THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

The vocalisations and behaviour of chickens in anticipation of rewards

Nicola Anne McGrath

Bachelor of Arts, Spanish and French (Honours)
Masters of Science in Applied Animal Behaviour and Welfare

A thesis submitted for the degree of Doctor of Philosophy at
The University of Queensland in 2018
School of Veterinary Science

Abstract

The vocalisations of non-human animals can serve as indicators of motivational or emotional state. The anticipation of rewards activates areas of the brain associated with reward and 'wanting', leading to consummatory behaviour and pleasure. Hence, anticipatory behaviour and vocalisations, prior to consumption of an item, could indicate whether animals are experiencing rewarding environments. This thesis aims to investigate whether domestic chickens *Gallus gallus* produce specific reward-related vocalisations, and whether they can be understood by humans. It first examines the behaviour of chickens in anticipation of different types of reward. It goes on to characterise the vocalisations made in anticipation of rewards, and explore variations in the acoustic structure of these calls. A playback paradigm is further used to study how other chickens respond to reward-related vocalisations, and whether there is any referential information encoded in these vocalisations. Finally, this project asks whether humans can identify chickens' 'reward' calls, and whether they can perceive the arousal levels or valence represented by these vocalisations.

Twelve hens were exposed to a Pavlovian conditioning paradigm, using sound cues to signal the availability of two food rewards (mealworms and the hens' normal food), one non-food reward (a container of dustbathing substrate) and a sound-neutral event (sound cue, no reward). A muted-neutral treatment (no sound cue, no reward) provided a baseline for behavioural and vocal observations. This study revealed that chickens increase their activity levels as a general response to reward. Quantifiable differences in the frequency and duration of goal-directed behaviours indicated that hens ranked the rewards differentially, and appeared to be more motivated to access the dustbathing reward.

Hens consistently produced a specific 'reward' call, described elsewhere as a 'food' call, in anticipation of all rewards, including the non-food reward. The cue signalling the dustbathing substrate elicited the highest proportion of these calls, suggesting that this reward induced the most arousal in hens. Variation in the acoustic structure of calls appeared to reflect differences in arousal. The peak frequency of reward calls made in anticipation of the dusty substrate was 45-75 Hz lower ($p=0.01$) than those made in anticipation of food rewards. This is the first study to reveal that chickens produce a 'reward' call. It also demonstrates that the frequency-related parameters of this call vary according to different contexts.

Referential information relating to environmental stimuli, such as a source of food or the presence of a predator, is sometimes encoded in the type, or structural variation, of animal acoustic signals. If referential information is encoded in reward calls transmitted by hens, receivers should respond as if they had experienced the stimuli themselves. In this phase of the project, twelve hens were exposed to a playback paradigm using calls recorded from other hens made when anticipating either a mealworm reward or a dustbathing substrate. A pure tone and a period of silence acted as controls. The behaviour of hens during sound playback was recorded during a 1 minute test period. Hens responded to all sound cues, including the pure tone, with short, sharp ‘alert’ head movements, including turning their head and neck in different directions in order to locate the source of the sound. Hens made ‘alert’ head movements for longer during the ‘mealworm’ sound treatment compared to the silent treatment. Hens did not perform any other behaviours that might indicate there was information contained in the calls. Therefore, hens’ reward calls appear to contain no referential information, but seem to induce an increased state of arousal and anticipation in receivers which is expressed in ‘alert’ head movements.

The final phase of this project tested whether humans could identify if calls made by chickens were made in rewarding or non-rewarding contexts, using an online survey. Participants listened to 16 calls, recorded when chickens were anticipating a reward or when no reward was available. They rated each call according to whether they thought the vocalising chicken was experiencing pleasure or displeasure, and high or low arousal. They were also asked to identify whether calls were made in a rewarding or non-rewarding context. Participants appeared to judge the valence and arousal levels of calls based on acoustic cues. Longer calls predicted ratings of higher displeasure and higher arousal. Older people were less adept at identifying the correct context of call production, and more likely to attribute higher valence ratings (higher pleasure) to the non-reward related calls. Importantly, this study showed that humans are able to identify chicken calls made in rewarding or non-rewarding contexts. This finding could have important ramifications for farm animal welfare assessments.

This research confirms that chickens produce ‘reward’ calls and, crucially, that humans are able to identify them. These findings are important for practical on-farm situations, as reward-related vocalisations could be used as ‘markers’ of welfare.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

I acknowledge that an electronic copy of my thesis must be lodged with the University Library and, subject to the policy and procedures of The University of Queensland, the thesis be made available for research and study in accordance with the Copyright Act 1968 unless a period of embargo has been approved by the Dean of the Graduate School.

I acknowledge that copyright of all material contained in my thesis resides with the copyright holder(s) of that material. Where appropriate I have obtained copyright permission from the copyright holder to reproduce material in this thesis and have sought permission from co-authors for any jointly authored works included in the thesis.

Publications included in this thesis

McGrath, N., Burman, O., Dwyer, C., Phillips, C.J.C. (2016) Does the anticipatory behaviour of chickens communicate reward quality? *Applied Animal Behaviour Science* **184**: 80-90 - incorporated as Chapter 2.

McGrath, N., Dunlop, R., Dwyer, C., Burman, O., Phillips, C.J.C.(2017) Hens vary their vocal repertoire and structure when anticipating different types of reward. *Animal Behaviour* **130**: 79-96 - incorporated as Chapter 3.

Submitted manuscripts included in this thesis

No manuscripts submitted for publication.

Other publications during candidature

Peer reviewed papers

Fordyce, G., McMillan, H., and **McGrath, N**. (2018) Post-operative healing and behaviour when surgical swabs are applied to calf dehorning wounds, *Australian Veterinary Journal* 96: 508-515

McGrath, N., Dunlop, R., Dwyer, C., Burman, O., Phillips, C.J.C. (2017) Hens vary their vocal repertoire and structure when anticipating different types of reward. *Animal Behaviour* 130: 79-96 DOI: 10.1016/j.anbehav.2017.05.025

McGrath, N., Burman, O., Dwyer, C., Phillips, C.J.C. (2016) Does the anticipatory behaviour of chickens communicate reward quality? *Applied Animal Behaviour Science* 184: 80-90 DOI: 10.1016/j.applanim.2016.08.010

Walker, J. K., **McGrath, N.,** Handel, I. G., Waran, N. K. and Phillips, C. J. C. (2014) Does owning a companion animal influence the belief that animals experience emotions such as grief?. *Animal Welfare*, 23: 71-79.

Walker, J. K., **McGrath, N.,** Nilsson, D. L., Waran, N. K. and Phillips, C. J. C. (2014) The role of gender in public perception of whether animals can experience grief and other emotions. *Anthrozoos*, 27: 251-266.

Fordyce, G., McMillan, H., and **McGrath, N**. (2014) Accelerating healing of calf frontal sinuses exposed by dehorning. Technical Report B.AWW.0227, QAAFI, The University of Queensland.

McGrath, N., Walker, J., Nilsson, D. and Phillips, C. (2013) Public attitudes towards grief in animals. *Animal Welfare*, 22 1: 33-47.

Book Chapters

Verrinder, J.M., Phillips, C.J.C. and **McGrath, N.** (2016) Science, Animal Ethics and the Law. In Cao D., White S. (eds) Animal Law and Welfare - International Perspectives. Ius Gentium: Comparative Perspectives on Law and Justice, vol. 53. Springer, Cham

McGrath, N., and Phillips, C.J.C. (2014). Emotions in animals. In Georgette Leah Burns and Mandy Paterson (Ed.), Engaging with Animals: Interpretations of a Shared Existence (pp. 225-250) Sydney, NSW, Australia: Sydney University Press

Conference Abstracts

McGrath, N., Burman, O., Dwyer, C., Phillips, C.J.C. (2016) Does the anticipatory behaviour of chickens communicate reward quality? ISAE 50th International Congress, Edinburgh, 2016

McGrath, N., Walker, J., Nilsson, D. and Phillips, C. (2012) Public attitudes towards grief in animals. ISAE Australasian Regional Meeting, Melbourne

McGrath, N., (2012) The behaviour and vocalisations of chickens in anticipation of rewards Australian College of Veterinary Science, Science Week, Gold Coast

Contributions by others to this thesis

Chapter 2

McGrath, N., Burman, O., Dwyer, C., Phillips, C.J.C. (2016) Does the anticipatory behaviour of chickens communicate reward quality? *Applied Animal Behaviour Science* 184: 80-90

CP, OB and CD contributed to the development of the methodology, assisted with interpretation of the results and edited the final manuscript. Dr. Rebecca Dunlop, who joined the advisory team after my mid candidature milestone, consulted on the statistics for this publication. NM contributed to the development of the research concept and methodology, performed the experiment, undertook data analysis and interpretation, and was responsible for the final manuscript.

Chapter 3

McGrath, N., Dunlop, R., Dwyer, C., Burman, O., Phillips, C.J.C. (2017) Hens vary their vocal repertoire and structure when anticipating different types of reward. *Animal Behaviour* 130: 79-96

CP, OB and CD contributed to the development of the methodology and helped to edit the final manuscript. RD assisted with the statistical analysis, interpretation of the results and was the main editor of the publication. NM contributed to the development of the research concept and methodology, performed the experiment, undertook data analysis and interpretation, and was responsible for the final manuscript.

Chapter 4

Do reward-related calls influence the behaviour of other hens in the flock? Contributions by Nicky McGrath, Rebecca Dunlop, Clive Phillips, Cathy Dwyer and Oliver Burman.

CP, OB and CD contributed to the development of the methodology. RD assisted with interpretation of the results and was the main editor of the chapter. NM contributed to the development of the research concept and methodology, performed the experiment, undertook data analysis and interpretation, and was responsible for the final chapter.

Chapter 5

Humans recognise the emotional content of chicken calls. Contributions by Nicky McGrath, Joerg Henning, Clive Phillips, Cathy Dwyer and Oliver Burman.

JH, CP, OB and CD contributed to the development of the methodology. CP also edited the manuscript. JH assisted with selection of statistical methods, interpretation of the results and was the main editor of the chapter. NM contributed to the development of the research concept and methodology, launched the survey, undertook data analysis and interpretation, and was responsible for the final chapter.

Statement of parts of the thesis submitted to qualify for the award of another degree

No works submitted towards another degree have been included in this thesis.

Research involving human or animal subjects

Chapter 2 and 3: Animal Ethics approval SVS/314/12
UQ Research and Innovation

Chapter 4: Animal Ethics approval CAWE/066/13/VOICELESS
UQ Research and Innovation

Chapter 5: Human Ethics approval 2016001225
UQ Human Research Ethics Committee.

All ethics approval certificates are provided in the Appendix.

Acknowledgements

First and foremost, I would like to thank my supervisors for being my patient and constant guides on this journey. Clive Phillips, you gave me all the encouragement I needed to start this PhD and wholeheartedly supported my choice of topic. I will forever be grateful to you for giving me the opportunity to follow my sometimes naïve, but always passionate, desire to work on vocalisations and emotions in animals. Cathy Dwyer, you have been a constant support from far away, especially during my pregnancy, and I always felt I could approach you with any PhD-related fears I had. Oliver Burman, you have been so enthusiastic about my chosen subject, and have encouraged me to look down paths that I would not have considered without your guidance, so thank you. Joerg Henning, thank you for stepping in with your support on my last chapter. You have always cheerfully answered any statistics-related question I have had, no matter how basic. Bec Dunlop, you have challenged me, listened to me, corrected me and have been a huge role model for me in the past three years. I am truly grateful for your honesty, your guidance and your patience. I have grown so much as a scientist through your feedback, and you have helped me feel ready for future challenges.

To the other researchers I have worked with and encountered along the way, you have all contributed to my growth during this journey. Jess Walker, you inspired me to start this journey. Thank you so much for letting me be a part of your PhD and igniting the belief that maybe I could do it too! Eduardo, Kris and Karen, it was always lovely to hang out with you in the White House when I was there, and great to bounce ideas off you. Anita and Jenny from the CEAL team in Gatton, thank you for helping me with some ideas for statistical analysis. Thank you also to Voiceless who have supported me with a grant during this project.

To the Ravenpro team at the Cornell Lab of Ornithology, especially Russ Charif and Liz Rowland: Thank you for teaching me all things Ravenpro and for welcoming my 9 month old baby into your fold while I was there!

To my amazing family who have never stopped believing in me over the past few years, and have been my constant source of strength, even from so far away, thank you so much. To my dad: I miss you enormously. I will always remember how quietly, but solidly, encouraging you were of this career change. You believed in me, and I know that if you were still here now you would be so proud of me. To my much loved friends who have kept me smiling, especially Suzi, Sarah Hornby, Susanne, Richie, Rosie, Jimbo, Sarah Harris, Helena and Katherine. Dr. Sarah Hornby, you have

been such a source of support and have always believed I could do this. Thank you so much for supporting me and looking after Katie while I tried out my presentation skills at the University of Newcastle.

Finally, I am dedicating this thesis to my beautiful little girl Katie. You are the reason I have kept my strength and determination. I love you with all my heart! Thank you for making me smile and giggle every day.

Financial support

This research project was partially funded by Voiceless.

Keywords

Animal vocalisations, animal behaviour, anticipation of rewards, motivation, emotion, referential, human perception, farm animal welfare.

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 060801, Animal Behaviour, 90%
ANZSRC code: 070299, Animal Production not elsewhere classified, 10%

Fields of Research (FoR) Classification

FoR code: 0608, Animal Behaviour, 100%

Table of Contents

Abstract ii

Declaration by author iv

Publications included in this thesis v

Submitted manuscripts included in this thesis vi

Other publications during candidature vi

Contributions by others to the thesis vii

Statement of part of the thesis submitted to qualify for the award of another degree ix

Research involving human or animal subjects ix

Acknowledgements x

Financial support xii

Keywords xii

Australian and New Zealand Standard Research Classifications (ANZSRC) xiii

Fields of Research (FoR) Classification xiii

Table of Contents xiv

List of Figures xix

List of Tables xx

List of Abbreviations xxii

CHAPTER 1 Introduction and Literature Review 1

1.1 Thesis overview and aims 1

1.2 Emotions in humans and animals 2

1.2.1 Measuring emotional states 3

1.3 The vocalisations of humans and non-human animals 5

1.3.1 Mechanisms 5

1.3.2 Signals 6

1.3.3 Receivers 8

1.3.4 Referential signals 10

1.3.5 The expression of emotion in vocalisations 11

1.3.6 Effect of vocalisations on emotional and sensory systems 12

1.4 Encoding and decoding of emotions in vocalisations 13

1.4.1 Acoustic features of emotions in humans 13

1.4.2 Decoding the acoustically-conveyed emotions of humans 15

1.4.3 Acoustic features of emotions in non-human animals 15

1.4.4 Decoding the acoustically-conveyed emotions of other animals 17

1.4.5 How do humans decode non-human animals' emotional cues? 18

1.4.6 Sensitivity to valence and arousal 22

1.5 The vocalisations of chickens 23

1.6 The behaviour of non-human animals in anticipations of rewards 24

1.6.1 The behaviour of chickens in anticipation of rewards 25

1.6.2 Expression of frustration in chickens 27

1.7 Conclusion and thesis outline 28

CHAPTER 2 Does the anticipatory behaviour of chickens communicate reward quality? 30

2.1 Abstract 31

2.2 Introduction 32

2.3 Methods 35

2.3.1 Subjects and housing 35

2.3.2 Treatment groups 35

2.3.3 Experimental apparatus 36

2.3.4 Training procedure 37

2.3.5 Test procedure 38

2.3.6 Behavioural recording 39

2.3.7 Statistical analysis 40

2.4 Results 43
2.4.1 Effect of signalled rewards compared with neutral treatments 43
2.4.1.1 Behavioural transitions 43
2.4.1.2 ‘Motivated’ behaviour 43
2.4.2 Effect of different signalled rewards 43
2.4.2.1 ‘Motivated’ behaviour 44
2.4.2.2 Standing 44
2.4.2.3 Latency to access the rewards 45
2.4.3 Effect of signalled neutral event 45
2.4.3.1 Alert head movements 45
2.4.4 Locomotion 46
2.5 Discussion 49
2.5.1 Effect of sound cues 49
2.5.2 Increase in activity / behavioural transitions 50
2.5.3 Behaviour reflects differences between signalled rewards 51
2.5.4 Behaviour reflects how birds rank rewards 51
2.5.5 Intensity of behavioural expression 51
2.5.6 Head movements 53
2.5.6.1 Sound cue effect – context-mediated equivalence? 54
2.6 Supplementary material 55
CHAPTER 3 Hens vary their vocal repertoire and structure when anticipating different types of reward 57
3.1 Abstract 58
3.2 Introduction 59
3.3 Methods 61
3.3.1 Test procedure 62
3.3.2 Acoustic analysis 63

3.3.3 Call classification 64

3.3.4 Statistical analyses of call types 66

3.4 Results 68

3.4.1 Call classification 68

3.4.2 Prevalence of call types 71

3.4.3 Differences in call parameters 72

3.5 Discussion 77

3.6 Supplementary material 82

CHAPTER 4 Do reward-related calls influence the behaviour of other chickens in the flock? 89

4.1 Abstract 90

4.2 Introduction 91

4.3 Methods 95

4.3.1 Subjects and housing 95

4.3.2 Experimental apparatus 95

4.3.3 Recordings and selection of calls 96

4.3.4 Design and test procedure 97

4.3.5 Ethogram 97

4.3.6 Behavioural recording 98

4.3.7 Statistical analyses 99

4.4 Results 100

4.5 Discussion 102

CHAPTER 5 Humans recognise the emotional content of chicken vocalisations 106

5.1 Abstract 107

5.2 Introduction 108

5.3 Methods 111

5.3.1 Experimental study to record chicken calls 111

5.3.1.1 Study design ... 111
5.3.1.2 Sound recordings ... 111
5.3.1.3 Acoustic analyses ... 112
5.3.2 Cross-sectional study on human recognition of chicken calls ... 112
5.3.2.1 Study design and participant selection ... 112
5.3.2.2 Questionnaire ... 113
5.3.2.3 Participants ... 114
5.3.2.4 Statistical analyses ... 115
5.4 Results ... 117
5.4.1 Valence and arousal ratings ... 117
5.4.2 Effect of acoustic variables on valence and arousal ratings ... 118
5.4.3 Effect of participant characteristics on valence and arousal ratings ... 119
5.4.4 Effect of demographic variables on context assignment ... 123
5.5 Discussion ... 123
5.6 Supplementary material ... 128
CHAPTER 6 General discussion and future directions ... 133
6.1 Discussion ... 133
6.2 Summary of main findings ... 139
6.3 Future directions ... 139
List of references ... 141
Appendix: Ethics approval certificates ... 165
Ethics approval certificate: Chapters 2 and 3 ... 165
Ethics approval certificate: Chapter 4 ... 166
Ethics approval certificate: Chapter 5 ... 167

