **13**, 248–253.

Morris, M.R., Batra, P. & Ryan, M.J. (1992). Male-Male Competition and Access to Females in the Swordtail *Xiphophorus nigrensis*. *Copeia*, **1992**, 980–986.

Nicholls, E.H., Burke, T. & Birkhead, T.R. (2001). Ejaculate allocation by male sand martins, *Riparia riparia*. *Proceedings of the Royal Society of London B: Biological Sciences*, **268**, 1265–1270.

Osorio-Beristain, M. & Drummond, H. (1998). Non-aggressive mate guarding by the blue-footed booby: a balance of female and male control. *Behavioural Ecology and Sociobiology*, **43**, 307–315.

Parker, G.A. (1970). Sperm Competition and Its Evolutionary Consequences in the Insects. *Biological Reviews*, **45**, 525–567.

Parker, T.H. & Ligon, D.J. (2002). Dominant male red junglefowl (*Gallus gallus*) test the dominance status of other males. *Behavioural Ecology and Sociobiology*, **53**, 20–24.

Petrie, M. (1994). Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature*, **371**, 598–599.

Petrie, M., Tim, H. & Carolyn, S. (1991). Peahens prefer peacocks with elaborate trains. *Animal Behaviour*, **41**, 323–331.

Petrie M. & Williams A. (1993). Peahens lay more eggs for peacocks with larger trains. *Proceedings of the Royal Society of London B: Biological Sciences*, **251**, 127–131.

Pizzari, T. (2001). Indirect partner choice through manipulation of male behaviour by female fowl, *Gallus gallus domesticus*. *Proceedings of the Royal Society of London B: Biological Sciences*, **268**, 181–186.

Pizzari, T. (2003). Food, vigilance, and sperm: the role of male direct benefits in the evolution of female preference in a polygamous bird. *Behavioural Ecology*, **14**, 593–601.

Pizzari, T. & Birkhead, T.R. (2000). Female feral fowl eject sperm of subdominant males. *Nature*, **405**, 787–789.

Pizzari, T., Cornwallis, C.K., Løvlie, H., Jakobsson, S. & Birkhead, T.R. (2003). Sophisticated sperm allocation in male fowl. *Nature*, **426**, 70–74.

Pöysä, H. (1987). Feeding-Vigilance Trade-Off in the Teal (*Anas crecca*): Effects of Feeding Method and Predation Risk. *Behaviour*, **103**, 108–122.

Price, C.S.C., Dyer, K.A. & Coyne, J.A. (1999). Sperm competition between Drosophila males involves both displacement and incapacitation. *Nature*, **400**, 449.

Prior, K.M., Adams, D.C., Klepzig, K.D. & Hulcr, J. (2018). When does invasive species removal lead to ecological recovery? Implications for management success. *Biological Invasions*, **20**, 267–283.

Pryke, S.R., Andersson, S., Lawes, M.J. & Piper, S.E. (2002). Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color. *Behavioural Ecology*, **13**, 622–631.

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Rodríguez-Muñoz, R., Bretman, A. & Tregenza, T. (2011). Guarding Males Protect Females from Predation in a Wild Insect. *Current Biology*, **21**, 1716–1719.

Rosher, C., Favati, A., Dean, R. & Løvlie, H. (2017). Relatedness and age reduce aggressive male interactions over mating in domestic fowl. *Behavioural Ecology*, **28**, 760–766.

Sansom, A., Lind, J. & Cresswell, W. (2009). Individual behavior and survival: the roles of predator avoidance, foraging success, and vigilance. *Behavioural Ecology*, **20**, 1168–1174.

Setchell, J.M., Charpentier, M. & Wickings, E.J. (2005). Mate guarding and paternity in mandrills: factors influencing alpha male monopoly. *Animal Behaviour*, **70**, 1105–1120.

Sheldon, B.C., Räsänen, K. & Dias, P.C. (1997). Certainty of paternity and paternal effort in the collared flycatcher. *Behavioural Ecology*, **8**, 421–428.

Shine, R., Olsson, M.M., Moore, I.T., Lemaster, M.P., Greene, M. & Mason, R.T. (2000). Body size enhances mating success in male garter snakes. *Animal Behaviour*, **59**, F4–F11.

Shutler, D. & Weatherhead, P.J. (1990). Targets of Sexual Selection: Song and Plumage of Wood Warblers. *Evolution*, **44**, 1967–1977.

Sullivan, M.S. (1994). Mate choice as an information gathering process under time constraint: implications for behaviour and signal design. *Animal Behaviour*, **47**, 141–151.

Sullivan, S.M.P., Watzin, M.C. & Hession, W.C. (2006). Differences in the Reproductive Ecology of Belted Kingfishers (*Ceryle alcyon*) Across Streams with Varying Geomorphology and Habitat Quality. *Waterbirds*, **29**, 258–270.

Takahashi, M., Arita, H., Hiraiwa-Hasegawa, M. & Hasegawa, T. (2008). Peahens do not prefer peacocks with more elaborate trains. *Animal Behaviour*, **75**, 1209–1219.

Thomas, R. & Lello, J. (2017). *Data Analysis with R Statistical Software: A Guidebook for Scientists*. Eco-explore.

Vincent, A., Ahnesjö, I., Berglund, A. & Rosenqvist, G. (1992). Pipefishes and seahorses: Are they all sex role reversed? *Trends in Ecology & Evolution*, **7**, 237–241.

Vincent, A.C.J. (1994). Seahorses Exhibit Conventional Sex Roles in Mating Competition, despite Male Pregnancy. *Behaviour*, **128**, 135–151.

Ward, E.J. (2008). A review and comparison of four commonly used Bayesian and maximum likelihood model selection tools. *Ecological Modelling*, **211**, 1–10.

Whittingham, L.A., Dunn, P.O. & Robertson, R.J. (1993). Confidence of paternity and male parental care: an experimental study in tree swallows. *Animal Behaviour*, **46**, 139–147.

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*, Use R! New York: Springer-Verlag.

Wilson, D.R., Bayly, K.L., Nelson, X.J., Gillings, M. & Evans, C.S. (2008). Alarm calling best predicts mating and reproductive success in ornamented male fowl, *Gallus gallus*. *Animal Behaviour*, **76**, 543–554.

Wilson, D.R. & Evans, C.S. (2008). Mating success increases alarm-calling effort in male fowl, *Gallus gallus*. *Animal Behaviour*, **76**, 2029–2035.

Wilson, D.R. & Evans, C.S. (2010). Female fowl (*Gallus gallus*) do not prefer alarm-calling males. *Behaviour*, **147**, 525–552.

Wilson, D.R., McDonald, P.G. & Evans, C.S. (2010). Mechanisms of Mate Investment in the Polygamous Fowl, *Gallus gallus*. *Ethology*, **116**, 755–762.

Zuk, M. & Johnsen, T.S. (2000). Social environment and immunity in male red junglefowl. *Behavioural Ecology*, **11**, 146–153.

Zuk, M., Johnsen, T.S. & Maclarty, T. (1995a). Endocrine-Immune Interactions, Ornaments and Mate Choice in Red Junglefowl. *Proceedings of the Royal Society of London B: Biological Sciences*, **260**, 205–210.

Zuk, M., Ligon, J.D. & Thornhill, R. (1992). Effects of experimental manipulation of male secondary sex characters on female mate preference in red junglefowl. *Animal Behaviour*, **44**, 999–1006.

Zuk, M., Popma, S.L. & Johnsen, T.S. (1995b). Male Courtship Displays, Ornaments and Female Mate Choice in Captive Red Junglefowl. *Behaviour,* **132**, 821–836.

Zuk, M., Thornhill, R., Ligon, J.D. & Johnson, K. (1990a). Parasites and mate choice in red junglefowl. *American Zoologist,* **30**, 235–244.

Zuk, M., Thornhill, R., Ligon, J.D., Johnson, K., Austad, S., Ligon, S.H., Thornhill, N.W. & Costin, C. (1990b). The Role of Male Ornaments and Courtship Behavior in Female Mate Choice of Red Junglefowl. *The American Naturalist,* **136**, 459–473.

APPENDIX

	Female Biased Total Number of Harassments			Even Ratio Total Number of Harassments			Male Biased Total Number of Harassments		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	64.07	26.99 – 152.09	**<0.001**	2.68	0.67 – 10.61	0.161	20.82	8.59 – 50.46	**<0.001**
Total Number of Aerial Alarm Calls Initiated	1.01	1.00 – 1.02	**0.046**	1.04	1.02 – 1.05	**<0.001**	0.98	0.94 – 1.02	0.284
Total Time Spent Vigilant (seconds)	1.00	0.99 – 1.00	**0.005**	1.00	1.00 – 1.00	**0.013**	1.00	1.00 – 1.01	**<0.001**
Age 2011				9.10	0.99 – 83.42	0.051			
Age 2012				15.63	3.16 – 77.21	**0.001**			
Age 2013				9.13	2.15 – 38.72	**0.003**			
Random Effects									
σ^2	0.03			0.54			1.29		
τ_{00}	0.36_{ID}			0.29_{obs_effect}			1.04_{obs_effect}		
	0.38_{GROUP}			0.43_{ID}			0.88_{ID}		
				0.09_{GROUP}			0.17_{GROUP}		
ICC	0.96			0.49			0.45		
N	13_{ID}			26_{ID}			27_{ID}		
	5_{GROUP}			5_{GROUP}			5_{GROUP}		
				30_{obs_effect}			45_{obs_effect}		
Observations	15			30			45		
Marginal R^2 / Conditional R^2	0.187 / 0.964			0.509 / 0.750			0.253 / 0.587		

Supplementary Material A. Model outputs for Poisson generalized linear mixed models exploring the relationship between vigilance and alarm calling with the total number of harassments performed dependant variable for Female Biased, Even Ratio and Male Biased groups separately. Male ID and group ID were random effects in all three models along with the total time spent vigilant (seconds) and the total number of aerial alarm calls initiated as fixed effects. The (1|obs_level) random effect was added to combat overdispersion in the Even Ratio and Male Biased models. The Male Biased models used the square-root link, whereas the other sex ratios used log-linked models. The model AIC is 145.1, 200.9 and 352.5 for the Female Biased, Even Ratio and Male Biased models respectively. The Female Biased model BIC is 148.7, loglikelihood is -67.6 and deviance is 135.1. The Even Ratio model BIC is 213.5, loglikelihood is -91.5 and deviance is 182.9. The Male Biased model BIC is 365.1, loglikelihood is -169.2 and deviance is 338.5.

	Female Biased Interruptions Received			Even Ratio Interruptions Received			Male Biased Interruptions Received		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	7.97	1.97 – 32.26	**0.004**	3.02	1.41 – 6.46	**0.004**	3.14	2.07 – 4.77	**<0.001**
Total Number of Aerial Alarm Calls Initiated	1.00	0.99 – 1.01	0.601	0.99	0.98 – 1.01	0.335	1.00	0.98 – 1.02	0.892
Total Time Spent Vigilant (seconds)	1.00	0.99 – 1.00	0.144	1.00	1.00 – 1.00	**0.034**	1.34	1.00 – 1.78	**0.046**
Random Effects									
σ^2	0.29			0.25			0.30		
τ_{00}	0.78_{ID}			0.97_{ID}			0.05_{obs_effect}		
	0.78_{GROUP}						0.17_{ID}		
							0.06_{GROUP}		
ICC	0.84			0.80			0.43		
N	13_{ID}			26_{ID}			27_{ID}		
	5_{GROUP}						5_{GROUP}		
							45_{obs_effect}		
Observations	15			30			45		
Marginal R^2 / Conditional R^2	0.059 / 0.852			0.081 / 0.812			0.147 / 0.516		

Supplementary Material B. Poisson generalized linear mixed model outputs for the total number of copulation interruptions received dependant variable for Female Biased, Even Ratio and Male Biased groups separately. The Even Ratio and Male Biased models used the square-root link, but the Female Biased model used the log-link. The total time spent vigilant (seconds) and the total number of aerial alarm calls initiated were fixed effects with male ID as a random effect in all three models. Group ID was removed as a random effect from the Even Ratio model because it had a variance of zero. The (1|obs_level) random effect was added to combat overdispersion in the Male Biased model. The model AIC is 93.1, 151.8 and 170.8 for the Female Biased, Even Ratio and Male Biased models respectively. The Female Biased model BIC is 96.6, loglikelihood is -41.5 and deviance is 83.1. The Even Ratio model BIC is 160.2, loglikelihood is -69.9 and deviance is 139.8. The Male Biased model BIC is 181.7, loglikelihood is -79.4 and deviance is 158.8.

	Female Biased Interruptions Performed			Even Ratio Interruptions Performed			Male Biased Interruptions Performed		
Coefficient	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	2.55	0.68 – 9.56	0.164	1.30	0.69 – 2.47	0.419	1.86	1.18 – 2.93	**0.007**
Total Number of Aerial Alarm Calls Initiated	1.04	1.03 – 1.05	**<0.001**	1.04	1.03 – 1.05	**<0.001**	1.01	0.99 – 1.04	0.168
Total Time Spent Vigilant (seconds)	1.00	0.99 – 1.00	0.163	1.00	1.00 – 1.00	0.167	1.00	1.00 – 1.00	0.188
Random Effects									
σ^2	0.25			0.29			0.31		
τ_{00}	0.87_{GROUP}			0.04_{obs_effect}			0.06_{obs_effect}		
				0.33_{ID}			0.36_{ID}		
				0.00_{GROUP}					
ICC	0.78			0.53			0.53		
N	5_{GROUP}			26_{ID}			27_{ID}		
				5_{GROUP}			45_{obs_effect}		
				30_{obs_effect}					
Observations	15			30			45		
Marginal R^2 / Conditional R^2	0.526 / 0.895			0.596 / 0.812			0.133 / 0.596		

Supplementary Material C. Poisson (square-root link) generalized linear mixed model outputs for the total number of copulation interruptions performed dependant variable for Female Biased, Even Ratio and Male Biased groups separately. The total time spent vigilant (seconds) and the total number of aerial alarm calls initiated were fixed effects in all three models. The Even Ratio model used both male ID and group ID as random effects. However male ID was removed from the Female Biased model, and group ID was removed from the Male Biased model because they both had a variance of zero and were not needed. The (1|obs_level) random effect was added to combat overdispersion in the Even Ratio and Male Biased models. The model AIC is 70.0, 139.1 and 174.9 for the Female Biased, Even Ratio and Male Biased models respectively. The Female Biased model BIC is 73.5, loglikelihood is -30.0 and deviance is 60.0. The Even Ratio model BIC is 147.5, loglikelihood is -63.5 and deviance is 127.1. The Male Biased model BIC is 185.8, loglikelihood is -81.5 and deviance is 162.9.

	Standardized Total Number of Matings		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	0.81	0.71 – 0.91	**<0.001**
Standardized Total Time Spent Vigilant	0.20	0.12 – 0.27	**<0.001**
Standardized Total Number of Aerial Alarm Calls Initiated	0.09	0.02 – 0.15	**0.007**
Standardized Total Time Spent Vigilant:Standardized Total Number of Aerial Alarm Calls Initiated	-0.04	-0.08 – -0.00	**0.032**
Random Effects			
σ^2	0.05		
$\tau_{00\ ID}$	0.03		
$\tau_{00\ GROUP}$	0.01		
ICC	0.43		
N_{ID}	31		
N_{GROUP}	15		
Observations	90		
Marginal R^2 / Conditional R^2	0.359 / 0.634		

Supplementary Material D. Linear mixed model output for the adjusted log transformed z -score standardized total number of successful matings dependant variable. The z-score standardized total time spent vigilant and z-score standardized total number of aerial alarm calls initiated were fixed effects with a two-way interaction between the two variables. Male ID and group ID are random effects. All z-score standardized variables were standardized within each sex ratio to control for sex ratio effects (see the Methods for details). The model AIC is 40.1, BIC is 57.6, loglikelihood is -13.0 and deviance is 26.1.

	Standardized Total Number of Females Mated		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	0.03	-0.22 – 0.29	0.805
Standardized Total Time Spent Vigilant	0.55	0.35 – 0.74	**<0.001**
Standardized Total Number of Aerial Alarm Calls Initiated	0.18	0.02 – 0.35	**0.032**
Standardized Total Time Spent Vigilant:Standardized Total Number of Aerial Alarm Calls Initiated	-0.15	-0.25 – -0.05	**0.004**
Random Effects			
σ^2	0.36		
$\tau_{00\ ID}$	0.19		
$\tau_{00\ GROUP}$	0.08		
ICC	0.42		
N_{ID}	31		
N_{GROUP}	15		
Observations	90		
Marginal R^2 / Conditional R^2	0.360 / 0.630		

Supplementary Material E. Linear mixed model output for the z-score standardized total number of females mated with dependant variable. The z-score standardized total time spent vigilant and z-score standardized total number of aerial alarm calls initiated were fixed effects with a two-way interaction between the two variables. Male ID and group ID are random effects. All z-score standardized variables were standardized within each sex ratio to control for sex ratio effects (see the Methods for details). The model AIC is 211.4, BIC is 228.9, loglikelihood is -98.7 and deviance is 197.4.

	Standardized Average Remating Rate Per Female		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	0.40	-0.00 – 0.81	0.051
Age 2011	0.44	-0.07 – 0.94	0.090
Age 2012	0.19	-0.24 – 0.62	0.383
Age 2013	0.33	-0.07 – 0.73	0.110
Female Biased	-0.03	-0.31 – 0.26	0.858
Male Biased	-0.08	-0.34 – 0.17	0.526
Standardized Total Time Spent Vigilant	0.11	-0.00 – 0.22	0.059
Standardized Total Number of Aerial Alarm Calls Initiated	0.12	0.02 – 0.21	**0.018**
Random Effects			
σ^2	0.12		
$\tau_{00\ ID}$	0.02		
$\tau_{00\ GROUP}$	0.02		
ICC	0.21		
N_{ID}	27		
N_{GROUP}	15		
Observations	64		
Marginal R^2 / Conditional R^2	0.261 / 0.419		

Supplementary Material F. Linear mixed model output for the adjusted log transformed z-score standardized average remating rate per female dependant variable. Age, sex ratio, the z-score standardized total time spent vigilant and z-score standardized total number of aerial alarm calls initiated were fixed effects. Male ID and group ID are random effects. All z-score standardized variables were standardized within each sex ratio to control for sex ratio effects (see the Methods for details). The model AIC is 77.6, BIC is 112.2, log-likelihood is -22.8 and deviance is 45.6.

	Standardized SCI		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	-0.06	-0.52 – 0.40	0.801
Standardized Total Time Spent Vigilant	-0.28	-0.54 – -0.02	**0.032**
Standardized Total Number of Aerial Alarm Calls Initiated	-0.09	-0.28 – 0.10	0.357
Random Effects			
σ^2	0.33		
$\tau_{00\ ID}$	0.23		
$\tau_{00\ GROUP}$	0.59		
ICC	0.71		
N_{ID}	27		
N_{GROUP}	15		
Observations	64		
Marginal R^2 / Conditional R^2	0.081 / 0.738		

Supplementary Material G. Linear mixed model output for the z-score standardized SCI dependant variable. The z-score standardized total time spent vigilant and z-score standardized total number of aerial alarm calls initiated were fixed effects. Male ID and group ID are random effects. All z-score standardized variables were standardized within each sex ratio to control for sex ratio effects (see the Methods for details). The model AIC is 171.8, BIC is 184.7, loglikelihood is -79.9 and deviance is 159.8.

	Standardized Total Number of Solicitations			
Fixed Effects	Estimate	Standard Error	t Value	p Value
Intercept	0.52669	0.07077	7.443	**<0.001*** **
Standardized Total Time Spent Vigilant	0.08076	0.04356	1.854	0.064
Standardized Total Number of Aerial Alarm Calls Initiated	0.09619	0.03069	3.134	**0.002****
Log Likelihood		0.056		
Akaike Inf. Crit.		11.888		
Bayesian Inf. Crit.		26.752		
Random Effects	Variance	Standard Deviation	Number	
ID	0.02675	0.1635	30	
GROUP	0.01271	0.1127	15	
Residual	0.30468	0.5520		

Supplementary Material H. Gamma (identity) generalized linear mixed model output for the adjusted log transformed z-score standardized total number of female solicitations received as the dependant variable. The z-score standardized total time spent vigilant and z-score standardized total number of aerial alarm calls initiated were fixed effects. Male ID and group ID are random effects. All z-score standardized variables were standardized within each sex ratio to control for sex ratio effects (see the Methods for details). The model AIC is 11.9, BIC is 26.8, loglikelihood is 0.1 and deviance is -0.1.

	Standardized Total Number of Courtship Waltzes		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	0.66	0.57 – 0.75	**<0.001**
Year 2017	0.36	0.20 – 0.51	**<0.001**
Standardized Total Time Spent Vigilant	0.07	0.00 – 0.14	**0.043**
Standardized Total Number of Aerial Alarm Calls Initiated	0.15	0.08 – 0.21	**<0.001**
Random Effects			
σ^2	0.06		
$\tau_{00\ ID}$	0.03		
$\tau_{00\ GROUP}$	0.00		
ICC	0.34		
N_{ID}	31		
N_{GROUP}	15		
Observations	90		
Marginal R^2 / Conditional R^2	0.371 / 0.586		

Supplementary Material I. Linear mixed model output for the adjusted log transformed z-score standardized total number of courtship waltzes performed dependant variable. Year, the z-score standardized total time spent vigilant and z-score standardized total number of aerial alarm calls initiated were fixed effects. Male ID and group ID are random effects. All z-score standardized variables were standardized within each sex ratio to control for sex ratio effects (see the Methods for details). The model AIC is 36.0, BIC is 53.5, loglikelihood is -11.0 and deviance is 22.0.

	Standardized Total Number of Harassments		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	0.70	0.49 – 0.91	**<0.001**
Female Biased	-0.03	-0.33 – 0.27	0.849
Male Biased	0.03	-0.25 – 0.30	0.846
Standardized Total Time Spent Vigilant	-0.09	-0.27 – 0.08	0.284
Standardized Total Number of Aerial Alarm Calls Initiated	0.20	0.09 – 0.31	**<0.001**
Female Biased:Standardized Total Time Spent Vigilant	-0.08	-0.33 – 0.16	0.497
Male Biased:Standardized Total Time Spent Vigilant	0.23	0.04 – 0.43	**0.017**
Female Biased:Standardized Total Number of Aerial Alarm Calls Initiated	0.02	-0.18 – 0.22	0.862
Male Biased:Standardized Total Number of Aerial Alarm Calls Initiated	-0.22	-0.36 – -0.08	**0.002**
Random Effects			
σ^2	0.06		
$\tau_{00\ ID}$	0.03		
$\tau_{00\ GROUP}$	0.04		
ICC	0.57		
N_{ID}	31		
N_{GROUP}	15		
Observations	90		
Marginal R^2 / Conditional R^2	0.171 / 0.640		

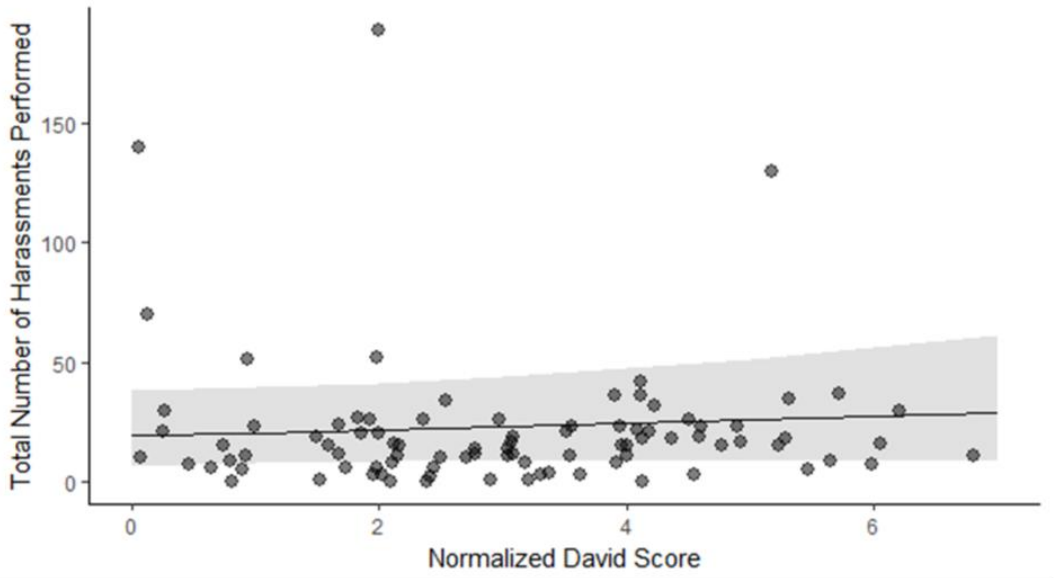
Supplementary Material J. Linear mixed model output for the adjusted log transformed z-score standardized total number of harassments performed dependant variable. Sex ratio, the z-score standardized total time spent vigilant and z-score standardized total number of aerial alarm calls initiated were fixed effects along with the two-way interaction between sex ratio and vigilance, and sex ratio and alarm calling. Male ID and group ID are random effects. All z-score standardized variables were standardized within each sex ratio to control for sex ratio effects (see the Methods for details). The model AIC is 62.9, BIC is 92.9, loglikelihood is -19.4 and deviance is 38.9.

	Standardized Total Number of Interruptions Received		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	0.42	0.26 – 0.57	**<0.001**
2017	0.30	0.05 – 0.55	**0.017**
Standardized Total Time Spent Vigilant	0.17	0.07 – 0.26	**0.001**
Standardized Total Number of Aerial Alarm Calls Initiated	-0.06	-0.15 – 0.03	0.176
Random Effects			
σ^2	0.09		
$\tau_{00\ ID}$	0.10		
$\tau_{00\ GROUP}$	0.01		
ICC	0.56		
N_{ID}	31		
N_{GROUP}	15		
Observations	90		
Marginal R^2 / Conditional R^2	0.097 / 0.602		

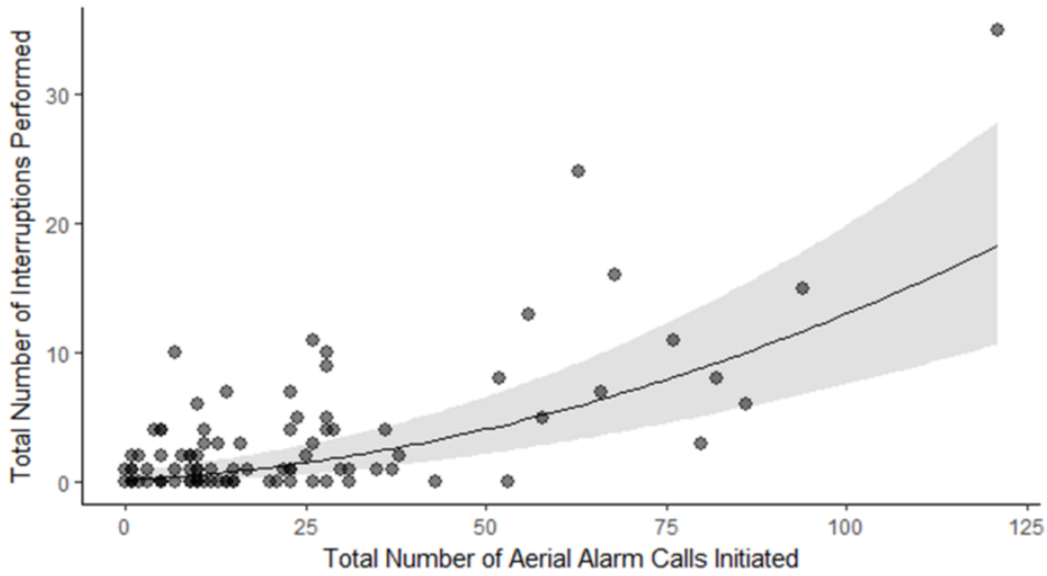
Supplementary Material K. Linear mixed model output for the adjusted log transformed z-score standardized total number of interruptions received dependant variable. Year, the z-score standardized total time spent vigilant and z-score standardized total number of aerial alarm calls initiated were fixed effects. Male ID and group ID are random effects. All z-score standardized variables were standardized within each sex ratio to control for sex ratio effects (see the Methods for details). The model AIC is 94.2, BIC is 111.8, loglikelihood is -40.1 and deviance is 80.2.

	Standardized Total Number of Interruptions Performed		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	0.37	0.22 – 0.52	**<0.001**
Year 2017	0.22	0.01 – 0.43	**0.044**
Female Biased	-0.05	-0.26 – 0.15	0.618
Male Biased	-0.02	-0.18 – 0.13	0.792
Standardized Total Time Spent Vigilant	0.15	0.00 – 0.30	**0.048**
Standardized Total Number of Aerial Alarm Calls Initiated	0.22	0.10 – 0.35	**<0.001**
Female Biased: Standardized Total Time Spent Vigilant	-0.32	-0.57 – -0.07	**0.013**
Male Biased: Standardized Total Time Spent Vigilant	0.01	-0.17 – 0.19	0.910
Female Biased: Standardized Total Number of Aerial Alarm Calls Initiated	0.14	-0.09 – 0.38	0.221
Male Biased: Standardized Total Number of Aerial Alarm Calls Initiated	-0.16	-0.31 – 0.00	0.055
Random Effects			
σ^2	0.09		
$\tau_{00\ ID}$	0.04		
$\tau_{00\ GROUP}$	0.00		
ICC	0.35		
N_{ID}	31		
N_{GROUP}	15		
Observations	90		
Marginal R^2 / Conditional R^2	0.335 / 0.569		

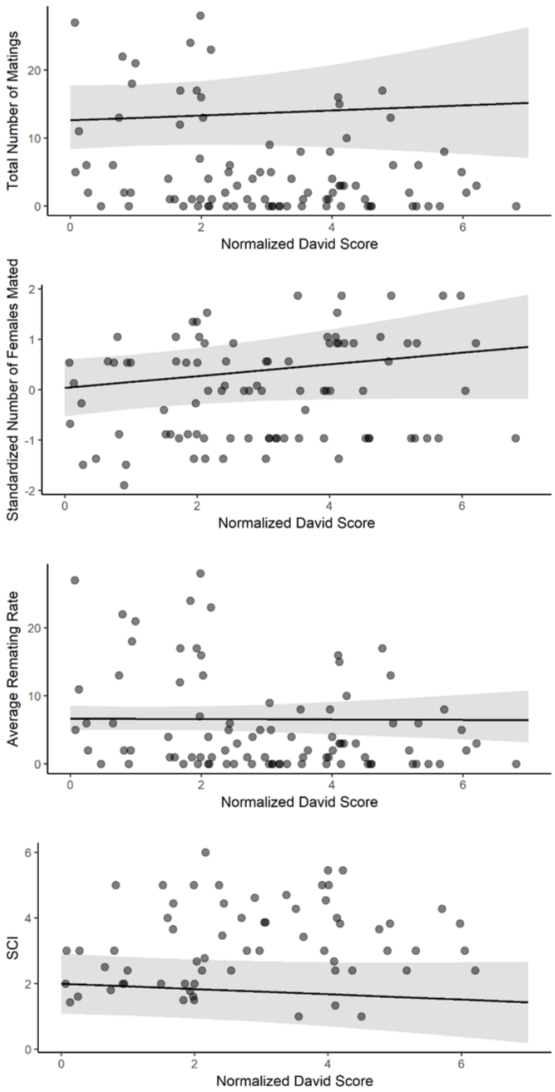
Supplementary Material L. Linear mixed model output for the adjusted log transformed z-score standardized total number of interruptions performed dependant variable. Year, sex ratio, the z-score standardized total time spent vigilant and z-score standardized total number of aerial alarm calls initiated were fixed effects along with the two-way interactions between sex ratio and vigilance, and sex ratio and alarm calling. Male ID and group ID are random effects. All z-score standardized variables were standardized within each sex ratio to control for sex ratio effects (see the Methods for details). The model AIC is 79.0, BIC is 111.5, loglikelihood is -26.5 and deviance is 53.0.



Supplementary Material M. Normalized David score against the total number of harassments performed. The line of best fit is the predicted line from the Poisson model using a square-root link. Year, sex ratio, normalized David score, z-score standardized total time spent vigilant and the two-way interaction between vigilance and sex ratio are fixed effects. Male ID and group ID are random effects with the observation-level added as a random effect to control for overdispersion. Normalized David score had no significant effect on the number of harassments performed (LRT: X^2 (1) =0.20, p=0.655). The grey shaded area represents confidence intervals.



Supplementary Material N. The total number of aerial alarm calls initiated against the total number of copulation interruptions performed. The line of best fit is the predicted line from the log-linked Poisson model. The grey shaded area represents confidence intervals.



Supplementary Material O. Normalized David score against a) the total number of matings, b) the z-score standardized total number of females mated with, c) the average remating rate per female and d) SCI score. The line of best fit is the predicted line from the a) square-root linked Poisson model, b) linear mixed model, c) log-transformed linear mixed model, and d) linear mixed model. The grey shaded area represents confidence intervals. All four models contained year, age, sex ratio, normalized David score and z-score standardized total time spent vigilant as fixed effects. All four models had male ID and group ID as random effects. An observation-level random effect was added in the Poisson model to combat overdispersion.

Dominance had no significant effect on the four mating dependant variables (Number of Matings: LRT: X^2 (1) =0.03, p=0.872; Standardized Number of Females Mated: LRT: X^2 (1) =2.53, p=0.111; Remating Rate: LRT: X^2 (1) =0.04, p=0.839; SCI: LRT: X^2 (1) =0.63, p=0.426).

Chapter 4

Testing Hypotheses of Male Vigilance and Alarm Calling Investment

Heather Kazara and Tommaso Pizzari

ABSTRACT

Male and females differ in their vigilance and alarm calling behaviours. Several functional hypotheses have been proposed to explain these differences. For example, males are often more conspicuous than females so may invest in higher vigilance to reduce predation risk (male constraints). Females may benefit from male vigilance (female benefits). Similarly, males could increase vigilance to decrease the risk of sperm competition by guarding females from competitors (mate guarding). Finally, males may preferentially invest in vigilance and alarm calling in the presence of females with whom they have previously mated to protect their future offspring (mate investment). In red junglefowl, *Gallus gallus*, and the related domestic fowl, *G. domesticus*, males are more vigilant than females and only males produce alarm calls for aerial predators. Wilson and Evans (2008) found that in domestic fowl males increased aerial predator alarm calling efforts after mating with a female, consistent with the mate investment hypothesis. A critical prediction of this hypothesis is that a male's investment should decrease when perception of sperm competition reduces his confidence in paternity. This chapter tested this prediction by experimentally parsing the effect of mating and perceived risk of sperm competition on male investment in vigilance and alarm calling in red junglefowl. Focal males were exposed to a female with or without the opportunity to mate, and with or without exposure to a male competitor. Males increased vigilance and alarm calling in treatments where they could mate with the female, regardless of the presence of a male competitor. Males in mating treatments also increased vigilance and alarm calling on the days when they mated, indicating that mating is temporally linked with these anti-predator behaviours. These results suggest that male investment is associated with mating but not paternity confidence.

INTRODUCTION

Vigilance is defined as the interruption of activity, usually foraging, to scan the surrounding environment (Hart & Lendrem, 1984; Elgar, 1989; Beauchamp, 2015), and is generally interpreted as an anti-predator behaviour (FitzGibbon, 1989; Cresswell *et al.*, 2003; Beauchamp, 2015). Given this naturally selected function, one would expect vigilance investment to be similar across males and females. Yet several species display marked sex differences in vigilance (Lendrem, 1983; Mayhew, 1987; Childress & Lung, 2003; Pizzari, 2003; Cameron & du Toit, 2005; Squires *et al.*, 2007). These differences are puzzling and demand a functional explanation. Four hypotheses have been put forward to explain differences in anti-predation behaviours: i) the male constraints hypothesis (Lendrem, 1983), ii) females benefit hypothesis (Squires *et al.*, 2007; Portugal & Guillemain, 2011), iii) mate guarding hypothesis (Guillemain *et al.*, 2003), and iv) the mate investment hypothesis (Alatalo & Helle, 1990; Wilson & Evans, 2008).

The male constraints hypothesis states that in sexually dimorphic species, the more conspicuous sex will suffer higher predation risk and thus invest preferentially in vigilance (Guillemain *et al.*, 2003; Squires *et al.*, 2007; Portugal & Guillemain, 2011). In species with traditional 'sex roles' (fowl (Zuk *et al.*, 1990b); peacocks (Petrie, 1994); mallards (Omland, 1996)), males are typically more conspicuous than females (Slagsvold *et al.*, 1995; Ligon *et al.*, 1998; Stuart-Fox *et al.*, 2003). More conspicuous phenotypes may suffer increased predation risk (Stuart-Fox *et al.*, 2003; Cain *et al.*, 2019). For example, models of conspicuous male collared lizards, *Crotaphytus collaris*, received significantly more predation than female models (Husak *et al.*, 2006). However, this is not

limited to visually dimorphic species. In some sexually monomorphic species males attract females through song and increased song effort can lead to higher predation risk for male passerines (Ward & Slater, 2005; Møller *et al.*, 2006). Field crickets, *Teleogryllus oceanicus*, living in certain populations suffer death from a parasitoid fly, *Ormia ochracea*, which locates males using their courtship song; leading to differences in call structure and behaviour across populations with and without parasites (Zuk *et al.*, 1993; Zuk *et al.*, 1998; Lewkiewicz & Zuk, 2004). One study examined male calling behaviours in a highly parasitized population finding that the majority of males had lost the apparatus needed to produce songs (Zuk *et al.*, 2006). Females in this population still mated with males despite the lack of courtship song, suggesting the predation costs associated with male courtship song in this population was too high, leading to the selection of silent males (Zuk Marlene *et al.*, 2006). Túngara frogs, *Engystomops pustulosus*, have a variable song structure composed of whine and chuck sounds, with chucks relaying information about the signaller's body size to females during mate choice (Ryan *et al.*, 1981; Ryan *et al.*, 1982). Males do not always produce calls with chucks, suggesting that more complex calls may increase predation risk (Ryan *et al.*, 1982). This was tested by exposing fringe-lipped bats, *Trachops cirrhosis*, to whine-only and chuck containing calls via two separate speakers, finding that bats significantly preferred to target speakers playing the complex call (Ryan *et al.*, 1982). If males with conspicuous phenotypes are more at risk from predation then males should be more vigilant than females regardless of mating status (Guillemain *et al.*, 2003; Squires *et al.*, 2007; Portugal & Guillemain, 2011). When looking at sleep and vigilance trade-offs in the mallard, *Anas platyrhynchos*, males were significantly more vigilant and slept less than females

when adopting the conspicuous breeding plumage (Lendrem, 1983). Males displayed higher vigilance regardless of mate presence, suggesting females are not benefitting nor is it being used to guard/protect mates. Furthermore male and female vigilance differences disappeared once males adopted the cryptic eclipse plumage (Lendrem, 1983). Thus, sexual dimorphism can place different constraints on the more conspicuous sex, and in some sexually dimorphic birds, males are more vigilant than females (Lendrem, 1983; Guillemain *et al.*, 2003; Pizzari, 2003; Squires *et al.*, 2007).

The female benefit hypothesis states that females gain benefits, such as increased predator protection, from pairing with vigilant males (Squires *et al.*, 2007). Vigilance is costly; reducing time spent foraging or resting (Elgar, 1989; Rasa, 1989; Cowlishaw *et al.*, 2004). In females greater foraging efforts may be needed to increase nutritional status and fecundity, therefore males could increase vigilance to allow females to spend more time foraging; thus increasing female reproductive success (Guillemain *et al.*, 2003; Squires *et al.*, 2007; Portugal & Guillemain, 2011). Males could gain direct reproductive benefits by allowing females to reduce vigilance and increase foraging effort because this would lead to better egg production, directly increasing the male's reproductive success. Males could also attract multiple partners by providing females with better protection from predators. In addition, females could prefer vigilant males because vigilance is a condition-dependant trait. Females prefer vigilant males in one bird species, and high vigilance effort is costly to maintain due to reduced foraging time (Dahlgren, 1990; Fusani *et al.*, 1997). To prevent poor quality individuals from cheating, the handicap principle states that reliable signals advertising male quality should be costly to maintain (Zahavi, 1975). Furthermore

vigilance effort has been linked to testosterone in one species, so high vigilance levels could reflect male genetic quality (Fusani *et al.*, 1997). Testosterone has been linked to sexually selected traits in several species (Owens & Short, 1995; Zuk *et al.*, 1995; Fusani *et al.*, 1997; Duckworth *et al.*, 2001; Hagelin & Ligon, 2001; Rantala *et al.*, 2003; Pérez-Rodríguez *et al.*, 2006); and high testosterone levels can cause compromised immune systems, as proposed under the immunocompetence handicap hypothesis (Owens & Short, 1995; Peters, 2000; Rantala *et al.*, 2003; Pérez-Rodríguez *et al.*, 2006). The immunocompetence handicap hypothesis was explored in male red grouse, *Lagopus lagopus scotica*, by testing the relationship between male comb size, body condition and immunocompetence in addition to manipulating testosterone levels to test whether higher testosterone causes enhanced ornamentation at the cost of reduced immunity (Mougeot *et al.*, 2004). Males with larger combs had better body conditions than males with smaller combs, and testosterone treated males had greater ornamentation but suffered reduced immunocompetence (Mougeot *et al.*, 2004). Mougeot et al. (2004) concluded that comb size is a reliable indicator of male quality because males with naturally larger combs were able to cope with higher levels of immunosuppressive testosterone, indicating that females would gain genetic benefits from selecting males using this trait. Only certain genotypes can afford costly traits or behaviours, like large ornaments and vigilance. If vigilance is a condition-dependant trait, then under this scenario females would gain genetic benefits by selecting vigilant males through higher viability of offspring from inheriting the paternal genes associated with high vigilance.

A study of European wigeons, *Anas penelope,* found evidence for the female benefits hypothesis (Portugal & Guillemain, 2011). Observations were made

when wigeon foraged on dry land both before and after pair formation. There was no difference in male vigilance before and after pairing with a female, however there was a significant decrease in female vigilance after pair formation. Female vigilance remained low even when foraging in evermore risky situations such as smaller groups or being further from the water. This suggests that paired females benefit from male presence, allowing them to lower vigilance and forage more efficiently (Portugal & Guillemain, 2011). A similar pattern was found in Harlequin ducks, *Histrionicus histrionicus*, with paired males being four times more vigilant than unpaired males and their mates; suggesting that males are not at higher risk due to brighter plumage (Squires *et al.*, 2007). Female vigilance was potentially influenced by male vigilance efforts, with females paired to more vigilant males engaging in more foraging activity; which is consistent with the idea that females benefit from pairing with vigilant males (Squires *et al.*, 2007).

The mate guarding hypothesis states that to increase reproductive success males should reduce sperm competition through guarding females around the time of fertilization to decrease the chance of extra-pair copulations (Møller, 1985; Birkhead & Hunter, 1990; Artiss & Martin, 1995; Kokko & Morrell, 2005). Sperm competition occurs when two or more males mate with a female, with paternity share in offspring declining with increasing number of competitors (Parker, 1970; Birkhead & Hunter, 1990). Males should aim to decrease competition and could use vigilance to guard females whilst simultaneously monitoring competitor males (Osorio-Beristain & Drummond, 1998; Kokko & Morrell, 2005; Girard-Buttoz *et al.*, 2014). Male mate guarding can cause conflict with females by restricting female movements or increasing male-female aggression, and this was explored using the field cricket, *Gryllus campestris*

(Rodríguez-Muñoz *et al.*, 2011). There was no predation difference between lone males and females however females benefitted from a significant reduction in predation when paired whereas males were 3.9 times more likely to be predated in pairs. This study suggested that mate guarding could evolve to benefit males only by preventing females from mating with others. However for this to be effective fertility windows for guarding females would need to be short or predictable (Rodríguez-Muñoz *et al.*, 2011). Yet when mate guarding is costly mate guarding could still evolve but females would also need to benefit from this behaviour (Rodríguez-Muñoz *et al.*, 2011). Female crickets gained two benefits from male mate guarding behaviour: first, reduced predation risk, second, privileged access to preferred males leading to higher remating rates between the pair. Furthermore females incurred no costs from increased aggression nor did males restrict their movements (Rodríguez-Muñoz *et al.*, 2011). Mate guarding for males provided increased mating and reproductive success although males suffered costs through higher predation (Rodríguez-Muñoz *et al.*, 2011).

A study on pairs of European wigeon found support for the mate guarding hypothesis acting on vigilance (Guillemain *et al.*, 2003). Paired males were significantly more vigilant than females but vigilance in unpaired males did not differ between the cryptic plumage and breeding plumage; implying that the conspicuous breeding plumage had no higher predation risk. There was no evidence in support of the female benefit hypothesis in this study since there was no difference in vigilance between paired and unpaired females. However paired females spent less time interacting with conspecifics, suggesting that male wigeon invest in vigilance to reduce the chance of extra-pair copulations by guarding females, increasing mating success (Guillemain *et al.*, 2003).

The mate investment hypothesis states that males should invest in the protection of his own reproductive partners up to the point when their offspring, that the male has sired, are independent of maternal care (Alatalo & Helle, 1990; Wilson & Evans, 2008). When paternity confidence is high males typically invest more provisions into mates and offspring to further increase reproductive success. For example, confidence of paternity was positively correlated with male investment in offspring feeding, after controlling for mating systems, across 52 bird species (Møller & Birkhead, 1993). Therefore, when paternity confidence is reduced males should reduce investment in provisions to both mates and offspring. In a recent meta-analysis, paternal offspring care was examined across 48 species in four taxa (insects, fish, birds and mammals) finding that males in 81% of species adjusted their paternal care with reduced paternity confidence (Griffin *et al.*, 2013). This was also explored in collared flycatchers, *Ficedula albicollis*, by detaining females immediately after egg laying, exploiting the post-egg laying insemination window (Sheldon *et al.*, 1997). During this window the female reproductive tract is empty, allowing newly inseminated sperm to gain immediate access to the next egg post-ovulation (Sheldon *et al.*, 1997). Males in this species intensively mate guard during this window, so removal of females should cause males to assume reduced paternity. When females were removed males significantly reduced feeding efforts to offspring, demonstrating how perceived reduced paternity confidence leads to males reducing referential provisions (Sheldon *et al.*, 1997).

The red junglefowl, *Gallus gallus*, and the related domestic fowl, *Gallus gallus domesticus*, are ideal species for studying these hypotheses on male anti-predator behaviour because males are more vigilant than females (Pizzari, 2003). Males

and females display sexual dimorphism, with males possessing colourful, conspicuous plumage with fleshy red ornaments that signify parasite resistance (Ligon *et al.*, 1990; Zuk *et al.*, 1990a, 1990b; Zuk *et al.*, 1995). Furthermore fowl produce both aerial and terrestrial alarm calls, with both calls being functionally referential for avian and terrestrial predators (Gyger *et al.*, 1987; Evans *et al.*, 1993; Wilson *et al.*, 2008). When hearing the terrestrial call fowl adopt an alert upright posture with increased vigilance, whereas aerial calls cause individuals to look upwards, crouch and seek shelter (Evans *et al.*, 1993). Interestingly the aerial alarm call is only produced by males, leading to the suggestion that it could play a role in male reproductive strategies.

A key study by Wilson and Evans (2008) manipulated male mating success in a population of domestic fowl to examine whether male alarm calling effort changed accordingly. Male and female pairs were housed in six adjacent outdoor pens and pairs were assigned to either the control or experimental treatment (Wilson & Evans, 2008). The experiment ran across four weeks and during week one pairs were kept separated in both the control and experimental treatment. Male crowing and aerial alarm calling behaviour was recorded daily across the four-week period. During weeks two and three, males in the experimental treatment were provided with the opportunity to mate. This was done by removing the partition separating the pair in the experimental treatment while in the control treatment the partition remained to prevent mating (Wilson & Evans, 2008). In the fourth week, pairs in the experimental treatment were separated again using the partition, allowing all pairs to be restored to the original conditions. Crowing and alarm calling efforts during weeks two and three were compared between the two treatments, finding that males in the experimental treatment increased alarm

calling efforts by approximately 30% during this two-week period. Furthermore, males in the experimental treatment called 20% more than control males during the fourth week, despite no longer having the opportunity to mate (Wilson & Evans, 2008). Additionally, mating success had no effect on male crowing behaviour, suggesting that mating does not cause a general increase in male vocal activity. Wilson and Evans (2008) concluded that mating behaviour directly affects aerial alarm calling in fowl and that this is probably because of the mate investment hypothesis. Males could increase investment in alarm calling to protect reproductive partners from predation. This would increase the likelihood of these mates surviving and siring his offspring; increasing his reproductive success (Wilson & Evans, 2008). In addition, a more recent study on domestic fowl found that more dominant males obtained more matings than subordinates, and that these dominant males also engaged in more risk-taking behaviours in relation to aerial alarm calling by producing longer calls (Kokolakis *et al.*, 2010). This could suggest that dominant males increase call length to better protect reproductive partners and future offspring (Kokolakis *et al.*, 2010). Wilson and Evans (2008) experimental set-up provided males with no sperm competitors, which vastly increased paternity confidence but may not accurately reflect natural mating behaviour in fowl. To fully test the mate investment hypothesis paternity confidence should be explored.

This study builds on the findings of Wilson and Evans (2008) by experimentally testing whether reducing paternity confidence results in males decreasing investment in vigilance and aerial alarm calling. Four treatments were used: mating without competition, mating with competition, no mating without competition and no mating with competition. The predictions for how the three

hypotheses will influence vigilance and aerial alarm calling are outlined below. Female vigilance was not measured in this study, so the female benefits hypothesis is excluded.

Predictions:

1. Male Constraints Hypothesis. If male junglefowl suffer greater predation risk due to brighter plumage, then male vigilance and alarm calling effort should be lowest in the competition treatments and higher when males have no competitors. This is because the addition of a second male should reduce predation risk through dilution effects (Foster & Treherne, 1981). Plus a second male should allow focal males to reduce individual vigilance and alarm calling efforts because there is now a second individual scanning for predators (Pulliam, 1973; Beauchamp, 2001) and able to produce male-specific aerial alarm calls (Evans *et al.*, 1993).
2. Mate Guarding Hypothesis. If male junglefowl use vigilance primarily for guarding females, then vigilance should be significantly higher in the competition treatments and low when males have no competitors. Alarm calling however is unlikely to be used for guarding females from rival males. Although males could produce a dishonest call to disrupt or prevent mating. Fork-tailed drongos, *Dicrurus adsimilis*, are renowned vocal mimics that frequently steal food from other species (Flower, 2011). Individuals produce both drongo-specific and mimicked alarm calls that are dishonest causing the target species to flee, allowing drongos to kleptoparasitize (Flower, 2011). Therefore, male fowl could increase dishonest alarm calling effort to disrupt the competitor male's copulations.

3. Mate Investment Hypothesis. If males invest more protection into females with whom they have mated to protect the offspring that they have sired, then both vigilance and alarm calling effort should be highest in the mating without competition treatment. When paternity confidence is reduced in the mating with competition treatment males should reduce their investment in vigilance and alarm calling. Investment should be lowest when the focal male has had no opportunity to mate with the female and there is competition from another male.

METHODS

Experimental Design

There were four treatments: mating with and without competition, and no mating with and without competition from one another male (Figure 1). All four treatments were run simultaneously, taking 7 days to complete. In mating treatments males were given the opportunity to mate on four evenings per week on days 1, 3, 5 and 7. Females were herded into the male's half pen through a door in the dividing wall. To control for the effect of an observer entering the pen a sham manipulation was done in the no mating treatments by opening and closing the divider door whilst not allowing the female through. Competition males were placed into the female's half pen on the other three evenings per week (days 2, 4 and 6). Competition males were always the same age as the focal male to control for age effects and were kept constant across the week. Competition males were placed into cardboard boxes and then released (See Appendix, Supplementary Material A). A sham manipulation was done in the no-competition trials by opening an empty box in the pen. Observations took place for three hours each evening across the 7-day period, ending 30 minutes before sunrise. Each focal male was observed for a total of 30 minutes each day. The order in which males were observed was rotated daily.

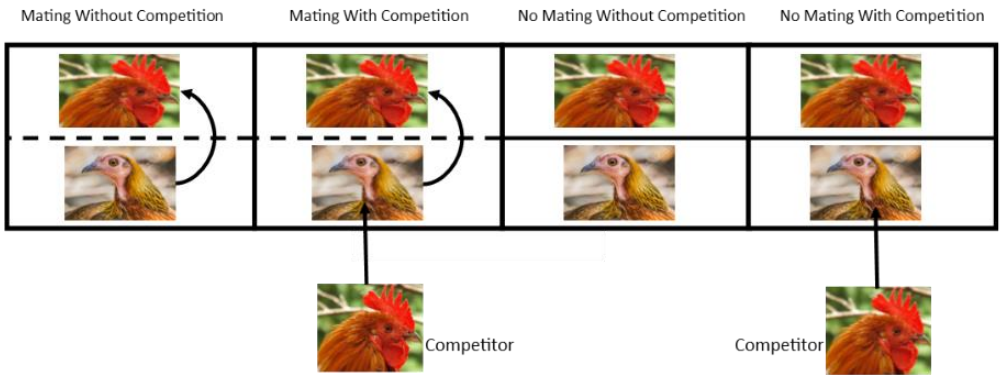


Figure 1. Experimental set up for experiment. Each focal male was housed in a pen with visual contact with a female. In mating treatments (dashed partition), the female could join the male and mate on days 1, 3, 5 and 7 out of the 7-day trial. In competition treatments a competitor male joined the female on days 2, 4 and 6 in a trial.

The experiment took place on a captive population of red junglefowl at the John Krebs's Field Station in Wytham during May-July 2017 and 2018. This experiment used a total of 16 males, 8 in 2017 and 8 in 2018, all aged between 4-5 years at the time they were used. Each male was used four times, once per treatment, and was rotated around treatments across a four-week period. The same four outdoor pens measuring 6x1.5m were used throughout the entire experiment. Each pen was subdivided into two equal sized half pens using a wooden and chicken wire divider, which allowed birds visual contact through the partition (See Appendix, Supplementary Material A). For each four-week period the treatment remained in the assigned pen. Like males, 16 females, 8 in each year, were used in total. Four females were used simultaneously, one placed into each front half pen. These females were placed into the pen in the morning of day 1 at the start of the four-week period and remained in the same half pen throughout those four weeks. Once the four weeks ended those females were removed and a new four were added. The four pens would then be assigned a different treatment for the new four weeks.

Four males were placed into the back halves of the pens in the mornings at approximately 11am on day 1. Males were placed into the back halves due to these being more open and exposed, increasing the likelihood of aerial alarm calls (See Appendix, Supplementary Material A). If this was the start of the four-week period, then females were also released at approximately 11am on day 1. During the four weeks females were left in their half pens and not caught and re-released along with the males. This was done to reduce stress in the females.

Behavioural Data

Anti-predation Behaviours

Vigilance was recorded *ad libitum* during 30-minute focal watches using a stopwatch. Birds were classed as being vigilant when they adopted an alert, upright posture with necks stretched upwards and both eyes open; often accompanied by moving the head from side-to-side (Inglis & Lazarus, 1981; Jacobsen & Ugelvik, 1994; Robinette & Ha, 2001; Pizzari, 2003; Randler, 2005; Squires *et al.*, 2007; Beauchamp, 2015). Birds with heads down exhibiting fixation on the ground, or birds with eyes closed were not counted as being vigilant. The number of aerial and terrestrial alarm calls produced were also recorded *ad libitum* during the 30 minutes spent with the focal male.

Male-Female Interactions

Male and female interactions were recorded *ad libitum* and included male harassment (chases, lunges and aggressive pecks), successful and unsuccessful matings, female solicitation and male courtship waltzing (lowering one wing while circling the female; Zuk *et al.*, 1992; Zuk *et al.*, 1995b). Successful copulations were defined by the male mounting females followed by either cloacal contact or the lowering of the males tail over the cloaca (Pizzari, 2003).

All matings were recorded as forced unless females solicited (crouched in front of male; Pizzari, 2003). All focal males mated at least once in the mating treatments, with the average number of matings across the 7-day period being 5.91 ± 0.40 (Standard Error). The average number of matings obtained across the 7-day period for the competition males in the competition treatments was 4.97 ± 0.76 (SE). However, three competition males failed to successfully mate across the 7-day trial.

Data Analysis

To test whether treatment had an effect on vigilance and aerial alarm calling two mixed models were created, one looking at vigilance and one for alarm calling, using the "lme4" package (version 1.1.21; Bates *et al.*, 2015) in R (version 1.1.423; R Core Team, 2017). To explore whether focal males were more vigilant and/or produced more alarm calls on mating days compared to no-mating days four mixed models were used. For mating treatments only, the effect of mating vs no-mating day was tested using two mixed models, one for vigilance and one for alarm calling. The final two models explored mating vs no-mating day effects on vigilance and alarm calling in no-mating treatments only. Analysis used 60 data points when looking at treatment. 224 data points were used for models exploring mating day effects.

The vigilance dependant variable was created by compiling the seconds spent vigilant for each male from each of the 7 evenings into one combined total number of seconds devoted to vigilance. The aerial alarm call dependent variable was created by adding the total number of aerial alarm calls produced for each male across the 7 observation sessions; giving an overall total number of aerial calls produced during a treatment.

To explore the effects of treatment on vigilance and aerial alarm calling two separate mixed models were created. The total number of seconds spent vigilant was entered as the dependent variable in the first model, with the total number of aerial alarm calls produced across the treatment as the dependent variable for the second model. The vigilance model used a square-root transformed linear mixed model, whereas the alarm calling model was a Poisson generalized linear mixed model using the square-root link. Treatment (four-levels) along with the four-week observation period (1-4) and the mean weekly temperature (ºC) were entered as fixed effects in both models. During the 2018 breeding season the United Kingdom experienced abnormally high temperatures during a heat wave that lasted several months. Due to this, the experimental pens were warmer than usual during this period, potentially leading to altered behaviours in the birds. Therefore, average weekly temperature was added to control for any temperature related differences on male behaviour. Male ID was added as a random effect in both models to control for repeated measures. Year was not included in the final models due to the overlap with the period variable. Since period incorporated year along with pens and females used this was retained. Furthermore, models were originally created using year instead of period and they were compared graphically to the period models to see which gave a better fit, with period fitting better.

Tukey's Post-Hoc tests were done on treatment for both the vigilance and aerial alarm calling model. These tests were done using the "multcomp" package (version 1.4.8; Hothorn *et al.*, 2008).

Due the results from the first models finding an effect of mating on vigilance and alarm calling, an additional analysis was run to test whether males increased their

vigilance or alarm calling efforts on mating days compared to no-mating days. This analysis tested mating treatments and no-mating treatments separately. Two Poisson (log-link) generalized linear mixed models were used for alarm calling and two square-root transformed linear mixed models for vigilance. Two models used data from males in mating treatments only, while the other two models looked at no-mating treatments only. All models looked at alarm calling and vigilance on each day rather than the combined weekly total. Period (1-4), treatment (two-levels) and whether it was a mating/no mating day were entered as fixed effects into all four models, with male ID as a random effect.