List of Figures

Figure 2.1 The experimental pen ... 36

Figure 2.2 Mean frequency of behavioural transitions ... 43

Figure 2.3 Mean frequency and duration of motivated behaviour ... 44

Figure 2.4 Mean frequency and duration of behavioural response 'Stand' ... 44

Figure 2.5 Mean duration of latency periods by treatment ... 45

Figure 2.6 Mean frequency and duration of 'Alert Head Movements' ... 46

Figure 2.7 Mean frequency and duration of behavioural response 'Locomotion' ... 46

Figure 3.1 Number of calls made and number of calls analysed by treatment ... 63

Figure 3.2 Classification and regression tree of vocalisations made by chickens in anticipation of rewards ... 69

Figure 3.3 Model predictions of call rate per hen for Double clucks, Fast clucks, Food calls and Single clucks in anticipation of five different treatments ... 71

Figure 3.4 Model predictions of call rate per hen for Mixed calls, Whines, Gakel calls and Singing calls in anticipation of five different treatments ... 72

Figure 3.5 Variation in the peak frequency of 'Food calls' by reward ... 73

Figure 3.6 Spectrograms of (a) 'Food call', (b) 'Fast cluck', (c) 'Single cluck' and (d) 'Double cluck' ... 76

Figure 3.7 Example spectrograms of (a) 'Whine' and (b) 'Gakel call' ... 77

Figure 5.1 Valence and arousal ratings provided by participants for 16 chicken calls ... 117

Figure 5.2 Correlation between peak frequency and call duration for 4 call types with ... mean valence and arousal ratings ... 118

Figure 5.3 Mean valence and arousal ratings with 95% confidence limits for each call type by age group ... 119

Figure 5.4 Food call and fast cluck context assignments by age group ... 122

Figure 5.5 Whine and Gakel call context assignments by age group ... 123

Figure 5.6 Caterpillar plot of random-effects of 'participant' on Valence ... 128

Figure 5.7 Caterpillar plot of random-effects of 'participant' on Arousal ... 128

Figure 5.8 Caterpillar plot of random-effects of 'participant' on Correct context ... 128

List of Tables

Table 2.1 Hen cue groups and combinations of CS and US 36

Table 2.2 Ethogram for behaviours displayed by hens during the experiment........................ 40

Table 2.3 Behavioural responses subjected to analysis.. 40

Table 2.4 Behavioural responses' lsmeans, 95% confidence limits and significance levels ... 47

Table 2.5 Medians, interquartile ranges and significance levels for 'Motivated' bouts, 'Alert head movements' frequency, and the latency of birds to enter the reward room once the door had been opened.. 47

Table 2.6 Comparison of the different linear mixed effects models (LMMs) for behavioural responses and latency time periods 48

Table 2.7 Comparison of the different generalised linear mixed effects models (GLMMs) for behavioural responses.. 49

Table 2.8 Final model outputs from LMMs for behavioural responses and latency time periods 55

Table 2.9 Final model outputs from GLMMs for behavioural responses and Behavioural Transitions variable.. 56

Table 3.1 Description of the measurements used in the quantitative classification of call types 64

Table 3.2 Mean values of acoustic parameters by call type.. 65

Table 3.3 Number of call types made by individual hens.. 65

Table 3.4 Random Forest confusion matrix and classification error for each call type........... 70

Table 3.5 Gini index showing importance of predictor variables in the Random Forest Analysis... 70

Table 3.6 Vocal parameters' lsmeans, 95% confidence limits and significance levels........... 74

Table 3.7 Comparison of the different linear mixed-effects models (LMMs) for behavioural responses 75

Table 3.8 Food call: Final model output from LMM.. 82

Table 3.9 Fast cluck: Final model output from LMM... 83

Table 3.10 Whine: Final model output from LMM 84

Table 3.11 Gakel call: Final model output from LMM.. 85

Table 3.12 Single cluck: Final model output from LMM 86

Table 3.13 Double cluck: Final model output from LMM........ 87

Table 3.14 Call-type prevalence model output from GLMM 88

Table 4.1 Ethogram 98

Table 4.2 Comparison of the different models for the behavioural responses........ 99

Table 4.3 Lsmeans, 95% confidence limits and significance levels from the final model associated with the effect of sound treatments on the behavioural response ‘Alert head movements’ 101

Table 4.4 Final linear mixed effects model output showing the effects of playback of different sound treatments and a silent treatment on alert head movements in hens........ 101

Table 4.5 Final mixed-effects, multivariable logistic-regression model of effect of treatment and phase on the standing behaviour of chickens 102

Table 5.1 Comparison of the different mixed effects models for Valence, Arousal and Correct Context 116

Table 5.2 Kendall’s tau-b results between acoustic variables and valence and arousal ratings 119

Table 5.3 Final mixed-effects, multivariable linear model of participant factors associated with valence ratings of chicken calls 120

Table 5.4 Final mixed-effects, multivariable linear model of participant factors associated with arousal ratings of chicken calls........ 121

Table 5.5 Final mixed-effects, multivariable logistic-regression model of participant factors associated with identifying correctly if chicken calls were made in a rewarding or non-rewarding context........ 122

List of abbreviations used in this thesis

AIC	Akaike information criterion
ANS	Autonomic nervous system
CART	Classification and regression trees
CG	Cue group
CS	Conditioned stimulus
DFT	Discrete Fourier transform
FA	Factor analysis
FO	Fundamental frequency
GLMM	Generalised linear mixed model
KMO	Kaiser-Meyer-Olkin
LMM	Linear mixed model
MN	Muted neutral
MS	Motivation-structural
MSA	Measure of sampling adequacy
OOB	Out-of-bag error rate
SEC	Stimulus evaluation check
SN	Sound neutral
SNS	Somatic nervous system
US	Unconditioned stimulus

Chapter 1: Introduction and Literature Review

1.1 Thesis overview and aims

The vocalisations of animals, including humans, are modulated by different states of arousal. Physiological changes induced by emotional arousal affect the mechanisms of voice production (Titze 1994), and the resulting changes in the acoustic structure of vocalisations may convey an animal's emotional state (Morton 1977; Briefer 2012). The anticipation of rewards is associated with appetitive and 'wanting' behaviour, which has been linked to dopamine neurotransmitter activation in the brain (Berridge 2007, Berridge & Kringelbach 2008, Kringelbach & Berridge 2017), and a corresponding increase in emotional arousal (Löw et al. 2008). Vocalisations may therefore be useful indicators of what non-human animals find rewarding.

Understanding what animals want or find rewarding is central to the welfare of animals under human management (Dawkins 2012). We are compelled to provide animals with what they physically need in order for them to be able to survive, grow, reproduce and be healthy. However, 'wanting' certain things may also indirectly cause health and welfare issues. Many animals have evolved to possess mechanisms that help them anticipate threats to survival (Dawkins 2012), and some of these residual mechanisms continue to govern behaviour. Some behaviours appear to be 'hard-wired' into animals, such that they 'want' to forage for food, or perch, or dustbathe, even though the outcomes of these behaviours will no longer affect their survival (Hughes & Duncan 1988; Dawkins 2012). Studies on some animals have shown that the instinct to perform certain behaviours, such as foraging, can be greater than their need for direct access to food (Inglis et al. 1997; Vasconcellos et al. 2012). The inability to perform particular behaviours, which would normally fulfil a need that may now be redundant, could result in frustration. This outcome may be detrimental to an animal's health, and their welfare may be compromised.

Studies have been conducted on chickens to investigate the behaviour they exhibit during non-reward situations that induce frustration (Zimmerman & Koene 1998; Zimmerman et al. 2000). During the first study, one particular vocalisation, the gakel call, was correlated with the behavioural expression of frustration (Zimmerman & Koene 1998), and follow-up studies supported this finding (Zimmerman et al. 2000; Zimmerman et al. 2003). The gakel call, therefore, could be viewed as a 'marker' for frustration in chickens. However, vocal indicators of reward-related, positively valenced states are lacking in the literature on chickens. Therefore, following a similar vein, this thesis investigates whether chickens produce specific reward-related vocalisations, which may be correlated with other behaviour shown in anticipation of rewards.

My thesis will determine what vocalisations chickens produce in rewarding contexts using different types of reward, and whether these vocalisations have referential qualities. In addition, this thesis will reveal whether humans can correctly identify chicken vocalisations made in rewarding contexts, and how humans judge the valence and arousal level (intensity) of these vocalisations. Correlating vocalisations with other behaviour can help deduce 'meaning' in vocalisations, and therefore Chapter 2 of this thesis looks at the behaviour of chickens in anticipation of food and non-food rewards. Chapter 3 characterises the vocalisations made in anticipation of rewards, and investigates the structural variation within these vocalisations, to determine whether they may encode information relating to different types of reward. Chapter 4 will investigate whether reward-related vocalisations are functionally referential, using a playback paradigm to demonstrate whether they influence the behaviour of other chickens. Vocalisations can be used as 'markers' of motivational or internal states, and if there are distinctive vocalisations elicited by rewarding situations, these could contribute to a useful method of assessing welfare. Chapter 5, therefore, will report on how humans perceive reward-related vocalisations produced by chickens, in order to establish whether humans can discriminate between vocalisations produced in rewarding or in non-rewarding contexts.

The following literature review provides some of the theoretical background needed for the study of vocalisations in anticipation of rewards. Firstly, it discusses emotions in humans and non-human animals and how they may be measured. It then reviews the structure and mechanisms behind the production of vocalisations in humans and animals, followed by the theory supporting vocalisations as signals. Subsequently, it focuses on the effects of vocalisations on receivers, and receiver responses. Then, it examines the vocal correlates of emotions in humans and non-human animals, and how information contained within affective vocalisations may be decoded. Finally, it looks at studies that have provided evidence of behaviour in anticipation of rewards in non-human animals, with a specific focus on chickens.

1.2. Emotions in humans and animals

Emotions are generally said to be about something, or to have an 'object' (Hacker 1999). The object can be any external or internal stimulus that elicits an emotional response. Anderson & Adolphs (2014) suggest that emotions in humans and non-human animals are mediated by central emotional states, evoked by internal or external stimuli, leading to behavioural responses and, in humans, subjective ‘feelings’. The latter are currently only identifiable in humans due to our ability to verbally report on them. In evolutionary terms, emotions are adaptive responses that enable animals to solve problems they would be unable to with simple automatic reflexes. Therefore, emotions offer behavioural flexibility, often through learning. Most emotions are short-term and intense, mobilizing physical responses that enable animals to avoid harm or to approach resources that will improve their fitness (Rolls 2000; Boissy et al. 2007). In simple terms, they provide an adaptive benefit (Bekoff 2007).

Emotional systems coordinate both behavioural and physiological processes (Panksepp 1998). Damasio’s (1998) somatic marker hypothesis suggests that associations between reinforcing stimuli induce a physiological affective state. A network of ‘markers’ influences decision-making in situations that may involve conflicting messages or stimuli, and directs the animal’s attention towards advantageous options, thereby simplifying the decision process. In this way, emotions play a critical role in the ability to make fast, rational decisions in complex and uncertain situations. Indeed, some behavioural responses, such as jumping away from what is perceived to be a snake before realising it is a stick, are innate in humans and non-human animals (Panksepp 1998).

Neuroscientific studies have found that there are many homologous neural structures in the brains of all mammals, especially in the subcortical areas (Panksepp 1998). Techniques used in affective neuroscience include electrical stimulation of the brain (ESB), creating brain lesions, lateralization studies, pharmacological activation or inhibition of neurotransmitter receptors, monitoring of neuronal firing patterns using microelectrodes, and gene expression measurement. By manipulating the brain using these techniques, and correlating the outcomes with observation of approach and avoidance behaviours, Panksepp (1998) suggested that five basic emotions (fear, anger, separation distress, play and seeking) exist universally in the mammalian brain. These emotions, he says, create ‘action tendencies’ which are flexible responses to situations where animals need to learn strategies for survival. Further proof that these emotions exist in non-humans becomes evident when key neurochemistries and brain activation patterns in animals are correlated with predicted emotional responses (Panksepp 2005).

1.2.1 Measuring emotional states

Emotions are experienced by humans both on a physiological level (generated by the subcortical part of the brain), and on a subjective psychological level (generated by the neocortex) (Panksepp 2011). However, the intrinsic ‘pleasantness’ of emotions cannot be easily inferred in non-human animals. Therefore, one of the challenges experimenters face when evaluating emotional states in animals is how to experimentally achieve this using a comparative framework.

Darwin (1872) was the first to describe the biological expression of emotion in both humans and non-human animals. He cited anger, happiness, sadness, disgust, fear and surprise as universal core emotions. This view of emotions as discrete entities was later supported by Ekman (1999) who maintained that individual emotions have universal antecedent causes as well as other characteristic hallmarks. Scherer (1984) outlined a more complex theory of emotions in his component process theory, which suggests that an emotion comprises an episode of temporary synchronisation of all major subsystems of organismic functioning represented by five components (cognition, physiological regulation, motivation, motor expression and monitoring-feeling). This synchronisation occurs as part of the evaluation of an external or internal stimulus event relevant to the central concerns of the organism. Such appraisals are called ‘stimulus evaluation checks’ (SECs) (Scherer 1984). Scherer (1986) went on to make predictions (based on his component theory) about the patterns of voice cues associated with various emotions. The outcome of an SEC has an effect on the somatic nervous system (SNS), which affects the muscle tension associated with voice production. SECs also affect the ANS (mucous and saliva production) which, in turn, affects voice production.

A dimensional approach to describing emotions was initially proposed by Wundt (1905), using a model integrating the dimensions of valence (positive-negative), arousal (calm-excited) and tension (relaxed-tense). Valence refers to the intrinsic attractiveness (positive valence) or the intrinsic aversiveness (negative valence) of an event, object or situation leading to an emotion. Arousal represents the degree of alertness, excitement or engagement produced by the object of emotion. This approach assumed that emotional states were on a continuum. Following on from this, various other dimensional models were proposed, including a circumplex model advanced by Russell (1980), which suggested that dimensions are interrelated and can be represented by a spatial model. A bi-phasic theory of emotions (Lang et al. 1992) suggested that two motivational systems in the brain govern responses to appetitive or aversive stimuli, by evaluating the valence of emotions (pleasant or unpleasant) or the intensity or activation stimulate (high arousal – excitation or low arousal – calm) (Lang et al. 1998).

Mendl et al. (2010) also proposed a dimensional approach to evaluating short-term emotional states and longer-term mood. In this approach, a core-affective framework locates discrete emotions within a two-dimensional space characterised by levels of valence (positive or negative) and arousal (high or low). Emotions may be positively valenced but represent low or high arousal (e.g. contentment vs. joy). Equally, emotions may be negatively valenced but also express either low or high arousal (e.g. depression vs. fear). These components of discrete emotions serve to prioritise action. Discrete emotions arising from events can have short-term effects on the overall mood-state (core affect), and also a cumulative effect on longer-term mood. For example, persistent exposure to negative events may result in a high-arousal, negative mood state. Therefore mood reflects past experiences, but also constantly changes according to individual experiences. Mood-states also have a reciprocal effect on discrete emotions and decision making.

Measuring emotions based on this framework involves making predictions about the types of situation that will generate a particular affective state, predicting the types of decisions animals will make in certain states and assessing underlying mood states by identifying discrete emotions associated with these states. This approach has the potential to identify positive affective states and discrete emotions (Mendl et al. 2010). However, as in humans, non-human animals may not experience all emotions as positive or negative and may transition quickly between them.

1.3 The vocalisations of humans and non-human animals

1.3.1. Mechanisms

The range of vocalisations in any animal is constrained by the mechanisms used to produce them. Human speech is a product of the integrated action of the lungs, larynx, supralaryngeal vocal tract, and articulators. Airflow from the lungs passes into the larynx, where vibrations in the vocal folds convert this airflow into a sound, known as the source signal or ‘glottal wave’. These vibrations determine the fundamental frequency (pitch) and harmonics of the sound (Fant 1960; Titze 1994; Taylor & Reby 2010). The vocal tract then acts as a ‘filter’, honing the sound by softening some frequencies and amplifying other frequencies which become ‘formants’ (Titze et al. 2015). In humans, articulators such as the tongue, lips, teeth, hard and soft palate and jaw, enable complex modifications of sounds by changing the size of the pharyngeal cavity and the mouth. The source-filter theory of animal vocal production has been generalised across all non-human mammals (Fitch & Suthers 2016), and also extended to cover avian species (Elemans et al. 2008; Ohms et al. 2010). In birds, the vocal organ is the syrinx, and air pressure built up in the bronchi and air sacs causes tympaniformic membranes to be brought closer together and vibrate, thus producing sound in a column of air (Gaunt et al. 1976). Vocal parameters such as frequency, amplitude, rate, call duration and energy distribution are dependent on the anatomy and physiology of the animal.

The acoustic structure of human speech and animal vocalisations encodes information within parameters such as frequency (Townsend et al. 2014), amplitude (Gustison & Townsend 2015; Reichard & Anderson 2015), rate of production (Clay et al. 2012) duration (Dentressangle et al. 2012) and energy distribution (Linhart et al. 2015). This information may be static and relate to individual identity (Vannoni & McElligott 2007; Favaro et al. 2015), age, body size and sex (Briefer & McElligott 2011a), geographical discrimination (Hahn et al. 2016) or recognition systems between mothers and offspring (Briefer & McElligott 2011b). Flexible traits, such as signaller motivation, may be reflected in vocal frequency, amplitude, duration and rate in both humans (Scherer 1986) and animals (Taylor & Reby 2010; Briefer 2012), as shown in animals such as meerkats (*Suricata suricatta)* (Hollén & Manser 2007) and rats *(Rattus norvegicus)* (Knutson et al. 2002).

1.3.2 Signals

Vocalisations produced under certain criteria can be constituted as signals, meaning they convey information to other animals (Font & Carazo 2010; Bradbury & Vehrencamp 2011). By definition, signals influence the behaviour of receivers. Maynard Smith and Harper (2003) define a signal as 'any act or structure which alters the behaviour of other organisms, which evolved because of that effect, and which is effective because the receiver's response has also evolved'. Seyfarth & Cheney (2003b) claim that signallers rarely call unless there is a potential audience present, even if the calls seem only to be involuntary expressions of arousal. Vervet monkeys (*Chlorocebus pygerythrus*) tend not to call when they are alone (Maynard Smith & Harper 2003). Chicken (*Gallus gall*us) food-calls are also mediated by an audience (Marler et al. 1986b; Evans & Marler 1994).

Some scientists have debated whether there is information in animal vocalisations that can be accessed by receivers (Dawkins & Krebs 1978; Owings & Morton 1998; Rendall & Owren 2002; Rendall et al. 2009). Other research on signalling does not attribute intentionality to signallers: signals, in this sense, do not transmit a message, nor does the signaller need to ascribe any thoughts or beliefs to the receiver (Font & Carazo 2010). Current scientific evidence suggests that most animals have no theory of mind, that is, they cannot attribute mental states to others (Seyfarth & Cheney 2003a; Shettleworth 2010; Dawkins 2012), and there is no systematic evidence that individuals try to instruct others, or that they are aware of the ignorance or knowledge of others. So, although calls may convey information, some vocalising animals may not intend to send this information. It has been suggested that these passive 'signals' are in fact cues - passive traits that inherently provide information to the observer rather than being intentional (Maynard Smith &

Harper 2003; Bradbury & Vehrencamp 2011). Examples of unintentional cues are involuntary sounds that reflect arousal and motivational states.