All three Poisson models were overdispersed so an observation-level random effect with a unique value for each observation (1: nrow(data)) was created; allowing incorporation of overdispersion into the models by adding (1|obs_effect) as a random effect. This is commonly used in ecological studies to combat overdispersion in Poisson and binomial models (Richards, 2008; Harrison, 2014, 2015).

To test that all models adhered to assumptions they were graphically tested using qqnorm and residual vs fitted plots. Furthermore, normality was tested using Shapiro-Wilk normality tests in the "stats" package (version 3.4.3; R Core Team, 2017), with all models expressing normality. To better assess the fit of the Poisson models the "DHARMa" package (version 0.2.0; Hartig, 2018) was used, which is commonly used to assess Poisson models in the ecological literature (Dorning & Harris, 2017; Rodríguez *et al.*, 2017; Rodríguez *et al.*, 2018; Walker *et al.*, 2017; Bowerman *et al.*, 2018; Eveson *et al.*, 2018).

Results were analysed through Likelihood Ratio Tests (LRT) using the "anova" function in the "lmerTest" package (version 2.0.36; Kuznetsova *et al.*, 2017). All graphs were created using the "ggplot2" package (version 2.2.1; Wickham, 2009). For visually displaying both vigilance and aerial alarm calling both variables were standardized using z-scores to allow easier interpretation of the strength of the effect of treatment and mating vs no-mating day on these behaviours.

RESULTS

Does treatment affect vigilance and alarm calling behaviour?

Males spent longer being vigilant when exposed to both mating treatments than when in the no-mating treatments (LRT X^2 (3) =22.28, p<0.001; Figure 2; Tables 1 and 2).

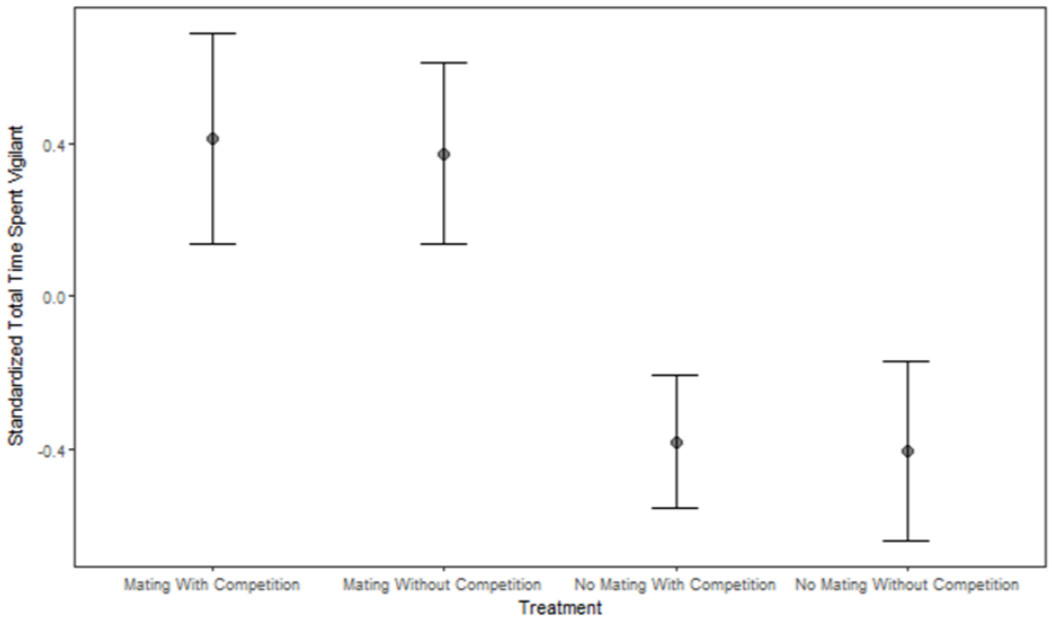


Figure 2. Average z-score standardized total time spent vigilant for each treatment. Error bars represent standard error.

Table 1. Model output for the square-root transformed linear mixed model for the total time spent vigilant (seconds), and the square-root linked Poisson generalized linear mixed model for the total number of aerial alarm calls produced. Models test the effect of treatment on vigilance and alarm calling. Average weekly temperature, period (1-4) and treatment are fixed effects with male ID as a random effect. In the alarm calling model the (1|obs_level) random effect was added to help overdispersion. The model AIC is 935.8 and 432.1 for the vigilance and alarm calling model respectively. Model BIC is 956.8, loglikelihood is -457.9 and deviance is 915.8 for the vigilance model. Model BIC is 453.1, loglikelihood is -206.1 and deviance is 412.1 for the alarm calling model.

	Total Time Spent Vigilant (seconds)			**Total Number of Aerial Alarm Calls Produced**		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	38.87	21.63 – 56.11	**<0.001**	11.50	0.81 – 163.14	0.071
Average Temperature	0.19	-0.45 – 0.83	0.559	1.11	1.00 – 1.23	0.057
Period 2	-11.96	-21.40 – -2.51	**0.013**	0.98	0.43 – 2.23	0.957
Period 3	-4.94	-14.41 – 4.54	0.307	0.26	0.11 – 0.60	**0.002**
Period 4	-5.54	-15.26 – 4.17	0.263	0.27	0.11 – 0.67	**0.005**
Mating Without Competition	-0.80	-5.16 – 3.56	0.720	1.24	0.61 – 2.51	0.555
No Mating With Competition	-8.54	-12.90 – -4.18	**<0.001**	0.34	0.17 – 0.70	**0.003**
No Mating Without Competition	-8.38	-12.74 – -4.02	**<0.001**	0.64	0.31 – 1.29	0.211
Random Effects						
σ^2	36.46			0.97		
τ_{00}	35.51_{ID}			0.72_{obs_effect}		
				0.06_{ID}		
ICC	0.49			0.06		
N	16_{ID}			16_{ID}		
				60_{obs_effect}		
Observations	60			60		
Marginal R^2 / Conditional R^2	0.327 / 0.659			0.396 / 0.432		

Table 2. Tukey's Post-Hoc Comparison Test for the total time spent vigilant (seconds) across treatments.

Treatment Comparison	Coefficient Estimate	SE	Z Value	*p* Value
Mating Without Competition - Mating With Competition	-0.03794	0.13495	-0.281	0.992
No Mating With Competition - Mating With Competition	-0.52421	0.13508	-3.881	< **0.001***
No Mating Without Competition - Mating With Competition	-0.54868	0.13494	-4.066	< **0.001***
No Mating With Competition - Mating Without Competition	-0.48628	0.13499	-3.602	**0.002***
No Mating Without Competition - Mating Without Competition	-0.51074	0.13498	-3.784	<**0.001***
No Mating Without Competition - No Mating With Competition	-0.02447	0.13516	-0.181	0.998

Similarly, mating treatments were associated with higher aerial alarm calling than no-mating treatments (LRT X^2 (3) =13.15, p=0.004; Figure 3; Tables 1 and 3). Although, the difference between the 'no mating without competition' and either mating treatments was not significant (Table 3).

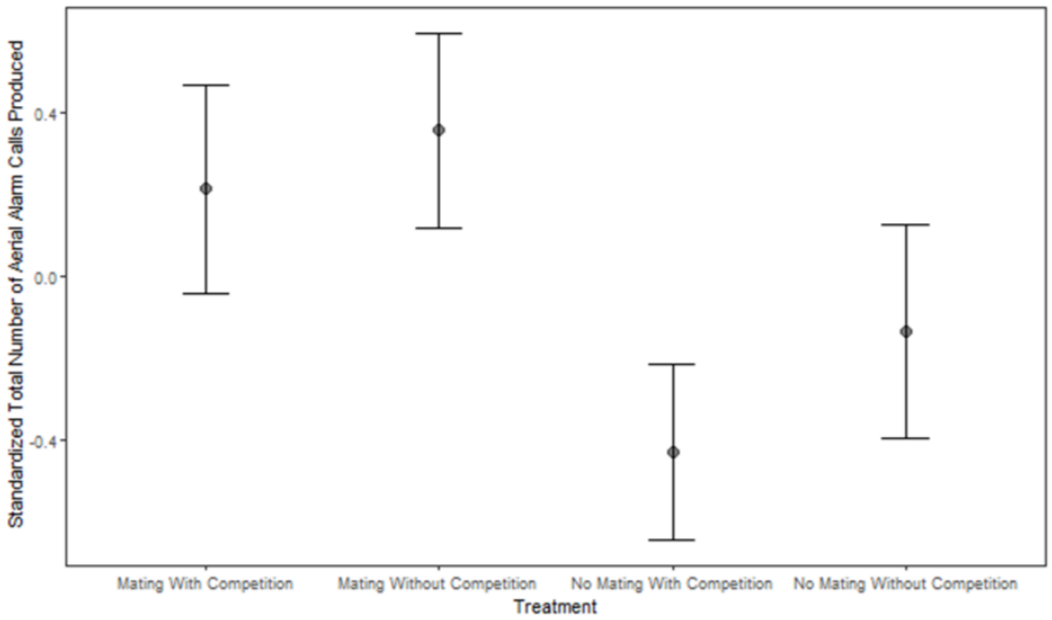


Figure 3. Average z-score standardized total number of aerial alarm calls produced that a male gave across the 7-day observation period for each treatment. Error bars represent standard error.

Table 3. Tukey's Post-Hoc Comparison Test for the total number of aerial alarm calls produced across treatments.

Treatment Comparison	Coefficient Estimate	SE	Z Value	*p* Value
Mating Without Competition - Mating With Competition	0.2132	0.3605	0..591	0.93469
No Mating With Competition - Mating With Competition	-1.0732	0.3609	-2.973	**0.01564***
No Mating Without Competition - Mating With Competition	-0.4528	0.3605	-1.256	0.59100
No Mating With Competition - Mating Without Competition	-1.2864	0.3607	-3.566	**0.00224***
No Mating Without Competition - Mating Without Competition	-0.6661	0.3606	-1.847	0.25134
No Mating Without Competition - No Mating With Competition	0.6203	0.3612	1.717	0.31464

Does mating day affect vigilance and aerial alarm calling?

Males in both mating treatments were more vigilant and produced more calls on 'mating' days when they were given the opportunity to mate with a female (i.e. days 1, 3, 5 and 7) than on alternate days when they were not given the opportunity to mate (i.e. days 2, 4 and 6). Analysing mating treatments exclusively found that both vigilance (LRT X^2 (1) =27.29, p<0.001; Figure 4; Table 4) and alarm calling (LRT X^2 (1) =18.28, p<0.001; Figure 5; Table 4) significantly increased on mating days compared to no-mating days. When testing the no-mating treatments separately, neither vigilance (LRT X^2 (1) =1.09, p=0.296; Figure 4; Table 5) nor alarm calling (LRT X^2 (1) =0.26, p=0.610; Figure 5; Table 5) were significantly influenced by 'mating' days compared to 'no-mating' days. However, the significant effect of mating days on vigilance and alarm calling in the mating treatments may be largely driven by the first few days in the trial (See Appendix, Supplementary Materials B and C).

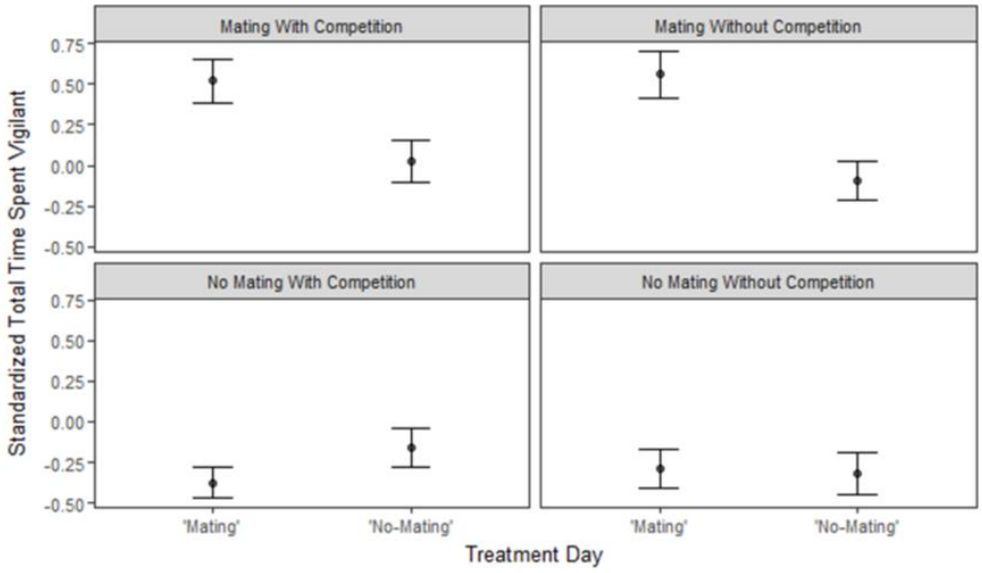


Figure 4. Average z-score standardized total time spent vigilant on 'mating' days (1, 3, 5 and 7) and 'no-mating' days (2, 4 and 6) for each treatment. In the mating treatments females were placed into the focal male's pen on 'mating' days, providing the opportunity to mate. In the no-mating treatments males and females remained separated on 'mating' days, preventing mating. Error bars represent standard error.

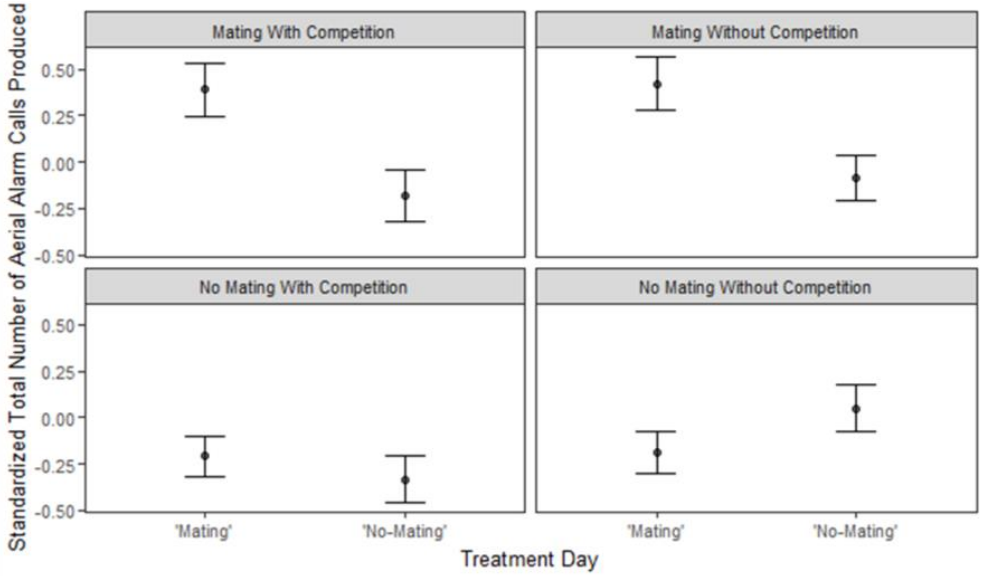


Figure 5. Average z-score standardized total number of aerial alarm calls produced that a male gave on 'mating' days (1, 3, 5 and 7) and 'no-mating' days (2, 4 and 6). In the mating treatments females were placed into the focal male's pen on 'mating' days, providing the opportunity to mate. In the no-mating treatments males and females remained separated on 'mating' days, preventing mating. Error bars represent standard error.

Table 4. Model output for the square-root transformed linear mixed model for the total time spent vigilant (seconds), and the log linked Poisson generalized linear mixed model for the total number of aerial alarm calls produced for mating treatments only. Models test whether 'mating' or 'no-mating' day influenced vigilance and alarm calling. Period (1-4), treatment and whether it was a 'mating' or 'no-mating' day are fixed effects with male ID as a random effect. In the alarm calling model the (1|obs_level) random effect was added to help overdispersion. The model AIC is 1246.6 and 960.1 for the vigilance and alarm calling model respectively. Model BIC is 1273.9, loglikelihood is -615.3 and deviance is 1230.6 for the vigilance model. Model BIC is 987.4, loglikelihood is -472.1 and deviance is 944.1 for the alarm calling model.

	Total Time Spent Vigilant (seconds)			**Total Number of Aerial Alarm Calls Produced**		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	13.84	11.11 – 16.57	**<0.001**	2.19	1.51 – 3.16	**<0.001**
Period 2	-4.24	-7.98 – -0.51	**0.026**	0.75	0.48 – 1.16	0.199
Period 3	-0.61	-4.34 – 3.13	0.750	0.55	0.35 – 0.86	**0.009**
Period 4	-1.89	-5.62 – 1.85	0.322	0.58	0.37 – 0.91	**0.017**
Mating Without Competition	-0.17	-1.10 – 0.76	0.723	1.09	0.86 – 1.38	0.485
Mating Day	2.58	1.64 – 3.52	**<0.001**	1.71	1.34 – 2.19	**<0.001**
Random Effects						
σ^2	12.64			0.75		
τ_{00}	6.36 $_{ID}$			0.37 $_{obs_effect}$		
				0.05 $_{ID}$		
ICC	0.33			0.06		
N	16 $_{ID}$			16 $_{ID}$		
				224 $_{obs_effect}$		
Observations	224			224		
Marginal R^2 / Conditional R^2	0.185 / 0.458			0.140 / 0.192		

Table 5. Model output for the square-root transformed linear mixed model for the total time spent vigilant (seconds), and the log linked Poisson generalized linear mixed model for the total number of aerial alarm calls produced for no-mating treatments only. Models test whether 'mating' or 'no-mating' day influenced vigilance and alarm calling. Period (1-4), treatment and whether it was a 'mating' or 'no-mating' day are fixed effects with male ID as a random effect. In the alarm calling model the (1|obs_level) random effect was added to help overdispersion. The model AIC is 1259.5 and 823.5 for the vigilance and alarm calling model respectively. Model BIC is 1286.8, loglikelihood is -621.8 and deviance is 1243.5 for the vigilance model. Model BIC is 850.7, loglikelihood is -403.7 and deviance is 807.5 for the alarm calling model.

	Total Time Spent Vigilant (seconds)			**Total Number of Aerial Alarm Calls Produced**		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*	*Incidence Rate Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	12.15	9.35 – 14.94	**<0.001**	1.44	0.90 – 2.31	0.125
Period 2	-3.37	-7.18 – 0.44	0.083	1.52	0.86 – 2.66	0.146
Period 3	-1.47	-5.28 – 2.34	0.449	0.41	0.22 – 0.75	**0.004**
Period 4	-0.04	-3.85 – 3.77	0.984	0.91	0.51 – 1.61	0.739
No Mating Without Competition	-0.18	-1.14 – 0.78	0.717	1.29	0.98 – 1.70	0.065
Mating Day	-0.51	-1.48 – 0.45	0.298	0.93	0.71 – 1.22	0.610
Random Effects						
σ^2	13.40			0.97		
τ_{00}	6.61_{ID}			0.42_{obs_effect}		
				0.10_{ID}		
ICC	0.33			0.09		
N	16_{ID}			16_{ID}		
				224_{obs_effect}		
Observations	224			224		
Marginal R^2 / Conditional R^2	0.090 / 0.390			0.187 / 0.262		

DISCUSSION

The key aim of this study was to test whether male fowl increase vigilance and alarm calling effort in a way that is consistent with the mate investment hypothesis, as suggested by Wilson and Evans (2008). We found that mating treatments influenced aerial alarm calling, with a particularly strong effect on vigilance. However, competition had no effect on vigilance or aerial alarm calling, suggesting that mate investment is not the cause (Table 6).

Table 6. Summary of the predictions outlined in the introduction and whether the results support these predictions.

Predictions	*Results*
Male Constraints- males are at increased risk of predation due to plumage but should reduce vigilance/alarm calling when another male is present.	Presence of a competitor male had no effect. Prediction not supported.
Mate Guarding- males use vigilance to guard females from rivals, so vigilance should increase in competition treatments	Presence of a competitor male had no effect. Prediction not supported.
Mate Investment- males invest vigilance/alarm calling into mated females to increase offspring success. Vigilance/alarm calling should be highest in the mating without competition treatment, then reduce in the mating with competition due to decreased paternity confidence. Both behaviours should be lowest in the no mating treatments.	Presence of a competitor male had no effect, with vigilance/alarm calling being equally high in both mating treatments. Prediction not supported.

Paternity Confidence and Mate Investment

The results of this chapter indicate that competition had no significant effect on either male vigilance or aerial alarm calling. By providing no support for the mate investment hypothesis, this finding is important in explaining what may be driving the link between male mating success and anti-predation behaviour in fowl. A reduction in male provisions has been experimentally tested in swallows, *Hirundo rustica*, with males reducing help with offspring feedings with increasing numbers of extra-pair matings (Møller, 1988). Male provisions in the face of reduced paternity confidence was explored in the dung beetle, *Onthophagus taurus*, finding that when paternity confidence was reduced males were significantly more likely to abandon females or reduce provision investment (Hunt & Simmons, 2002). Whereas studies of the Eastern bluebird, *Sialia sialis,* (Kempenaers *et al.*, 1998) and the tree swallow, *Tachycineta bicolor*, (Whittingham *et al.*, 1993; Kempenaers *et al.*, 1998) have found no effect of paternity confidence on male investment in offspring care. Both these studies suggested a paternity confidence threshold, with males only reducing investment once past this threshold but maintaining investment when above the threshold (Whittingham *et al.*, 1993; Kempenaers *et al.*, 1998). Our results support the findings of Whittingham et al. (1993) and Kempenaers et al. (1998) that reduced paternity confidence does not affect male investment. A paternity confidence threshold may explain why competition had no effect on either vigilance or alarm calling in our study since males only had one sperm competitor, so male paternity confidence may still be above the acceptable threshold. However, Whittingham et al. (1993) and Kempenaers et al. (1998) used the time separated from females during fertile periods as the measure of paternity confidence, assuming that males use female

absence as a cue for paternity assessment. Therefore, these studies may have found no effect on paternal investment because this may not be an accurate measure of paternity confidence. Like the beetle study by Hunt and Simmons (2002), this chapter did not make these assumptions because focal males could see their mate copulating with the competition male; making it a truer representation of reduced paternity confidence. However, it could also be argued that the use of only one competition male in our study is a weak test of mate investment because a single male likely only reduces paternity confidence by a marginal amount. A better approach may have been to use multiple competition males, perhaps even having various treatments with different numbers of competition males to see at what number of sperm competitors, if any, males reduce investment in vigilance and/or aerial alarm calling. This approach would not have been feasible at the time experiments were conducted due to a limited number of males being available for use. Furthermore, additional competition treatments would have required a larger sample size, which again was not feasible due to limited males.

Mate Guarding

The presence of a competitor male had no significant effect on time devoted to vigilance, suggesting that male vigilance in this species is not primarily used to guard females from rival males. In long-tailed macaques, *Macaca fascicularis*, male vigilance increased with increased time spent mate guarding, indicating that vigilance is used to guard promiscuous females (Girard-Buttoz *et al.*, 2014). In our experimental design, males in two treatments were provided with a single male competitor, so if males used vigilance to guard females and monitor rivals then vigilance should have been higher in the competition treatments, which was

not shown. Therefore, the results suggest that mate guarding is not primarily influencing vigilance in fowl.

Male Compensation

The results from this chapter suggest that neither the male constraints, mate guarding, or mate investment hypotheses are driving male vigilance and aerial alarm calling behaviour in fowl. Vigilance was significantly different in mating treatments compared to no-mating treatments. Yet there was no significant difference in alarm calling in mating treatments compared to the no mating without competition treatment. Males presented with competitors did produce slightly fewer alarm calls and since aerial alarm calling is a male-only behaviour (Gyger *et al.*, 1987; Evans *et al.*, 1993; Evans, 1997) males could have reduced alarm calling efforts when the competitor male was present. Therefore, aerial alarm calling may be predominantly influenced by mating behaviour, yet in no-mating treatments alarm calling could have been driven more by anti-predation with males compensating by increasing efforts when alone. Overall mating treatments did cause an increase in both vigilance and alarm calling, suggesting alternative mechanisms surrounding mating activity could be the result.

Mating vs No-mating Days

This chapter found that overall, mating days were associated with a significant increase in both vigilance and alarm calling compared to no-mating days for males in the mating treatments. Wilson et al. (2010) explored whether males can selectively invest in mated females by allowing males to mate with one of two females, then either removing the mated or non-mated female. No significant effect from removing mated females was found, implying that male fowl do not discriminate between mated and non-mated females. (Wilson *et al.*, 2010). Not

selectively investing could be costly because males could invest provisions, like alarm calling, into promiscuous or non-mated females; reducing his fitness. Conversely, under natural conditions male fowl fight for territories during the breeding season, with territorial males gaining harems of females (Collias & Collias, 1967; McBride *et al.*, 1969; Wilson *et al.*, 2010). Under these conditions increasing alarm calling effort in response to recent mating success could benefit males because all the females residing within his territory are likely his mates (Wilson *et al.*, 2010). Like Wilson et al. (2010), the increase in anti-predation behaviours on mating days could be the result of males increasing investment as a response to recent mating success.

Alternatively, vigilance and alarm calling could have increased on mating days because males shared their half pen with the female, whereas males in no-mating treatments never physically interacted with females. However, this seems unlikely because males could see and interact with females through the pen divider. If these behaviours increased to allow females to decrease vigilance, then there should be no clear difference across days because males could always visually monitor females regardless of whether they were separated or shared a pen. Furthermore female fowl have been shown to decrease vigilance in the presence of males even when in adjacent pens (Pizzari, 2003), therefore sharing a pen should have no effect.

Anti-predator Behaviours and Sexual Selection

An alternative explanation for why mating treatments and mating day had an effect, but competition did not, is that these behaviours are preferred by females during mate selection. In the grey partridge vigilance is a testosterone dependant trait that has been demonstrated to be sexually selected by females (Dahlgren,

1990; Fusani *et al.*, 1997). Furthermore, in fowl anti-predator behaviours have been linked to mating, with one study finding aerial alarm calling rate to be the best predictor of mating and reproductive success (Wilson *et al.*, 2008). If males increased vigilance and alarm calling to attract females on mating evenings, then perhaps there should have been a high number of female solicitations. Overall there were 229 successful copulations from the focal males across the entire experiment, and only 47 of these were solicited with the rest being forced, suggesting that these behaviours did not increase to attract females. In addition, when females were placed into the male's half pen in most cases males would immediately harass and chase the female, forcing a copulation within the first few minutes. This implies that females had little choice over whether to copulate with the focal male, again suggesting that male vigilance and alarm calling do not increase to increase female propensity to mate. Finally, observations on male anti-predator behaviours began after females were released into their half pen in mating treatments, and as stated previously, most males mated within the first five minutes. This adds support to Wilson et al. (2010) conclusions that males increase anti-predator behaviours post-mating.

Conclusions

The main findings of this study do not support the conclusions of Wilson and Evans (2008) because the results found no difference in vigilance or alarm calling investment when paternity confidence was reduced, suggesting mate investment is not causing an increase in these behaviours. Due to the overall effect of mating treatments, and the effect of mating days, the results may suggest that the act of mating itself is the driving force behind increased efforts in anti-predation behaviours in red junglefowl, agreeing with the conclusions of Wilson et al.

(2010). Although it is also possible that these behaviours increase pre-copulation to attract females, though more recent studies do not support this idea (Wilson & Evans, 2008, 2010; Wilson *et al.*, 2010). Future studies should further explore the relationship between mating and anti-predator behaviours because the underlying mechanisms causing these behaviours remain unclear. Future work could also explore female vigilance and foraging activity to test whether females do benefit from increased male vigilance and alarm calling effort.

REFERENCES

Alatalo, R.V. & Helle, P. (1990). Alarm calling by individual willow tits, *Parus montanus*. *Animal Behaviour,* **40**, 437–442.

Artiss, T. & Martin, K. (1995). Male vigilance in white-tailed ptarmigan, *Lagopus leucurus*: mate guarding or predator detection? *Animal Behaviour,* **49**, 1249–1258.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1–48.

Beauchamp, G. (2001). Should vigilance always decrease with group size? *Behavioural Ecology and Sociobiology*, **51**, 47–52.

Beauchamp, G. (2015). *Animal Vigilance: Monitoring Predators and Competitors*. Academic Press.

Birkhead, T.R. & Hunter, F.M. (1990). Mechanisms of sperm competition. *Trends in Ecology & Evolution,* **5**, 48–52.

Bowerman, T., Roumasset, A., Keefer, M.L., Sharpe, C.S. & Caudill, C.C. (2018). Prespawn Mortality of Female Chinook Salmon Increases with Water Temperature and Percent Hatchery Origin. *Transactions of the American Fisheries Society*, **147**, 31–42.

Cain, K.E., Hall, M.L., Medina, I., Leitao, A.V., Delhey, K., Brouwer, L., Peters, A., Pruett-Jones, S., Webster, M.S., Langmore, N.E. & Mulder, R.A. (2019). Conspicuous Plumage Does Not Increase Predation Risk: A Continent-Wide Test Using Model Songbirds. *The American Naturalist,* **193**, 359–372.

Cameron, E.Z. & du Toit, J.T. (2005). Social influences on vigilance behaviour in giraffes, *Giraffa camelopardalis*. *Animal Behaviour,* **69**, 1337–1344.

Childress, M.J. & Lung, M.A. (2003). Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Animal Behaviour,* **66**, 389–398.