According to the differentiation made above, signals have a function for the sender and receivers must respond through changes in their behaviour. Examples of signals are contact calls that facilitate group cohesion (Leighty et al. 2008; King & Sueur 2011). Other calls appear to signal possession of a resource or a claim to territory, and may function to prevent conflict (Brumm & Todt 2004; Clay et al. 2012). Further calls function as devices to attract a potential mate, or to attract conspecifics to food sources where feeding alone may be less advantageous than feeding with others (Elgar 1986; Musolf et al. 2010). All these calls confer an advantage on the sender by altering the behaviour of the receivers to be favourable to the sender. However, signalling doesn't always imply communication between two or more parties. For example, baboon (*Papio cynocephalus ursinus)* contact calls appear to reflect the signaller's own state and position, but the signallers don't seem to be influenced by the knowledge the receiver might possess (Seyfarth & Cheney 2003b). Vervet monkeys continue to give alarm calls even when all others in their group are giving the alarm call (Cheney & Seyfarth 1985). In addition, even in cases where there is a specific audience, signalling may fail and not induce a response (as a result of excessive ambient noise, for example) (Scott-Phillips 2008). Communication, by contrast, refers to successful completion of the signalling act (Scott-Phillips 2008).

The reliability of signals has been the subject of debate. Dawkins and Krebs (1978) initially urged scientists to disregard the concept of information in animal signals. They believed that signals were attempts on behalf of the sender to manipulate the receiver's behaviour to their own advantage and were therefore likely to be sending incorrect information, in order to mislead the receiver. Some theorists claim that a response from another individual implies that the system has some level of reliability, and that the signal is 'honest on average' (Searcy & Nowicki 2005). Others have asked how signals could be reliable when evolution produced individuals that acted in their own self-interest (Smith 1994; Searcy & Nowicki 2005). If evolutionary interests were likely to be identical for individuals (e.g. genetically identical individuals) then honest signalling, without incurring any costs, could take place. However, in most situations, even in mother-offspring situations, there may be divergent, if not conflicting, interests. Scientists generally agree that the honesty of signals in these situations is maintained through the imposition of costs, and this is referred to as the 'handicap principle' (Grafen 1990; Zahavi & Zahavi 1997; Searcy & Nowicki 2005). These costs may be development costs (paid at the time the signal develops), production costs (paid at the time of giving the signal) and maintenance costs (present even if the signal is not being made). The various costs may have several different components: for example energy may be expended during production of the signal or during development of the capacity for signalling, and time spent signalling rather than foraging may force a compromise between mating and searching for food (Searcy & Nowicki 2005). Although we cannot measure whether these costs have a direct impact on fitness, we can reasonably assume that they have an indirect impact on fitness. Reliability can be inferred by correlating the attributes of a signal with whatever it is that the receiver would benefit from knowing in a certain situation (Searcy & Nowicki 2005). Situations that seem to elicit reliable calls are the presence of predators (Seyfarth & Cheney 2003a; Seyfarth et al. 2010), and the presence of food (Evans & Evans 1999; Evans & Evans 2007).

Deception does sometimes occur, where competition between individuals can mean there is a strong temptation to cheat. For example male chickens were demonstrated to give food calls to attract females when there was no food available (Marler et al. 1986b). Females were shown to discriminate against males who gave these unreliable calls, and cease to respond to these individuals, thereby foiling the male's potential opportunity to mate (Evans & Evans 2002 in Searcy & Nowicki 2005). This discrimination is also shown by vervet monkeys, with individuals ignoring any calls made by the individual who gave the deceptive signal (Cheney & Seyfarth 1988). This individually-directed discrimination puts pressure on senders to produce reliable calls.

Signals are also subject to eavesdropping, as they occur not only in dyadic interactions, but often in groups (Bradbury & Vehrencamp 2011). Eavesdropping may be a factor in the evolution of calls. Certainly some animals appear to benefit from the ability to eavesdrop on other animals in order to determine their responses to threats or to forage more efficiently and reduce their vigilance of predators (Ridley et al. 2014; Wunder 2014). Other animals appear to have developed strategies to counteract eavesdropping. Song birds, for example, may sing quietly, or vary their song repertoire and the time between repetition of a song, to interfere with recognition by heterospecifics (Bayly & Evans 2003; Dabelsteen 2004; Riesch & Deecke 2011).

1.3.3 Receivers

It is difficult to prove that receivers acquire information from signals, and even more difficult to ascertain what type of information they acquire. Some scientists have suggested that signal design is the key to receiver response (Rendall & Owren 2002; Rendall et al. 2009). Rendall et al. (2009) suggest that some courtship signals stimulate 'pre-existing sensory biases' in females which they are incapable of ignoring, including biases in how they detect, perceive and react to these signals. They also theorise that basic alarm call structures tap into the auditory system, which links with neuroendocrine processes that regulate affect and motivation. In this sense receivers cannot control their response. Other research has also linked the effects vocalisations have on conspecifics with acoustic quality (e.g. Kent 1989; Puppe et al. 2005; Pongrácz et al. 2006; Bright 2008; Düpjan et al. 2011). For example, Collias and Joos (1953) found that calls known to attract chicks have certain features, which include repetitiveness and brief duration of components. In some cases, the acoustic properties of calls appear to mediate the responses of animals through effects on the nervous system or neuroendocrine processes (Rendall et al. 2009; Wheeler & Fischer 2012). For example, squirrel monkeys (*Saimiri sciureus*) exhibit a generalised startle response and avoidance behaviour as a reaction to their own species' alarm calls (Herzog & Hopf 1984). Rendall & Owren (2002) say that this startle reflex is an involuntary response triggered by abrupt sounds, which produces immediate attentional shifts and interruption of activity, concurrently with nervous-system responses. These responses do not require any experience of the predator, but they may facilitate appropriate learned responses to future encounters with predators (Rendall & Owren 2002).

Some types of infant-directed speech in humans use this ability to incite a reflexive response, and interrupt unsafe or unwanted behaviour in infants or children (Bryant 2013). Infants respond by stopping their activity and orienting towards the sound source, and this same principle is used by animal trainers who wish to initiate a specific activity or interrupt certain behaviours (McConnell 1991). 'Motherese' is the soft but high-pitched speech mothers use with their infants, which has been suggested to have a function of attracting the attention of infants (Lang et al. 1998) as well as helping infants to learn language (Nelson et al. 1989). Similarly 'Doggerel' is the type of speech humans use when interacting with their dogs (Hirsh-Pasek & Treiman 1982), which appears to function to help dogs learn from humans (Gergely et al. 2017). In addition, dogs appear to have learned how to get humans to respond to them by varying the acoustic quality of their vocalisations (Miklosi et al. 2000).

However, Seyfarth & Cheney (2010) maintain that a focus on signal acoustic properties and their arousal-inducing effects does not explain how some species can have multiple alarm calls, and that responses to them are so different and flexible. Selection has produced receivers whose qualitatively different responses reflect their own interests, and will depend not only on the acoustic properties of signals, but on the information content too. This suggests that animal vocal signals encode information, and receivers are able to decode this. This ability appears to extend to heterospecific calls. Aubin (1991) studied the reactions of five species of birds to a synthetically produced alarm call. He noted behavioural responses in all five species, which he did not attribute to the acoustic quality of the call, but rather he suggested that the birds were decoding the calls as if they conveyed information that a predator was in the area.

Some animals exhibit behaviour that implies that their knowledge of their external environment has been altered. Domestic chicks respond to food calls as if those calls have conveyed information about the presence of food (Evans & Evans 1999). They increase their rate of pecking when their mother gives a food call, and adults increase foraging behaviour and tend to look downwards more towards substrate (Evans & Evans 1999; Wauters & Richard-Yris 2002). Other calls have been demonstrated to be reliable indicators of a level of need: for example, isolation calls in piglets express need at tangibly different levels, and sows exhibit a stronger response to more extreme calls (Weary & Fraser 1995; Searcy & Nowicki 2005). Some calls may be vague in terms of the information receivers may acquire from them. Seyfarth & Cheney (2003b) distinguish between the context-specific information that can be acquired from baboon 'move' and infant grunts. Move grunts convey individual identity, location, and that the group is about to move (groups seldom move without grunts being emitted), while infant grunts, which are given during friendly social interactions, and may or may not predict infant handling (some infant handling occurs without the presence of grunts), do not precisely convey what type of social interaction will occur. Some receiver reactions to calls, however, indicate that very specific information has been acquired.

1.3.4 Referential signals

A signal can be considered referential if it conveys 'sufficient information about an event for receivers to select appropriate responses' without the aid of contextual information (Macedonia & Evans 1993). The signal evokes an adaptive response as if the receivers had actually experienced the eliciting stimuli themselves (Clay et al. 2012). The information acquired may be very specific, but the call must also accurately predict the circumstances providing the eliciting stimulus (e.g. the presence of a predator) and the breadth of stimuli eliciting the call must be narrow (Seyfarth & Cheney 2003b; Clay et al. 2012). Referential calls do not have to have intention on the signaller's part (Seyfarth & Cheney 2003a). Theoretically, they can be entirely based on arousal in the sender. However, they may still be referential if there is a narrow range of eliciting stimuli which are also predictable. Hence, calls can be purely affective on the sender's part, but referential or semantic as a result of the listener's ability to detect a pattern in the other's vocalisation (Seyfarth & Cheney 2003b)

Much recent research has focussed on whether animals make calls that can be classified as functionally referential (e.g. Evans & Evans 2007; Seyfarth et al. 2010; Wheeler & Fischer 2012). Some primates make calls that fulfil the above criteria; vervet monkeys, Diana monkeys (*Cercopithecus Diana*) and other primates give different alarm calls for different predators (Zuberbühler 2000; Seyfarth & Cheney 2010). Receivers of these calls behave as if they have knowledge of a type of predator when they hear alarm-calls from other members of their group, performing a different adaptive response for each call (Zuberbühler 2000; Seyfarth & Cheney 2003a). For example, when a vervet monkey hears an eagle alarm call, it looks up and runs into cover, whereas when it hears a leopard alarm call, it runs up a tree (Seyfarth et al. 1980). Domestic chickens also possess acoustically distinct alarm calls for aerial or terrestrial predators, and these two alarm calls elicit different postures or behaviours in receivers (Gyger et al. 1987; Evans et al. 1993).

Some food calls are also thought to have referential properties. Food calls typically attract others to food sources, and some reliably indicate the quality or quantity of food available (Marler et al. 1986a; Evans & Evans 1999). In some cases, the senders and receivers of these calls are related, for example, mother and offspring. However, in other cases, these calls may attract unrelated individuals as well as kin. The possible benefits that calling may confer in these situations are to decrease the risk of predation, or to form a coalition to compete for a resource (Searcy & Nowicki 2005). Food calls may also be used by males to attract females for mating. Cockerels exhibit this behaviour, and hens respond by approaching the male and the food source (Marler et al. 1986b).

1.3.5 The expression of emotion in vocalisations

Vocalisations may also encode information about an animal's emotional state. The literature on how emotion is expressed through vocalisations is diverse. Goodall (1985) proposed that some calls are affective reflexive reactions to a stimulus. Darwin initially believed that animal vocalisations were involuntary consequences of other bodily movements, but he later proposed that vocalisations were associated with the emotional state of the animal (Darwin 1872). Owings and Morton (1998) proposed that the food-calls of domestic chickens are an example of vocalisations that reflect the motivational state of the signaller. They maintain that a chicken's varied rate of calling reflects the motivation to feed, either due to perception of quality of food (excitement about the food), or from their level of hunger (Owings & Morton 1998). Some scientists believe that vocalisations reflect distinct inner states in animals e.g. fear, pain, hunger or sexual drive

(Manteuffel et al. 2004; Tallet et al. 2010), while others believe that vocally expressed emotions are better described in terms of arousal and valence

Animals often produce vocalisations when they are experiencing negative emotional states. Calls may be elicited by social isolation and separation of animals, and may be observed alongside other behaviour such as increased activity or locomotion, escape attempts, increased heart-rate or hypothalamic-pituitary activity (Seay et al. 1962; Weary et al. 1999; Watts et al. 2001; Siebert et al. 2011; Ungerfeld et al. 2011). These reactions indicate distress and a desire for reunion (Hofer 1984; Topal et al. 2005; Hofer 2006). Qualitatively differing levels of need may also be expressed in distress vocalisations (Weary & Fraser 1995; Weary & Fraser 1997). Animals also sometimes vocalise in positive situations. For example, rats produce 50 kHz ultrasonic vocalisations in anticipation of play or during tickling from a familiar human (Knutson et al. 1998; Panksepp & Burgdorf 2000), while cats purr in affiliative contexts (Peters 2002).

1.3.6 Effect of vocalisations on emotional and sensory systems

Darwin recognised that sounds could be used to induce affect (Darwin 1872). The ‘affect induction’ perspective also suggests that the primary function of signalling is not to express emotion but instead to influence the listener by inducing an emotional response, resulting in a desired behaviour (Bachorowski 1999; Rendall & Owren 2002). Inferences about the signaller’s emotional state are a secondary outcome and reflect attributions that listeners base on their own affective emotional responses to the sound, their past experience with the sounds, and the context in which the signal is being produced (Bachorowski 1999). This view contrasts with the view that acoustic cues encoded in vocalisations represent the emotional states of the signaller (e.g. Juslin and Laukka, 2001; Scherer, 2003).

Other scientists concur with the idea that the emotional or sensory system of receivers is affected by vocalisations. Owings and Morton (1998) suggest that ‘vocal signals should affect the behaviour of others in part by evoking emotional states and thereby motivating strong behavioural reactions’. Indeed, other types of signals appear to affect the emotional state of receivers. For example human infants exhibit social referencing behaviour. When babies encounter new stimuli, they will often fixate on their mother’s face and mirror their mother’s facial expression. Research suggests that if the mother’s facial expression indicates a positive rather than a negative emotion, infants are more likely to make contact with this unfamiliar stimulus (Klinnert 1984). In another study, vocal cues appeared to be even more effective than facial cues at guiding infant behaviour (Vaish & Striano 2004). While research on social referencing in non-human animals has only produced ambiguous results (Russell et al. 1996; Merola et al. 2012), examples of less cognitive-oriented research on animals have demonstrated that the heart-rates of heifers increase when their calves vocalisations are played back to them (Marchant-Forde 2002), and dogs respond to conspecific distress vocalisations with stress-related behaviours (Quervel-Chaumette et al. 2016)

Certain calls may evoke emulative vocalizations in conspecifics which may ‘virtually by positive feedback, adjust and synchronize the emotional states of the animals so that an entire group may be evaluated’ (in terms of welfare) (Manteuffel et al. 2004). This ‘emotional contagion’ functions to improve cohesion in groups, and regulates social interactions (Panksepp 2010). Preston and de Waal (2002) cite a number of experiments that suggest that apes and humans show contagion-like responses to the distress of a conspecific. Burman et al. (2007) suggest that playback of rat ultrasonic vocalisations may induce a negative emotional state in conspecifics. Dupjan et al. (2011) also believe that emotional contagion can take place, and use an example of distress calls evoking distress responses in conspecifics, as ‘transmission of distress’. However, in an experiment to discover if pigs’ responses to conspecific distress calls indicated emotional contagion, they found no evidence of such transmission of emotional state. (Düpjan et al. 2011). This result could have been due to the fact that the calls were made by unfamiliar conspecifics, and therefore may not have been relevant to the receivers. Emotional contagion may depend on the relationship between the senders and receivers. Indeed, Briefer et al. (2017) found that horses responded differentially, both behaviourally and physiologically, to differently valenced whinnies given by familiar conspecifics, suggesting that emotional contagion was at play. Evidence of emotional contagion in non-human animals’ is relatively poor (Edgar et al. 2012), despite the potential for transfer of affective states between animals (de Waal 2008). As a result, Briefer (2018) has proposed that future research should focus on strengthening evidence on the vocal contagion of emotions in non-human animals. To achieve this, researchers could perform playback experiments that use similarly valenced vocalisations which indicate different levels of arousal, or differently valenced vocalisations indicating similar levels of arousal (Briefer 2018).

1.4 Encoding and decoding of emotions in vocalisations

1.4.1 Acoustic features of emotions in humans

Speech and music, as well as human non-verbal acoustic communication, have the capacity to convey emotional state (Banse & Scherer 1996; Juslin & Laukka 2003; Sauter et al. 2010). For example, faster and more intense music is often associated with happiness, while slower and less intense music is frequently associated with sadness. The emotional cues emitted in vocalisations are mainly involuntary. Changes in affective state induce the nervous-system to produce changes in respiration, activity and tension in the diaphragm, intercostal and vocalis muscles. This activity modifies vocal parameters (Scherer 2003).

'Affective prosody' refers to the melodic or rhythmic aspects of human vocalisations, including the changes in pitch, tone, timbre, pauses and loudness of vocalisations that give human vocalisations their affective quality (Murray & Arnott 1993; Banse & Scherer 1996; Ilie & Thompson 2006). Speakers across different cultures convey emotion using similar prosodic cues (Bachorowski & Owren 1995; Calder et al. 2001), and people of different languages and cultures have interpreted the same emotions from these paralinguistic vocal cues (Scherer et al. 2001; Sauter et al. 2010). In this way, the acoustic profile of several discrete emotions has been decoded (Hammerschmidt & Jürgens 2007). Detection of emotion in human vocal cues occurs very rapidly (Sauter & Eimer 2009), and the cross-cultural evidence above suggests that it is an innate mechanism. This mechanism may have pre-human origins, as complex pitch perception mechanisms have been shown to be shared by humans and primates (Song et al. 2016).

Some human studies have provided evidence for acoustic links to discrete emotions (Banse & Scherer 1996; Hammerschmidt & Jürgens 2007). Scherer (2003) posited that discrete affective states experienced by the vocaliser are reflected in the particular patterns of acoustic cues in their speech. Banse & Scherer (1996) analysed vocalisations produced by 12 actors portraying 14 emotions. The fundamental frequency (*F0)* and amplitude of the vocalisations showed the strongest correlations with the emotions being portrayed. However, demonstrating acoustic links to discrete emotions presents difficulties, as descriptions of emotions may be imprecise or differ between languages (Murray & Arnott 1993; Briefer 2012).

Evidence of vocal parameters linked to emotions has been shown in various studies using a dimensional approach (Bänziger & Scherer 2005; Goudbeek & Scherer 2010). Several acoustic parameters have been associated with the arousal dimension of emotional expression, although some evidence suggests that low levels of arousal are hard to identify (Juslin & Laukka 2001). Physiological arousal is mainly reflected in *F0*, amplitude, energy distribution, harmonic to noise ratio, *F0* contour, *F0* range, duration, rate, and decrease in inter-vocalisation interval, Conversely, there is less evidence for how the valence of an emotion is expressed vocally, although it may be detectable in intonation patterns and voice quality (Scherer 1986; Briefer 2012). Goudbeek and Scherer (2010) found that the shape of the spectrum, vocalisation rate, and amplitude also expressed valence accurately, but cautioned that it is important to control for arousal levels to be able to tease out the relevant acoustic correlates of valence. Bachorowski (1999) suggested that speech sounds primarily signal the sender's arousal state but only communicate the valence of their emotional state to a small degree. Banziger and Scherer (2005) claimed that measuring the mean *F0* and *F0* range (range of *F0* contours) was enough to account for the most important variation between emotions in a range of studies they reviewed.

1.4.2 Decoding the acoustically-conveyed emotions of humans

Emotional cues may be perceived by others. Perception of the internal state of others forms an important part of social communication in humans (Bachorowski 1999), and is linked to empathy. Empathy is the capacity to perceive, understand and respond emotionally to the unique affective state of another person or animal (Hammerschmidt & Jürgens 2007; Edgar et al. 2012). From an evolutionary perspective, the ability to identify an emotional state in others allows receivers to perceive threats or danger, thereby increasing survival opportunities (Anderson & Adolphs 2014; Filippi et al. 2017a). Some situations where receivers or senders would benefit from the accurate perception of emotional states within species are territory disputes, avoidance of a predator, social interactions and the survival of newborns (Owings & Morton 1998; Gogoleva et al. 2010). In addition, being able to identify the social intentions and motivations of others may confer biological fitness benefits for both signaller and receiver (Schmidt & Cohn 2001).

In a meta-analysis of studies examining vocal expressions and music, Juslin & Laukka (2003) proposed that expressions of emotion are processed by general purpose brain mechanisms that respond to acoustic features, regardless of whether the stimulus input is speech or music. Listeners decode emotional meaning in music, and also report experiencing physical sensations such as lacrimation, changes in heart rate and blood pressure as well as skin conductance levels, which appear to be emotionally induced physiological changes (Laukka et al. 2005; Altenmüller et al. 2013). Music is often used to change mood (Laukka et al. 2005) and manipulation of the acoustic properties of both music and speech was found to influence affective judgements (Lang et al. 1998). This suggests that emotional processing in music and speech lies within similar neural systems.

1.4.3 Acoustic features of emotions in non-human animals

Darwin was one of the first scientists to describe how the production of vocalisations seemed to be associated with other behavioural signs of emotion in animals (Darwin 1872). Following on from this, one study provided an early framework for categorising mammal vocalisations according to the motivation underlying their production. Morton (1977) proposed that the vocalisations of birds and mammals tend to be subject to certain 'Motivation-Structural' (MS) rules. According to this concept, vocalisations produced in one motivational context (e.g. a hostile situation) should differ in structure from vocalisations produced in a very different motivational context (e.g. fearful interactions) (Morton 1977). In mammals, low frequency and broadband sounds are produced in aggressive contexts, whereas higher frequency, tonal sounds are produced in fearful contexts. This theory has been validated in many species: in dogs, chimpanzees, coatis, elks and other animals low frequency calls are common in hostile contexts, whereas in fearful contexts, high tonal calls are produced (for a review of studies conducted on acoustic parameters of non-human animal vocalisations see Briefer 2012). Therefore, in some animals, it may be possible to guess their motivational state from the structure of their calls. For example, the vocalisations of squirrel monkeys show an increase in peak frequency, and frequency range, in response to more aversive stimuli (Fichtel et al. 2001).

Briefer's (2012) comprehensive review of vocal communication in mammals extended the scope of Morton's contexts by suggesting that if an animal experiences a negative high-arousal state in a hostile context, then a friendly context would be likely to induce a positive low-arousal state. If negative emotional states are characterised by low-frequency calls, then positive emotional states should, logically, be characterised by high-frequency sounds. However, Briefer (2012) cautions that more acoustic parameters need to be studied to separate high-frequency sounds that represent negative emotions (fear) from those representing positive emotion. She cites several studies where vocal correlates of valence have been investigated in negative (situations of need, presence of threats) or positive contexts (during play or in affiliative situations). However, the contexts are often not clearly positive or negative (e.g. Yeon et al. 2011), and acoustic parameters indicating valence have proved difficult to pinpoint (Briefer 2012). Individual differences in reactions to assumed positive and negative contexts would also make it difficult to generalize that animals may be experiencing the same emotion.

The most common acoustic features of arousal in non-human animals are peak frequency, amplitude contour, energy distribution, harmonic to noise ratio, *F0* contour, *F0* range, formant contour vocalisation / element duration and rate, and decrease in inter-vocalisation interval, with other potential measures being jitter, spectral noise, and time of peak frequency (Briefer 2012; Linhart et al. 2015). Increases in arousal, therefore, produce vocalisations that are longer, harsher, louder, faster, and with higher frequency and a wider frequency range. These are very similar to human vocal correlates of arousal, and it has been suggested that these acoustic features may be universal across primates, stemming from shared mechanisms of vocal emotional expression (Hammerschmidt & Jürgens 2007; Filippi et al. 2017a).

As in humans, acoustic links to valence in non-human animals are less easy to define. Vocalisations signalling negative emotions (cries, alarm calls) are much more common in the animal world. In addition, as Briefer (2012) points out, it sometimes proves difficult to separate the effects of arousal and valence. Notwithstanding this challenge, some studies have attempted to do this. Positive vocalisations of dogs are characterised by shorter vocalisations, higher *F0*, and a shorter inter-call interval (Yin & McCowan 2004; Taylor et al. 2009), and cat vocalisations in positive situations (affiliative) are characterised by higher peak frequency and energy distribution than those produced in negative situations. Some animals produce low frequency calls in positive situations (Scheumann et al. 2007; Gogoleva et al. 2010; McGrath et al. 2017). Positive vocalisations, then, appear to vary in frequency. Call duration appears to be a more robust measure, with positive situations mainly eliciting shorter call durations (Brudzynski 2007; Taylor et al. 2009; McGrath et al. 2017). However, Briefer (2012) urges that future research should investigate acoustic links to valence using more acoustic parameters, such as shifts in energy distribution, frequency ranges, spectral slopes, formants and spectral noise.

1.4.4 Decoding the acoustically-conveyed emotions of other animals

The ability to evaluate the emotional state of another species may increase survival opportunities (Nesse 1990). Decoding vocal expressions of emotion provides a method of evaluating internal states. Not surprisingly, many animals have a hierarchical structure in their auditory systems, optimised for conspecific sound processing. In birds and mammals there also appear to be neural preferences for conspecific vocalisations over the vocalisations of other species, which has been shown in gene expression in canaries and zebra finches (Mello et al. 1992). Preference for conspecific vocalisations has also been found in mammals such as Mexican free-tailed bats (*Tadarida brasiliensis*) (Pollak 2013), mice (*Mus musculus)* (Holmstrom et al. 2010), guinea pigs (*Cavia porcellus*) (Šuta et al. 2013), chimpanzees (*Pan troglodytes)* (Taglialatela et al. 2008) and also in humans (Andics et al. 2010). However, eavesdropping occurs across a wide range of species, with birds eavesdropping on other birds and mammals, and mammals eavesdropping on other mammals, birds or lizards (Magrath et al. 2015). Some animals may assimilate information extracted from heterospecific vocalisations and conspecific behaviour to determine their own response to potential threats (Filippi et al. 2017b). For example, one species' alarm call may alert another species to an impending threat, whether the call is acoustically similar (Aubin 1991), or not (Templeton et al. 2005).

Superb Fairy wrens (*Malurus cyaneus*) respond to unknown but acoustically similar calls, and their response is more marked with increasing acoustic similarity (Fallow et al. 2011) The peak frequency and number of frequency cycles in calls are the acoustic features that govern how wrens respond (Fallow et al. 2011). However, learning also appears to play a part in the responses of some species, with fairy wrens also responding to alarm calls similar to ones they have learned (Fallow et al. 2011). Learning may play an important role in whether animals respond when calls are not acoustically similar to their own. For example, vervet monkeys learn to respond to the alarm calls of starlings (*Sturnus vulgaris*), and learning is hastened with more exposure to starling alarm calls (Hauser 1988). Acoustic similarity may also not be necessary for heterospecific recognition if an alarm call contains acoustic features that are inherently arousing or frightening, such as non-linear components (broadband or harsh sounds) which direct attention to the sound (Magrath et al. 2015).

Examples of responses to heterospecific vocalisations are becoming more common in the literature. Mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) respond to infant distress vocalisations of humans and other animals if the fundamental frequency of the vocalisation falls within the deer frequency range; mothers living with young fawns approached distress calls of marmots (*Marmota flaviventris)*, cats (*Felis catus*) and other species in the same way they approached the distress calls from their own young (Lingle & Riede 2014). This ability to decode distress calls from diverse species suggests that there is a homologous origin to distress calls among mammals. There also appear to be cross-taxa similarities in how emotions are conveyed acoustically across different mammalian groups (Maruščáková et al. 2015).

Interspecific communication appears to exist between humans and their pets, and potentially between humans and other domesticated animals, such as pigs (*Sus scrofa*) (Tallet et al. 2010). Dogs (*Canis lupus familiaris)* appear to discriminate between both humans' and dogs' positive or negative emotions using visual and auditory cues (Albuquerque et al. 2016) and dogs approach with nuzzling and licking behaviour when a human pretends to cry (Custance & Mayer 2012). This response is not just behavioural but also physiological. Dog cortisol levels increase when they hear a human baby crying (Yong & Ruffman 2014). Certain cat vocalisations are produced more with a human audience than a conspecific audience, and this has been interpreted as communication from cats to humans (Nicastro & Owren 2003).

1.4.5 How do humans decode non-human animals' emotional cues?

Decoding the emotional states of others is crucial for communication between humans. Cross-taxa studies have attempted to pin down the biological roots of this capacity in humans, to establish whether emotional prosody is related to specialised areas of the human brain, or whether precursors in other mammals can be traced (Filippi et al. 2017a). Basic spectral and temporal cues, and similarities in vocal expressions of emotion, may support cross-species emotional recognition (Andics et al. 2014). If humans also have the ability to perceive the emotional state of non-human animals, this could help improve welfare in animals under human management (Manteuffel et al. 2004). Identifying reliable ‘markers’ of internal states within vocalisations could lead to automated identification of compromised or good welfare states within management systems. Welfare assessments in management systems are currently performed by humans, and therefore it is important to understand how humans perceive and classify the vocalisations of animals.

Neuroimaging studies of human brains have shown that affective information in human non-speech sounds is processed differently from speech perception (Sander & Scheich 2001; Grandjean et al. 2005). Belin et al. (2008) investigated activation of areas in the brain in response to playback of human, rhesus macaque (*Macaca mulatta*) and cat vocalisations given in a positively or negatively valenced context. The results showed that, while participants in the study stated they could not identify the valence of the contexts for non-human animals through their behaviour, neural mechanisms within participants’ brains responded to the valence of animal vocalisations at an unconscious level. Cerebral activity related to the valence of the calls was dissociated from the response to arousal (Belin et al., 2008). Andics et al. (2014) found that human brains tune into heterospecific sounds (dog vocalisations) but not to non-vocal environmental sounds. The authors suggest that this may be because dog vocalisations are ‘relevant’. Humans are also able to extract information about the emotional content of heterospecific vocalisations in the same way they do with conspecific vocalisations, and acoustic cues related to valence are processed using similar brain mechanisms in dogs and humans (Andics et al. 2014). These results suggest that voice areas of the brain may date back to the common ancestor of dogs and humans, or that the evolution of both species was convergent.

Studies investigating which animal vocalisations can be decoded by humans have mainly used a two-dimensional approach to the rating of emotions. This approach positions emotions in terms of valence and intensity / arousal, and can be applied across different taxa, allowing for comparative studies (Mendl et al. 2010).

A large body of evidence suggests that the humans use basic acoustic rules to assess the emotional content of conspecific and other species’ vocalisations. In a study using pig vocalisations, humans were asked to listen to calls and place them into the correct context the calls were produced in (Tallet et al. 2010). The authors attributed the success of participants in correctly classifying calls to use of Morton’s Motivation-Structural rules. This result was supported in a further study that found that personality, empathy and attitudes to animals did not influence evaluations of the emotional content of pig calls from different contexts, but these evaluations were based on the acoustic properties of the vocalisations (Maruščáková et al. 2015). Two sequential studies on dogs investigated how humans classify dog barks, with the first suggesting that acoustic features predicted how humans classified dogs barks (Pongrácz et al. 2005). A subsequent study specifically examined whether the pattern of Morton's MS rules was used by humans to describe emotionality in dog barks, and found that scoring of bark sequences did indeed follow these rules (Pongrácz et al. 2006). It was hypothesised that the human preference for vocalisations with a specific acoustic structure may have been a selective pressure in the development of bark production (Pongrácz et al. 2010). In another study, humans also rated the emotional intensity and valence of both human non-verbal vocalisations and dog vocalisations using the same acoustic rules (Faragó et al. 2014). Negatively-valenced arousal in silver foxes was identified by humans using frequency-based acoustic parameters (Filippi et al. 2017b). Two studies have suggested that cat vocalisations influence human receivers by the acoustic cues. In one, the authors concluded that the 'miaow' has evolved to provoke a nervous-system and affective response in humans, without providing contextually-specific information (Nicastro & Owren 2003). Cats have also been found to introduce a high-frequency component into their purr when soliciting food from humans, which adult humans rated as more 'urgent', and both cat owners and non-owners were highly sensitive to (McComb et al. 2009). This call may benefit the sender by taking advantage of the mammalian sensitivity to high frequency cries (McComb et al. 2009). However, this sensitivity may not always induce emotion in receivers. In another study, infant and chimpanzee cries were played to human infants, who responded by crying only in response to the infant cries, not the chimpanzees' (Martin & Clark 1982).

Shared acoustic mechanisms of emotional expression are not restricted to mammals. One study found that humans use the same frequency-related parameters to identify levels of arousal in the vocalisations of non-mammalian vertebrates including amphibians, reptiles and birds (Filippi et al. 2017a). The case for automated classification of the emotional content of calls has also been made. A recent study by Cummins et al. (2017) found that affective computing-based acoustic feature sets (used to capture emotional information in human vocalisations) could be used to correctly classify either the context or the valence of dog barks.

Some studies have examined whether human experience with a type of animal may affect recognition of the context a call is made in. Scheumann et al. (2014) asked whether the correct identification of emotional cues occurs because the acoustic cues induce emotion in the receiver (human), or whether they have learned the association between a call type and the eliciting context, and can therefore correctly identify the context. The authors tested emotional recognition of four species (human infant, dog, tree shrew, chimpanzee) according to three possible explanatory factors: a) induced emotional state b) familiarity with acoustic stimuli and c) phylogenetic distance to species. All calls were recorded in either a positive or a negative valenced context. If animals were not labelled as their correct species, the authors expected low recognition of the valence of the call, but if the animal producing the call was correctly identified, the authors expected high recognition of the valence of the call. Cross-taxa emotional recognition was highest for negative contexts, which suggested that there may be an evolutionary link to the ability to identify calls made in contexts bearing high survival costs. The authors found that cognitive experience-based mechanisms or familiarity with the species predicted emotional recognition rather than phylogeny. In other words, human listeners needed to be familiar with the respective sounds and contexts to be able to classify calls correctly.

Experience with the vocalising species does not always influence recognition of acoustically conveyed emotions. Listeners experienced with dogs were unable to correctly classify the valence of affiliative dog vocalisations (Scheumann et al. 2014). Pongrázc et al. (2005) also found that experience did not affect categorisation of dog barks. Dogs bark in both affiliative and agonistic contexts and this may be why humans find it difficult to discriminate valence, despite the differences in the acoustic structure of the barks (Yin & McGowan). In another study it was found that blind people with no experience of dogs were equally accurate at categorising dog barks made in different contexts as sighted people (Molnár et al. 2010). Children aged 10 and under were also able to correctly classify dog barks according to the context they were recorded in (Pongrácz et al. 2011). Experience with dogs did not influence this ability, which suggests that other animals' acoustic signals may be interpreted by humans from a young age based on their acoustic structure (Pongrácz et al. 2011). However, in contrast to their previous study using dog barks, Faragó et al. (2017) found that experience or learning influenced recognition of emotional content in dog growls. They also found that, in agreement with a study by Taylor et al. (Taylor et al. 2009), the temporal structure (rather than frequency-based parameters) of the growl sequence affected how listeners rated the emotionality of growls (Faragó et al. 2017). Correct classification of cat vocalisations moderately improves with experience of cats, although experience was not a factor in whether listeners were more likely to be able to assign the valence of a vocalisation correctly (Nicastro & Owren 2003). Experience of having pets at home did not influence children's interpretation of monkey vocalisations (Linnankoski et al. 1994). Instead the authors found that children were able to recognise the context of macaque voices correctly, and this ability increased with age. The authors attributed this to a natural development of the capacity to interpret human and non-human emotional behaviour.

1.4.6 Sensitivity to valence and arousal

A growing body of research demonstrates that humans are able to accurately perceive the context some animal vocalisations are made in, and the arousal level of other animals, but less accurately the valence. Humans differentiate very quickly between affective and non-affective vocalisations of other humans, with evaluations being made around 150ms after the onset of the sound (Sauter & Eimer 2009). However, when humans listen to animal sounds, activation of areas of the brain sometimes contradicts conscious judgements of valence. As discussed previously, Belin et al. (2008) found a dissociation between overt judgements of valence and corresponding brain activation. Brain regions sensitive to emotional valence in vocalisations show stronger neural activity for more negatively-valenced vocalisations (Belin et al. 2008). Evolution may play a part in this process. Scheumann et al. (2017) measured brain responses to negative and positive vocalisations of a variety of animals from different taxa. The authors found there was a biphasic response, with an early negative response and a late positive response, when comparing aversive and affiliative novel sounds respectively. They suggested that these early negative brain responses are important for survival as they engage the limbic system and auditory cortex, whereas late positive responses to affiliative vocalisations may reflect cognitive evaluation of the stimulus.

Vocal cues related to valence have been suggested to be more independent of physiological changes than cues that correlate with arousal. Valence is often associated with cues that develop over time, and is better perceived during longer phrases in human speech (Laukka et al. 2005). Certainly, animal studies have shown that call duration is the only consistent parameter to be associated with valence (Briefer 2012; Maruščáková et al. 2015; Faragó et al. 2017). Briefer (2012) recommends that multiple indicators or parameters, including formant frequencies, should be considered when attempting to find correlates of valence. Studies have found that arousal, in contrast to valence, is reliably correlated with frequency-related acoustic parameters, amplitude contours, vocalisation rate, and temporal interval between bouts (Morton 1977; Briefer 2012; Faragó et al. 2014; Maruščáková et al. 2015). One study narrows it down by pointing out that in all human perception studies of acoustic correlates of arousal, listeners mainly rely on increases in fundamental frequency to rate both human and heterospecific vocalisations as expressing heightened levels of arousal (Filippi et al. 2017a). However, there are sometimes individual differences in the acoustic profiles of calls, and individual variation in calls demonstrably affects human perception of animal vocalisations (Riede et al. 2005).