Collias, N.E. & Collias, E.C. (1967). A Field Study of the Red Junglefowl in North-Central India. *The Condor,* **69**, 360–386.

Cowlishaw, G., Lawes, M.J., Lightbody, M., Martin, A., Pettifor, R. & Rowcliffe, J.M. (2004). A simple rule for the costs of vigilance: empirical evidence from a social forager. *Proceedings of the Royal Society of London B: Biological Sciences*, **271**, 27–33.

Cresswell, W., Lind, J., Kaby, U., Quinn, J.L. & Jakobsson, S. (2003). Does an opportunistic predator preferentially attack nonvigilant prey? *Animal Behaviour*, **66**, 643–648.

Dahlgren, J. (1990). Females choose vigilant males: an experiment with the monogamous grey partridge, *Perdix perdix*. *Animal Behaviour,* **39**, 646–651.

Dorning, J. & Harris, S. (2017). Dominance, gender, and season influence food patch use in a group-living, solitary foraging canid. *Behavioural Ecology,* **28**, 1302–1313.

Duckworth, R.A., Mendonça, M.T. & Hill, G.E. (2001). A Condition Dependent Link between Testosterone and Disease Resistance in the House Finch. *Proceedings of the Royal Society of London B: Biological Sciences,* **268**, 2467–2472.

Elgar, M.A. (1989). Predator Vigilance and Group Size in Mammals and Birds: A Critical Review of the Empirical Evidence. *Biological Reviews,* **64**, 13–33.

Evans, C.S. (1997). Referential Signals. In *Communication*, Perspectives in Ethology: 99–143. Owings, D.H., Beecher, M.D. & Thompson, N.S. (Eds.). Springer US.

Evans, C.S., Evans, L. & Marler, P. (1993). On the meaning of alarm calls: functional reference in an avian vocal system. *Animal Behaviour,* **46**, 23–38.

Eveson, J.P., Bravington, M.V. & Farley, J.H. (2018). Accounting for environmental and observer effects in estimating abundance of southern bluefin tuna from aerial survey data. *PLoS ONE,* **13**, e0207790.

FitzGibbon, C.D. (1989). A cost to individuals with reduced vigilance in groups of Thomson's Gazelles hunted by Cheetahs. *Animal Behaviour,* **37**, 508–510.

Flower, T. (2011). Fork-tailed drongos use deceptive mimicked alarm calls to steal food. *Proceedings of the Royal Society of London B: Biological Sciences,* **278**, 1548–1555.

Foster, W.A. & Treherne, J.E. (1981). Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature,* **293**, 466–467.

Fusani, L., Beani, L., Lupo, C. & Dessì-Fulgheri, F. (1997). Sexually selected vigilance behaviour of the grey partridge is affected by plasma androgen levels. *Animal Behaviour,* **54**, 1013–1018.

Girard-Buttoz, C., Heistermann, M., Rahmi, E., Agil, M., Ahmad Fauzan, P. & Engelhardt, A. (2014). Costs of mate-guarding in wild male long-tailed macaques (*Macaca fascicularis*): Physiological stress and aggression. *Hormones and Behavior,* **66**, 637–648.

Griffin, A.S., Alonzo, S.H. & Cornwallis, C.K. (2013). Why Do Cuckolded Males Provide Paternal Care? *PLoS Biology,* **11**, e1001520.

Guillemain, M., Caldow, R.W.G., Hodder, K.H. & Goss-Custard, J.D. (2003). Increased vigilance of paired males in sexually dimorphic species: distinguishing between alternative explanations in wintering Eurasian wigeon. *Behavioural Ecology*, **14**, 724–729.

Gyger, M., Marler, P. & Pickert, R. (1987). Semantics of an Avian Alarm Call System: The Male Domestic Fowl, *Gallus domesticus*. *Behaviour*, **102**, 15–40.

Hagelin, J.C. & Ligon, J.D. (2001). Female quail prefer testosterone-mediated traits, rather than the ornate plumage of males. *Animal Behaviour*, **61**, 465–476.

Harrison, X.A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, **2**, e616.

Harrison, X.A. (2015). A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology & evolution. *PeerJ*, **3**, e1114.

Hart, A. & Lendrem, D.W. (1984). Vigilance and scanning patterns in birds. *Animal Behaviour*, **32**, 1216–1224.

Hartig, F. (2018). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.2.0. http://florianhartig.github.io/DHARMa/.

Hothorn, T., Bretz, F. & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Hunt, J. & Simmons, L.W. (2002). Confidence of paternity and paternal care: covariation revealed through the experimental manipulation of the mating system in the beetle *Onthophagus taurus*. *Journal of Evolutionary Biology*, **15**, 784–795.

Husak, J.F., Macedonia, J.M., Fox, S.F. & Sauceda, R.C. (2006). Predation Cost of Conspicuous Male Coloration in Collared Lizards (*Crotaphytus collaris*): An Experimental Test Using Clay-Covered Model Lizards. *Ethology*, **112**, 572–580.

Inglis, I.R. & Lazarus, J. (1981). Vigilance and Flock Size in Brent Geese: The Edge Effect. *Zeitschrift für Tierpsychologie*, **57**, 193–200.

Jacobsen, O.W. & Ugelvik, M. (1994). Grazing and vigilance behaviour of breeding Eurasian Wigeon *Anas penelope* in relation to distance from water. *Wildfowl*, **45**, 119-123.

Kempenaers, B., Lanctot, R.B. & Robertson, R.J. (1998). Certainty of paternity and paternal investment in eastern bluebirds and tree swallows. *Animal Behaviour*, **55**, 845–860.

Kokko, H. & Morrell, L.J. (2005). Mate guarding, male attractiveness, and paternity under social monogamy. *Behavioural Ecology*, **16**, 724–731.

Kokolakis, A., Smith, C.L. & Evans, C.S. (2010). Aerial alarm calling by male fowl (*Gallus gallus*) reveals subtle new mechanisms of risk management. *Animal Behaviour*, **79**, 1373–1380.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, **82**, 1–26.

Lendrem, D.W. (1983). Sleeping and vigilance in birds. I. Field observations of the mallard (*Anas platyrhynchos*). *Animal Behaviour*, **31**, 532–538.

Lewkiewicz, D.A. & Zuk, M. (2004). Latency to resume calling after disturbance in the field cricket, *Teleogryllus oceanicus*, corresponds to population-level differences in parasitism risk. *Behavioural Ecology and Sociobiology*. **55**, 569–573.

Ligon, J.D., Kimball, R. & Merola-Zwartjes, M. (1998). Mate choice by female red junglefowl: the issues of multiple ornaments and fluctuating asymmetry. *Animal Behaviour*, **55**, 41–50.

Ligon, J.D., Thornhill, R., Zuk, M. & Johnson, K. (1990). Male-male competition, ornamentation and the role of testosterone in sexual selection in red junglefowl. *Animal Behaviour*, **40**, 367–373.

Mayhew, P.W. (1987). Vigilance levels in European Wigeon - sexual differences. *Wildfowl*, **38**, 77–81.

McBride, G., Parer, I.P. & Foenander, F. (1969). The Social Organization and Behaviour of the Feral Domestic Fowl. *Animal Behaviour Monographs*, **2**, 125–181.

Møller, A.P. (1985). Mixed reproductive strategy and mate guarding in a semi-colonial passerine, the swallow *Hirundo rustica*. *Behavioural Ecology and Sociobiology*, **17**, 401–408.

Møller, A.P. (1988). Paternity and paternal care in the swallow, *Hirundo rustica*. *Animal Behaviour*, **36**, 996–1005.

Møller, A.P. & Birkhead, T.R. (1993). Certainty of paternity covaries with paternal care in birds. *Behavioural Ecology and Sociobiology*, **33**, 261–268.

Møller, A.P., Chabi, Y., Cuervo, J.J., Lope, F.D., Kilpimaa, J., Kose, M., Matyjasiak, P., Pap, P.L., Saino, N., Sakraoui, R., Schifferli, L. & Hirschheydt, J.V. (2006). An Analysis of Continent-Wide Patterns of Sexual Selection in a Passerine Bird. *Evolution*, **60**, 856–868.

Mougeot, F., Irvine, J.R., Seivwright, L., Redpath, S.M. & Piertney, S. (2004). Testosterone, immunocompetence, and honest sexual signaling in male red grouse. *Behavioural Ecology,* **15**, 930–937.

Omland, K.E. (1996). Female mallard mating preferences for multiple male ornaments. *Behavioural Ecology and Sociobiology*, **39**, 353–360.

Osorio-Beristain, M. & Drummond, H. (1998). Non-aggressive mate guarding by the blue-footed booby: a balance of female and male control. *Behavioural Ecology and Sociobiology*, **43**, 307–315.

Owens, I.P.F. & Short, R.V. (1995). Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. *Trends in Ecology & Evolution,* **10**, 44–47.

Parker, G.A. (1970). Sperm Competition and Its Evolutionary Consequences in the Insects. *Biological Reviews,* **45**, 525–567.

Pérez-Rodríguez, L., Blas, J., Viñuela, J., Marchant, T.A. & Bortolotti, G.R. (2006). Condition and androgen levels: are condition-dependent and testosterone-mediated traits two sides of the same coin? *Animal Behaviour,* **72**, 97–103.

Peters, A. (2000). Testosterone Treatment Is Immunosuppressive in Superb Fairy-Wrens, Yet Free-Living Males with High Testosterone Are More Immunocompetent. *Proceedings of the Royal Society of London B: Biological Sciences,* **267**, 883–889.

Petrie, M. (1994). Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature,* **371**, 598–599.

Pizzari, T. (2003). Food, vigilance, and sperm: the role of male direct benefits in the evolution of female preference in a polygamous bird. *Behavioural Ecology,* **14**, 593–601.

Portugal, S.J. & Guillemain, M. (2011). Vigilance patterns of wintering Eurasian Wigeon: female benefits from male low-cost behaviour. *Journal of Ornithology,* **152**, 661–668.

Pulliam, H.R. (1973). On the advantages of flocking. *Journal of Theoretical Biology*, **38**, 419–422.

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Randler, C. (2005). Coots *Fulica atra* reduce their vigilance under increased competition. *Behavioural Processes,* **68**, 173–178.

Rantala, M.J., Vainikka, A. & Kortet, R. (2003). The role of juvenile hormone in immune function and pheromone production trade-offs: a test of the

immunocompetence handicap principle. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 2257–2261.

Rasa, O.A.E. (1989). The costs and effectiveness of vigilance behaviour in the Dwarf Mongoose: implications for fitness and optimal group size. *Ethology Ecology & Evolution*, **1**, 265–282.

Richards, S.A. (2008). Dealing with overdispersed count data in applied ecology. *Journal of Applied Ecology*, **45**, 218–227.

Robinette, R.L. & Ha, J.C. (2001). Social and ecological factors influencing vigilance by northwestern crows, *Corvus caurinus*. *Animal Behaviour*, **62**, 447–452.

Rodríguez, A., Dann, P. & Chiaradia, A. (2017). Reducing light-induced mortality of seabirds: High pressure sodium lights decrease the fatal attraction of shearwaters. *Journal for Nature Conservation*, **39**, 68–72.

Rodríguez, A., Holmberg, R., Dann, P. & Chiaradia, A. (2018). Penguin colony attendance under artificial lights for ecotourism. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, **329**, 457–464.

Rodríguez-Muñoz, R., Bretman, A. & Tregenza, T. (2011). Guarding Males Protect Females from Predation in a Wild Insect. *Current Biology*, **21**, 1716–1719.

Ryan, M.J., Tuttle, M.D. & Rand, A.S. (1982). Bat Predation and Sexual Advertisement in a Neotropical Anuran. *The American Naturalist*, **119**, 136–139.

Ryan, M.J., Tuttle, M.D. & Taft, L.K. (1981). The costs and benefits of frog chorusing behavior. *Behavioural Ecology and Sociobiology*, **8**, 273–278.

Sheldon, B.C., Räsänen, K. & Dias, P.C. (1997). Certainty of paternity and paternal effort in the collared flycatcher. *Behavioural Ecology*, **8**, 421–428.

Slagsvold, T., Dale, S. & Kruszewicz, A. (1995). Predation favours cryptic coloration in breeding male pied flycatchers. *Animal Behaviour*, **50**, 1109–1121.

Squires, K.A., Martin, K., Goudie, R.I. & Yasukawa, K. (2007). Vigilance Behavior in the Harlequin Duck (*Histrionicus histrionicus*) during the Preincubation Period in Labrador: Are Males Vigilant for Self or Social Partner? *The Auk*, **124**, 241–252.

Stuart-Fox, D.M., Moussalli, A., Marshall, N.J. & Owens, I.P.F. (2003). Conspicuous males suffer higher predation risk: visual modelling and experimental evidence from lizards. *Animal Behaviour*, **66**, 541–550.

Walker, R.H., King, A.J., McNutt, J.W. & Jordan, N.R. (2017). Sneeze to leave: African wild dogs (*Lycaon pictus*) use variable quorum thresholds

facilitated by sneezes in collective decisions. *Proceedings of the Royal Society B: Biological Sciences,* **284**, 20170347.

Ward, S. & Slater, P.J.B. (2005). Raised thermoregulatory costs at exposed song posts increase the energetic cost of singing for willow warblers *Phylloscopus trochilus*. *Journal of Avian Biology*, **36**, 280–286.

Whittingham, L.A., Dunn, P.O. & Robertson, R.J. (1993). Confidence of paternity and male parental care: an experimental study in tree swallows. *Animal Behaviour,* **46**, 139–147.

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*, Use R! New York: Springer-Verlag.

Wilson, D.R., Bayly, K.L., Nelson, X.J., Gillings, M. & Evans, C.S. (2008). Alarm calling best predicts mating and reproductive success in ornamented male fowl, *Gallus gallus*. *Animal Behaviour,* **76**, 543–554.

Wilson, D.R. & Evans, C.S. (2008). Mating success increases alarm-calling effort in male fowl, *Gallus gallus*. *Animal Behaviour*, **76**, 2029–2035.

Wilson, D.R. & Evans, C.S. (2010). Female fowl (*Gallus gallus*) do not prefer alarm-calling males. *Behaviour,* **147**, 525–552.

Wilson, D.R., McDonald, P.G. & Evans, C.S. (2010). Mechanisms of Mate Investment in the Polygamous Fowl, *Gallus gallus*. *Ethology,* **116**, 755–762.

Zahavi, A. (1975). Mate selection- A selection for a handicap. *Journal of Theoretical Biology*, **53**, 205–214.

Zuk, M., Johnsen, T.S. & Maclarty, T. (1995a). Endocrine-Immune Interactions, Ornaments and Mate Choice in Red Junglefowl. *Proceedings of the Royal Society of London B: Biological Sciences,* **260**, 205–210.

Zuk, M., Ligon, J.D. & Thornhill, R. (1992). Effects of experimental manipulation of male secondary sex characters on female mate preference in red junglefowl. *Animal Behaviour,* **44**, 999–1006.

Zuk, M., Popma, S.L. & Johnsen, T.S. (1995b). Male Courtship Displays, Ornaments and Female Mate Choice in Captive Red Junglefowl. *Behaviour,* **132**, 821–836.

Zuk, M., Rotenberry, J.T. & Simmons, L.W. (1998). Calling Songs of Field Crickets (*Teleogryllus oceanicus*) with and Without Phonotactic Parasitoid Infection. *Evolution,* **52**, 166–171.

Zuk, M., Thornhill, R., Ligon, J.D. & Johnson, K. (1990a). Parasites and mate choice in red junglefowl. *American Zoologist*, **30**, 235–244.

Zuk, M., Thornhill, R., Ligon, J.D., Johnson, K., Austad, S., Ligon, S.H., Thornhill, N.W. & Costin, C. (1990b). The Role of Male Ornaments and

Courtship Behavior in Female Mate Choice of Red Junglefowl. *The American Naturalist*, **136**, 459–473.

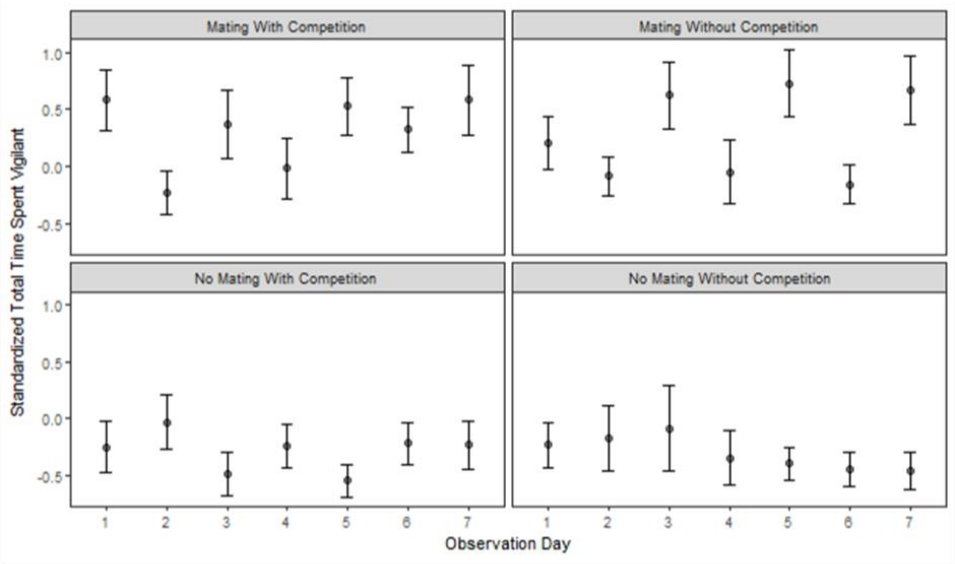
Zuk Marlene, Rotenberry John T & Tinghitella Robin M. (2006). Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. *Biology Letters*, **2**, 521–524.

Zukl, M., Simmons, L.W. & Cupp, L. (1993). Calling characteristics of parasitized and unparasitized populations of the field cricket *Teleogryllus oceanicus*. *Behavioural Ecology and Sociobiology*, **33**, 339–343.

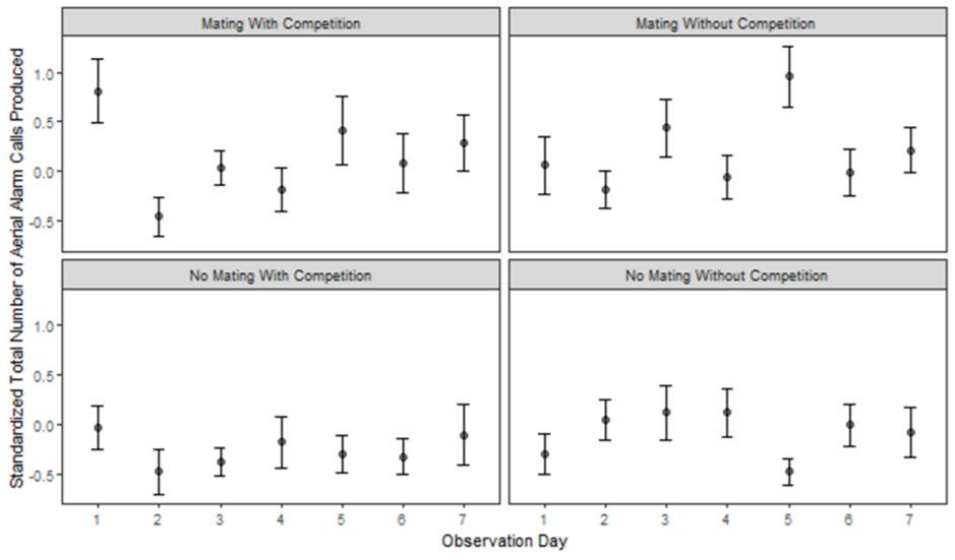
APPENDIX



Supplementary Material A. A panel of four photograph taken in 2017 showing the experimental pens and set-up. The first photograph (a) shows the pen set up with males and females separated by a pen divider. The second photograph (b) again shows the pen divider, with a male junglefowl in the back half pen, and a female in the front. Individuals can see one another and are interacting. The third photograph (c) shows the back half pens of the 4 pens used. The final photograph (d) shows an experimental pen with a male and a female separated. The image shows a cardboard box used for transporting competition males.



Supplementary Material B. Average z-score standardized total time spent vigilant for each observation day across each treatment. On days 1, 3, 5 and 7 focal males in the mating treatments were given the opportunity to mate. Males in the no-mating treatments remained separated from females on these days, preventing mating. On days 2, 4 and 6 a male was placed into the half pen with the female in competition treatments. On these days for no-competition treatments no male was placed into the pen with the female. Error bars represent standard error.



Supplementary Material C. Average z-score standardized total number of aerial alarm calls produced that a male gave on each observation day across each treatment. On days 1, 3, 5 and 7 focal males in the mating treatments were given the opportunity to mate. Males in the no-mating treatments remained separated from females on these days, preventing mating. On days 2, 4 and 6 a male was placed into the half pen with the female in competition treatments. On these days for no-competition treatments no male was placed into the pen with the female. Error bars represent standard error.

Chapter 5

Exploring Vigilance Behaviour in Female Red Junglefowl, *Gallus gallus*

Heather Kazara and Tommaso Pizzari

ABSTRACT

In species with sex differences in vigilance behaviour, studies have largely focused on the sex making the largest investment, neglecting the role of vigilance in the other sex. In fowl, where males are often found to be more vigilant than females, female vigilance has received little attention. This study explores the role of vigilance in female fowl. In the first part of the study, we analysed patterns of female vigilance in mixed-sex groups and investigate the effects of sex ratio and social dominance on female vigilance. While we confirm that females are less vigilant than males, we did not find any evidence that individual variation in vigilance is explained by group sex ratio or female social status. In the second part of this study, we experimentally tested whether vigilance may enable a female to obtain information on the location of food by observing another female. Specifically, we explored how food knowledge impacted vigilance by assigning pairs of females to one of three treatments, one in which the focal female (but not the companion) had prior information, one in which the focal was naïve and the companion informed, and the third one in which both birds were naïve. We found no evidence that focal females were more vigilant when they were naïve, and that vigilance enabled the focal female to locate the food more quickly by monitoring the informed companion. Collectively, the results of this study suggest that vigilance is limited and does not appear to play a role in female social competition and foraging strategies. Female vigilance in fowl therefore likely plays a traditional role by reducing predation risk.

INTRODUCTION

Vigilance is the ceasing of activity, usually foraging, to scan the surrounding environment for predators (Elgar, 1989; Beauchamp, 2001, 2007). There are numerous factors that influence vigilance behaviour with the most investigated being group size (Godin *et al.*, 1988; Burger & Gochfeld, 1992; Beauchamp, 2008). As group size increases individual vigilance is expected to decrease as there are more eyes scanning the surroundings (Pulliam, 1973; Beauchamp & Livoreil, 1997). This group size effect has been demonstrated in multiple species including ostriches, *Struthio camelus*, where birds in groups of three or four spend 85% of their time foraging compared to only 65% when alone due to reduced vigilance (Bertram, 1980). As well as group size, the spatial position within the group can influence the vigilance effort of an individual. Hamilton's Selfish Herd Theory (1971) states that individuals can reduce "domains of danger" by reducing distance to neighbours. Edge individuals typically display higher levels of vigilance due to this increased predation risk (Hamilton, 1971; Beauchamp, 2007), as shown in Brent geese, *Branta bernicla*, and pronghorns, *Antilocapra americana* (Inglis & Lazarus, 1981; Lipetz & Bekoff, 1982). Predation risk is not always equal among group members, for example sex could cause some individuals to be at greater risk despite group size and flock position. In sexually dimorphic species the more conspicuous sex could suffer from higher predation risk (Stuart-Fox *et al.*, 2003; Husak *et al.*, 2006), so changing group size and position may not dilute the risk as much as in the cryptic sex.

The effect of sex on vigilance behaviour is less well studied than group size and group position. Sex differences have been shown in house sparrows, *Passer domesticus*, and chaffinches, *Fringilla coelebs*, with females scanning

significantly more frequently irrespective of flock size (Beveridge & Deag, 1987). Concurringly, female white-breasted nuthatches, *Sitta carolinensis*, have also been shown to scan more often than males (Waite, 1987). Increased female vigilance can be explained in terms of maternal investment; for example, in elks, *Cervus canadensis*, mothers with nursing calves spent significantly less time feeding and significantly more time engaged in vigilance than females without calves, even when group size increased (Childress & Lung, 2003). In this species, nursing calves are very vulnerable to predation so increased vigilance could be used to monitor the calves' location (Childress & Lung, 2003). In contrast, no sex differences in vigilance behaviour have been found in some species (McDonough & Loughry, 1995), while other species are characterised by higher vigilance in males (Bertram, 1980; Hogstad, 1988; Westcott & Cockburn, 1988). Higher male vigilance could reflect increased predation pressures. In sexually dimorphic bird species males are usually bright and colourful, potentially increasing predation risk (Ligon & Zwartjes, 1995; Javůrková *et al.*, 2011). Male mallards, *Anas platyrhynchos*, in the breeding plumage were more vigilant than males in eclipse plumage (Lendrem, 1983). Male Eurasian wigeon, *Mareca penelope*, are also twice as vigilant as females when paired; which could be due to increased predation risk (Guillemain *et al.*, 2003), although another explanation is that male wigeons increase vigilance to guard their mate from competitors, or to search for extra-pair mating opportunities (Guillemain *et al.*, 2003; Portugal & Guillemain, 2011). Vigilance behaviour can therefore have social uses, as well as avoiding predation.

Social vigilance can also be used to gather information on food location and foraging opportunities (Fernández-Juricic *et al.*, 2004, 2005). Few studies

however have examined the potential link between vigilance behaviour and the ability to secure better and more profitable food.

Foraging and vigilance are often considered mutually exclusive since both require time and attention, producing trade-offs (Metcalfe & Furness, 1984; Duriez *et al.*, 2005; Beauchamp, 2009). A study on Richardson's ground squirrels, *Spermophilus richardsonii*, investigated this trade-off by assigning juveniles to two treatments, one provided with high energy food and the other with low energy. Juveniles in the higher energy food treatment were able to decrease foraging effort and dedicate more time to vigilance whereas individuals consuming lower energy food decreased vigilance efforts in favour of increased foraging (Arenz & Leger, 2000). Wading birds typically flock in large numbers, reducing predation risk through dilution effects and allowing individuals to decrease vigilance (Vines, 1980; Goss-Custard *et al.*, 1982; Wrona & Dixon, 1991; Cresswell, 1994). Large groups however, can reduce individual food intake through increased competition (Vahl *et al.*, 2005b, 2005a; Sansom *et al.*, 2008). The Eurasian oystercatcher, *Haematopus ostralegus*, uses three different feeding techniques; continuously pecking at food items found (visual-feeding), continuous probing with the bill while walking (tactile), and scanning the environment to locate food items done in a stop-run-stop manner (pause-travel) (Harris, 1967; Goss-Custard *et al.*, 1999). The foraging strategy chosen can influence vigilance since individuals need to keep heads down when using continuous techniques (visual or tactile) whereas birds using pause-travel foraging can scan for predators and look for food simultaneously (Goss-Custard *et al.*, 1999). Therefore, vigilance behaviour and foraging are not always mutually exclusive.

Another exception to the trade-off between foraging and vigilance occurs when scanning the environment provides opportunities to scrounge or steal food (kleptoparasitize) from other individuals. When foraging in large groups, individuals can choose to be either a producer, where they find food independently, or a scrounger, exploiting food patches used by others (Vahl *et al.*, 2005a; Beauchamp, 2009, 2014). In north-western crows, *Corvus caurinus*, scrounging individuals spent more time vigilant than producers, and vigilance was not higher when predators were present, suggesting vigilance in this species has primarily social uses (Robinette & Ha, 2001). Along with foraging strategy and technique the way food is distributed could affect vigilance. If food is highly dispersed in an environment then it would be difficult for individuals to defend all items, whereas if food is clumped together in one area then individuals should be more willing to engage in aggressive encounters to defend highly profitable clumps of food (Vahl *et al.*, 2005a; Beauchamp, 2009, 2014). In ruddy turnstones, *Arenaria interpres*, dominant and subdominant individuals had similar intake rates when food was evenly dispersed in the experiment however when food was clumped subdominants had less food intake when a dominant bird was present (Vahl *et al.*, 2005a). Clumped food patches favour scrounging, leading to vigilance remaining high even in large groups. Whereas more even food distribution can allow vigilance to decrease with increasing group size (Beauchamp, 2009, 2014). In some species individuals forage for very large and profitable food items that are easier to kleptoparasitize. More vigilant birds had a higher likelihood of retaining mussels from conspecifics in Eurasian oystercatchers (Goss-Custard *et al.*, 1999). Dominant individuals were the most successful at defending mussels, despite being the least vigilant. This could be due

to dominants being larger and so more likely to win in aggressive encounters. Yet subordinate birds detected twice the number of attacks as the dominants because of increased vigilance. Subordinates were less likely to win in any aggressive encounters, but increasing vigilance allowed individuals to detect attacks early enough for the mussel to be taken away (Goss-Custard *et al.*, 1999).

The relationship between food and vigilance is more complex than a mutually exclusive trade-off. Vigilance can be influenced by a combination of food availability (Beauchamp, 2009), food dispersal (Vahl *et al.*, 2005a) and kleptoparasitism (Goss-Custard *et al.*, 1999); potentially leading to an increase in vigilance to monitor neighbours. Females can experience greater nutritional demands because of egg production or gestation, therefore females could use vigilance to gain information related to food scrounging/stealing opportunities.

This study investigates the role of vigilance in female red junglefowl, *Gallus gallus*. Red junglefowl live in small groups consisting of a dominant male, subordinate males and females with a polygynandrous mating system (Pizzari & Birkhead, 2000; Johnsen *et al.*, 2001; Parker & Ligon, 2002; Pizzari & McDonald, 2019) , and where sex-specific social hierarchies modulate access to resources in males and females (Pizzari & McDonald, 2019). Red junglefowl are strongly sexually dimorphic, females are smaller with a cryptic plumage whereas males are larger and display bright, conspicuous plumage (Ligon *et al.*, 1990; Ligon & Zwartjes, 1995). In fowl both vigilance and aerial alarm calling are sexually dimorphic, with males being more vigilant than females and only males producing aerial alarm calls (Pizzari, 2003; Wilson *et al.*, 2008; Wilson *et al.*, 2010; Wilson & Evans, 2008). Vigilance effort has been linked to male social status in multiple studies, as more dominant males spend more time vigilant and

perform more aerial alarm calls (Pizzari & Birkhead, 2000; Pizzari, 2003; Wilson *et al.*, 2008). Male vigilance was examined in a feral population of domestic fowl by Pizzari (2003) by placing male pairs in visual proximity to an isolated female; finding that females were less vigilant in the presence of a male compared to alone. Females may be able to forage more efficiently by relying on males to monitor predators (Pizzari, 2003). Consistent with this idea, an experiment examining female vigilance in female-only flocks against mixed flocks in domestic fowl found that females in female-only groups spent more time vigilant (65.6 seconds per 30 minutes) compared to females in mixed groups (27.7 seconds per 30 minutes; Odén *et al.*, 2005), suggesting that females plastically reduce their vigilance effort in response to the number of males in a group. However, females are strongly food-driven, spending much time foraging for protein-rich food. Activity budgets in fowl have shown that hens spend 60% of their time ground pecking and 34% ground scratching (Dawkins, 1989). Thus, vigilance can in principle also allow females to obtain information on food availability by monitoring foraging neighbours (Robinette & Ha, 2001). Access to resources may be modulated by social status in this species, so investment in vigilance may have greater returns for dominant females, and female vigilance effort may be linked to status, which has been reported for male vigilance. Yet the effect of dominance on female vigilance is unknown. Generally, since vigilance effort is more marked in male than female fowl, most studies have focused on male anti-predator behaviours (Gyger *et al.*, 1987; Pizzari, 2003; Kokolakis *et al.*, 2010), and little is known about patterns of individual variation in female vigilance and the functional significance of female vigilance in this species.

The present study explores female vigilance using two approaches. The first part of the study tests whether female vigilance varies in response to the number of males in a group, by monitoring female vigilance in groups of four birds across three sex ratios: Female Biased, Even Ratio and Male Biased. We predicted that if the function of female vigilance is primarily predator avoidance, females decrease vigilance with increasing numbers of males present, i.e. as the group becomes male-biased because males are thought to be the more vigilant sex. We further considered the role of female social status and predicted female investment in vigilance to be positively correlated with female social status.

The second part of the study examines experimentally whether females use vigilance to gather information on food location. Females were paired, resulting in a dominant and subordinate, and assigned to one of three experimental treatments in which information on food location available to the dominant and the subdominant females was manipulated as follows: (i) the focal dominant female is informed about food location and the subordinate is naïve (informed dominant), (ii) the subordinate female is informed and the focal dominant is naïve (informed subordinate), and (iii) both the dominant focal and the subdominant female are naïve (control). If vigilance in females is mainly used for avoiding predation then food knowledge should have no effect across treatments since predation risk has not changed. However, if females primarily use vigilance to monitor neighbours to gain information on food then vigilance should differ across treatments. For example, when the focal female is informed about food location, she should be less vigilant as she already knows where the food is and does not need to monitor conspecifics. Conversely, when focal female is naïve vigilance effort should be higher. Similarly, the time taken to locate food for the dominant focal should be

reduced when the subordinate female is informed compared to when both are naïve in the control. This is because the dominant female could monitor the subordinate to gain information on food location, but this will be on average more advantageous when the subordinate is informed. The final prediction is that the focal dominant females that are more vigilant, locate the food fastest.

.

METHODS

Study System

Experiments took place at the John Krebs's Field Station in Wytham during March-April and August-September 2017; March, July-August 2018; and February 2019.

Part 1: Female vigilance and group sex ratio

Experimental Setup

This part of the study used a subset of groups from the experiment presented in Chapter 2. This chapter used a total of 14 females in 2017 and 10 in 2018, all aged between 2-6. Each female was repeated no more than twice. A total of 21 males were used across the two years, all aged between 4-7 years of age at the time they were used. A total of 13 groups of 4 were created including three Male Biased, five Even Ratio and five Female Biased. Male Only groups of 4 from Chapter 2 were excluded because they contained no females. Furthermore, all groups of 12 from Chapter 2 were also excluded because female vigilance was not recorded in these groups. Each group took six days to run, with four days of observations. Males were released into the pen on day 1 at approximately 1pm. Females were released on day 2 at the same time and observations began on day 3. All birds were released together rather than individually to reduce the chance of birds becoming more dominant through the founder effect. Observations took place for 8 hours a day in the morning and evening. Observations started 30 minutes after sunrise for 4 hours. Evening observations ended 30 minutes before sunset.

Behavioural Data

Females were defined as vigilant when adopting an upright posture while actively scanning the environment (Odén *et al.*, 2005). Vigilance was recorded via 10-

minute focal watches of each individual group member, using a stopwatch during the first 2.5 hours in the morning, and the last 2.5 hours in the evening. The order of focal watches was randomised for each session using a random number generator in R (version 1.1.423) (R Core Team, 2017). Female dominance behaviours included avoidances (bird A moved away from bird B within 1 minute) (Odén *et al.*, 2005), chases (Kim & Zuk, 2000), and dominance/aggressive pecks (Odén *et al.*, 2005). These were recorded *ad libitum* (for more details on the methods and behaviours recorded see Chapter 2).

Data Analysis

Data were analysed using two mixed models in the "lme4" package (version 1.1.21; Bates *et al.*, 2015) in R (version 1.1.423; R Core Team, 2017). The first model used both males and females using 52 data points, whereas observations were from 28 data points for the second model, which analysed females only. All dominance interactions across the 4-day period were scored with a winner and a loser in each interaction. A dominance matrix was then produced for each group using the number of winnings and losses for everyone within the group. These matrixes were entered R and used to calculate normalised David scores using the "Steepness" package (version 0.2.2; Leiva & de Vries, 2014). A dominance rank was then created using these scores, ranking females from one (top-ranking) to three (bottom-ranking). This was done to compare across the three sex ratios, which varied in the number of females (from one in the Male Biased, to three in Female Biased). Normalized David scores could not be computed for single females in the Male Biased groups. However, having no competitors means that a bird adopts a socially dominant behaviour, and can be considered socially

dominant by default. Additionally, social hierarchies were very clear between two and three individuals, so the use of dominance rank seems appropriate.

To test the effect of sex on vigilance behaviour a linear mixed model with a square-root transformed dependant variable was produced. The total number of seconds spent vigilant was entered as the dependent variable. Sex was entered as a fixed effect along with dominance rank (three-levels), sex ratio (three-levels), and year (two-levels). Bird ID and group ID were added as random effects however group ID was removed after finding it explained no variation in the model. To explore the effects of sex ratio and female social status on female vigilance effort, a linear mixed model with the total number of seconds spent vigilant as the dependent variable was created. The same fixed and random effects as described in the previous model were entered here except for sex. This model used data from females only (28 data points). Year was not significant and was removed from the model to improve model fit.

To test that the models met the assumptions they were graphically tested using qqnorm and residual vs fitted plots along with performing Shapiro-Wilk normality tests. The models met the assumptions. Results were analysed through Likelihood Ratio Tests (LRT) using the "anova" function within the "lmerTest" package (version 2.0.36; Kuznetsova *et al.*, 2017) .

Part 2: Food knowledge and female vigilance

Experimental Set Up

This experiment used a total of 19 focal females, 12 in March 2018 and 11 in February 2019, with four females being reused across the two seasons. This study also used 12 subordinate females in 2018 and 11 in 2019, four of which were also utilised in 2019. All dominant and subordinate females that were repeated were assigned to a different treatment in the following season. All females were aged between 2-7 years of age at the time they were used.

Females were housed in three groups of 12 during 2018, and two groups of 12 in 2019. Prior to the experiment each group of females was observed for two hours per day for 7 days to record dominance interactions. All dominance interactions across the 7-day period were scored with a winner and a loser in each interaction. A dominance matrix was then produced for each group using the number of winnings and losses for everyone within the group. These matrixes were entered into R and used to calculate normalized David scores using the "Steepness" package (version 0.2.2; Leiva & de Vries, 2014).

Experimental pairs of females were assembled using the normalized David scores. For this experiment the most dominant females represented the focal and were paired with the least dominant females from the same group. Dominant females only were examined to control for potential dominance effects on vigilance.

The experimental pen measured 6m x 16m. Six translucent plastic trays were placed evenly around three sides of the pen, affixed to the pen walls using zip ties (Figure 1). One side of the pen was avoided because it was adjacent to other pens housing other fowl, therefore females would naturally gravitate towards this side.

The observer sat out of sight in a sectioned off area in the pen (Figure 1; See Appendix, Supplementary Material A).

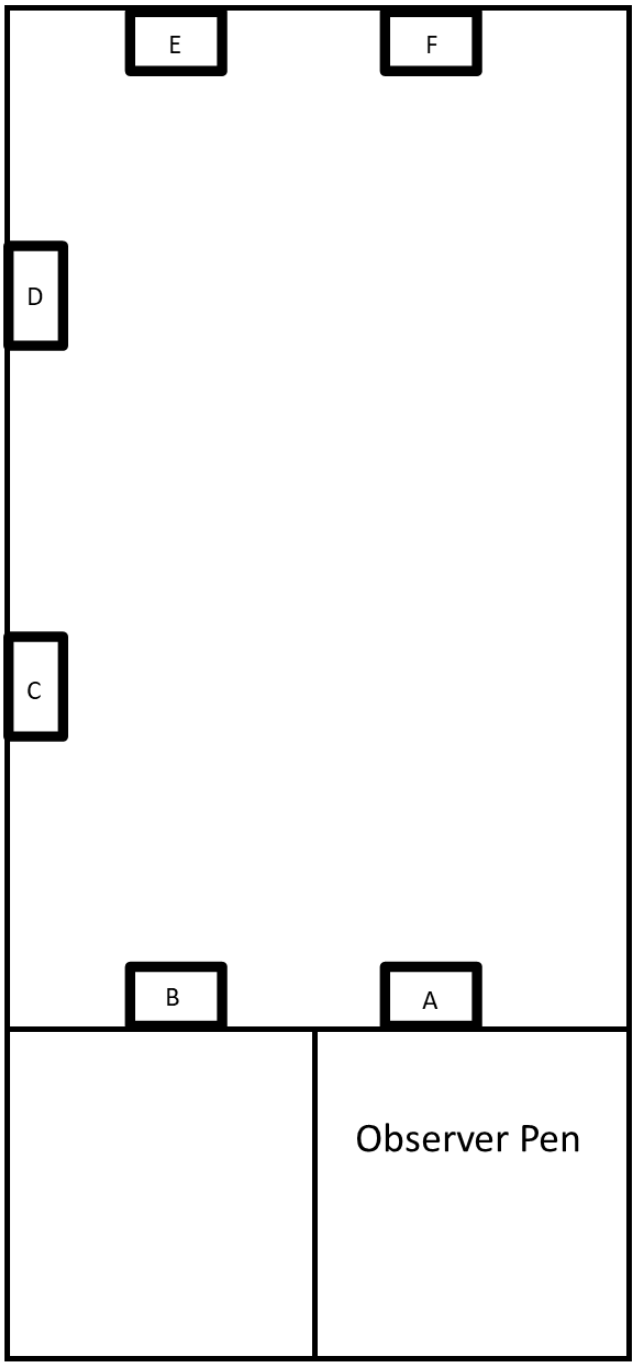


Figure 1. A diagram showing the layout of the experimental pen. The black outlined boxes represent the plastic trays used. The sectioned off area that the observer hid in to observe the females is labelled. Birds were housed in pens adjacent to the right-hand side of the pen.

Experimental Procedure

Training could only start once dominance hierarchies had been established. These pairs were assigned to one of three treatments:

1) Informed/naïve. Focal dominant female is informed about food location, the subordinate is naïve (i.e. uninformed about the location of mealworms).
2) Naïve/informed. Focal, dominant female is naïve, the subordinate is informed
3) Naïve/naïve. Both focal dominant and subordinate are naïve.

Training Phase

For the informed dominant and informed subordinate treatments (i.e. informed/naïve, and naïve/informed, respectively), one of the six plastic trays chosen at random was filled with mealworms (See Appendix, Supplementary Material A). The female (either a dominant or subordinate depending on the treatment) was placed into the arena and given a maximum of 30 minutes to locate and begin eating the mealworms, or as soon as a female located and ate the food. This was repeated three times over three successive days between 8am to 5pm. The naïve female in the pair was also placed into the pen alone across the same three days however, the mealworms were placed into a different tray on each day. This was to prevent the naïve female from learning to expect mealworms from a specific tray. On days 4-6, the same protocol was utilised but sawdust was added to all the trays. Again, mealworms were placed in the same tray for the 'informed' birds, and in a randomly chosen tray for the 'uninformed' birds (See Appendix, Supplementary Material A). The time taken to locate the food by each bird was measured.

Experimental Phase

Females were tested on the 7th day. Both members of a pair were simultaneously released in the arena and observed for a maximum of 30 minutes. In each treatment, the dominant female of each pair was selected as the focal bird. The vigilance effort for the focal female was recorded in seconds with a stopwatch. The time taken for the focal female to locate the mealworms was also recorded. For the females in 2019, time spent foraging was also recorded, which was defined as scratching and/or pecking at the ground (Beveridge & Deag, 1987; Dawkins, 1989; Andersson *et al.*, 2001). The trial ended once the focal female was within 1 body length of the box containing the food, which we assumed to reflect the focal female being aware of the mealworms. However, females were allowed to eat the mealworms before being removed from the pen.

Data Analysis

To assess the effect of treatment on the vigilance effort of the focal bird, and on the time taken to locate the food, two mixed models were created using the "lme4" package (version 1.1.21; Bates *et al.*, 2015) in R (version 1.1.423; R Core Team, 2017).

Both models included treatment (three-levels) as a fixed effect with female ID and the plastic tray used as random effects. The vigilance model also included whether the focal found the food first (yes/no) as a fixed effect and used the binomial family (logit) with the proportion of time spent vigilant as the dependant variable. This was used rather than time spent vigilant in seconds to control for the difference in trial lengths since trials ended once food was found. The second model added the proportion of time spent vigilant as a fixed effect and used the time taken to find food in seconds as the dependant variable. Both random effects

in the model exploring the time taken to find food had estimates near to zero. Therefore, random effects were removed from this model, running a linear model instead using the same fixed effects. This linear model was created using the "lm" function in the "stats" package (version 3.4.3; R Core Team, 2017). Both models used 23 data points.

Two further linear models were created to test whether foraging time affected vigilance and the time taken to locate the mealworms. Time spent foraging was not recorded during 2018, so these models used data collected in 2019 only (11 data points). Since each female in 2019 was only used once during this period ID was not needed as a repeated measure. Both linear models included the proportion of time spent foraging (covariate) as a fixed effect, with the proportion of time spent vigilant and the time taken to locate the food (seconds) as the response variables. Due to there only being 11 data points only the proportion of time spent foraging was added to these models with no other fixed effects. To further explore the relationship between foraging and vigilance/time taken to find the food Pearson's product-moment correlations were calculated. These were completed using the "cor.test" function in the "stats" package (version 3.4.3; R Core Team, 2017).

To test that the models fitted the data they were tested using the Shapiro-Wilk test from the "stats" package (version 3.4.3; R Core Team, 2017) and graphically explored using qqnorm and residual vs fitted plots. The linear models were also tested for heteroscedacity using the Breusch–Pagan test in the "lmtest" package (version 0.9.36; Zeileis & Hothorn, 2002). All four models displayed normality. Additionally the "DHARMa" package (version 0.2.0; Hartig, 2018) was used to assess the fit of the binomial model.

Results for the mixed effects models were analysed through Likelihood Ratio Tests using the "anova" function within the "lmerTest" package (version 2.0.36; Kuznetsova *et al.*, 2017). Results for the non-mixed models were achieved through F Tests using the "anova" function within the "stats" package (version 3.4.3) in R.

All the graphs displayed were produced using the "ggplot2" package (version 2.2.1; Wickham, 2009).

RESULTS

Part 1: Exploring female vigilance in mixed sex groups

Males were on average more than three times more vigilant than females (Males: 307.00 ± 51.30 (Standard Error); Females: 80.40 ± 10.70 (SE); LRT: X^2 (1) =18.79, p<0.001; Table 1; Figure 2).

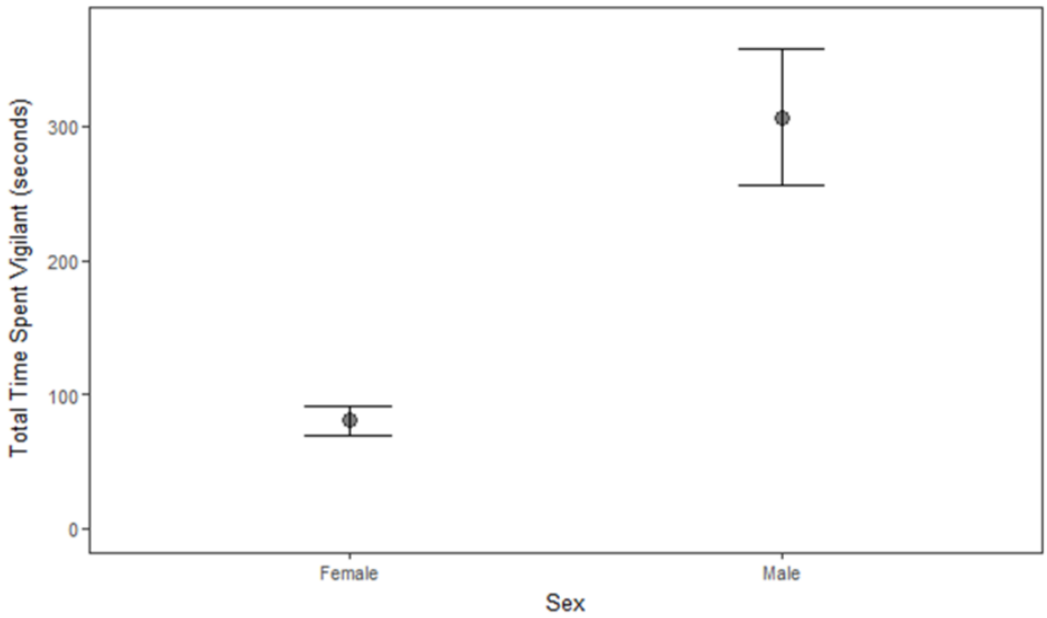


Figure 2. Average total time spent vigilant (seconds) by females and males across the 4-day observation period. Error bars represent standard error.

Table 1. Model output of the square-root transformed linear mixed model exploring male and female differences in vigilance. Year, dominance rank, sex ratio and sex were fixed effects with bird ID as a random effect. Model AIC is 326.8. Model BIC is 346.3, loglikelihood is –153.4 and deviance is 306.8.

	Total Time Spent Vigilant (seconds)		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	9.31	5.91 – 12.71	**<0.001**
Year 2018	2.57	-0.60 – 5.73	0.112
Dominance Rank 2	-5.67	-8.73 – -2.62	**<0.001**
Dominance Rank 3	-4.05	-7.73 – -0.38	**0.031**
Female Biased	1.60	-0.75 – 3.96	0.182
Male Biased	-0.89	-3.88 – 2.10	0.560
Male	8.21	4.90 – 11.53	**<0.001**
Random Effects			
σ^2	3.64		
$\tau_{00\ ID}$	26.72		
ICC	0.88		
N_{ID}	45		
Observations	52		
Marginal R^2 / Conditional R^2	0.446 / 0.934		

Sex ratio did not have a significant effect on the time spent vigilant by females (LRT: X^2 (2) =4.02, *p*=0.134; Table 2; Figure 3a). Similarly, female vigilance effort was not linked to female social status (LRT: X^2 (2) =1.24, *p*=0.538; Table 2; Figure 3b).

Table 2. Model output of the square-root transformed linear mixed model exploring the effect of dominance and sex ratio on female vigilance. Dominance rank and sex ratio are fixed effects with female ID and group ID as random effects. Model AIC is 153.0. Model BIC is 163.7 loglikelihood is –68.5 and deviance is 137.0.

	Total Time Spent Vigilant (seconds)		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	8.05	5.60 – 10.50	**<0.001**
Dominance Rank 2	-1.30	-4.04 – 1.44	0.351
Dominance Rank 3	-0.77	-4.23 – 2.68	0.661
Female Biased	2.00	-0.66 – 4.66	0.141
Male Biased	-1.76	-6.00 – 2.48	0.417
Random Effects			
σ^2	5.07		
$\tau_{00\ ID}$	3.80		
$\tau_{00\ GROUP}$	0.94		
ICC	0.48		
N_{ID}	24		
N_{GROUP}	13		
Observations	28		
Marginal R^2 / Conditional R^2	0.146 / 0.559		

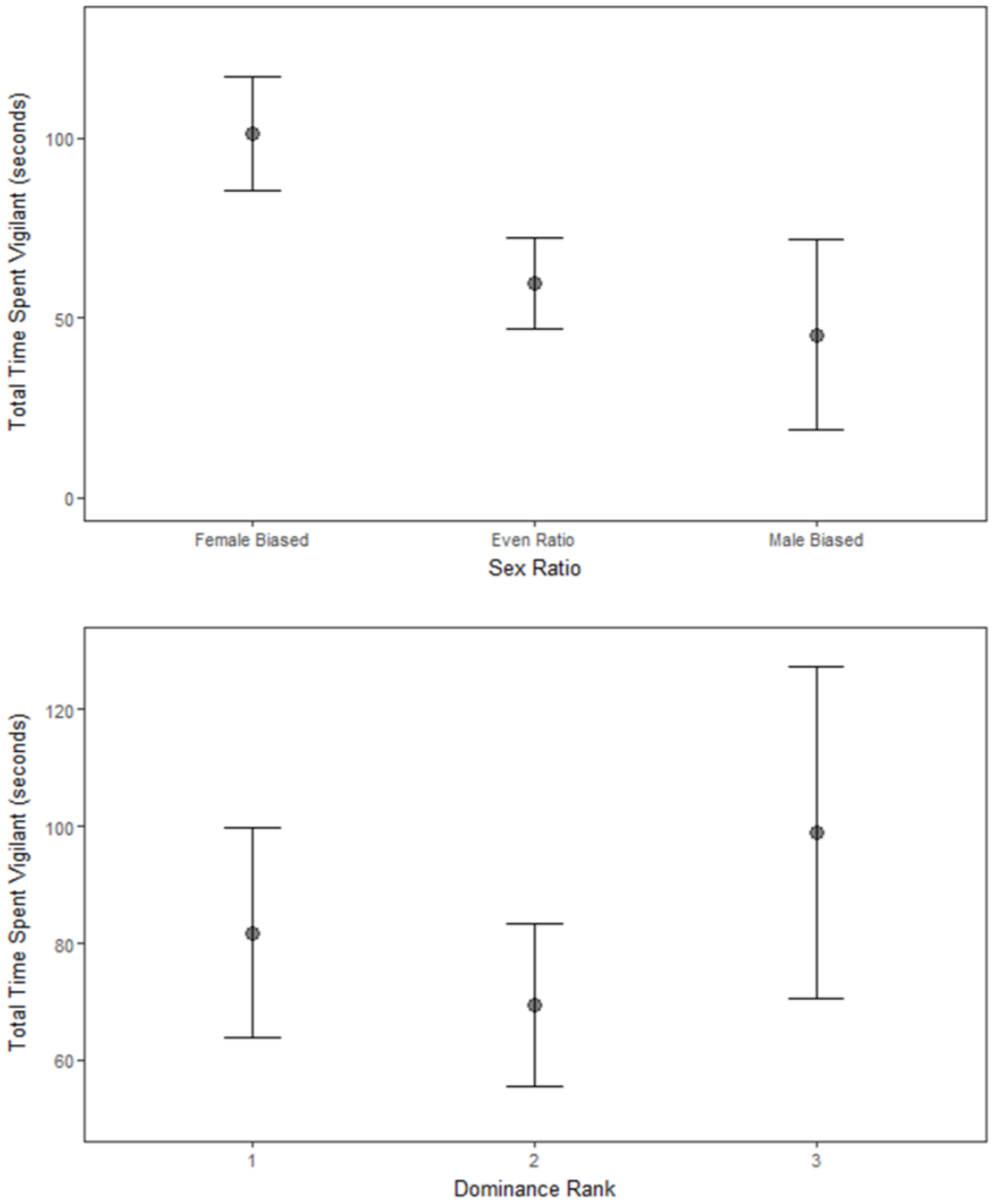


Figure 3. Average total time spent vigilant (seconds) by females across the 4-day observation period for a) each sex ratio and b) female dominance rank. Error bars represent standard error.

Part 2: Food knowledge and female vigilance

There was no significant effect of treatment on the proportion of time spent vigilant by the focal female (LRT: X^2 (2) =3.02, *p*=0.221; Table 3; Figure 4). There was also no significant effect regarding whether the focal female found the food first on the proportion of time spent vigilant (LRT: X^2 (1) =2.24, *p*=0.135; Table 3; Figure 5).

Table 3. Model output for the binomial generalized linear mixed model exploring the effect of treatment and whether food was found first by the focal female on the proportion of time spent vigilant. Whether the food was found first and the treatment were entered as fixed effects. Female ID and the tray used were random effects. Model AIC is 176.0. Model BIC is 182.8, loglikelihood is –82.0 and deviance is 164.0.

	Proportion of Time Spent Vigilant		
Coefficient	*Odds Ratios*	*Conf. Int (95%)*	*P-Value*
Intercept	0.06	0.03 – 0.10	**<0.001**
Found First Yes	0.71	0.47 – 1.09	0.114
Informed Dominant	1.52	0.96 – 2.42	0.076
Informed Subordinate	1.29	0.74 – 2.22	0.367
Random Effects			
σ^2	3.29		
$\tau_{00\ ID}$	0.15		
$\tau_{00\ TRAY_USED}$	0.31		
ICC	0.12		
N_{ID}	19		
N_{TRAY_USED}	6		
Observations	23		
Marginal R^2 / Conditional R^2	0.013 / 0.134		

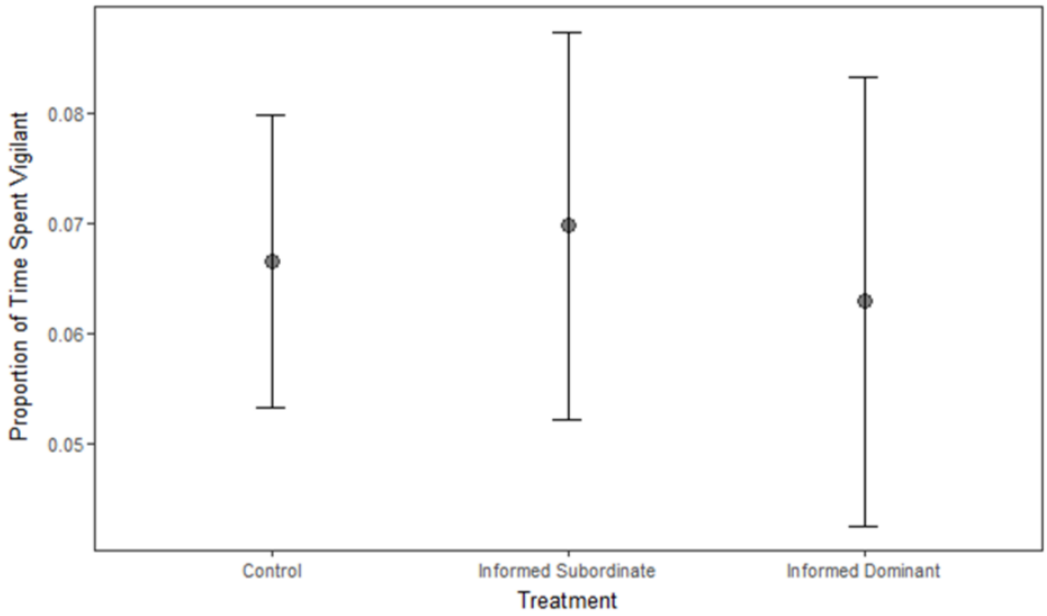


Figure 4. Average proportion of time that a focal female spent vigilant for each treatment: i.e. 'naïve/naïve' (control), 'naïve/informed' (informed subordinate), and 'informed/naïve' (informed dominant). Error bars represent standard error.