1.5 The vocalisations of chickens

Domestic chickens are very social animals, and their main method of communication is through vocalisations. One of their calls, the gakel call, has been identified as a reliable indicator of frustration during thwarting of access to rewards. Other specific vocalisations have referential qualities which enable conspecifics to extract information about the environment around them; for example, whether a predator is present, or whether there is food available nearby.

Collias (1987) documented 24 separately distinguishable calls in the Red Junglefowl (*Gallus gallus spadiceus*), the wild ancestor of the domestic chicken, while Wood-Gush (1971) made a more conservative estimate of 19 discrete types of calls in the domestic fowl. Confusion around the exact number of calls made by domestic chickens stems from the interpretation of calls by different researchers, and analysis of the literature indicates that some individual calls appear to have been given multiple names. For example, a call given by the mother hen to her chicks, which stimulates them to follow her, has been called an ‘attraction’ call, a ‘follow-me’ call or ‘clucking’ by separate researchers (Collias & Joos 1953; Field et al. 2007; Edgar et al. 2011). Despite these discrepancies, there appear to be between 20-25 discrete calls made by the domestic fowl.

Vocalisations are thought to be the main method of communication in the domestic fowl, as is characteristic of forest-living social animals (Woodgush 1971). Research conducted on the eliciting stimuli of vocalizations, the behaviour of the caller, and the behaviour of conspecifics has enabled classification of calls. The domestic chicken appears to have different food calls: e.g. from mother to chick (Collias & Joos 1953; Moffatt & Hogan 1992; Field et al. 2007); from male to female (Woodgush 1971; Marler et al. 1986b; Collias 1987); and distinctive predator alarm calls: e.g. alarms indicating aerial (Collias 1987; Evans et al. 1993) or ground predators (Collias & Joos 1953; Evans et al. 1993). Other calls function as attraction, courtship or roosting calls, facilitating social contact (Collias & Joos 1953; Collias 1987; Edgar et al. 2011), while others appear to indicate arousal such as distress or fear (Kruijt 1964; Marx et al. 2001; Brumm et al. 2009). Recently, research has found that the gakel call, normally produced prior to egg-laying, is also an indicator of frustration in the domestic fowl (Zimmerman et al. 2000).

It has also been suggested that chickens give functionally referential calls. Their aerial predator and ground predator alarm calls have been suggested to reliably indicate the presence of these different types of predators (Gyger et al. 1987; Evans et al. 1993), and food calling was thought to reliably indicate the presence of food (Evans & Evans 1999).

1.6 The behaviour of non-human animals in anticipation of rewards

When animals cannot access what they want, they sometimes exhibit behavioural and physiological signs of disturbance. For example, the inability to access rewards may lead to agitation, aggression, increased escape behaviour and increased stress response (Duncan & Wood-Gush 1972; Dantzer et al. 1987) which may imply frustration. Similarly, deprivation of what an animal wants may lead to the performance of vacuum behaviours or stereotypies, which may indicate long-term adaptation to a stress response (Broom 1991; Fraser 1993; Waran 2001). To improve welfare, therefore, the first task is to identify what particular animals find rewarding.

A reward is anything an animal will work for (Rolls 2000). Outcomes from neuroscientific experiments indicate that the period between a signal indicating the arrival of a reward, and the actual presentation of the reward, is the period in which dopaminergic activity can be observed in the brain (Berridge 1996). This brain activity is linked to a state of appetitive ‘wanting’ and may be correlated with overt behavioural responses, which comprise anticipatory behaviour (Boissy et al. 2007). Behaviour in anticipation of rewards is defined as ‘responses elicited by rewarding stimuli that lead to, and facilitate, consummatory behaviour’ (Spruijt et al. 2001). Various accounts of anticipatory behaviour have described it as ‘preparatory behaviour’ (Matthews et al. 1996), or goal-directed behaviour (Wit & Dickinson 2009). Consumption of the reward then leads to ‘pleasure’ and activation of the mesolimbic opioid system, or hedonic ‘hotspots’ in the brain (Waugh & Gotlib 2008, Kringelbach & Berridge 2017). However, if access to the reward is prevented, this may lead to frustration (Amsel et al. 1992). In addition, if an animal has no control over when a reward may be accessed, for example in captive animals that have no control over when they are fed, the period prior to being fed may induce stress responses (Waitt & Buchanan-Smith 2001).

Increased activity during anticipation of a reward is thought to represent activation of the reward centres of the brain (Spruijt et al. 2001), and has been documented in rats (van der Harst et al. 2003), mink (Vinke et al. 2006), pigs (Dudink et al. 2006) and silver foxes (Moe et al. 2006). Some animals, however, do not always conform to this tendency. Cats sometimes show decreased behaviour in response to anticipation, due to differences in behavioural strategy relating to ecology (i.e. opportunistic vs. predator forager) (van den Bos et al. 2003).

Consumer demand, preference and choice tests have been devised to establish what animals find rewarding (Dawkins 1983; Kirkden & Pajor 2006). Analysing behaviour in anticipation of, and during thwarting of access to, rewards gives us a clear indicator of what animals really want. When presented with multiple rewards, animals may also indicate how much they prioritise one reward over another (Dawkins 2012). Moe et al (2009) believe that analysis of the strength and frequency of anticipatory behaviour can lead to the ability to develop an index of sensitivity to reward, and therefore an indication of the animal's general state of welfare. Sensitivity to reward is the way animals respond to differences in quality or quantity of a reward, or how they respond to the same reward after exposure to different environments. This has been investigated in various animals such as rats and hens (van der Harst & Spruijt ; van der Harst et al. 2003; Wichman et al. 2012) In addition, the efficacy of inducing anticipatory behaviour through exposure to reward, or reward-associated stimuli, as a method of reducing stress, has also been explored (van der Harst et al. 2005). Being able to form declarative representations (being able to predict outcomes based on stored general information relating to past events, which is not directly linked to any given situation) and showing object permanence awareness are considered pre-requisites for anticipatory behaviour (Etienne 1984; Forkman 2000).

Experimentally inducing anticipatory behaviour can be achieved using a Pavlovian conditioning paradigm in which a conditioned stimulus (which is initially neutral) is repeatedly paired with the arrival of a reward (Holland 1980)). This stimulus then serves to announce the reward. Inserting a delay between presentation of the conditioned stimulus and the presentation of the reward provides a period of time when anticipatory behaviour could be recorded (Moe et al. 2009). Consistent intervals between the stimulus announcing the reward and the arrival of the reward allow animals to form a temporally-based expectation of the unconditioned stimulus (reward) (Balsam & Gallistel 2009). The associations between stimulus and reward have been demonstrated to be resilient to change in the interval time (Balsam & Gallistel 2009). However, for some individual animals, anticipatory behaviour, or positively valenced arousal, may shift into frustration due to lack of arrival of the expected reward. Therefore, caution must be used when deciding on the interval between conditioned stimulus and the unconditioned stimulus (the reward), as delaying access to rewards may lead to frustration (Amsel 1992).

1.6.1 The behaviour of chickens in anticipation of rewards

Much research has been done to establish what chickens find rewarding. Chickens have been demonstrated to work for food (Olsson et al. 2002), nest boxes (Cooper & Appleby 1996; Cooper & Appleby 2003) dustbaths (Widowski & Duncan 2000) and optimal space according to their needs (Faure 1994), and have also shown motivation to perch (Olsson & Keeling 2000). Other experiments have shown that mealworms are more rewarding to chickens relative to other foods (Bruce et al. 2003).

Moe et al (2009) documented the behaviour of laying hens in anticipation of rewards. In their study, the hens' lack of activity is more akin to the response shown by cats. Hens stood still, or walked slowly with body and neck stretched upwards, with their heads sometimes oriented towards the reward source (Moe et al. 2009). However, in a study conducted by Kostal et al (1992), broilers showed increased activity (walking) before they received their daily food allowance. Zimmerman et al (2011) also found that hens took fewer steps in anticipation of positive, rather than negative, stimuli, but concluded that locomotion is not a good indicator of anticipation in hens. Increased attentiveness towards the expected reward location was found by Moe et al (2009) and Zimmerman et al (2011). Hens also exhibited less foraging behaviour in anticipation of a positive food reward in comparison with neutral trials (Zimmerman et al. 2011).

Interpretation of behaviour in anticipation of rewards may be difficult. If experiments are trying to compare the value of rewards in choice tests or in anticipatory situations, the motivational state of the animal (e.g. whether they are hungry or satiated) needs to be taken into account. Forkman et al. (2012) demonstrated recently that this consideration also needs to apply to the motivational state the animal was in when it first encountered the reward. Goal-directed behaviour (approaching the reward) can partly be attributed to motivational state. However, it can also be associated with whether the outcome of the goal has an incentive value. Incentive values are probably learned on first encounter with a specific reward (e.g. a food item may have been assigned a higher reward (or incentive) value than a different food item if the animal was first exposed to it when food deprived) (Dickinson & Balleine 1995; Forkman et al. 2012).

As previously discussed, some animals still possess residual mechanisms that govern their behaviour. One example is pre-laying behaviour in hens. Pre-laying behaviour is dependent on the appropriate hormones being present as a result of ovulation (Wood-Gush & Gilbert 1964). It is characterised by increased locomotion and exploratory behaviour, and performance of nest-building activity at a specifically chosen site (Freire et al. 1996). On the other hand, nesting behaviour (sitting on the nest) is contingent on external environmental cues (Cooper & Appleby 1996).

Fixed behavioural patterns, such as those demonstrated by hens in pre-laying / nest-building behaviours, or during dustbathing, appear to be something hens need to do (Weeks & Nicol 2006). That they perform these behaviours in the absence of the relevant stimuli is suggested to indicate a high motivation to perform them (Hughes & Duncan 1988). Freire et al (1996) suggested that a failure to express appetitive behaviour fully may interfere with the performance of consummatory behaviour. Also, if external cues are absent, animals may redirect their appetitive behaviour towards other stimuli. However, although it has been suggested that the performance of vacuum behaviours may actually satisfy the motivational needs of animals (Dawkins 1983), the inability to perform these 'hard-wired' behaviours could lead to poor welfare.

1.6.2 Expression of frustration in chickens

Frustration often occurs when access to rewards is thwarted, and has been defined as 'an aversive motivational state that results from non-reward, reduced reward or delayed reward in the presence of a history of reward' (Amsel 1992). Behavioural and physiological indicators of frustration have been documented in many studies on domestic chickens, where access to a reward was prevented (Duncan & Wood-Gush 1972; Meijsser & Hughes 1989; Freire et al. 1996; Zimmerman & Koene 1998; Zimmerman et al. 2000).

When access to food was prevented in an experiment by Duncan & Wood-Gush (1972), hens exhibited stereotyped pacing behaviour which the authors suggested was an extension, or replacement, for escape behaviour. Stereotypies are fixed behavioural sequences performed repetitively with no obvious function (Dawkins 1990). Displacement behaviours are also responses to frustration, and often occur often in social situations. Bird feeders in which food is temporarily unavailable induce displacement behaviour, such as preening (Maynard Smith & Harper 2003). Displacement preening in hens was documented when access to food was thwarted (Duncan & Woodgush 1972).

Wood-Gush and Guiton (1967) also studied the behaviour of hungry hens when thwarted in their attempts to get food. The birds were trained to expect food in the test cage. However, in the test condition, a glass cover prevented access to the food. The hens immediately responded to being unable to access the food by trying to escape, a behaviour which was not observed in the control situation. Displacement preening was initially infrequent. By the fourth test the number of escapes had declined while preening increased in frequency. Escape behaviour is probably a primary reaction to thwarting which becomes replaced by other activities such as preening. The repetition of thwarting may lead to a reduction of its aversive effects as well as a reduction in stimulation to access the reward.

Frustration is sometimes expressed vocally by hens. Hens produce a 'gakel' call, which is a long, harsh sounding call, when prevented from accessing rewards such as food or a dustbath (Zimmerman & Koene 1998; Zimmerman et al. 2000). Hens also exhibit other frustration-related behaviour. For example, deprivation of access to a dustbathing substrate leads to 'sham' dustbathing, which means the behaviour is performed in the absence of relevant stimuli (Olsson and Keeling, 2005). Sham dustbathing may occur as a result of irregular dustbathing behaviour in the absence of a suitable substrate (Wichman and Keeling 2008). Vestergaard (1980) suggest that the tendency to sham dustbath is an indicator of high levels of motivation to perform this activity. In addition, an increased stress response, in the form of increased corticosterone, was found in hens that were deprived of dustbaths (Vestergaard et al. 1997). Other circumstances have also been demonstrated to induce frustration in hens. Prolonged conditioned stimulus (CS) - unconditioned stimulus (US) intervals may be experienced as a lack of reward in studies of anticipatory behaviour (Moe et al. 2009).

1.7. Conclusion and thesis outline

The vocalisations of non-human animals convey information that may be decoded by conspecific and heterospecific receivers. This information may relate to static traits about the animal, such as age, sex or body size. However, vocalisations may also convey the emotional state of the sender, and the mechanisms for doing this appear to be shared across mammals. Humans are often able to accurately decode these vocalisations using acoustic cues. This ability could advance animal welfare assessments, and automated systems that accurately classify calls could provide a convenient way of doing this.

The review above has explored some of the subjects which provide the background to this project. It reviewed some of the literature on emotions in humans and non-human animals, and how emotions may be measured. It has provided a basis for understanding how vocalisations may convey an animal's emotional state within their acoustic structure. It has also discussed how the valence or intensity of human and non-human animal emotional states may be predicted by variations in specific acoustic parameters. In addition, the literature on how humans decode the emotional content of non-human animal vocalisations has been reviewed. Finally, this review has provided background information on chicken vocalisations, and what is currently known about the behaviour of chickens and other animals in anticipation of rewards.

Chickens have been demonstrated to produce a certain, very distinctive call (the gakel call) in situations of frustrative non-reward (Zimmerman et al. 2000). Accurate identification of this call (made in frustrative non-reward contexts rather than pre-laying contexts) may alert caretakers to potentially poor welfare situations. Therefore, if calls made in anticipation of rewards can be accurately identified, these could be used to identify potentially rewarding situations for chickens, and enable us to more confidently provide them with what they want.

There is currently no evidence available on whether chickens produce reward-related vocalisations, and how they may be perceived by other chickens, or by humans. One of the aims of this thesis is to ascertain whether anticipation of different rewards elicits consistent vocalisations that can be considered reward-related. If ‘reward’ calls exist, it is also important to investigate whether they convey information that affects the behaviour other chickens in the flock. Receiver responses to vocalisations may indicate that referential information about the rewards may be conveyed within the calls. Finally, no study has examined how humans perceive chicken vocalisations made in anticipation of rewards. This thesis will address these gaps in knowledge through a sequence of investigations.

In order to substantiate the hypothesis that specific vocalisations represent a certain motivational state, it is crucial to correlate vocalisations with other behavioural responses, Chapter 2, therefore, investigates the behaviour of domestic chickens in anticipation of different types of reward. Previous studies have examined how chickens behave in anticipation of food rewards, and this chapter will extend on, and challenge, current knowledge on anticipatory behaviour in chickens. It provides evidence for behaviour in anticipation of both food and non-food rewards. It also examines whether the quality of the reward is expressed in differential behaviour. Chapter 3 then characterises the vocalisations of chickens in anticipation of rewards, and also explores variations in the acoustic structure of those calls. In Chapter 4, the responses of other chickens to reward-related calls are investigated using a playback paradigm. Chapter 5 goes on to examine how humans decode the vocalisations of chickens, and provides evidence for human perception of arousal and valence levels in these vocalisations. Finally, the general discussion chapter brings together the project findings, and discusses their wider implications.

CHAPTER 2

McGrath, N., Burman, O., Dwyer, C., Phillips, C.J.C. (2016) Does the anticipatory behaviour of chickens communicate reward quality? *Applied Animal Behaviour Science* 184: 80-90

Contributor	**Statement of contribution**
McGrath, N	Experimental design (70%) Conducted experiment (100%) Statistical analysis of data (80%) Wrote the paper (100%)
Burman, O	Experimental design (10%) Edited paper (30%)
Dwyer, C	Experimental design (10%) Edited paper (30%)
Phillips, C	Experimental design (10%) Edited paper (40%)
Dunlop, R	Statistical analysis of data (20%)

2.1. Abstract

The anticipatory behaviour of animals has been credited with enabling scientists to more closely infer what an animal wants. From a welfare perspective, this knowledge could improve how we care for animals under our management, as information about how animals prioritise rewarding items may guide how we allocate resources effectively. This study's goal was to determine if the behaviour of chickens *Gallus gallus* in anticipation of different types of reward was differentially expressed. It was investigated whether certain behaviours were characteristic of anticipation of both food and non-food rewards, and whether signals indicating rewards led to increased activity levels. Twelve laying hens experienced a Pavlovian conditioning paradigm using sound cues to signal the availability of two different food rewards (Mealworms, Normal Food), one non-food reward (a container of mixed soil and sand substrate suitable for foraging and dustbathing (Dustbathing substrate)) and a sound-neutral event, which was signalled by a sound, but no reward was given. A muted-neutral treatment (no reward and no sound cue) controlled for any specific behaviour as a result of the sound cues. Behavioural responses and the number of transitions between behaviours were measured during a 15 second anticipatory period, before birds accessed rewards in an adjoining compartment by pushing through a door. These responses and latency to access the rewards were analysed using linear and generalised linear mixed models. Differences in pushing and pecking at the door (frequency: Dustbathing substrate 4.87^{a}, Mealworm 3.18^{b}, Normal Food 2.23^{b}, Sound Neutral 0.30^{c}, Muted Neutral 0.03^{d}, $\chi^2(4)=228.99$, $p<0.001$) and standing (not walking) (duration (s): Sound Neutral 9.92^{c}, Muted Neutral 7.49^{bc}, Normal Food 7.39^{bc}, Mealworm 7.05^{b}, Dustbathing substrate 3.06^{a}, $\chi^2(4)=36.28$, $p<0.001$), reflected the perceived value of the rewards, with birds appearing to be more motivated to access the dustbathing substrate compared with the food rewards. Rewarded sound cues elicited increased transitions between behaviours, compared with neutral events (Dustbathing substrate 10.16^{a}, Mealworm 10.13^{a}, Normal Food 9.22^{ab}, Sound Neutral 7.89^{bc}, Muted Neutral 6.43^{c}, $\chi^2(4)=72.05$, $p<0.001$). The sound-neutral treatment induced increased head movements, previously associated with anticipation of rewards (duration (s): Sound Neutral 1.58^{b}, Muted Neutral 0.58^{ab}, Normal Food 0.48^{a}, Mealworm 0.27^{a}, Dustbathing substrate 0.00^{a}, $\chi^2(4)=25.56$, $p<0.001$). Latency to access rewards conveyed the relative value of rewards (Dustbathing substrate 7.30^{a}, Mealworm 10.06^{ab}, Normal Food 16.53^{b}, $\chi^2(2)=10.88$, $p=0.004$).These outcomes indicate that, under certain conditions, hens increase their activity levels (behavioural responses and transitions) in anticipation of rewards. Importantly, this study demonstrates that this response is not food specific, but rather a general response to both food and non-food rewards. This study extends our knowledge of reward-related anticipatory behaviour, and of how hens rank rewards of contrasting incentive value, which may have implications for the methods and environments applied to improve the welfare of laying hens in managed systems.