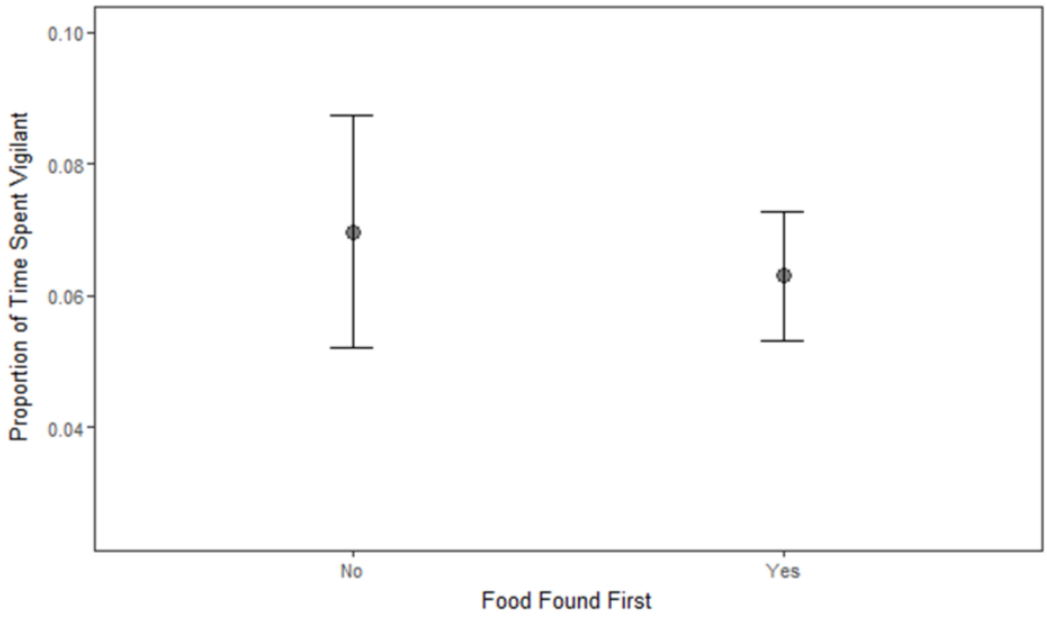


Figure 5. Average proportion of time that a female spent vigilant against whether she located the food before the subordinate. Error bars represent standard error.

Focal females in the 'naïve/informed' treatment, where the subordinate was informed about food location, found the food the fastest; although this was not significant (ANOVA: F (2,19) =0.69, *p*=0.515; Table 4; Figure 6). The proportion of time spent vigilant had no significant effect on the time taken to locate the food (ANOVA: F (1,19) =0.63, *p*=0.439; Table 4; Figure 7).

Table 4. Model output for the linear model exploring the effect of treatment and the proportion of time spent vigilant on the time taken to find the food (seconds). Model AIC is 363.7. Model BIC is 369.4 and log-likelihood is –176.8.

	Time Taken To Find Food (seconds)		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	839.20	304.44 – 1373.96	**0.006**
Proportion of Time Vigilant	-2115.42	-7405.05 – 3174.22	0.443
Informed Dominant	-269.07	-839.07 – 300.93	0.366
Informed Subordinate	-324.22	-914.13 – 265.70	0.295
Observations	23		
R^2 / adjusted R^2	0.095 / -0.048		

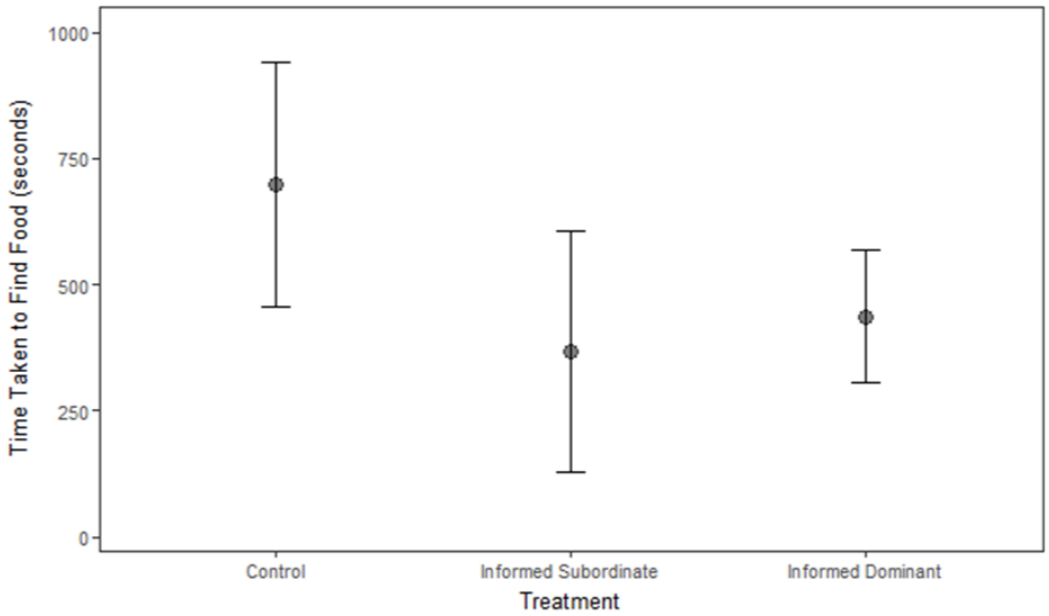


Figure 6. Average total time taken for a focal female to locate the food (seconds) for each treatment: i.e. 'naïve/naïve' (control), 'naïve/informed' (informed subordinate), and 'informed/naïve' (informed dominant). Error bars represent standard error.

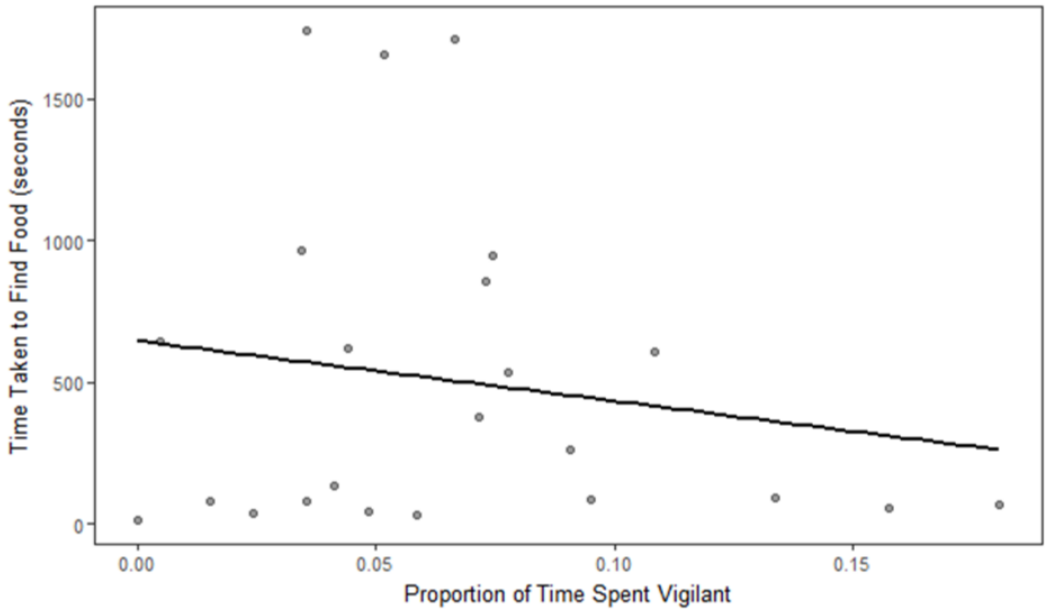


Figure 7. Proportion of time spent vigilant for each focal female against the time taken to find the food (seconds).

The proportion of time that a focal female spent foraging had no effect on the time taken to find the mealworms (ANOVA: F (1,9) =0.29, *p*=0.605; Table 5; Figure 8). However, the proportion of time spent foraging had a slight significant positive correlation with the proportion of time spent vigilant within these 11 females (ANOVA: F (1,9) =6.36, *p*= 0.033; Table 5; Figure 9). This correlation was also confirmed via a significant Pearson's product-moment correlation (r= 0.64, *p*= 0.034, n=11).

Table 5. Model output for the linear models exploring the effect of the proportion of time spent foraging on the proportion of time spent vigilant and the time taken to find the food (seconds).

	Proportion of Time Spent Vigilant			**Total Time Taken To Find Food (seconds)**		
Coefficient	*Estimates*	*Conf. Int (95%)*	*P-Value*	*Estimates*	*Conf. Int (95%)*	*P-Value*
Intercept	0.03	0.00 – 0.07	0.075	984.62	157.58 – 1811.67	**0.044**
Proportion of Time Spent Foraging	0.14	0.03 – 0.25	**0.033**	-741.53	-3450.99 – 1967.93	0.605
Observations	11			11		
R^2 / adjusted R^2	0.414 / 0.349			0.031 / -0.077		

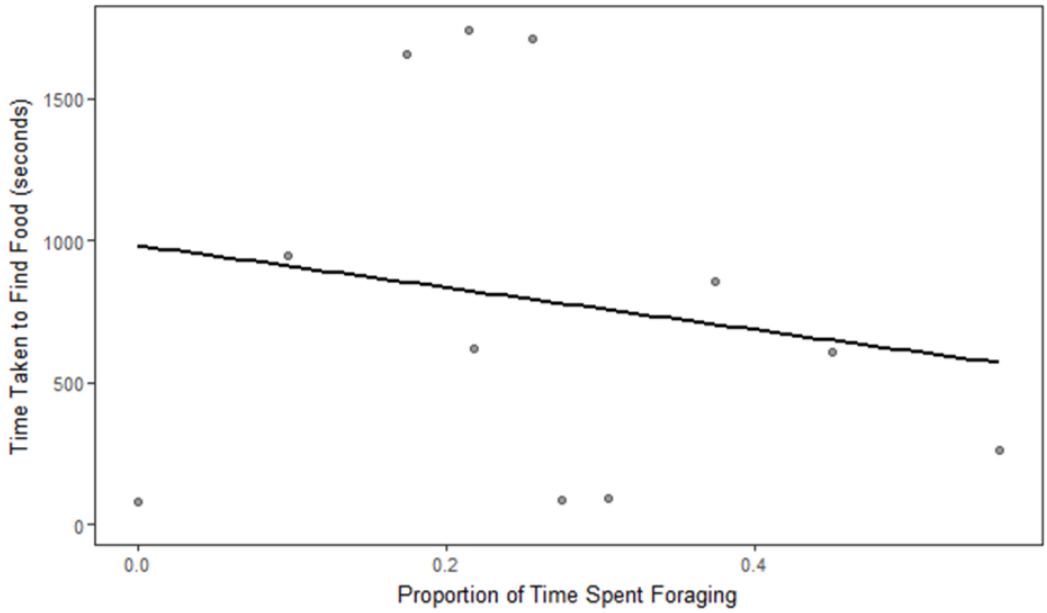


Figure 8. Proportion of time that each focal female spent foraging against the time taken to find the food (seconds).

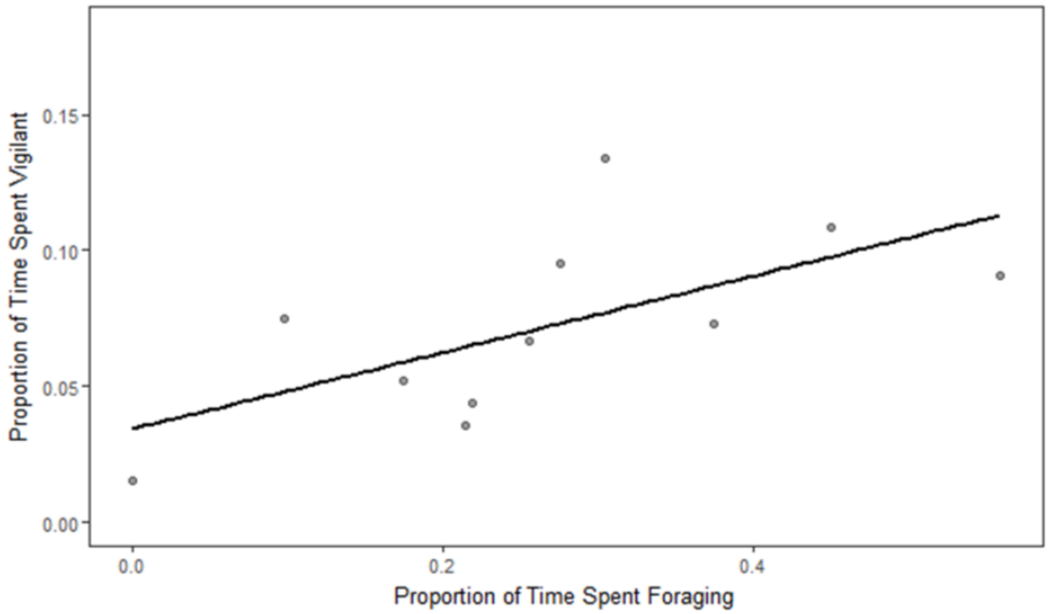


Figure 9. Proportion of time that each focal female spent foraging against the proportion of time spent vigilant.

DISCUSSION

Few studies have explored female vigilance behaviour in fowl, with this study aiming to expand the knowledge surrounding female vigilance. Females within the groups of 4 spent a very low proportion of time vigilant, spending only approximately 2% of their time engaged in this behaviour. These results are consistent with the findings of Odén et al. (2005), which found that females in mixed sex flocks also spent approximately 2% of their time vigilant. Females spent a higher proportion of time vigilant during the food experiment, spending approximately 6% of their time vigilant. During this experiment females were paired and placed into a large pen. Therefore, this increase in vigilance is likely caused by females having only one conspecific in such a large area. In addition, the lower half of the experimental pen used in the food experiment was more exposed than the pen used for the groups of 4, so females may have increased vigilance due to a perceived increased risk of predation. Although sample sizes were small there were several interesting findings albeit most lacking statistical significance. The key finding was a definite difference in vigilance behaviour between the sexes (Table 6).

Table 6. Summary of the predictions outlined in the introduction and whether the results support these predictions. Bold indicates that the prediction was supported.

	Predictions	*Results*
Part 1- Groups of 4	Sex Differences- males should be more vigilant than females, as shown by previous research.	Females were significantly less vigilant than males. **Prediction supported.**
	Sex Ratio Effects- females should reduce vigilance as groups become more male-biased because males are more vigilant.	Females were less vigilant in more male-biased groups but this lacked significance. Prediction not supported.
	Dominance- female vigilance should be positively correlated with social status, like in male fowl.	Dominance had no strong effect. Prediction not supported.
Part 2- Food Knowledge	If vigilance is primarily used to avoid predation then vigilance should not differ across treatments.	Treatment had no effect on vigilance. **Prediction supported.**
	If vigilance is used to gather information on food then it should be lowest when the focal is informed on food location and higher when she is naïve.	Treatment had no effect on vigilance. Prediction not supported.
	If vigilance is used to find food then there should be a negative correlation between time spent vigilant and time taken to locate the food.	Vigilance had no significant correlation with time taken to find the food. Prediction not supported.

Part 1: Exploring female vigilance in mixed sex groups

Sex Differences in Vigilance

The findings of this study show a significant difference between male and female vigilance, which could help explain sex differences in vigilance in other sexually dimorphic species. Some studies show that males are more vigilant than females for example, male ostriches are significantly more vigilant than females when alone (Bertram, 1980). However, males are not always the more vigilant sex, with females scanning significantly more frequently in house sparrows and chaffinches (Beveridge & Deag, 1987). In sexually dimorphic birds, males are usually more conspicuous, potentially leading to increased predation risk; thus higher vigilance in males compared to cryptic females (Lendrem, 1983; Mayhew, 1987; Guillemain *et al.*, 2003). Flashy plumage has long been considered a handicap in relation to predation risk though some studies have shown no increased risk from bright colouration (Götmark, 1993; Cain *et al.*, 2019). However, other research suggests that males might increase vigilance efforts to invest in mated females through increased protection from predation and/or guarding from male rivals (Guillemain *et al.*, 2003; Squires *et al.*, 2007; Portugal & Guillemain, 2011). Conversely, higher vigilance in males might allow females to reduce individual vigilance and increase time spent foraging allowing better egg production (Squires *et al.*, 2007; Portugal & Guillemain, 2011). Both male investment in mated females and increased female benefits through more foraging might explain why females were significantly less vigilant than males in this experiment.

Sex Ratio and Female Vigilance

Although sex ratio had no significant effect, female vigilance was lower when more males were present. Previous work has shown that male fowl who mate

increase both the number of alarm calls initiated (Wilson & Evans, 2008) and time spent vigilant (See Chapter 4). Hence increased vigilance post-copulation may explain why male vigilance was on average significantly higher than in females. However higher male vigilance could lead to females benefitting through lower vigilance and increased foraging as a by-product. Since foraging behaviour was not recorded, nor were any female-only groups of 4 tested, it cannot be known whether females in mixed sex groups were less vigilant and/or foraged more than if males were absent.

More studies should re-evaluate sex differences in vigilance within sexually dimorphic species by examining whether males are targeted more frequently by predators along with whether female vigilance greatly decreases when grouped with males compared to females and when solitary.

Dominance and Female Vigilance

No significant effect of social status on vigilance was found in female fowl despite higher social status being linked to increased vigilance and alarm calling effort in males (Pizzari, 2003; Wilson *et al.*, 2008). Although, vigilance was slightly higher in the lowest ranking female. Bottom ranking females could receive more aggressive interactions from the more dominant females, leading to this higher vigilance to avoid encounters. Research has shown that male presence reduces female aggression, with aggressive interactions in ISA Brown laying hens being 63% higher in single sex groups (Odén *et al.*, 1999). Therefore, more males in a group may lead to less female-female aggression so reduce the need for vigilance to avoid dominant females.

Alternatively, males have been shown to prefer mating and investing sperm in females with larger combs (Pizzari *et al.*, 2003). In domestic fowl, *Gallus gallus domesticus*, comb size and dominance appear to be linked in females, with females becoming subordinate after combs had been experimentally altered (Marks *et al.*, 1960). As comb length is linked with dominance in males, and females with larger combs are preferred by males (Cornwallis & Birkhead, 2007), it is possible that comb length and dominance are linked in female red junglefowl. If this was true then higher ranking females in this study were potentially selected more for copulations and sperm investment, potentially leading to increased benefits from male vigilance efforts. This may explain the lower vigilance in first and second ranking females compared to third ranking. However, it is important to note that dominance had no significant effect on female vigilance within this study, so no concrete conclusions can be drawn here.

Part 2: Food knowledge and female vigilance

In this study the proportion of time invested in vigilance was not affected by treatment suggesting that females do not use vigilance to gain food information. Although the proportion of time spent vigilant was lowest when dominants were informed of food location yet highest when subordinate females were knowledgeable, which would be expected if females use vigilance to detect food by exploiting subordinates. In barnacle geese, *Branta leucopsis*, dominant individuals engaged in more vigilant behaviour than subordinates despite foraging time being similar, and exploited food plots discovered by exploratory subordinates (Stahl *et al.*, 2001). Unlike with barnacle geese, our study did not find any evidence to support the assumption that female fowl use vigilance to aid in food scrounging. Since predation risk across treatments was equal, the lack of

effect from treatment could indicate that females primarily use vigilance to avoid predation, not to monitor neighbours. The sample size for this experiment was very low using only 23 data points and 19 females, thus the lack of significance is possibly due to sample size issues. Yet this experiment shows an interesting pattern in vigilance behaviour and food knowledge which should be explored further in future studies.

Dominance and Exploration

There was a non-significant trend for focal females in the informed subordinate ('naïve/informed') treatment to locate the food the fastest. In some bird species, dominant individuals are less exploratory (Wiley, 1991; Stahl *et al.*, 2001). For example, less exploratory individuals were more likely to win in dominance interactions against more exploratory birds in mountain chickadees, *Poecile gambeli* (Fox *et al.*, 2009). Therefore, if subordinates were indeed more exploratory then this would explain why females in treatments with informed subordinates found food the quickest, as dominant females could exploit the greater exploratory ability in subordinates to locate better food patches. However, like with vigilance, treatment had no significant effect on the time taken to find the food.

Contrafreeloading

Dominant focal females in the 'informed/naïve' treatment were trained across the 6-day period to always expect food from the same plastic tray. Therefore, on the test day these females should have gone towards the tray they knew contained the mealworms, yet these females took longer to approach the food than females in the informed subordinate treatment. This could be a form of contrafreeloading, which refers to animals choosing to work for food rather than selecting the food

freely provided (Lindqvist *et al.*, 2006; Lindqvist & Jensen, 2008, 2009). Furthermore, red junglefowl have been shown to spend 60-70% of their time engaged in contrafreeloading (Schütz & Jensen, 2001; Lindqvist *et al.*, 2002), likely explaining why informed females did not immediately go to the known food source.

Foraging and Vigilance

Unexpectedly there appears to be a strong positive relationship between the proportion of time spent foraging and the proportion of time dedicated to vigilance, which was significant. Most studies suggest that foraging and vigilance behaviour are mutually exclusive requiring trade-offs, with increased time foraging leading to less vigilance (Metcalfe & Furness, 1984; Pöysä, 1987; Elgar, 1989; Arenz & Leger, 2000; Duriez *et al.*, 2005). However a positive relationship between vigilance and peck rate during foraging was shown in chaffinches with individuals that foraged faster needing less time with heads down, increasing predation detection (Cresswell *et al.*, 2003). Exposure to a hawk model revealed that individuals performing more head-ups detected the model quicker. So faster more efficient foragers can increase intake rate whilst also increasing avian predator detection by raising heads during seed handling (Cresswell *et al.*, 2003). Fowl do not have long food handling times for mealworms so increasing vigilance while handling food does not explain the relationship here. An increase in scanning may aid in finding scrounging opportunities, leading to increased foraging; potentially explaining this positive relationship.

The experimental pen used was adjacent to pens containing males, and both males and the females used were in visual contact during this experiment. Females have been shown to decrease vigilance in the presence of males (Pizzari, 2003).

Therefore, females may have increased foraging activity by relying on males for additional vigilance, potentially overcoming the trade-off between vigilance and foraging. The models examining foraging behaviour used a subset of 11 females because foraging was not recorded during the first year of study, so it is impossible to draw any definitive conclusions on foraging behaviour as this pattern could simply be an anomaly in this subset. Future studies should explore the relationship between foraging and vigilance in female fowl to test whether this positive relationship remains; contradicting research showing vigilance decreasing with increased foraging (Metcalfe & Furness, 1984; Arenz & Leger, 2000; Duriez *et al.*, 2005). Future work should also examine female vigilance and foraging without males present to see whether females only by-pass this trade-off due to additional vigilance from males.

Conclusions

Vigilance in female fowl has been largely ignored, with very few studies exploring this behaviour. The results from the first experiment support previous findings that female are significantly less vigilant than males (Pizzari, 2003). Despite social dominance being linked to vigilance behaviour in males vigilance was not influenced by dominance rank in females. However male presence can mediate aggression between females, so it is possible that this affected the relationship between female dominance and vigilance. Future studies should examine vigilance and dominance in female-only groups.

Due to female fowl spending much of their time foraging it was plausible that females primarily used vigilance to gain information on food. However female vigilance was not significantly affected by food knowledge in this study. Sample size was an obvious concern with the sample size for the second experiment being

particularly low, even more so when exploring foraging behaviour. The low sample size likely reduced the statistical power of the analysis. Rather than combining these two studies into one chapter, it may have been better to separate them to allow a more detailed examination of female vigilance in fowl, particularly when looking at social information. Although, due to both time limitations and a lack of females, a more sophisticated approach was not feasible. More studies should focus on female vigilance in fowl to address the unanswered questions surrounding its function and potential relationship to food finding.

REFERENCES

Andersson, M., Nordin, E. & Jensen, P. (2001). Domestication effects on foraging strategies in fowl. *Applied Animal Behaviour Science,* **72**, 51–62.

Arenz, C.L. & Leger, D.W. (2000). Antipredator vigilance of juvenile and adult thirteen-lined ground squirrels and the role of nutritional need. *Animal Behaviour,* **59**, 535–541.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software,* **67**, 1–48.

Beauchamp, G. (2001). Should vigilance always decrease with group size? *Behavioural Ecology and Sociobiology,* **51**, 47–52.

Beauchamp, G. (2007). Vigilance in a selfish herd. *Animal Behaviour*, **73**, 445–451.

Beauchamp, G. (2008). What is the magnitude of the group-size effect on vigilance? *Behavioural Ecology,* **19**, 1361–1368.

Beauchamp, G. (2009). How does food density influence vigilance in birds and mammals? *Animal Behaviour,* **78**, 223–231.

Beauchamp, G. (2014). A field investigation of scrounging in semipalmated sandpipers. *Behavioural Ecology and Sociobiology,* **68**, 1473–1479.

Beauchamp, G. & Livoreil, B. (1997). The effect of group size on vigilance and feeding rate in spice finches (*Lonchura punctulata*). *Canadian Journal of Zoology,* **75**, 1526–1531.

Bertram, B.C.R. (1980). Vigilance and group size in ostriches. *Animal Behaviour,* **28**, 278–286.

Beveridge, F.M. & Deag, J.M. (1987). The Effects of Sex, Temperature and Companions on Looking-Up and Feeding in Single and Mixed Species Flocks of House Sparrows (*Passer domesticus*), Chaffinches (*Fringilla coelebs*), And Starlings (*Sturnus vulgaris*). *Behaviour*, **100**, 303–319.

Burger, J. & Gochfeld, M. (1992). Effect of group size on vigilance while drinking in the coati, *Nasua narica* in Costa Rica. *Animal Behaviour,* **44**, 1053–1057.

Cain, K.E., Hall, M.L., Medina, I., Leitao, A.V., Delhey, K., Brouwer, L., Peters, A., Pruett-Jones, S., Webster, M.S., Langmore, N.E. & Mulder, R.A. (2019). Conspicuous Plumage Does Not Increase Predation Risk: A Continent-Wide Test Using Model Songbirds. *The American Naturalist,* **193**, 359–372.

Childress, M.J. & Lung, M.A. (2003). Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Animal Behaviour,* **66**, 389–398.

Cornwallis, C.K. & Birkhead, T.R. (2007). Changes in Sperm Quality and Numbers in Response to Experimental Manipulation of Male Social Status and Female Attractiveness. *The American Naturalist,* **170**, 758–770.

Cresswell, W. (1994). Flocking is an effective anti-predation strategy in redshanks, *Tringa totanus*. *Animal Behaviour,* **47**, 433–442.

Cresswell, W., Quinn, J.L., Whittingham, M.J. & Butler, S. (2003). Good foragers can also be good at detecting predators. *Proceedings of the Royal Society of London B: Biological Sciences,* **270**, 1069–1076.

David Leiva & Han de Vries. (2014). Steepness: testing steepeness of dominance hierarchies. R package version 0.2.2. Available at: http://CRAN.R-project.org/package=steepness.

Dawkins, M.S. (1989). Time budgets in Red Junglefowl as a baseline for the assessment of welfare in domestic fowl. *Applied Animal Behaviour Science*, **24**, 77–80.

Duriez, O., Fritz, H., Binet, F., Tremblay, Y. & Ferrand, Y. (2005). Individual activity rates in wintering Eurasian woodcocks: starvation versus predation risk trade-off? *Animal Behaviour,* **69**, 39–49.

Elgar, M.A. (1989). Predator Vigilance and Group Size in Mammals and Birds: A Critical Review of the Empirical Evidence. *Biological Reviews,* **64**, 13–33.

Fernández-Juricic, E., Erichsen, J.T. & Kacelnik, A. (2004). Visual perception and social foraging in birds. *Trends in Ecology & Evolution,* **19**, 25–31.

Fernández-Juricic, E., Smith, R. & Kacelnik, A. (2005). Increasing the costs of conspecific scanning in socially foraging starlings affects vigilance and foraging behaviour. *Animal Behaviour,* **69**, 73–81.

Fox, R.A., Ladage, L.D., Roth, T.C. & Pravosudov, V.V. (2009). Behavioural profile predicts dominance status in mountain chickadees, *Poecile gambeli*. *Animal Behaviour,* **77**, 1441–1448.

Godin, J.-G.J., Classon, L.J. & Abrahams, M.V. (1988). Group Vigilance and Shoal Size in a Small Characin Fish. *Behaviour,* **104**, 29–40.

Goss-Custard, J.D., Cayford, J.T. & Lea, S.G. (1999). Vigilance during food handling by Oystercatchers *Haematopus ostralegus* reduces the chances of losing prey to kleptoparasites. *Ibis,* **141**, 368–376.

Goss-Custard, J.D., Sitters, H.P. & Swinfen, R. (1982). Age-structure and survival of a wintering population of Oystercatchers. *Bird Study,* **29**, 83–98.

Götmark. (1993). Conspicuous coloration in male birds is favoured by predation in some species and disfavoured in others. *Proceedings of the Royal Society of London B: Biological Sciences,* **253**, 143–146.

Guillemain, M., Caldow, R.W.G., Hodder, K.H. & Goss-Custard, J.D. (2003). Increased vigilance of paired males in sexually dimorphic species: distinguishing between alternative explanations in wintering Eurasian wigeon. *Behavioural Ecology*, **14**, 724–729.

Gyger, M., Marler, P. & Pickert, R. (1987). Semantics of an Avian Alarm Call System: The Male Domestic Fowl, *Gallus domesticus*. *Behaviour*, **102**, 15–40.

Hamilton, W.D. (1971). Geometry for the selfish herd. *Journal of Theoretical Biology*, **31**, 295–311.

Harris, M.P. (1967). The Biology of Oystercatchers *Haematopus Ostralegus* on Skokholm Island, S. Wales. *Ibis*, **109**, 180–193.

Hartig, F. (2018). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.2.0. http://florianhartig.github.io/DHARMa/.

Hogstad, O. (1988). Advantages of social foraging of Willow Tits *Parus montanus*. *Ibis*, **130**, 275–283.

Husak, J.F., Macedonia, J.M., Fox, S.F. & Sauceda, R.C. (2006). Predation Cost of Conspicuous Male Coloration in Collared Lizards (*Crotaphytus collaris*): An Experimental Test Using Clay-Covered Model Lizards. *Ethology*, **112**, 572–580.

Inglis, I.R. & Lazarus, J. (1981). Vigilance and Flock Size in Brent Geese: The Edge Effect. *Zeitschrift für Tierpsychologie*, **57**, 193–200.