2.2. Introduction

An important goal of farmers, welfare scientists, and those who create the legal frameworks for food production, is to find an optimal environment that balances production and welfare. One key aspect of animal welfare is to provide an environment in which animals' wants and needs can be satisfied (Dawkins 2012). Measuring what animals want, therefore, is crucial to developing animal management systems that provide good welfare. One such method is to investigate the anticipatory behaviour of animals in order to examine how they perceive potential stressors or rewards.

A reward is defined as anything that an animal will work for (Rolls 2000), in contrast to a punisher, which is defined as a stimulus that decreases the probability of actions on which it is contingent (Rolls 2005). Neuroscience experiments have revealed that the period between a signal indicating the arrival of a reward and the actual presentation of the reward is when behavioural activity correlates with appetitive, or 'wanting' (dopaminergic), activity in the brain (Berridge 1996, 2007). Various accounts of anticipatory behaviour have described it as 'preparatory behaviour' (Matthews et al. 1996), or goal-directed behaviour (Wit & Dickinson 2009), leading to and facilitating consummatory behaviour (Berridge 1996). Importantly, this state of 'wanting' can be directly observed, potentially providing a means of measuring how animals prioritise one reward over another (Dawkins 2012). Anticipatory behaviour may also demonstrate how sensitive animals are to a reward (Spruijt et al. 2001; van der Harst et al. 2003), delivering insight into their current welfare state, although caution must be used as the correlation between choices animals make and welfare indicators is not always clear (Nicol et al. 2009).

In a rewarding environment, animals often exhibit appetitive and consummatory behaviour around certain resources (Spruijt et al. 2001). Anticipation requires the ability to make contingent the association that one event precedes another (Greiveldinger et al. 2011), and, therefore, in order to investigate behaviour in anticipation of rewards, one approach is to train animals to associate a stimulus with the arrival of a particular reward. Presentation of the stimulus should subsequently elicit a behavioural response which is reward-related, and therefore may indicate excitement or arousal. This type of research has enabled scientists to characterise reward-related anticipatory behaviour in chickens, horses, pigs and lambs (Moe et al. 2009; Peters et al. 2012; Reimert et al. 2013; Anderson et al. 2015). Elicited behaviours vary, with increased activity and more frequent transitions between different behaviours being characteristic of some animals such as pigs (Imfeld-Mueller & Hillmann 2012) horses (Peters et al. 2012) and mink (Vinke et al. 2004), while cats appeared to show a decrease in activity (van den Bos et al. 2003).

Previous research has produced some conflicting results in terms of characterising anticipatory behaviour in chickens. Kostal et al. (1992) found that broilers showed increased walking prior to their scheduled feeding time, which they interpreted as appetitive foraging behaviour, shown in anticipation of the arrival of food. This increase in activity is reminiscent of the activity shown by mammals as described above. However, in a study by Zimmerman et al. (2011), hens showed no increase in locomotory activity in anticipation of a palatable food reward (mealworm), but increased their activity prior to a negative event (being squirted with water) and during a control treatment. The authors concluded that locomotory activity was not a good indicator of anticipation of a positive event in chickens. In other studies, Moe et al. (2009; 2011; 2013) defined anticipatory behaviour in laying hens, based on a description previously made by Buijs et al. (2006), as arousal-related behaviours, performed in sequence, specifically ‘standing still or taking slow steps, with legs, body and neck stretched upwards and eyes open, and frequent head movements’. After part of this display was attenuated by administration of a dopamine antagonist, the authors concluded that frequent head movements ‘in any direction’ were under dopaminergic control (Moe et al. 2011), and suggested that head movements may represent the activation of the dopaminergic reward system in hens. However, the behaviour described was characterised by slow and measured movements rather than by the increased locomotory activity seen in the study done by Kostal et al.(1992).

Thus, the research on chickens has not been able to definitively clarify whether chickens demonstrate high or low activity levels in anticipation of rewards. In addition, there is ambiguity around the contexts that elicit head movements; in the study by Zimmerman et al.(2011), the negative event elicited an increase in the frequency of head movements compared with the positive and the neutral event, a result which seems to contradict the proposal by Moe et al.(2011) that head movements represent activation of the internal reward system in hens. Indeed, Zimmerman et al. (2011) concluded that head movements could express anticipation of a negative event in general, or of their specific negative event, and they also suggested that head movements could indicate increased vigilance, or an effort to locate the source of the sound cue. They also found that comfort behaviour was associated with anticipation of a positive event, and suggested that this behaviour may reflect positive affect in laying hens. These differences in behavioural expression between studies could result from variations in experimental procedure; the study by Kostal et al. (1992) observed broiler chickens in their home environment; the experiment conducted by Zimmerman et al. (2011) recorded the behaviour of laying hens in an experimental anticipation compartment connected to a reward compartment; and Moe et al. (2009; 2011; 2013) observed singly housed laying hens, using an automated system to deliver rewards.

The conditioning paradigm studies cited above focussed on using food as an unconditioned stimulus to induce anticipatory behaviour. Moe et al. (2009) investigated whether different trace intervals following a conditioned stimulus would induce differential displays of behaviour in anticipation of a mealworm reward . In a subsequent study, again using mealworms as the reward, Moe et al. (2011) investigated whether a dopamine D2-like receptor antagonist would decrease displays of anticipatory behaviour without affecting consumption of the reward. Their next study explored whether anticipatory behaviour reflected the incentive value of two food rewards (mealworm and whole-wheat) (Moe et al. 2013), and a further study compared the behaviour of domestic fowl in anticipation of a mealworm reward with that of the Red Jungle Fowl (Moe et al. 2014). Zimmerman et al. (2011) also used mealworms as their reward, when comparing behaviour in anticipation of a positive (rewarding) or a negative event.

These studies illustrate a lack of certainty over what constitutes reward-related anticipatory behaviour in chickens - whether it is characterised by an increase in activity, or by slow steps, accompanied by head movements. In addition, it is not known how chickens behave in anticipation of non-food rewards, such as prized environmental resources like a substrate suitable for dustbathing or perches (Olsson & Keeling 2000, 2005).

Therefore, the goal of this study was to characterise the behaviour of laying hens in anticipation of different types of reward, and, more specifically, to investigate whether hens differentially express the quality of rewards in their behaviour. In order to provide more conclusive evidence of the general types of behaviour hens exhibit when they are in a state of ‘wanting’, it was important to investigate whether anticipatory behaviours shown in our experiment were simply food-related or could be generalised to other rewarding items. To achieve this, anticipation of rewards was experimentally induced using a Pavlovian conditioning paradigm. Items that are known to be rewarding to hens, including two food items (mealworm and normal food) and a tray containing a topsoil/sand substrate suitable for dustbathing (Bruce et al. 2003; Olsson & Keeling 2005) were used as the rewards.

It was predicted that the frequency and duration of behaviours in response to sound cues signalling the rewards would reflect the perceived quality of the different rewards, and that cues signalling food rewards would induce a higher intensity of behavioural expression. It was expected that the latency to access the rewards, as a proxy of motivation, would provide a further indicator of the quality of the reward as ranked by the birds. It was also reasoned that, if anticipation of rewards elicits appetitive behaviour and ultimately consumption of the reward, then hens would show behaviour that indicates readiness for accessing the reward. Therefore, as hens were able to see the reward location and had to push through a door to reach the rewards, it was expected that they would demonstrate increased activity when rewards were signalled.

2.3. Methods

2.3.1. Subjects and Housing

Twelve ISA Brown hens, approximately 18 weeks old, were obtained from the University of Queensland's poultry unit. The hens were housed in groups of three birds in pens measuring 266 cm x 266 cm x 133 cm (height). The floor of the home pen was shredded rubber chip, and each pen contained a perch at height of 41cm, (length 149cm, width 119cm), and two nest-boxes (35 cm x 40 cm x 45 cm (height)). Food (standard layer pellets) and water were available ad libitum in the home pens. The housing had natural light as well as artificial light (on between 06:00 and 18:00h). There was no temperature control, however all experimental work was conducted between 08:00 and 12.30 h to standardise the conditions. Hens were individually identifiable to the experimenter based on plumage colouring, marking and comb size, avoiding the need for individual marking or ringing. The methods used in this study were approved by the University of Queensland Animal Ethics Committee (Ref. SVS/314/12).

2.3.2. Treatment Groups

Hens were subjected to a Pavlovian conditioning paradigm as used by Zimmerman et al. (2011) and Moe et al. (2009). An initially neutral stimulus (conditioned stimulus, CS) was repeatedly paired with the presentation of one of three different rewards (mealworm, normal food, or dustbathing substrate) or a sound neutral (SN) event (an empty compartment) which served as the unconditioned stimuli (US). Different sound cues were used as conditioned stimuli, all of five seconds duration: 'ring' (ringing of an old fashioned telephone), 'beep' (an alarm-clock style beep) 'buzz' (a buzz sound as in a game show) and 'horn' (an old-fashioned car horn sound). A 'muted neutral' (MN) treatment (five seconds of 'nothing', no CS or US) was used to control for the effect of sound in the other treatments. The sound cues were played from a computer at a sound pressure level of 75 dBA. Each of the four sound cues was used to signal the presence of each type of US. Consequently four cue groups of three hens (from the same home pen) each experienced different combinations of CS and US (Table 2.1).

Table 2.1 Hen cue groups and combinations of CS and US

Groups	Mealworm	Dusty Substrate	Normal Food	Sound Neutral (SN)
Cue group 1 (n=3)	Ring	Beep	Buzz	Horn
Cue group 2 (n=3)	Horn	Ring	Beep	Buzz
Cue group 3 (n=3)	Buzz	Horn	Ring	Beep
Cue group 4 (n=3)	Beep	Buzz	Horn	Ring

2.3.3. Experimental Apparatus

An experimental pen (200 cm long x 125 cm wide x 60 cm high) was located in a room lined with sound proofing foam (Broadband Studio Acoustic Foam, Swamp Industries Pty Ltd, NSW) adjacent to the room in which the birds were housed. Sound proofing was tested by playing sounds (White noise, loud music, alarm clock sound) in the adjacent room and recording in the test chamber. The pen contained two compartments of equal size - a waiting compartment and a reward compartment, separated by a wire-mesh partition and connected by a swing door in the middle of the partition (Fig. 2.1). The door could be locked and released by increasing or decreasing an electrical current going through an electromagnet attached to it, and only opened in the direction of the reward compartment. Three of the four walls of the experimental pen were made of plywood and one was made of wire-mesh to allow video recordings of both compartments. A lamp, secured to the middle of the outer wall at 60 cm from the floor could be operated by the experimenter who sat behind a screen out of sight of the hens during tests. This light shone into the reward compartment and was used to highlight the reward and indicate that the door was open.

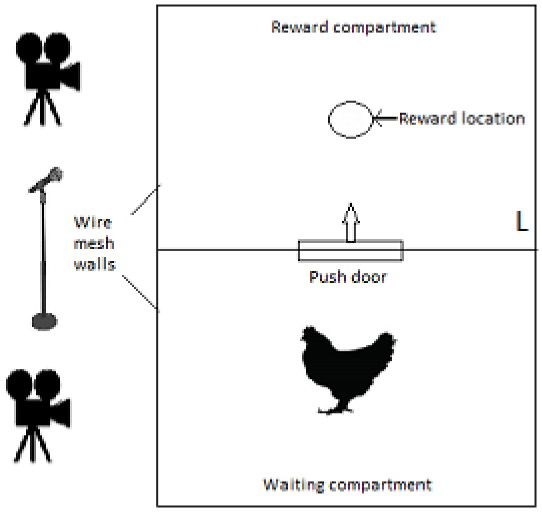

Figure 2.1 The experimental pen. L shows where the lamp was positioned.

The apparatus used for rewards were a white food bowl, a tray filled with topsoil / sand mix, and the birds' normal feeders. The topsoil/sand mix was chosen after a review of the literature on functional substrates for dustbathing, and its dry crumbly texture made it an ideal substrate for this purpose (Olsson & Keeling 2005). Duplicates of the white food bowl containing food and a white tray filled with the topsoil /sand mix were put in the home pens three days before training started to allow birds to become accustomed to them.

2.3.4. Training Procedure

The training consisted of several phases similar to those used by Zimmerman et al. (2011) with adjustments in the length of each phase due to the number of conditioned stimuli used. As a result of the hens learning to enter the reward compartment more slowly, training took place over 25 days (compared with 22 days in Zimmerman et al. (2011)). In phase 1 (Days 1 to 7) birds were trained to use the swing door. This initial training was done in groups of three to increase the speed of training. During the first two days the door was kept fully open, and a trail of sunflower seeds led through the door into the reward compartment where the white bowl held more sunflower seeds. Each home pen group of three birds was allowed to accustom themselves to moving from the waiting compartment to the reward compartment following the trail of seeds. In the following five days, home pen groups were trained to go through the door, the opening width of which was gradually reduced more on each day. During this period, the birds were food deprived for an average of two hours and mealworms were placed in the white bowl in the reward compartment to incentivise the birds to go through the door. Each group of three birds experienced eight consecutive trials. On the final day the door was fully closed, but unlocked, so that the birds had to push through it to gain access to the mealworm.

In phase two (Days 8 to 13), the birds were individually trained to recognise the specific CS for each of the rewards. The containers containing the dustbathing substrate were removed from the home pens from this time. In this phase, the door was kept unlocked and birds were given 10 minutes to go through the swing door after their particular CS for mealworm, dustbathing substrate, or normal food was played and the light switched on. All birds entered the compartment within the 10 minute time limit. For the food rewards, birds either ate the mealworms or were allowed one minute to consume their normal food. For the dustbathing substrate, the birds were allowed to dustbathe or forage (with no food present) for five minutes. After consumption of the reward, the light was switched off and the birds were guided back into waiting area by the experimenter. Each cue group was trained for one stimulus on one day and each individual experienced three consecutive trials. Hence, during the six days, each hen experienced six trials of each stimulus with the door unlocked. In phase 3 (Days 14 – 17), the swing door was locked and the CS and light signal were made contingent on the behaviour of the bird. Birds were placed individually into the waiting compartment and allowed to try to push through the locked door twice before the CS was played and the light switched on at the same time as the door was unlocked. This procedure was repeated three times and then the CS was played and the light switched on / door unlocked at random when the bird was not near the door. When the bird went through the door immediately after the CS/light was given in five consecutive trials, the training session was ended and birds were returned to their home pen. In phase 4 (Days 18-19), a trace conditioning procedure (Moe et al. 2009) was used to accustom the hens to an interval between the CS being played and activating the light signalling the door was unlocked. The CS was played for five seconds and the interval between the end of the CS and the light signal was gradually increased from 0 to 15 seconds over five consecutive trials for each individual bird on each day. Birds successfully reached the criterion for proceeding to the next stage when they went through the swing door within five seconds after the light had been switched on. In phase 5 (Days 20-22), all birds were introduced to their Sound Neutral CS. In these SN trials nothing happened after the light had been switched on. The light was kept on for 15 seconds and then switched off. In phase 6 (Days 23-25), rewarded (mealworm, dustbathing substrate, normal food) and SN trials were presented in a randomised order, with each cue being presented at least once to each bird on each day. Birds successfully reached the required criterion for proceeding to the test stage when they went through the swing door within five seconds on every rewarded trial.

2.3.5. Test Procedure

For testing, a bird was collected from her home pen and put in the experimental pen. The order of testing was determined using an orthogonal latin square design where every single condition follows another on two occasions. Each hen received one test session per day on five consecutive days. Birds were deprived of food for an average of 1.5 hours prior to testing, and deprived of a substrate suitable for foraging and dustbathing in their home pens for all five days of the test period. Each test session consisted of presentation of each of five stimuli; three reward treatments (mealworms, dustbathing substrate, normal food), one SN and one MN trial. At the start of each session a bird was allowed to habituate to the experimental pen for 30 seconds. Then the appropriate CS was given for five seconds, after which behaviour was recorded for 15 seconds before the light was switched on signalling the door was unlocked. There was no CS in the MN trial, but behaviour was recorded for 15 seconds from when the trial started. In the mealworm trial, after the CS and the 15 second anticipation period, the door was unlocked and the bird entered the reward compartment and ate the mealworm. Then the light was switched off and the bird was ushered gently into the waiting compartment by the experimenter who held the swing-door open. In the normal food trial, the same happened except that the birds were allowed one minute to feed before the light was switched off and the bird was returned to the waiting compartment. In the dustbathing substrate trial, the same process was followed except that the birds were allowed to dustbathe or forage (with no food present) for five minutes before the light was switched off and the bird was returned to the waiting compartment. If the birds stopped feeding or foraging / dustbathing and walked away from the stimulus, or engaged in other behaviour in other parts of the pen for a continuous period of 10 seconds, then the trial was ended. In a SN trial, the CS was given and, after a 15 second anticipation period, the light was switched on but the door did not open. In all trials, between the end of the trial and the start of a new waiting period, there was an inter-trial interval of 10, 20 or 30 seconds (balanced between hens), to prevent hens from easily anticipating the start of the next trial.

During the testing procedure video recordings were made using 2 x K-32HCVF, (Kobi, Taiwan) cameras and recorded onto a K9 XQ H.264 DVR (Kobi, Taiwan). These were then transferred to a PC for analysis using Cowlog: Version 2.11 (Hänninen & Pastell 2009)

2.3.6. Behavioural recording

The frequencies and durations of selected behaviours (see ethogram, Table 2.2) were scored from video recordings. The duration of behaviours was recorded from the beginning of the behavioural sequence, until that behaviour ceased. For example, if a bird pecked three times against the door, the duration was measured from the beginning of the first peck until the end of the last peck. Comfort behaviour (as defined by Zimmerman et al. (2011), including preening, wing flapping, feather ruffling, scratching body, yawning and tail wagging) was only infrequently observed during the test periods and therefore was not included in the analysis. Other behaviours omitted from the analysis due to infrequent occurrence were ‘Pecking the cage’, ‘Putting head through side mesh’, ‘Scratching the side mesh’, ‘Explore Ground’, ‘Peck Ground’, ‘Peck Wall’, ‘Explore Object’ and ‘Scratch Ground’. For the final analysis, similar behaviours were merged into related groups of behaviour; ‘Locomotory behaviour’ included Walk, Step and Run, and ‘Motivational behaviour’ incorporated Peck Door and Push Door. The other behaviours included in the analysis were ‘Stand’, and ‘Alert Head Movements’ (see Table 2.3).

In addition, the following latency time periods were measured for all reward treatments: Time the door was opened to the time the bird entered the reward compartment (Door open to Enter), time the door was opened to the time the bird accessed the reward (Door open to Reward) and, finally, time the bird entered the reward compartment to the time the bird accessed or consumed the reward (Enter to Reward).

Table 2.2 Ethogram for behaviours displayed by hens during the experiment

Behaviour	Description
Walk	Walking in a continuous, fluid manner
Step	Lifting foot and putting it down again
Run	Fast, running movement
Stand	Shifting front of body upwards while neck is stretched upwards
Lying	Body touching the ground, either chest or side, legs bent under the body
Look towards reward room	Alert, with fixed gaze towards the reward compartment
Feed	Eating from food container, food hopper or other food source
Peck Ground	Pecks at items (visible or not) on the ground
Scractch ground	Scratching at ground, often intermittent during bouts of ground peck, often followed by one or two steps backwards after ground scratch
Explore ground	Walking or standing with head close to ground
Explore feed	Head close to ground, eyes focussing on feed or other edible objects
Explore object	Visual inspection or pecking at novel object
Push door	Pushing at the door separating the waiting and reward compartments with head, body or foot
Peck door	Pecking at the door separating the waiting and reward compartments
Peck wall	Pecking at walls
Peck mesh	Pecking at wire-mesh dividing the rooms
Put head through side mesh	Putting head through the mesh of the side walls
Scratch side mesh	Scratching at side mesh with feet
Preening neck/chest	Moving beak along feathers of neck and chest
Preening foot	Moving beak along foot
Preening other	Moving beak along feathers excluding neck and chest
Wing flapping	Lifting wings and flapping them
Feather ruffling	Stretching neck, raising ruff and ruffling feathers and body
Scratching body	Moving foot along feathers
Yawning	Opening mouth widely without vocalising
Tail wagging	Lowering tail and moving it rapidly from side to side in the horizontal plane
Head flicking	A short bout of rapid, vigorous side-to-side head movements (average 80ms)
Alert head movement	Head and neck stretched upwards, eyes open, with frequent head movements, turning head in various directions as if to locate an object or sound
Dustbathing	Vertical wing shake, rubbing body in litter, moving dust with wings and pecking at dust

Table 2.3 Behavioural responses subjected to analysis

Behaviour	Frequency	Duration
Standing	Stand F	Stand D
Locomotory	Loco F	Loco D
Motivated	Mot F	Mot D
Alert Head Movements	Alert F	Alert D

2.3.7. Statistical analysis

Linear mixed effects models (LMMs) and generalised linear mixed effects models (GLMMs) were performed in R (R Core Team 2013) using the lme4 (Bates et al. 2015) and glmmADMB (Skaug et al. 2013) packages. Mixed models were used to account for the within-bird variance associated with repeated measurements. Standard statistical models assume independence of residuals, but when measurements are taken from the same individual they are correlated. Mixed effects models allow us to include individual (bird) identity as a random factor, thus enabling us to separate the total variance in the response variable into a within-subject and between-subject variance component. Where LMMs were used, the assumptions of normal distribution, linearity and homoscedasticity of the residuals were checked by visual inspection of residual plots and by Shapiro-Wilks tests. Residuals that deviated from normality were corrected by log transformations. Parameter estimates were computed using the maximum likelihood method, and the significance of predictor variables were tested using maximum likelihood ratio tests, ('anova' function in R). For all LMMs and GLMMs the Chi-squared statistic (χ^2) and associated *P*-values are reported. Post hoc analyses were conducted using the lsmeans package (version 2.20-23) in R, applying the Tukey method to adjust *P*-values for multiple comparisons.

The effects of the different treatments on the duration of behaviours during the anticipation period were analysed using LMMs, with each response variable modelled separately. LMMs were also carried out on the three different latency periods. An initial model included Treatment (dustbathing substrate, mealworm, normal food, sound neutral, muted neutral), Cue Group (1-4), Day (1-5), Preceding Treatment (dustbathing substrate, mealworm, normal food, sound neutral, muted neutral, no preceding treatment) and the interaction between Cue Group and Treatment as fixed effects. However, as there were very few significant effects of Day and Preceding Treatment in the model outputs, the data were collapsed to give an average duration of each behaviour over the five days. Subsequently, models with the following predictor variables, 1) Treatment only, 2) Treatment and Cue Group and 3) Treatment, Cue Group and Treatment*Cue Group interaction, were compared using Akaike Information Criterion (AIC) scores and the anova() function, to detect if any models fitted significantly better than the other. The best fitting models, according to AIC scores, are detailed in Table 2.6 for behavioural responses and latency periods. In order to meet the assumptions of the linear mixed model, two behavioural variables (Mot D and Alert D) and two latency period variables (Door to Reward and Enter to Reward) were log (x+1) transformed. One latency period (Door to Enter) did not meet the assumption of normality of residuals, despite attempts at transformation. A Friedman test was therefore conducted in Minitab 17 (Statistical Software (2010). State College, PA: Minitab, Inc,) with Bird as a blocking factor, and post hoc analyses were performed using two-tailed Wilcoxon Signed Rank tests, applying a Bonferroni correction, resulting in a significance level set at *P*=0.02.

All behaviour frequency response variables (Stand F, Loco F, Mot F, and Alert F), and the Behavioural Transitions variable, consisted of count data. Poisson models, and other models in the family, may be used to analyse count data and generally require the data to be discrete, whole numbers. Therefore, the original data (not averaged over 5 days) was used to analyse these variables. Another important assumption of the Poisson distribution is that the mean and the variance of the sample are identical. Stand F and Loco F met this requirement and therefore were analysed using Poisson regression models. When the variance is greater than the mean, (e.g. the variance of Mot F was nearly five times greater than its mean), the data is said to be overdispersed which can result in biased standard errors if using a Poisson model. In this case, a negative binomial distribution was used, which accommodates overdispersion. An additional complication, common in count data regression, is having too many zeros, which was the case for 45% of the observations for Mot F. A zero-inflated negative binomial model was therefore used to account for this large amount of zeros. The 'Behavioural Transitions' variable contained no zeros, and AIC scores indicated a zero-truncated negative binomial model (type 1) was appropriate for the data. The function glmmADMB was used to run all Poisson and negative binomial models.

Model selection for GLMMs was carried out by comparing AIC scores, and by using the anova() function, to detect if any models fitted significantly better than the others. Models included the following sets of predictor variables: 1) Treatment only, 2) Treatment and Cue Group, 3) Treatment, Cue Group and Day, 4) Treatment, Cue Group, Day and Preceding Treatment, 5) Treatment, Cue Group, Day and Treatment*Cue Group Interaction, 6) Treatment, Cue Group and Treatment*Cue Group Interaction, and 7) Treatment, Cue Group, Day, Preceding Treatment and Treatment*Cue Group Interaction. Model comparison and AIC scores are detailed in Table 2.7. Residual plots were checked by running the models in lme4 and using the plot() function to check for any patterns in the data. Incident rate ratios and 95% confidence levels were extracted, and mean predictions were also checked against observed data to ensure they did not deviate, as deviation would indicate a poorly fitting model.

The variable Alert F was also count data. However, despite initial exploration indicating a negative binomial model might be appropriate, the model did not converge. A binomial model was not appropriate due to the fact that birds made no alert head movements during the dustbathing substrate treatment, and therefore one cell contained all zeros. It was decided that removing the dustbathing substrate treatment from the analysis and running the statistical analysis using the remaining treatment would result in an unacceptable loss of information. Therefore, a Friedman test was used for Alert F with bird as the blocking factor, and post hoc analyses were performed using two-tailed Wilcoxon Signed Rank tests applying a Bonferroni correction, resulting in a significance level set at P=0.005.

All final model outputs are detailed in Tables 2.8 and 2.9 (supplementary material). A Bonferroni correction was applied to account for the large number of variables being tested (12 in total) using the same data set, and therefore *P*-values of and below 0.004 were considered significant

2.4 Results

2.4.1 Effect of signalled rewards compared with neutral treatments

2.4.1.1 Behavioural transitions

All three rewarded sound cues (dustbathing substrate, mealworm and normal food) elicited a higher frequency of transitions between behaviours compared with the muted neutral (MN) treatment (Table 2.4 and Fig. 2.2). The dustbathing substrate and mealworm sound cues also elicited more behavioural transitions than the sound neutral (SN) treatment (Table 2.4 and Fig. 2.2).

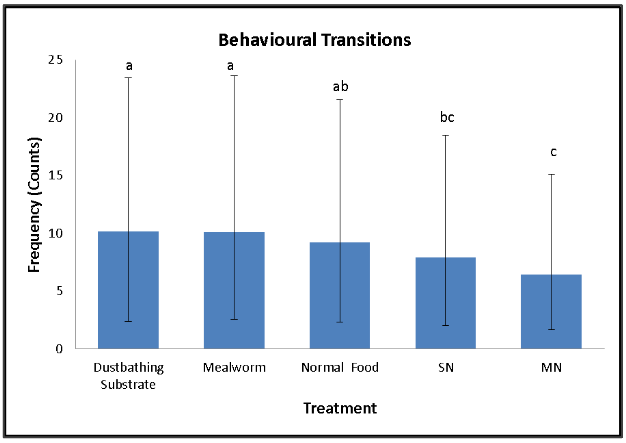

Figure 2.2 Mean frequency of behavioural transitions with lower and upper confidence limits. Different letters indicate statistically reliable differences between treatments (Tukey's HSD test).

2.4.1.2 'Motivated' behaviour

Birds also performed significantly higher frequencies and durations of 'motivated' behaviour (pushing and pecking at the door) following all three reward sound cues, compared with both the SN and MN treatments (Table 2.4 and Fig. 2.3). The frequency of motivated behaviour was also higher following the SN sound cue compared with during the MN treatment (Table 2.4 and Fig. 2.3).

2.4.2 Effect of different signalled rewards

Differential behaviour was elicited by the sound cues signalling the different rewards.

2.4.2.1. 'Motivated' behaviour

Birds performed 'motivated' behaviour (pushing and pecking at the door) significantly more frequently and for a longer duration following the cue signalling the dustbathing substrate compared with following the mealworm and normal food sound cues (Table 2.4 and Fig. 2.3).

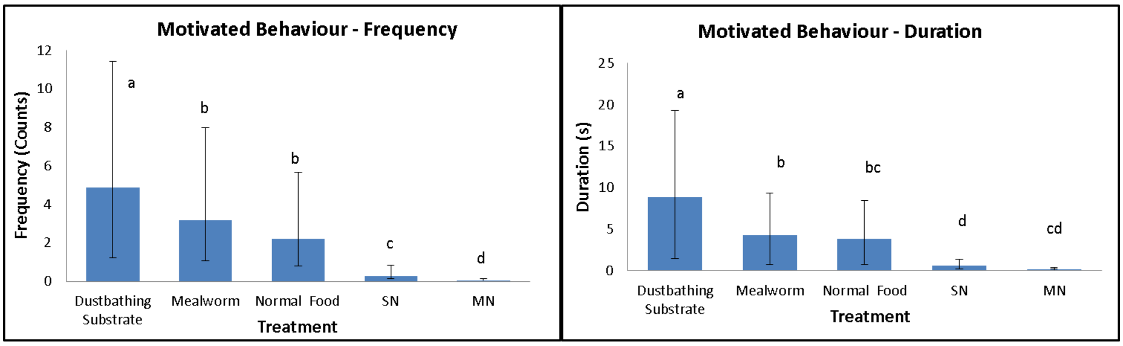

Figure 2.3 Mean frequency and duration of motivated behaviour. Error bars represent lower and upper confidence limits. Different letters indicate statistically reliable differences between treatments (Tukey's HSD test)

2.4.2.2. Standing

Birds stood still for less time in the period following the dustbathing substrate sound cue compared with all the other treatments (Table 2.4 and Fig. 2.4). The frequency of this behaviour was lower following the dustbathing substrate sound cue compared with all other treatments except the MN treatment (Table 2.4 and Fig. 2.4). The cue signalling mealworm led to birds standing still for less time compared with following the SN sound cue (Table 2.4 and Fig. 2.4).

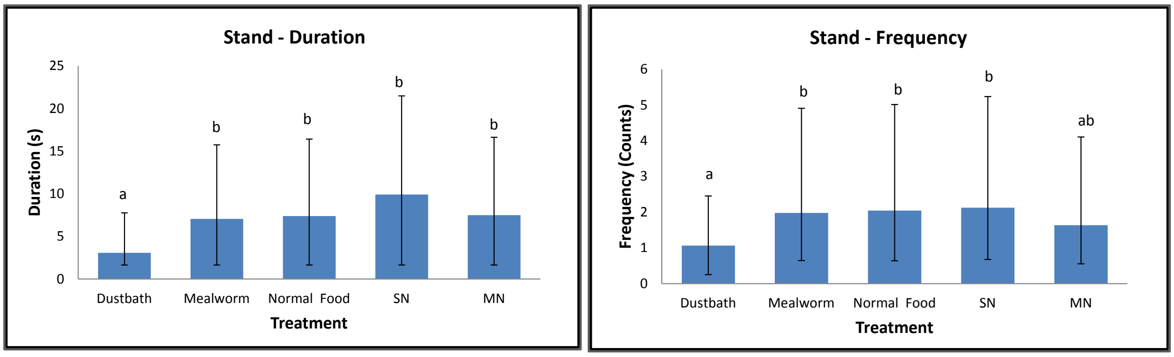

Figure 2.4 Mean frequency and duration of 'Stand'. Error bars represent lower and upper confidence limits. Different letters indicate statistically reliable differences between treatments (Tukey's HSD test)

2.4.2.3. Latency to access the rewards

After birds had entered the reward compartment, birds accessed/consumed the dustbathing substrate and mealworm rewards faster than their normal food (Enter to Reward), and accessed the dustbathing substrate faster than normal food once the door had been opened (Door open to Reward) (Table 2.4 and Fig 2.5). Treatment had no effect on the time birds took to enter once the door had been opened (Door Open to Enter) (Table 2.5 and Fig. 2.5).

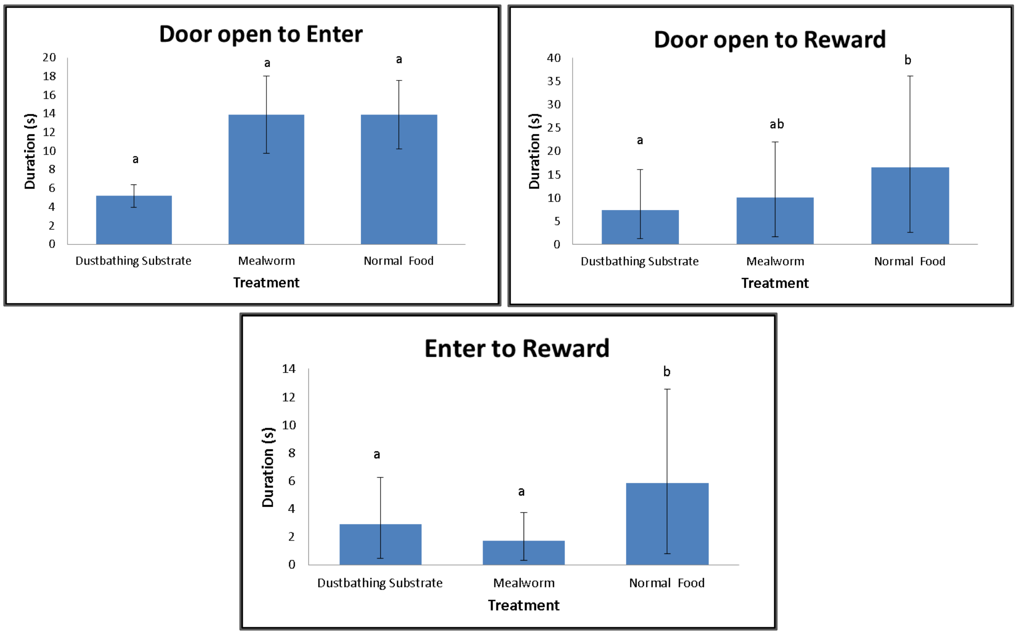

Figure 2.5 Mean duration in seconds of latency periods by treatment. For ‘Door to Reward’ and ‘Enter to Reward’ charts, error bars represent lower and upper confidence limits; For ‘Door to Enter’ chart, error bars represent standard error of the mean. Different letters indicate statistically reliable differences between treatments (Tukey’s HSD test and Wilcoxon Signed Rank Test).

2.4.3. Effect of signalled neutral event

2.4.3.1. Alert head movements

Birds exhibited significantly higher durations of alert head movements after the SN treatment had been signalled compared with after the sound cues for all three rewards (Table 2.4 and Fig. 2.6). The frequency of alert head movements was significantly higher following the SN sound cue compared to after the dustbathing substrate sound cue (Table 2.5 and Fig.2.6). (There were no alert head movements recorded during the dustbathing substrate treatment).

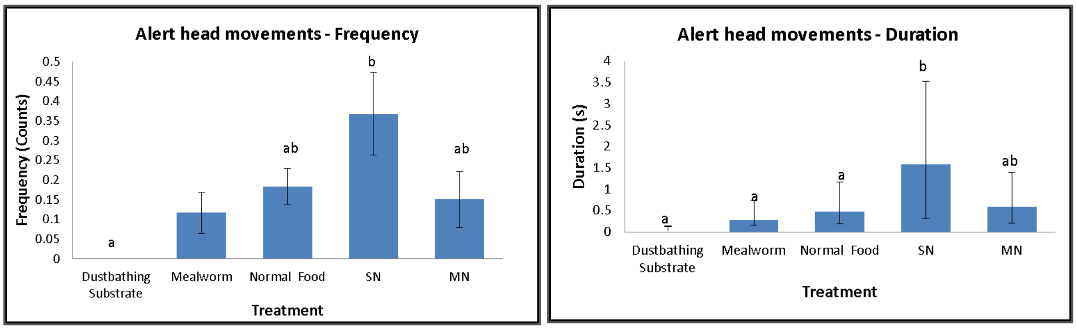

Figure 2.6 Mean frequency and duration of 'Alert Head Movements'. For frequency chart, error bars represent standard error of the mean; for duration chart, error bars represent lower and upper confidence limits. Different letters indicate statistically reliable differences between treatments (Alert D - Tukey's HSD test and Alert F - Wilcoxon Signed Rank Test)

2.4.4. Locomotion

The frequency and duration of locomotory behaviour were not affected by treatment (Table 2.4 and Figure 2.7).