Javůrková, V., Hořák, D., Kreisinger, J., Klvaňa, P. & Albrecht, T. (2011). Factors Affecting Sleep/vigilance Behaviour in Incubating Mallards. *Ethology*, **117**, 345–355.

Johnsen, T.S., Zuk, M. & Fessler, E.A. (2001). Social Dominance, Male Behaviour and Mating in Mixed-Sex Flocks of Red Junglefowl. *Behaviour*, **138**, 1–18.

Kim, T. & Zuk, M. (2000). The effects of age and previous experience on social rank in female red junglefowl, *Gallus gallus*. *Animal Behaviour*, **60**, 239–244.

Kokolakis, A., Smith, C.L. & Evans, C.S. (2010). Aerial alarm calling by male fowl (*Gallus gallus*) reveals subtle new mechanisms of risk management. *Animal Behaviour*, **79**, 1373–1380.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, **82**, 1–26.

Lendrem, D.W. (1983). Sleeping and vigilance in birds. I. Field observations of the mallard (*Anas platyrhynchos*). *Animal Behaviour*, **31**, 532–538.

Ligon, J.D., Thornhill, R., Zuk, M. & Johnson, K. (1990). Male-male competition, ornamentation and the role of testosterone in sexual selection in red junglefowl. *Animal Behaviour,* **40**, 367–373.

Ligon, J.D. & Zwartjes, P.W. (1995). Ornate plumage of male red junglefowl does not influence mate choice by females. *Animal Behaviour,* **49**, 117–125.

Lindqvist, C. & Jensen, P. (2008). Effects of age, sex and social isolation on contrafreeloading in red junglefowl (*Gallus gallus*) and White Leghorn fowl. *Applied Animal Behaviour Science,* **114**, 419–428.

Lindqvist, C. & Jensen, P. (2009). Domestication and stress effects on contrafreeloading and spatial learning performance in red junglefowl (*Gallus gallus*) and White Leghorn layers. *Behavioural Processes,* **81**, 80–84.

Lindqvist, C., Zimmerman, P. & Jensen, P. (2006). A note on contrafreeloading in broilers compared to layer chicks. *Applied Animal Behaviour Science,* **101**, 161–166.

Lindqvist, C.E.S., Schütz, K.E. & Jensen, P. (2002). Red Junglefowl Have More Contrafreeloading than White Leghorn Layers: Effect of Food Deprivation and Consequences for Information Gain. *Behaviour,* **139**, 1195–1209.

Lipetz, V.E. & Bekoff, M. (1982). Group Size and Vigilance in Pronghorns. *Zeitschrift für Tierpsychologie,* **58**, 203–216.

Marks, H.L., Siegel, P.B. & Kramer, C.Y. (1960). Effect of comb and wattle removal on the social organization of mixed flocks of chickens. *Animal Behaviour,* **8**, 192–196.

Mayhew, P.W. (1987). Vigilance levels in European Wigeon - sexual differences. *Wildfowl,* **38**, 77–81.

McDonough, C.M. & Loughry, W.J. (1995). Influences on Vigilance in Nine-banded Armadillos. *Ethology,* **100**, 50–60.

Metcalfe, N.B. & Furness, R.W. (1984). Changing priorities: the effect of pre-migratory fattening on the trade-off between foraging and vigilance. *Behavioural Ecology and Sociobiology,* **15**, 203–206.

Odén, K., Gunnarsson, S., Berg, C. & Algers, B. (2005). Effects of sex composition on fear measured as tonic immobility and vigilance behaviour in large flocks of laying hens. *Applied Animal Behaviour Science,* **95**, 89–102.

Odén, K., Vestergaard, K.S. & Algers, B. (1999). Agonistic behaviour and feather pecking in single-sexed and mixed groups of laying hens. *Applied Animal Behaviour Science*, **62**, 219–231.

Parker, T.H. & Ligon, D.J. (2002). Dominant male red junglefowl (*Gallus gallus*) test the dominance status of other males. *Behavioural Ecology and Sociobiology*, **53**, 20–24.

Pizzari, T. (2003). Food, vigilance, and sperm: the role of male direct benefits in the evolution of female preference in a polygamous bird. *Behavioural Ecology*, **14**, 593–601.

Pizzari, T. & Birkhead, T.R. (2000). Female feral fowl eject sperm of subdominant males. *Nature*, **405**, 787–789.

Pizzari, T., Cornwallis, C.K., Løvlie, H., Jakobsson, S. & Birkhead, T.R. (2003). Sophisticated sperm allocation in male fowl. *Nature*, **426**, 70–74.

Pizzari, T. & McDonald, G.C. (2019). Sexual selection in socially-structured, polyandrous populations: Some insights from the fowl. *Advances in the Study of Behavior*, **51**, 77–141.

Portugal, S.J. & Guillemain, M. (2011). Vigilance patterns of wintering Eurasian Wigeon: female benefits from male low-cost behaviour. *Journal of Ornithology*, **152**, 661–668.

Pöysä, H. (1987). Feeding-Vigilance Trade-Off in the Teal (*Anas crecca*): Effects of Feeding Method and Predation Risk. *Behaviour*, **103**, 108–122.

Pulliam, H.R. (1973). On the advantages of flocking. *Journal of Theoretical Biology*, **38**, 419–422.

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Robinette, R.L. & Ha, J.C. (2001). Social and ecological factors influencing vigilance by northwestern crows, *Corvus caurinus*. *Animal Behaviour*, **62**, 447–452.

Sansom, A., Cresswell, W., Minderman, J. & Lind, J. (2008). Vigilance benefits and competition costs in groups: do individual redshanks gain an overall foraging benefit? *Animal Behaviour*, **75**, 1869–1875.

Schütz, K.E. & Jensen, P. (2001). Effects of Resource Allocation on Behavioural Strategies: A Comparison of Red Junglefowl (*Gallus gallus*) and Two Domesticated Breeds of Poultry. *Ethology*, **107**, 753–765.

Squires, K.A., Martin, K., Goudie, R.I. & Yasukawa, K. (2007). Vigilance Behavior in the Harlequin Duck (*Histrionicus histrionicus*) during the Preincubation Period in Labrador: Are Males Vigilant for Self or Social Partner? *The Auk*, **124**, 241–252.

Stahl, J., Tolsma, P.H., Loonen, M.J.J.E. & Drent, R.H. (2001). Subordinates explore but dominants profit: resource competition in high Arctic barnacle goose flocks. *Animal Behaviour*, **61**, 257–264.

Stuart-Fox, D.M., Moussalli, A., Marshall, N.J. & Owens, I.P.F. (2003). Conspicuous males suffer higher predation risk: visual modelling and experimental evidence from lizards. *Animal Behaviour,* **66**, 541–550.

Vahl, W.K., Lok, T., van der Meer, J., Piersma, T. & Weissing, F.J. (2005a). Spatial clumping of food and social dominance affect interference competition among ruddy turnstones. *Behavioural Ecology,* **16**, 834–844.

Vahl, W.K., van der Meer, J., Weissing, F.J., van Dullemen, D. & Piersma, T. (2005b). The mechanisms of interference competition: two experiments on foraging waders. *Behavioural Ecology,* **16**, 845–855.

Vines, G. (1980). Spatial consequences of aggressive behaviour in flocks of oystercatchers, *Haematopus ostralegus*. *Animal Behaviour,* **28**, 1175–1183.

Waite, T.A. (1987). Vigilance in the White-Breasted Nuthatch: Effects of Dominance and Sociality. *The Auk,* **104**, 429–434.

Westcott, D.A. & Cockburn, A. (1988). Flock Size and Vigilance in Parrots. *Australian Journal of Zoology,* **36**, 335–349.

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*, Use R! New York: Springer-Verlag.

Wiley, R.H. (1991). Both High- and Low-Ranking White-Throated Sparrows Find Novel Locations of Food. *The Auk,* **108**, 8–15.

Wilson, D.R., Bayly, K.L., Nelson, X.J., Gillings, M. & Evans, C.S. (2008). Alarm calling best predicts mating and reproductive success in ornamented male fowl, *Gallus gallus*. *Animal Behaviour,* **76**, 543–554.

Wilson, D.R. & Evans, C.S. (2008). Mating success increases alarm-calling effort in male fowl, *Gallus gallus*. *Animal Behaviour,* **76**, 2029–2035.

Wilson, D.R., McDonald, P.G. & Evans, C.S. (2010). Mechanisms of Mate Investment in the Polygamous Fowl, *Gallus gallus*. *Ethology,* **116**, 755–762.

Wrona, F.J. & Dixon, R.W.J. (1991). Group Size and Predation Risk: A Field Analysis of Encounter and Dilution Effects. *The American Naturalist,* **137**, 186–201.

Zeileis, A. & Hothorn, T. (2002). Diagnostic Checking in Regression Relationships. R version 0.9.36. https://CRAN.R-project.org/doc/Rnews/

APPENDIX



Supplementary Material A. Four photographs taken in March 2018 showing the experimental setup. The first photograph (a) shows the size of the experimental pen while the 2nd photograph (b) is showing the sectioned off area that the observer hid in to observe the females. Photographs c and d show live mealworms inside translucent plastic trays, with d showing a sawdust filled tray.

Chapter 6

Discussion

The overall aim of my thesis was to explore the role of both vigilance and aerial alarm calling behaviour in the red junglefowl, finding sex differences in this species. Chapters 2-4 examined males by testing hypotheses related to sexual selection and reproductive strategies. Both vigilance and alarm calling differed across sex ratios in Chapter 2, with an overall decrease in these behaviours in more male-biased groups. The key findings include both vigilance and alarm calling being correlated with mating activity, with Chapter 3 showing positive correlations with the number of matings, number of females mated and average remating rates per female. While Chapter 4 experimentally tested three hypotheses finding that vigilance and alarm calling are unlikely to be correlated with mating due to the mate investment hypothesis. Chapter 5 explored the role of vigilance behaviour in females showing that females were significantly less vigilant than males however there was no evidence that females used vigilance to gain information on food location.

Vigilance, Alarm Calling and Mating

The key finding in this thesis is that both male vigilance and alarm calling efforts are positively related to different measures of male reproductive performance (e.g. mating success). Wilson et al. (2008) was the first study to show that alarm calling was a good predictor of male fowl mating success, suggesting either male attraction or mate investment as the cause. Wilson et al. (2008) suggested that alarm calling could be a sexually selected trait in fowl, proposing three potential explanations. First, females could gain direct benefits from selecting males that alarm call more frequently through reduced predation risk. Second, females could gain indirect genetic benefits by mating with alarm calling males because high alarm calling rates could advertise a male's ability to escape predation since only

males that were able to avoid an attack could afford to increase conspicuousness through frequent calling. Third, alarm calling in fowl is a testosterone-dependant trait, and high testosterone levels cause compromised immunity (Gyger *et al.*, 1988; Folstad & Karter, 1992; Peters, 2000; Mougeot *et al.*, 2004), therefore alarm calling could be an honest signal of male health (Zahavi, 1975). Alternatively, Wilson et al. (2008) suggested that alarm calling could be linked to mating success because males that have recently mated increase alarm calling investment to protect reproductive partners, and prospective offspring. Thus, Wilson & Evans (2008) aimed to test whether male alarm calling effort in a female increased post-mating by experimentally allowing males in one treatment to mate whilst males in the control treatment were exposed to a female but prevented from mating. Males that could mate with a female increased alarm calling effort by approximately 30% during the two-week period when males could mate. Yet, even when they were once again separated from females during the final week, males in the experimental treatment continued to produce 20% more alarm calls than control males. This was the first experimental evidence that mating has a causal effect on aerial alarm calling effort (Wilson & Evans, 2008). Wilson & Evans (2008) suggested that males could gain direct reproductive benefits from increasing the survival of reproductive partners that accompany males during nest building and egg laying periods (Wilson & Evans, 2008). Another study experimentally tested the male attractiveness hypothesis in fowl by experimentally increasing or decreasing males' aerial alarm calling efforts in three separate mate choice experiments. Wilson and Evans (2010) found that female fowl did not prefer better alarm callers, suggesting mate investment rather than male attractiveness. A fourth study built on the findings of Wilson and Evans

(2008), by testing the mechanisms causing increased alarm calling post-mating (Wilson *et al.*, 2010). Males were provided with two females, and during week one males could mate with only one female. Either the mated or the non-mated female was removed during the second week and alarm calling behaviour monitored. Male alarm calling effort did not change regardless of whether the mated or non-mated female was removed, suggesting that males do not invest selectively into females whom they have mated with (Wilson *et al.*, 2010). Wilson et al. (2010) proposed that the increase in alarm calling post-copulation could be explained by males using recent mating success as a cue to increase alarm calling efforts (Wilson *et al.*, 2010). In natural conditions, fowl form harems during the breeding season, therefore using recent mating activity as a cue could benefit males by further increasing the survival of his harem females; thus, increasing reproductive success. There were no endocrine changes shown pre-, during and post-mating, suggesting hormones are not causing this behaviour (Wilson *et al.*, 2010). The consensus from these four studies, and Chapters 3 and 4, is that male alarm calling is linked to mating behaviour, with recent mating success causing an increase, potentially due to mating acting as a cue for investment.

Chapter 4 was able to experimentally test mate investment. Wilson and Evans (2008) suggested mate investment as the most likely explanation of the patterns of male alarm calling observed, however they failed to test how paternity confidence influences this male alarm calling investment. If males increase investment in anti-predator protection for the offspring of their own reproductive partners, then males should reduce investment when their confidence in offspring paternity is reduced. This is because it would be maladaptive for males to invest in another male's offspring. Reduction in male investment in offspring provisions when

paternity confidence is reduced has been shown for paternal care in various species (Møller, 1988; Sheldon *et al.*, 1997; Kempenaers *et al.*, 1998). Chapter 4 tested this prediction by experimentally manipulating male mating success and paternity confidence by preventing or allowing sexual access to a female and a sperm competitor. Both vigilance and alarm calling increased in treatments where males were provided with the opportunity to mate, with reduced paternity confidence having no significant effect. Therefore, the findings from both Chapters 3 and 4 show that male vigilance and alarm calling are related to mating activity, yet this is unlikely to function as paternal protection of future offspring. Thus, vigilance and alarm calling may be linked to mating because of the male attractiveness hypothesis.

Anti-predator behaviours have only been linked directly to female choice in one species, the grey partridge, *Perdix perdix* (Dahlgren, 1990; Fusani *et al.*, 1997). In this monogamous species males dedicated most of their time to vigilance during the pre-hatching period, allowing females to reduce vigilance and increase foraging efforts (Fusani *et al.*, 1997). This allows females to gain direct benefits from increased foraging, potentially leading to better egg quality thus indirectly increasing male reproductive success. Both sexes are vigilant post-hatching, suggesting vigilance is important in offspring survival. Therefore by selecting vigilant males females gain additional reproductive benefits through increased offspring survival (Fusani *et al.*, 1997).

The selection of traits reflecting male condition is widespread (Zuk *et al.*, 1995; Omland, 1996; Doucet & Montgomerie, 2003; Loyau *et al.*, 2005). In a species of wolf spider, *Hygrolycosa rubrofasciata*, males attract females by performing costly drumming behaviours (Mappes *et al.*, 1996). Food intake was manipulated

in one study demonstrating that drumming is an honest signal of male quality. Furthermore, males with higher drumming rates suffered increased predation and increased weight loss compared to males with lower drumming rates (Mappes *et al.*, 1996). Intensity of colouration derived from carotenoids is food-dependant, with individuals consuming low carotenoid nutrient foods suffering from duller colour. Male house finches, *Haemorhous mexicanus*, show variation in carotenoid based plumage colouration (Hill, 1990). One study tested female preference for redder males by experimentally altering plumage colour using dyes and increasing or decreasing carotenoids consumption (Hill, 1990). In mate choice trials females significantly preferred the experimentally redder males, which could imply that male colouration is an honest signal of male quality (Hill, 1990). Therefore, it is possible that vigilance and alarm calling in fowl are honest signals used to advertise male quality to females. Vigilance typically reduces time spent foraging (Metcalfe & Furness, 1984; Duriez *et al.*, 2005; Beauchamp, 2007; Blanchard & Fritz, 2007), so males with high vigilance could be fitter males advertising their ability to maintain vigilance levels despite reduced food intake. Whereas increasing alarm calling could be an honest reflection of a male's ability to escape predation since alarm calling reveals the signaller's location to the predator. Crowing in fowl is energetically inexpensive (Wilson & Evans, 2008), so it is probable that aerial alarm calls are not costly to produce however increasing individual predation risk is costly. For females to gain genetic benefits from selecting vigilant and alarm calling males these behaviours must be partly additive, allowing females to benefit by producing offspring that inherit genes for superior viability (Arnold & Wade, 1984; Houde, 1992; Pizzari & McDonald, 2019). Alternatively, these behaviours may not be condition dependant traits used

in mate selection. Females could prefer vigilant males because they could gain direct benefits through reducing individual vigilance thus increasing foraging efforts. Females would also gain direct benefits from associating with better alarm callers due to males acting as the sentinel by warning his females of potential avian predators.

It remains unclear as to whether these behaviours are truly sexually selected by females, however, Chapter 4 does experimentally show that males are not selectively investing into females they have mated with. Chapter 4 does not conclude the male attractiveness hypothesis is driving this because it is still unclear. Most of the vigilance and alarm calling behaviour recorded on these evenings was recorded post-mating because observations started immediately after females were released into the pen on mating days. On these days males would immediately attempt to mate with the female, with most males mating within the first five minutes. This implies that the strong effect on vigilance and alarm calling in mating treatments and on mating days found in Chapter 4 is caused by a rise in these behaviours post-copulation. Additionally, approximately 21% of the matings recorded by focal males across the experiment were solicited, with the remaining being forced. Females often attempted to escape males once released into the half pens, yet males would continue to harass females until they obtained a successful copulation. This indicates that females were not attracted to these males, suggesting alternative mechanisms.

To summarise, Chapters 3 and 4 definitively show a link between male mating activity and vigilance and alarm calling in red junglefowl. Furthermore, Chapter 4 experimentally showed that reduced paternity confidence had no effect on either vigilance or alarm calling, concluding that males do not increase these behaviours

post-copulation to selectively invest in females they have mated with. The link between these anti-predator behaviours and mating success could be because of the male attractiveness hypothesis. Yet, the findings from Chapter 4, and those in Wilson et al. (2010), could suggest an increase in these behaviours post-copulation. This could be driven by endocrine changes post-mating, or the behaviour of mating itself could act as a cue to increase investment in females, as suggested in Wilson et al. (2010). Wilson et al. (2010) found that males do not invest selectively into females with whom they have recently mated but that alarm calling is a general response to recent mating success. Wilson et al. (2010) suggested a simple rule of thumb mechanism whereby males use recent mating success as a cue to increase alarm calling investment in nearby females.

Group Size and Sex Ratio Effects on Vigilance and Alarm Calling

The strong effect of sex ratio on both male vigilance and alarm calling could be the result of higher mating success in more female-biased groups. Since both vigilance and alarm calling are linked to mating success in Chapters 3 and 4 it seems possible that the strong result of sex ratio reflects this. However, Chapters 3 and 4 explores individual variation within groups in relation to mating success whereas Chapter 2 examines group-level variation. Alarm calling was significantly higher in the Female Biased groups of 4 compared to the groups of 12. If sex ratio had a strong effect on alarm calling because of male mating success then alarm calling should be highest in the groups with the most matings, yet alarm calling was much higher in Female Biased groups of 4 despite both group sizes having a similar average number of matings. Therefore, the pattern does not reflect male mating success but suggests natural selection pressures. Alarm calling is significantly higher in groups of 4 and significantly higher in

more female-biased groups, which suggests that males compensate for females not producing aerial alarm calls. Only males produce aerial calls (Gyger *et al.*, 1987; Evans, 1997) and males in Female Biased groups of 4 likely increased calling efforts in response to this. Males in more male-biased groups were able to decrease individual efforts and rely on other males.

Like alarm calling, vigilance was also significantly affected by sex ratio. Vigilance was significantly higher in Even Ratio groups of 12 compared to the groups of 4, which reflects the difference in average matings obtained in this sex ratio (Even Ratio 4: 2 ± 0.8 (SE), Even Ratio 12: 7 ± 1.3 (SE)). Yet vigilance was highest in the Even Ratio groups of 12 which does not reflect the overall number of matings because Female Biased groups had more matings. This suggests that, like with alarm calling, sex ratio differences are not solely driven by mating activity. Apart from the Even Ratio groups, all the other sex ratios follow the same pattern as alarm calling by decreasing with increasing number of males. In general, male vigilance likely decreases with increasing males because males can relax individual vigilance efforts and rely on neighbouring males. Even Ratio groups of 12 are significantly higher than the groups of 4 because of the results from the 2016 groups of 12. These 2016 groups were completed during a continuous four-week period, so possibly an abiotic factor or increased predator presence during this period caused higher vigilance. These 2016 Even Ratio groups of 12 caused vigilance to be significantly higher in groups of 12, with the exclusion of Even Ratio groups revealing that group size had no significant effect on vigilance. This is inconsistent with the large body of research on the group size effect, which states that individual vigilance should decrease with increasing group size because there are more eyes scanning the environment (Bertram, 1980;

Blumstein *et al.*, 1999; Beauchamp, 2001, 2008; Childress & Lung, 2003). Although not all studies support the group size effect, for example this was not shown in silvereyes *Zosterops lateralis* (Catterall *et al.*, 1992). This species is arboreal so it is possible that foliage prevented individuals from assessing group size, hence why it had no effect (Catterall *et al.*, 1992). Nearest neighbour distance was not recorded, and this has been shown to be an important measure for reducing vigilance and predation risk in numerous studies (Hamilton, 1971; Rolando *et al.*, 2001; Quinn & Cresswell, 2006; Fernández-Juricic *et al.*, 2007). Therefore, it is possible that neighbour distance is more important than group size in terms of reducing predation risk in this population, potentially explaining the lack of group size effect after removing Even Ratio groups.

Vigilance Behaviour in Female Red Junglefowl

Female vigilance behaviour receives little attention in fowl, so the final aim of my thesis was to add information to this understudied area. Firstly, males were significantly more vigilant than females, supporting previous work (Pizzari, 2003). Cryptic plumage often reduces predation risk (Götmark & Hohlfält, 1995; Slagsvold, Dale & Kruszewicz, 1995), so sex differences in vigilance may reflect reduced predation pressure on females. As discussed previously, females may select for more vigilant males to gain direct benefits through increased foraging. So, alternatively, the sex difference found in vigilance could reflect sexual selection pressures on males to increase vigilance to attract mates.

In the second part of Chapter 5, the relationship between food and vigilance in females was experimentally tested by placing females into one of three treatments: informed dominant, informed subordinate, and control. Dominant females in the informed subordinate and control treatments were naïve about

mealworm location whereas those in the informed dominant treatment were trained to always find mealworms in the same tray. Food information had no significant effect on the proportion of time spent vigilant, potentially due to very low sample sizes. However, there was a non-significant positive correlation between the proportion of time vigilant and the time taken to find the food, potentially suggesting that vigilance may aid in food searching. Female fowl could be using vigilance to scrounge profitable food items from conspecifics, which has been found in other species (Goss-Custard *et al.*, 1999; Robinette & Ha, 2001). In addition, despite the lack of significance, focal dominant females in the informed subordinate treatment tended to be the fastest at finding food. This adds potential support to the idea that females can use vigilance to exploit food finding efforts of neighbours. This may add insights into how social vigilance may be used in species with very high food competition and scrounging, such as in large flocks of waders (Ens & Goss-Custard, 1984; Vahl *et al.*, 2005; Beauchamp, 2009).

Potential Effects of Personality on Vigilance and Alarm Calling

Animal personality is not addressed in my thesis, yet it could influence anti-predator behaviour. Animal personality is loosely defined as inter-individual differences in behaviour that persist through time (Carter *et al.*, 2013). If the phenotypic plastic and behavioural repertoire of an individual is constrained by personality, then this might expose some individuals to an inherently greater predation risk. For example, bolder rainbow trouts, *Oncorhynchus mykiss*, are more likely to be caught by fishermen (Biro & Post, 2008). One study tested how boldness and exploratory behaviour correlated with response time to a simulated predator attack using juvenile convict cichlid fish, *Amatitlania nigrofasciata*

(Jones & Godin, 2009). Fish that increased exploration in novel environments took longer to react to predator attacks, with more exploratory individuals prioritising foraging over vigilance (Jones & Godin, 2009). In wild Eastern grey kangaroo females, *Macropus giganteus*, bolder individuals were also less vigilant than more risk-averse individuals (Edwards *et al.*, 2013). However in Trinidad guppies, *Poecilia reticulata*, bolder, more exploratory fish survived for longer periods when exposed to a predator (Smith & Blumstein, 2010).

Furthermore, some studies have linked personality type to mating success. For example, when presented with males of equal weights, female fiddler crabs, *Uca mjoebergi*, prefer bolder individuals (Reaney & Backwell, 2007). In zebra fish, *Danio rerio*, groups comprising of bolder individuals fertilized a higher proportion of eggs than groups with shyer males, so females gain direct reproductive benefits from bold males (Ariyomo & Watt, 2012). In addition, assortative pairing based on personality can affect offspring success. Exploratory zebra finch, *Taeniopygia guttata*, females produce better offspring when paired with males with a similar exploratory nature (Schuett *et al.*, 2011a). Similarly, in mate choice trials, female zebra finches observed two males explore a novel arena, with one male's exploration efforts being experimentally reduced (Schuett *et al.*, 2011b). Exploratory females significantly preferred more exploratory males whereas less exploratory females showed no preference (Schuett *et al.*, 2011b).

The relationship between personality and mate preference could mean that the correlations found in Chapter 3 between vigilance/alarm calling and mating success are the result of females selecting for certain personality traits, like boldness, rather than explicitly selecting for vigilance or alarm calling. This is

possible because boldness and exploratory behaviour have been linked to anti-predator behaviour in other species (Biro & Post, 2008; Jones & Godin, 2009).

Potential Effects of Captivity on Vigilance and Alarm Calling

The junglefowl population used in my thesis was a captive bred population that has been established at Wytham since 2006, with birds housed in outdoor aviaries. Although outdoor aviaries allowed birds to see potential avian and terrestrial predators, birds were fully protected in these aviaries which could have led to relaxed selection on anti-predator behaviours in this population. For example, in juvenile brown trout, *Salmo trutta*, simulated predators caused wild born individuals to increase the use of refuge whereas hatchery born fish did not increase refuge use, suggesting negative effects of captivity on predator response (Álvarez & Nicieza, 2003).

If captivity reduces predation response, then this could lead to lower survival when releasing captive populations into the wild. Captive bred grey partridges were released into the wild finding that individuals spent only 3.8-5.4% of their time vigilant compared to 43% in wild reared populations (Rantanen *et al.*, 2010). Furthermore released partridges did not decrease vigilance with increasing group size (Rantanen *et al.*, 2010), whereas this has been shown in wild populations (Watson *et al.*, 2007); suggesting captive populations do not behaviour adaptively. The released captive-bred population also suffered from high mortality from both mammalian and avian predators (Rantanen *et al.*, 2010). Reduced anti-predator behaviours in captive populations are a concern when planning to release animals into the wild so some studies have attempted to train individuals to recognise and respond to predators pre-release. This was done in juvenile prairie dogs, *Cynomys ludovicianus*, by exposing individuals in the experimental group to hawk models

as well as live snakes and ferrets whilst playing back conspecific alarm calls (Shier & Owings, 2006). Individuals that were trained using models/predators produced significantly more alarm calls and were more vigilant when tested with live/model predators than those in the control group (Shier & Owings, 2006). Additionally, more trained individuals survived to one year after being released from captivity than control individuals (Shier & Owings, 2006).

Furthermore, populations that have been in captivity longer can show a further reduction in predator response. In oldfield mice, *Peromyscus polionotus*, a higher number of generations in captivity causes individuals to be less likely to seek cover when predators are present (McPhee, 2004). Four different captive populations of red junglefowl have been compared to test whether anti-predator responses were reduced in populations which have been captive for longest and in populations that were the most protected from predation (Håkansson & Jensen, 2008). Two of the populations used were free-roaming, one population was held in outdoor aviaries and the final population was kept indoors. Individuals were exposed to aerial predator models finding that the population that had been captive for the least amount of time had the strongest response to the attack, suggesting increased time in captivity reduces selection pressures on individuals to avoid predators; leading to a reduced response (Håkansson & Jensen, 2008). Furthermore captive environment also had a strong effect, with the most protected population showing significantly less response and agitation to predator models, with 30% of individuals not reacting to models at all (Håkansson & Jensen, 2008). This study concluded that captive animals have reduced natural selection due to increased protection from predators (Håkansson & Jensen, 2008).

Based on the previous findings showing captivity effects on anti-predator behaviours and response in several species, it is possible that individuals in the Wytham population had reduced natural selection pressures leading to reduced investment in both vigilance and alarm calling. If selection pressures are lower than it is possible that individuals in this population could have devoted more vigilance towards social uses such as findings mates, scrounging food, avoiding aggressive encounters, etc rather than avoiding predation. Males in this population still produced aerial alarm calls in response to stimuli flying over the aviaries, however when aerial calls were heard in most cases individuals did not react adaptively by seeking cover or crouching. Håkansson & Jensen (2008) showed that red junglefowl populations that had been in captivity for longer periods had a reduced reaction to a simulated aerial predator attack. The Wytham population has been kept in outdoor aviaries and bred for several generations since 2006, therefore this might explain why most individuals do not respond to aerial calls by crouching or finding shelter. Captivity effects could explain why vigilance effort was extremely low in this population. Like Rantanen et al. (2010), finding that captive born released grey partridges spent up to 5% of their time vigilant, the average proportion of time spent vigilant in the groups of 4 was only 5%, with 8% in the groups of 12. Furthermore, in Chapter 4 males in the mating treatments only spent 11% of their time vigilant compared to 7% in the no-mating treatments. A small amount of time spent vigilant is maladaptive because reduced vigilance could increase predation risk. However, as this is a captive population that has never experienced real predation risk due to being protected, this may have caused reduced selection for vigilance. Vigilance can be costly, so males could reduce efforts since the predation risk in the aviaries is low. Additionally, males in the

groups of 4 initiated an average of 56 aerial alarm calls, with 19 initiated in groups of 12, across the entire 32-hour observation period. Again, in Chapter 4 males only produced an average of 4 calls in the mating treatments and 2 calls in the no-mating treatments across the entire 3.5-hour observation period. This highly suggests that males in this population are under reduced selection pressures to avoid predation, thus males have very low vigilance and alarm calling efforts. Plus, Håkansson and Jensen (2008) showed that the length of time in captivity causes individuals to have reduced responses to simulated aerial predator attacks, with individuals in non-free roaming populations also showing reduced responses. The Wytham population is not free roaming and they have been in captivity since 2006, therefore captivity effects most likely explain why alarm calling rate was low and why individuals often did not seek cover when hearing the alarm.

Nonetheless, all of the previous studies linking aerial alarm calling to mating success in fowl used captive populations (Wilson *et al.*, 2008, 2010; Wilson & Evans, 2008, 2010) and all experiments, except the first experiment in Wilson and Evans (2010), used outdoor aviaries similar to those at Wytham (Wilson *et al.*, 2008, 2010; Wilson & Evans, 2008, 2010). Therefore, the results found in my thesis are certainly comparable to the previous work exploring anti-predator behaviours and mating success in fowl. However, it is important to note that the implications of my findings in wild populations is unclear. The majority of studies on red junglefowl and the domestic fowl use captive populations in either indoor or outdoor aviaries (Zuk *et al.*, 1990; Zuk *et al.*, 1995b; Kim & Zuk, 2000; Johnsen *et al.*, 2001; Wilson *et al.*, 2008; Campler *et al.*, 2009). So, although my thesis is comparable with the large body of research on fowl, no studies have examined the link between anti-predator behaviours and mating behaviour in wild

populations. Future studies should aim to use birds in more natural settings to reduce the problems of captivity effects and to fully explore whether anti-predator behaviours play a role in mating success when populations suffer genuine predation risks.

Redefining Vigilance

Multiple studies have demonstrated various social uses of vigilance (Figure 1), with my thesis showing that anti-predation behaviour in fowl is linked to mating success and is influenced by both sex ratio and group size. The use of vigilance can be situational; for example, Chapter 2 demonstrated that vigilance was primarily used to detect predators within groups of 4 because of the strong correlation between time spent vigilant and the number of aerial alarm calls initiated. However, within the groups of 12 vigilance may primarily be used for social purposes since there were no strong correlations between vigilance and aerial alarm calling.

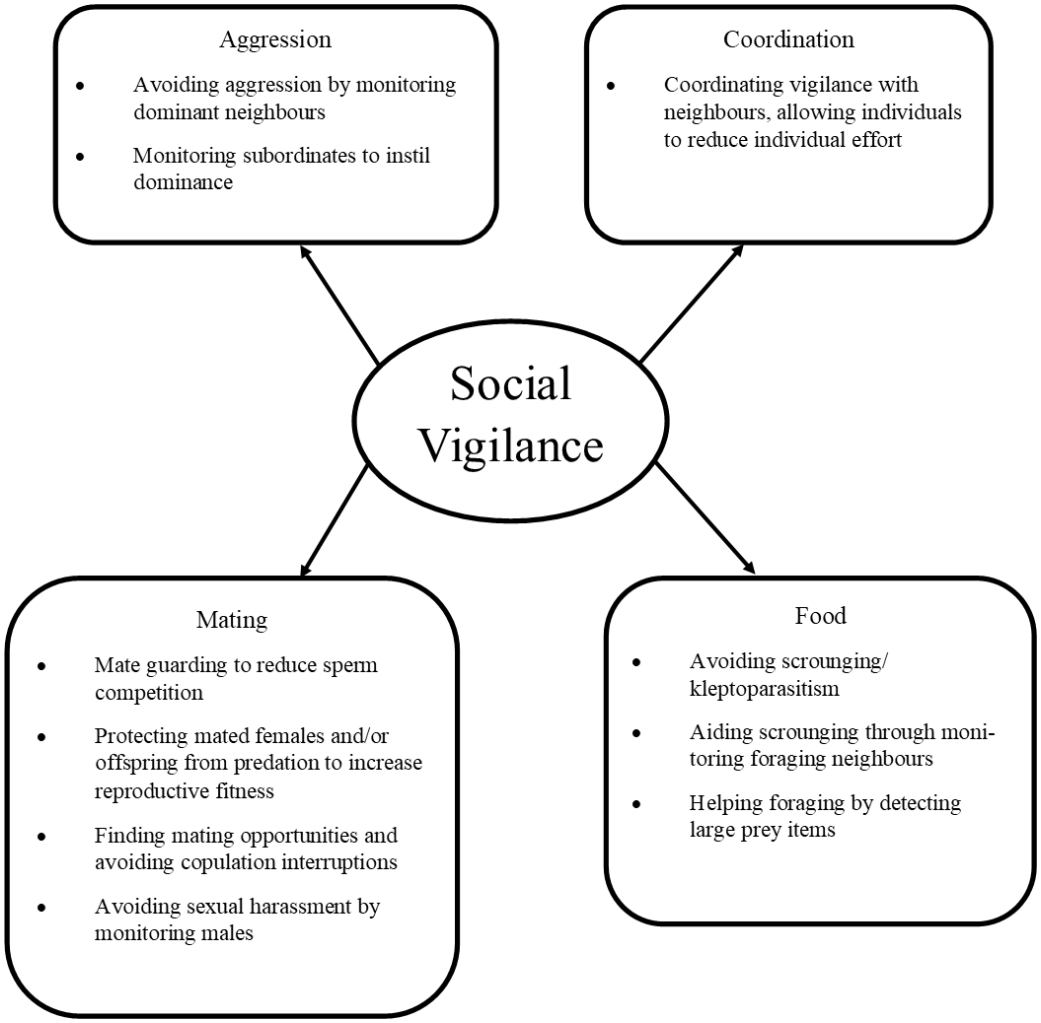


Figure 1. Summary of the key functions of social vigilance in the literature divided into four main categories of aggression, coordination, mating and food.

Early models of vigilance associated individuals in a head-down position with being non-vigilant. However dark-eyed juncos, *Junco hyemalis*, were able to detect an advancing raptor despite being in a head-down state. Implying that birds can trade-off costly, better quality head-up vigilance with less costly, but lower quality head-down vigilance (Lima & Bednekoff, 1999). Mammals often use olfactory senses to find food rather than only vision, potentially allowing greater scanning for predators while feeding despite lacking the advantages of avian eyes (Lima & Bednekoff, 1999; Bednekoff & Lima, 2005). This research expanded the

field of vigilance, causing future work to consider head-down to be a reduced vigilant state rather than non-vigilant. Similarly, there is ample evidence for alternative uses of vigilance (Waite, 1987; Roberts, 1988; Robinette & Ha, 2001; Cameron & du Toit, 2005; Squires *et al.*, 2007; Gaynor & Cords, 2012), so perhaps it is time to redefine vigilance to incorporate the multiple functions.

Alternatively, a new term could be used to describe all non-predator related uses of vigilance, or social vigilance could be captured within an already existing term like social observation. Social observation is used within social learning literature and it refers to naïve individuals observing an experienced individual (demonstrator) performing a novel behaviour leading to the naïve individual learning this behaviour through imitation (Laland & Plotkin, 1990; Fryday & Greig-Smith, 1994; Nicol & Pope, 1994, 1999; Lachlan *et al.*, 1998; Laland, 2004). Social vigilance will not necessarily fit within this definition because not all social uses involve learning. Individuals using vigilance to scrounge profitable food items from neighbours are not learning new behaviours through observation, nor are individuals learning from others when using vigilance to find mating opportunities.

While avoiding predation is likely still the primary function in most species, it is not the only role of vigilance and this must be incorporated into definitions of vigilance moving forwards. One question that arises from social vigilance is whether it is possible to discriminate between social and anti-predator vigilance. In some species discriminating between the two functions may be possible by monitoring glance rate towards conspecifics and general scanning of the environment (Alberts, 1994; Gaynor & Cords, 2012). Yet even when performing general scans individuals could still gather information on neighbours.

Furthermore fowl have laterally placed eyes leading to very large visual fields (Gyger *et al.*, 1987; Evans & Evans, 1999), thus when performing a scan for predators they may simultaneously be gathering information on food, mating opportunities etc. Since it is currently difficult to separate out social and anti-predator vigilance, particularly in species with lateral eyes, the field should incorporate social uses within the standard definition of vigilance. I propose the following updated definition of vigilance: the interruption of prior activity to scan the surrounding environment for predators and/or neighbours. The addition of neighbours into the definition incorporates all the potential social benefits of watching both conspecific and heterospecific neighbours including avoiding aggression, avoiding food theft, scrounging profitable food items, avoiding cuckoldry, finding mating opportunities, coordinating vigilance etc.

Future Work

Future work needs to determine the mechanisms causing the relationship between male vigilance/alarm calling and mating behaviour. Future studies could potentially investigate vigilance and alarm calling effort in males pre- and post-copulation. Potentially, males and females could be separated to prevent mating. During this non-mating period anti-predator behaviours would be recorded. Males and females could then be released, allowing them to mate, with birds separated immediately after a successful mating has taken place. Once males and females are separated again anti-predator behaviours could be recorded. This would allow studies to directly compare male vigilance and alarm calling effort immediately before and after mating. If these behaviours were significantly higher pre-copulation, then this would indicate that males increase efforts to attract females. However, if they significantly increased post-copulation then this suggests other

mechanisms. Additionally, future work could examine cryptic female choice. Female fowl can alter insemination success through cloacal contractions immediately after copulation, leading to the ejection of semen (Thornhill, 1983; Pizzari & Birkhead, 2000). Although females can choose males pre-copulation through solicitation, 'undesirable' males are still able to forcefully mate with females. If vigilance or alarm calling are attractive traits to females, then males that invest in these may suffer reduced sperm ejection if females gain genetic benefits from siring offspring with these males.

Conclusions

My thesis set out to explore the role of both vigilance and alarm calling behaviour and I feel that I have achieved this by exploring their role in male mating behaviour, how they are influenced by group size and sex ratio and the function of vigilance in females.

My key findings include sex ratio having a strong effect on male vigilance and alarm calling, with males typically increasing efforts with fewer males present; reflecting natural selection pressures due to low vigilance and no aerial calls from females. Unlike in males, sex ratio did not have a strong effect on female vigilance though it was lowest with increasing males present. Females were significantly less vigilant than males, potentially reflecting females decreasing vigilance when males are present. No female-only groups of 4 were conducted so my thesis cannot conclude whether male and female vigilance would differ when comparing female-only and male-only groups of 4. Although female-only pairs were used in part two of Chapter 5, and focal females used in this experiment spent an average of 7% of their time vigilant compared to 2% for females in groups of 4; which may suggest that females do indeed decrease vigilance when

males are present. However, the higher vigilance in these pairs could also be the result of smaller group size.

The most important finding of my thesis was the evidence for a link between both vigilance and alarm calling on male mating success. Not only were these behaviours correlated with male mating success in Chapter 3, but Chapter 4 directly tested the mate investment hypothesis finding that this does not adequately explain the link between vigilance, alarm calling and mating. Therefore, my thesis was able to conclude that males do not selectively invest in females that they have mated with to increase survival of those females, increasing male reproductive success. This leaves the male attractiveness hypothesis as one explanation for the link between anti-predator behaviours and mating. Future work should directly explore female preference potentially using post-copulatory cryptic choice to examine whether more vigilant males or those that initiate the most calls are less likely to have sperm ejected whereas poor callers/less vigilant males could receive significantly higher sperm ejection.

Anti-predator behaviours have only ever been directly linked to mating success in the grey partridge and the domestic fowl. However others species show sex differences in anti-predation behaviours and responses (Mayhew, 1987; Guillemain *et al.*, 2003; Squires *et al.*, 2007; Portugal & Guillemain, 2011). Therefore, it is possible that sex differences found in other species may also be related to male mating behaviour, with males increasing these behaviours to attract females, or these behaviours increasing post-mating due to hormones or mate investment. More studies need to be done addressing these questions.

Overall my thesis adds to the understudied body of research linking anti-predator behaviours to mating success, with Chapter 4 concluding that it is unlikely that males are selectively investing vigilance/alarm calling into reproductive partners. Therefore, these behaviours are either sexually selected traits preferred by females, or the act of mating itself causes a surge in these behaviours post-copulation. A rise post-copulation could be because of hormonal fluctuations or mating being a cue to increase general anti-predator investment in harem females, as suggested in Wilson et al. (2010).

REFERENCES

Alberts, S.C. (1994). Vigilance in young baboons: effects of habitat, age, sex and maternal rank on glance rate. *Animal Behaviour*, **47**, 749–755.

Álvarez, D. & Nicieza, A.G. (2003). Predator avoidance behaviour in wild and hatchery-reared brown trout: the role of experience and domestication. *Journal of Fish Biology*, **63**, 1565–1577.

Ariyomo, T.O. & Watt, P.J. (2012). The effect of variation in boldness and aggressiveness on the reproductive success of zebrafish. *Animal Behaviour*, **83**, 41–46.

Arnold, S.J. & Wade, M.J. (1984). On the Measurement of Natural and Sexual Selection: Theory. *Evolution*, **38**, 709–719.

Beauchamp, G. (2001). Should vigilance always decrease with group size? *Behavioural Ecology and Sociobiology*, **51**, 47–52.

Beauchamp, G. (2007). Exploring the role of vision in social foraging: What happens to group size, vigilance, spacing, aggression and habitat use in birds and mammals that forage at night? *Biological Reviews*, **82**, 511–525.

Beauchamp, G. (2008). What is the magnitude of the group-size effect on vigilance? *Behavioural Ecology*, **19**, 1361–1368.

Beauchamp, G. (2009). How does food density influence vigilance in birds and mammals? *Animal Behaviour*, **78**, 223–231.

Bednekoff, P.A. & Lima, S.L. (2005). Testing for peripheral vigilance: do birds value what they see when not overtly vigilant? *Animal Behaviour*, **69**, 1165–1171.

Bertram, B.C.R. (1980). Vigilance and group size in ostriches. *Animal Behaviour*, **28**, 278–286.

Biro, P.A. & Post, J.R. (2008). Rapid depletion of genotypes with fast growth and bold personality traits from harvested fish populations. *Proceedings of the National Academy of Sciences*, **105**, 2919–2922.

Blanchard, P. & Fritz, H. (2007). Induced or routine vigilance while foraging. *Oikos*, **116**, 1603–1608.

Blumstein, D.T., Evans, C.S. & Daniel, J.C. (1999). An experimental study of behavioural group size effects in tammar wallabies, *Macropus eugenii*. *Animal Behaviour*, **58**, 351–360.

Cameron, E.Z. & du Toit, J.T. (2005). Social influences on vigilance behaviour in giraffes, *Giraffa camelopardalis*. *Animal Behaviour*, **69**, 1337–1344.

Campler, M., Jöngren, M. & Jensen, P. (2009). Fearfulness in red junglefowl and domesticated White Leghorn chickens. *Behavioural Processes*, **81**, 39–43.

Carter, A.J., Feeney, W.E., Marshall, H.H., Cowlishaw, G. & Heinsohn, R. (2013). Animal personality: what are behavioural ecologists measuring? *Biological Reviews*, **88**, 465–475.

Catterall, C.P., Elgar, M.A. & Kikkawa, J. (1992). Vigilance does not covary with group size in an island population of silvereyes (*Zosterops lateralis*). *Behavioural Ecology*, **3**, 207–210.

Childress, M.J. & Lung, M.A. (2003). Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Animal Behaviour*, **66**, 389–398.

Dahlgren, J. (1990). Females choose vigilant males: an experiment with the monogamous grey partridge, *Perdix perdix*. *Animal Behaviour*, **39**, 646–651.

Doucet, S.M. & Montgomerie, R. (2003). Multiple sexual ornaments in satin bowerbirds: ultraviolet plumage and bowers signal different aspects of male quality. *Behavioural Ecology*, **14**, 503–509.

Duriez, O., Fritz, H., Binet, F., Tremblay, Y. & Ferrand, Y. (2005). Individual activity rates in wintering Eurasian woodcocks: starvation versus predation risk trade-off? *Animal Behaviour*, **69**, 39–49.

Edwards, A.M., Best, E.C., Blomberg, S.P. & Goldizen, A.W. (2013). Individual traits influence vigilance in wild female eastern grey kangaroos. *Australian Journal of Zoology*, **61**, 332–341.

Ens, B.J. & Goss-Custard, J.D. (1984). Interference Among Oystercatchers, *Haematopus ostralegus*, Feeding on Mussels, *Mytilus edulis*, on the Exe Estuary. *Journal of Animal Ecology*, **53**, 217–231.

Evans, C.S. (1997). Referential Signals. In *Communication*, Perspectives in Ethology: 99–143. Owings, D.H., Beecher, M.D. & Thompson, N.S. (Eds.). Springer US.

Evans, C.S. & Evans, L. (1999). Chicken food calls are functionally referential. *Animal Behaviour*, **58**, 307–319.

Fernández-Juricic, E., Beauchamp, G. & Bastain, B. (2007). Group-size and distance-to-neighbour effects on feeding and vigilance in brown-headed cowbirds. *Animal Behaviour*, **73**, 771–778.

Folstad, I. & Karter, A.J. (1992). Parasites, Bright Males, and the Immunocompetence Handicap. *The American Naturalist*, **139**, 603–622.

Fryday, S.L. & Greig-Smith, P.W. (1994). The Effects of Social Learning on the Food Choice of the House Sparrow (*Passer domesticus*). *Behaviour*, **128**, 281–300.

Fusani, L., Beani, L., Lupo, C. & Dessì-Fulgheri, F. (1997). Sexually selected vigilance behaviour of the grey partridge is affected by plasma androgen levels. *Animal Behaviour,* **54**, 1013–1018.

Gaynor, K.M. & Cords, M. (2012). Antipredator and social monitoring functions of vigilance behaviour in blue monkeys. *Animal Behaviour*, **84**, 531–537.

Goss-Custard, J.D., Cayford, J.T. & Lea, S.G. (1999). Vigilance during food handling by Oystercatchers *Haematopus ostralegus* reduces the chances of losing prey to kleptoparasites. *Ibis,* **141**, 368–376.

Götmark, F. & Hohlfält, A. (1995). Bright Male Plumage and Predation Risk in Passerine Birds: Are Males Easier to Detect Than Females? *Oikos,* **74**, 475–484.

Guillemain, M., Caldow, R.W.G., Hodder, K.H. & Goss-Custard, J.D. (2003). Increased vigilance of paired males in sexually dimorphic species: distinguishing between alternative explanations in wintering Eurasian wigeon. *Behavioural Ecology*, **14**, 724–729.

Gyger, M., Karakashian, S.J., Dufty Jr., A.M. & Marler, P. (1988). Alarm signals in birds: The role of testosterone. *Hormones and Behavior,* **22**, 305–314.

Gyger, M., Marler, P. & Pickert, R. (1987). Semantics of an Avian Alarm Call System: The Male Domestic Fowl, *Gallus domesticus*. *Behaviour*, **102**, 15–40.

Håkansson, J. & Jensen, P. (2008). A longitudinal study of antipredator behaviour in four successive generations of two populations of captive red junglefowl. *Applied Animal Behaviour Science,* **114**, 409–418.

Hamilton, W.D. (1971). Geometry for the selfish herd. *Journal of Theoretical Biology*, **31**, 295–311.

Hill, G.E. (1990). Female house finches prefer colourful males: sexual selection for a condition-dependent trait. *Animal Behaviour,* **40**, 563–572.

Houde, A.E. (1992). Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces: Poeciliidae) (guppies). *Heredity*, **69**, 229.

Johnsen, T.S., Zuk, M. & Fessler, E.A. (2001). Social Dominance, Male Behaviour and Mating in Mixed-Sex Flocks of Red Junglefowl. *Behaviour,* **138**, 1–18.

Jones, K.A. & Godin, J.-G.J. (2009). Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. *Proceedings of the Royal Society of London B: Biological Sciences,* **277**, 625-632.

Kempenaers, B., Lanctot, R.B. & Robertson, R.J. (1998). Certainty of paternity and paternal investment in eastern bluebirds and tree swallows. *Animal Behaviour,* **55**, 845–860.

Kim, T. & Zuk, M. (2000). The effects of age and previous experience on social rank in female red junglefowl, *Gallus gallus*. *Animal Behaviour,* **60**, 239–244.

Lachlan, R.F., Crooks, L. & Laland, K.N. (1998). Who follows whom? Shoaling preferences and social learning of foraging information in guppies. *Animal Behaviour,* **56**, 181–190.

Laland, K.N. (2004). Social learning strategies. *Animal Learning & Behavior,* **32**, 4–14.

Laland, K.N. & Plotkin, H.C. (1990). Social learning and social transmission of foraging information in Norway rats (*Rattus norvegicus*). *Animal Learning & Behavior,* **18**, 246–251.

Lima, S.L. & Bednekoff, P.A. (1999). Back to the basics of antipredatory vigilance: can nonvigilant animals detect attack? *Animal Behaviour,* **58**, 537–543.

Loyau, A., Jalme, M.S. & Sorci, G. (2005). Intra- and Intersexual Selection for Multiple Traits in the Peacock (*Pavo cristatus*). *Ethology,* **111**, 810–820.

Mappes, J., Alatalo, R.V., Kotiaho, J. & Parri, S. (1996). Viability costs of condition-dependent sexual male display in a drumming wolf spider. *Proceedings of the Royal Society of London B: Biological Sciences,* **263**, 785–789.

Mayhew, P.W. (1987). Vigilance levels in European Wigeon - sexual differences. *Wildfowl,* **38**, 77–81.

McPhee, M.E. (2004). Generations in captivity increases behavioral variance: Considerations for captive breeding and reintroduction programs. *Biological Conservation,* **115**, 71–77.

Metcalfe, N.B. & Furness, R.W. (1984). Changing priorities: the effect of pre-migratory fattening on the trade-off between foraging and vigilance. *Behavioural Ecology and Sociobiology,* **15**, 203–206.

Møller, A.P. (1988). Paternity and paternal care in the swallow, *Hirundo rustica*. *Animal Behaviour,* **36**, 996–1005.

Mougeot, F., Irvine, J.R., Seivwright, L., Redpath, S.M. & Piertney, S. (2004). Testosterone, immunocompetence, and honest sexual signaling in male red grouse. *Behavioural Ecology,* **15**, 930–937.

Nicol, C.J. & Pope, S.J. (1994). Social learning in small flocks of laying hens. *Animal Behaviour,* **47**, 1289–1296.

Nicol, C.J. & Pope, S.J. (1999). The effects of demonstrator social status and prior foraging success on social learning in laying hens. *Animal Behaviour,* **57**, 163–171.

Omland, K.E. (1996). Female mallard mating preferences for multiple male ornaments. *Behavioural Ecology and Sociobiology*, **39**, 353–360.

Peters, A. (2000). Testosterone Treatment Is Immunosuppressive in Superb Fairy-Wrens, Yet Free-Living Males with High Testosterone Are More Immunocompetent. *Proceedings of the Royal Society of London B: Biological Sciences*, **267**, 883–889.

Pizzari, T. (2003). Food, vigilance, and sperm: the role of male direct benefits in the evolution of female preference in a polygamous bird. *Behavioural Ecology*, **14**, 593–601.

Pizzari, T. & Birkhead, T.R. (2000). Female feral fowl eject sperm of subdominant males. *Nature*, **405**, 787–789.

Pizzari, T. & McDonald, G.C. (2019). Sexual selection in socially-structured, polyandrous populations: Some insights from the fowl. *Advances in the Study of Behavior*, **51**, 77–141.

Portugal, S.J. & Guillemain, M. (2011). Vigilance patterns of wintering Eurasian Wigeon: female benefits from male low-cost behaviour. *Journal of Ornithology*, **152**, 661–668.

Quinn, J.L. & Cresswell, W. (2006). Testing domains of danger in the selfish herd: sparrowhawks target widely spaced redshanks in flocks. *Proceedings of the Royal Society of London B: Biological Sciences*, **273**, 2521–2526.

Rantanen, E.M., Buner, F., Riordan, P., Sotherton, N. & Macdonald, D.W. (2010). Vigilance, time budgets and predation risk in reintroduced captive-bred grey partridges *Perdix perdix*. *Applied Animal Behaviour Science*, **127**, 43–50.

Reaney, L.T. & Backwell, P.R.Y. (2007). Risk-taking behavior predicts aggression and mating success in a fiddler crab. *Behavioural Ecology*, **18**, 521–525.

Roberts, S.C. (1988). Social influences on vigilance in rabbits. *Animal Behaviour*, **36**, 905–913.

Robinette, R.L. & Ha, J.C. (2001). Social and ecological factors influencing vigilance by northwestern crows, *Corvus caurinus*. *Animal Behaviour*, **62**, 447–452.

Rolando, A., Caldoni, R., De Sanctis, A. & Laiolo, P. (2001). Vigilance and neighbour distance in foraging flocks of red-billed choughs, *Pyrrhocorax pyrrhocorax*. *Journal of Zoology*, **253**, 225–232.

Schuett, W., Dall, S.R.X. & Royle, N.J. (2011a). Pairs of zebra finches with similar 'personalities' make better parents. *Animal Behaviour*, **81**, 609–618.

Schuett, W., Godin, J.-G.J. & Dall, S.R.X. (2011b). Do Female Zebra Finches, *Taeniopygia guttata*, Choose Their Mates Based on Their 'Personality'? *Ethology,* **117**, 908–917.

Sheldon, B.C., Räsänen, K. & Dias, P.C. (1997). Certainty of paternity and paternal effort in the collared flycatcher. *Behavioural Ecology*, **8**, 421–428.

Shier, D.M. & Owings, D.H. (2006). Effects of predator training on behavior and post-release survival of captive prairie dogs (*Cynomys ludovicianus*). *Biological Conservation,* **132**, 126–135.

Slagsvold, T., Dale, S. & Kruszewicz, A. (1995). Predation favours cryptic coloration in breeding male pied flycatchers. *Animal Behaviour,* **50**, 1109–1121.

Smith, B.R. & Blumstein, D.T. (2010). Behavioural types as predictors of survival in Trinidadian guppies (*Poecilia reticulata*). *Behavioural Ecology*, **21**, 919–926.

Squires, K.A., Martin, K., Goudie, R.I. & Yasukawa, K. (2007). Vigilance Behavior in the Harlequin Duck (*Histrionicus histrionicus*) during the Preincubation Period in Labrador: Are Males Vigilant for Self or Social Partner? *The Auk,* **124**, 241–252.

Thornhill, R. (1983). Cryptic Female Choice and Its Implications in the Scorpionfly *Harpobittacus nigriceps. The American Naturalist,* **122**, 765–788.

Vahl, W.K., Lok, T., van der Meer, J., Piersma, T. & Weissing, F.J. (2005). Spatial clumping of food and social dominance affect interference competition among ruddy turnstones. *Behavioural Ecology*, **16**, 834–844.

Waite, T.A. (1987). Vigilance in the White-Breasted Nuthatch: Effects of Dominance and Sociality. *The Auk,* **104**, 429–434.

Watson, M., Aebischer, N.J. & Cresswell, W. (2007). Vigilance and fitness in grey partridges *Perdix perdix*: the effects of group size and foraging-vigilance trade-offs on predation mortality. *Journal of Animal Ecology*, **76**, 211–221.

Wilson, D.R., Bayly, K.L., Nelson, X.J., Gillings, M. & Evans, C.S. (2008). Alarm calling best predicts mating and reproductive success in ornamented male fowl, *Gallus gallus*. *Animal Behaviour,* **76**, 543–554.

Wilson, D.R. & Evans, C.S. (2008). Mating success increases alarm-calling effort in male fowl, *Gallus gallus*. *Animal Behaviour,* **76**, 2029–2035.

Wilson, D.R. & Evans, C.S. (2010). Female fowl (*Gallus gallus*) do not prefer alarm-calling males. *Behaviour,* **147**, 525–552.

Wilson, D.R., McDonald, P.G. & Evans, C.S. (2010). Mechanisms of Mate Investment in the Polygamous Fowl, *Gallus gallus*. *Ethology*, **116**, 755–762.

Zahavi, A. (1975). Mate selection- A selection for a handicap. *Journal of Theoretical Biology*, **53**, 205–214.

Zuk, M., Johnsen, T.S. & Maclarty, T. (1995a). Endocrine-Immune Interactions, Ornaments and Mate Choice in Red Junglefowl. *Proceedings of the Royal Society of London B: Biological Sciences*, **260**, 205–210.

Zuk, M., Popma, S.L. & Johnsen, T.S. (1995b). Male Courtship Displays, Ornaments and Female Mate Choice in Captive Red Junglefowl. *Behaviour*, **132**, 821–836.

Zuk, M., Thornhill, R., Ligon, J.D. & Johnson, K. (1990). Parasites and mate choice in red junglefowl. *American Zoologist*, **30**, 235–244.