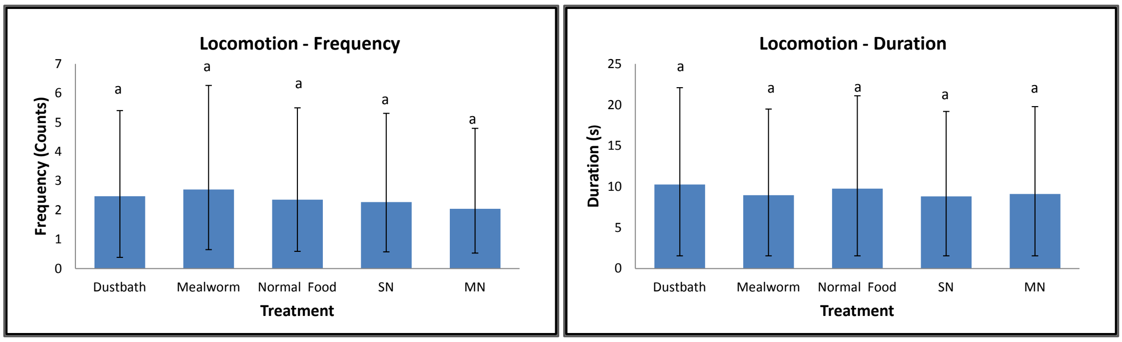

Figure 2.7. Mean frequency and duration of 'Locomotion'. Error bars represent lower and upper confidence limits. Different letters indicate statistically reliable differences between treatments (Alert D - Tukey's HSD test and Alert F - Wilcoxon Signed Rank Test)

Table 2.4: Behavioural responses' lsmeans, 95% confidence limits and significance levels

Behaviour	Dustbath			Mealworm			Normal Food			SN			MN					
	Lsmean	**Lower CL**	**Upper CL**	**Lsmean**	**Lower CL**	**Upper CL**	**Lsmean**	**Lower CL**	**Upper CL**	**Lsmean**	**Lower CL**	**Upper CL**	**Lsmean**	**Lower CL**	**Upper CL**	**X^2**	**X^2 d.f.**	***P* value**
Stand F	1.06^{a}	(0.81 ,	1.39)	1.98^{b}	(1.33 ,	2.93)	2.04^{b}	(1.40 ,	2.97)	2.12^{b}	(1.45 ,	3.11)	1.63^{ab}	(1.08 ,	2.47)	28.86	4	<0.001
Stand D	3.06^{a}	(1.42 ,	4.71)	7.05^{b}	(5.40 ,	8.70)	7.39^{bc}	(5.74 ,	9.03)	9.92^{c}	(8.27 ,	11.57)	7.49^{bc}	(5.84 ,	9.14)	36.28	4	<0.001
Loco F	2.47^{a}	(2.09 ,	2.93)	2.71^{a}	(2.06 ,	3.56)	2.36^{a}	(1.77 ,	3.14)	2.27^{a}	(1.70 ,	3.04)	2.04^{a}	(1.51 ,	2.76)	6.12	4	0.19
Loco D	10.26^{a}	(8.70 ,	11.83)	8.96^{a}	(7.39 ,	10.52)	9.77^{a}	(8.20 ,	11.34)	8.81^{a}	(7.25 ,	10.38)	9.10^{a}	(7.54 ,	10.67)	3.34	4	0.50
Mot F	4.87^{a}	(3.62 ,	6.55)	3.18^{b}	(2.10 ,	4.81)	2.23^{b}	(1.44 ,	3.46)	0.30^{c}	(0.16 ,	0.57)	0.03^{d}	(0.01 ,	0.14)	228.99	4	<0.001
Mot D *(Log(x+1) transformed)*	8.83^{a}	(7.43 ,	10.46)	4.23^{b}	(3.49 ,	5.10)	3.83^{bc}	(3.14 ,	4.63)	0.54^{d}	(0.32 ,	0.79)	0.13^{cd}	(-0.06 ,	0.28)	78.29	4	<0.001
Alert D *(Log(x+1) transformed)*	0.00^{a}	(-0.13 ,	0.14)	0.27^{a}	(0.11 ,	0.46)	0.48^{a}	(0.29 ,	0.69)	1.58^{b}	(1.25 ,	1.95)	0.58^{ab}	(0.38 ,	0.81)	25.56	4	<0.001
Behavioural Transitions	10.16^{a}	(7.77	13.29)	10.13^{a}	(7.59	13.52)	9.22^{ab}	(6.88	12.36)	7.89^{bc}	(5.87	10.60)	6.43^{c}	(4.75	8.70)	72.05	4	<0.001
Latency periods																		
Door Open - Access reward	7.30^{a}	(6.10 ,	8.71)	10.06^{ab}	(8.46 ,	11.93)	16.53^{b}	(14.00 ,	19.50)	-	-	-	-	-	-	10.88	2	0.004
Enter - Access reward	2.87^{a}	(2.434 ,	3.36)	1.69^{a}	(1.39 ,	2.03)	5.84^{b}	(5.07 ,	6.71)	-	-	-	-	-	-	23.77	2	<0.001

Superscript letters indicate statistically reliable group differences (Tukey's HSD test; p≤0.05)

Table 2.5: Medians, interquartile ranges and significance levels for 'Alert head movements' frequency, and the latency of birds to enter the reward room once the door had been opened

Behaviour	Dustbath		Mealworm		Normal Food		SN		MN				
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**S**	**d.f**	***P* value**
Alert F	0.00^{a}	(0.00 - 0)	0.00^{ab}	(0.00 - 0.20)	0.20^{ab}	(0.00 - 0.35)	0.20^{b}	(0.20 - 0.40)	0.00^{ab}	(0.00 - 0.20)	22.22	4	<0.001
Latency period													
Door Open - Enter	3.50^{a}	(3.00 - 6.89)	7.11^{a}	(4.30 - 20.28)	9.56^{a}	(6.04 - 13.82	-	- -	-	- -	6.50	2	0.04

Superscript letters indicate statistically reliable differences between treatments (Wilcoxon Signed Rank Test)

Table 2.6 Comparison of the different linear mixed effects models (LMMs) for behavioural responses and latency time periods

Behaviour / Latency period	**Fixed variables in model**	**Comparison**	**d.f.**	**AIC**	**X^2**	**X^2 d.f.**	***P*-value**
Stand D	**Treatment**		**7**	298			
	Treatment + CG	1 vs 2	10	301	2.8	3	n.s.
	Treatment * CG	2 vs 3	22	319	6.2	12	n.s.
Loco D	**Treatment**		7	294			
	Treatment + CG	1 vs 2	10	299	1.2	3	n.s.
	Treatment * CG	2 vs 3	22	314	8.9	12	n.s.
Mot D *(Log(x+1) transformed)*	**Treatment**		7	1			
	Treatment + CG	1 vs 2	10	5	2.9	3	n.s.
	Treatment * CG	2 vs 3	22	19	9.4	12	n.s.
Alert D *(Log(x+1) transformed)*	**Treatment**		7	-14			
	Treatment + CG	1 vs 2	10	-12	3.7	3	n.s.
	Treatment * CG	2 vs 3	22	7	5.0	12	n.s.
Enter - access reward	**Treatment**		5	-14			
(Log(x+1) transformed)	Treatment + CG	1 vs 2	8	-9	0.5	3	n.s.
	Treatment * CG	2 vs 3	14	-9	12.2	6	n.s.
Door open - access reward	**Treatment**		5	5			
(Log(x+1) transformed)	Treatment + CG	1 vs 2	8	7	3.7	3	n.s.
	Treatment * CG	2 vs 3	14	9	10.6	6	n.s.

Data includes model degrees of freedom (d.f.), Akaike information criterion (AIC), X^2, X^2 degrees of freedom (X^2 d.f.) and *P*-values
CG, Cue Group
n.s. indicates that the model is not significantly improved from the previous model

Table 2.7 Comparison of the different generalised linear mixed effects models (GLMMs) for behavioural responses

Behaviour	Fixed variables in model	Comparison	d.f.	AIC	Loglik	Deviance	*P*-value
Stand F	**Treatment**			**917**	**-453**		
	Treatment + CG	1 vs 2	3	919	-451	3.79	n.s.
	Treatment + CG + Day	1 vs 3	7	918	-446	12.75	n.s.
	Treatment + CG + Day + Preceding Treatment	1 vs 4	12	927	-445	14.33	n.s.
	Treatment * CG + Day	1 vs 5	19	932	-441	22.82	n.s.
	Treatment * CG	1 vs 6	15	933	-446	13.86	n.s.
	Treatment * CG + Day + Preceding Treatment	1 vs 7	24	941	-440	24.34	n.s.
Loco F	**Treatment**			**1012**	**-500**		
	Treatment + CG	1 vs 2	3	1016	-499	1.28	n.s.
	Treatment + CG + Day	1 vs 3	7	1015	-494	10.92	n.s.
	Treatment + CG + Day + Preceding Treatment	1 vs 4	12	1023	-494	12.37	n.s.
	Treatment * CG + Day	1 vs 5	19	1034	-492	15.94	n.s.
	Treatment * CG	1 vs 6	15	1035	-497	6.30	n.s.
	Treatment * CG + Day + Preceding Treatment	1 vs 7	24	1043	-491	17.20	n.s.
Mot F	**Treatment**			**939**	**-462**		
	Treatment + CG	1 vs 2	3	943	-460	2.24	n.s.
	Treatment + CG + Day	1 vs 3	7	947	-458	6.62	n.s.
	Treatment + CG + Day + Preceding Treatment	1 vs 4	12	951	-455	12.49	n.s.
	Treatment * CG + Day	1 vs 5	19	951	-448	26.56	n.s.
	Treatment * CG	n/a	n/a	n/a	n/a	n/a	n/a
	Treatment * CG + Day + Preceding Treatment	1 vs 7	24	957	-446	30.15	n.s.
Behavioural Transitions	Treatment			1524			
	Treatment + CG	1 vs 2	3	1527	-754	2.61	n.s.
	Treatment + CG + Day	**1 vs 3**	**7**	**1523**	**-748**	**14.47**	**0.04**
	Treatment + CG + Day + Preceding Treatment	1 vs 4	12	1531	-747	16.23	n.s.
	Treatment * CG + Day	1 vs 5	19	1541	-744	21.07	n.s.
	Treatment * CG	1 vs 6	15	1544	-750	9.21	n.s.
	Treatment * CG + Day + Preceding Treatment	1 vs 7	24	1549	-744	22.46	n.s.

Data includes model degrees of freedom (d.f.), Akaike information criterion (AIC), Deviance, X^2 degrees of freedom (X^2 d.f.) and *P*-values
CG, Cue Group
n.s. indicates that the model is not significantly improved from the previous model

2.5. Discussion

The results from this study confirm that hens differentially anticipate food and non-food rewards. Differences in the intensity of behaviours appear to reveal how birds ranked rewards, with the cue signalling the non-food reward (dustbathing substrate) consistently inducing higher frequencies and durations of certain behaviours compared with both mealworm and normal food. An increase in behavioural transitions, as opposed to an increase in head movements, appears to characterise the anticipation of all reward types.

2.5.1. Effect of Sound Cues

Consistent differences in behaviour elicited by the sound cues showed that birds learned to discriminate between cues signalling the imminent arrival of rewards and the cue or absence of a cue signalling a sound neutral or muted neutral treatment where nothing occurred. This confirms findings made by Zimmerman et al. (2011) and Moe et al. (2013) where laying hens were found to differentially anticipate different rewards or events.

2.5.2. Increase in activity / Behavioural transitions

It was predicted that anticipation of rewards would cause an increase in activity, which would suggest ‘preparatory behaviour’ (Spruijt et al. 2001) in order to access the resource. The results from this study supported this prediction, revealing that birds performed a higher frequency of behavioural transitions in anticipation of the three rewards compared with the neutral treatments. The number of transitions between behaviours is deemed to be a good indicator of activity (van der Harst et al. 2003; Vinke et al. 2004; Anderson et al. 2015), and changes in patterns of goal-directed behaviour are suggested to be an expression of ‘wanting’ (Boissy et al. 2007). This study’s results appear to contradict previous findings by Moe et al. (2009) in which hens demonstrated a lack of activity more akin to the response shown by cats when a food reward was signalled. An increase in activity in anticipation of rewards brings this study’s findings closer to those relating to many other animals such as rats (van den Bos et al. 2003), mink (Vinke et al. 2004), horses (Peters et al. 2012), and lambs (Anderson et al. 2015), and suggests preparation in order to facilitate consumption of the rewards. In the case of the non-food reward, the dustbathing substrate, this may reflect arousal in anticipation of being able to satisfy a hard-wired need (Wichman & Keeling 2008).

It is also worth considering, however, that the differences between the findings above relating to activity levels and the findings of Moe et al. (2009; 2011; 2013) may lie in the experimental procedure. In this study, hens had been trained to expect a reward in a specific location that they were able to see, whereas in experiments conducted by Moe et al. (2009; 2011; 2013) the reward was delivered into the birds’ home pen after a light cue, and the birds had no ability to see the reward or the location of potential rewards beforehand. Increased activity may therefore reflect the hens’ motivation to gain access to the reward location after a reward was signalled, rather than wait for a reward to be delivered.

It should also be noted that there is a distinction to be made between behavioural transitions and locomotory behaviour, both of which are indicators of activity. In this experiment, locomotory behaviour was not significantly affected by treatment. There are many possible reasons for locomotory behaviour in chickens. Zimmerman et al.(2011) found that birds took an increased number of steps in their negative treatment compared with their positive treatment (mealworm), and hypothesised that this could potentially be ‘pacing’ as a result of frustration, but they also suggested that increased locomotion is typical foraging behaviour in chickens. Kostal et al. (1992) also suggested that an increase in walking reflected a motivation to forage in anticipation of food. Hence, the lack of significance between rewards and neutral treatments could be because there were different motivations for locomotion in the different treatment. During the MN and SN treatment, the absence of a reward could have induced frustration leading to pacing, or hens may have been walking or foraging more because they were hungry, whereas in rewarded treatments, locomotion stemmed more from motivation to access the reward. Further investigations are needed to investigate the underlying motivation for locomotory behaviour during anticipation of rewards.

2.5.3. Behaviour reflects differences between signalled rewards

The analysis of ‘Motivated’ behaviour and ‘Standing’ revealed differences between reward treatments, which confirmed that birds were able to associate the respective sound cues with the different reward types. This outcome supported findings made by Zimmerman et al. (2011) that birds were able to learn associations between sound cues and positive, negative and neutral events, and also by Moe et al. (2013), that birds were able to associate two different light cues with two different food rewards. The findings above extend this knowledge, as behaviours in this study revealed differences between food and non-food rewards.

2.5.4. Behaviour reflects how birds rank rewards

The behavioural responses of this study appear to provide evidence that hens’ preferences for rewards are ranked. In a study on how food rewards are differentially expressed in hens, Moe et al. (2013) suggested that differences in the frequency of head movements reflected the incentive value of mealworms over wholewheat. The authors also suggested a need to investigate whether cue-induced behaviours may be food-reward specific. Here, this study reveals that there were quantitative rather than qualitative differences in behaviour between all three rewards, and therefore both food and non-food rewards appear to evoke a general anticipatory response in which the intensity of the behaviour (frequency, duration, etc.) differentiates the ranking of the reward but not the type.

2.5.5. Does the anticipation of food elicit a higher intensity of behavioural expression?

It was predicted that cues signalling food rewards would evoke a higher intensity of behavioural expression. The results of this study confirm that anticipation of different rewards is differentially expressed in the frequency and duration of some behaviours. However, it was the sound cue for the dustbathing substrate that elicited a higher duration of pushing and pecking at the door, and significantly less standing than following the signals of both the other rewards, results which do not support the above hypothesis.

If the higher intensity of behaviours induced by the signal for dustbathing substrate reflects the hens' greater motivation to access that reward compared with the others, then these results suggest that hens ranked the dustbathing substrate as more attractive than the food reward. This outcome does not support a previous study by Petherick et al (1993), where motivation to access a dustbathing substrate after deprivation was lower than motivation to access food. Furthermore, Dawkins (1983) demonstrated that access to litter under restricted time conditions was deemed by hungry birds to be of less value than food. However, in the same study, when birds had not been food restricted, they overwhelmingly chose access to litter over food. In my study, birds only had access to a dustbathing substrate during testing, and although birds were food restricted, the duration of this restriction did not exceed two hours. Therefore, it appears that hens may have felt satiated enough that a dustbathing substrate represented a more attractive reward than food. In concurrence with this idea, Widowski and Duncan (2000) proposed that dustbathing is motivated by pleasure, and if there is a low cost of performing dustbathing behaviour, then the fitness benefit increases. Fraser and Duncan (1998) laid out the framework for this idea, suggesting that positive affective or 'pleasure' states evolve in 'opportunity situations' rather than in 'need situations'. Therefore, although scientists suggest that dustbathing is important for animal welfare and the incidence of sham dustbathing in battery cages is deemed to be an indicator of high motivation to perform this behaviour (Olsson & Keeling 2005), it cannot be concluded from the above results that the apparent higher motivation to dustbathe, rather than access food, reflects a higher need to perform this behaviour.

Despite these equivocal results, it may be concluded that access to a dustbathing substrate is an attractive resource which is important to hens, even when birds have not been exposed to this kind of substrate before. The birds' original home environment was caged, with no access to a substrate for dustbathing. However, most hens performed a full sequence of dustbathing behaviour twice during the five test days. (The sequence consists of scratching and bill raking in the substrate, followed by the bird erecting its feathers, squatting down in the substrate, wing-shaking, head-rubbing, scratching with one leg, and side-lying or side-rubbing in the substrate). Chickens dustbathe every two days on average, however they tend not to dustbathe during the morning hours (Vestergaard 1982) which highlights the fact that birds in my experiment were highly motivated to perform this behaviour (testing had ended by 12.30pm on each day). If the birds did not perform the full sequence of dustbathing behaviours, they spent the majority of their time pecking in the substrate. Scratching, foraging and pecking in litter, as well as being precursors to dustbathing, are behaviours which chickens are motivated to perform in their own right (Olsson & Keeling 2005). In addition, although no hens appeared to perform nesting behaviour during the trials in this study, a dustbathing substrate may also be perceived as a potential nest site in a caged environment. (Smith et al. 1993). Thus, a dustbathing substrate can be a multi-faceted resource, and in this experiment, may have also represented the opportunity to be 'rewarded' for a longer period (as they were allowed access to the container full of substrate for five minutes, as opposed to one minute for normal food and mealworm respectively). However, the time limit imposed may also be a limitation, as bouts of dustbathing by chickens with unrestricted access to a suitable substrate last for, on average, 27 minutes (Vestergaard, 1982). Curtailing dustbathing behaviour before the bout has been completed may have the effect of devaluing the behaviour (Mason et al. 1998). Notwithstanding this possibility, a decline in motivation to access the dustbathing substrate was not observed over time. Indeed, other studies have found that hens worked for five minute access to litter (Matthews et al.1993, 1995), with a further study finding that hens worked for litter access as short as 150s (Matthews et al. 1998).

The latency of the chickens to access the rewards was also used as a proxy of motivation. The results show differences between the times taken to access the rewards, and also provide evidence that suggests consistent preferences between the two food rewards. Chickens appeared to consistently rank the dustbathing substrate as more attractive than their normal food, but, once in the reward compartment, they reached the mealworm reward quickest. This could be due to the fact that the mealworms represented a live target which could potentially disappear, and the movement of the mealworms incentivised them to consume the reward quickly. The difference in speed of accessing the mealworm reward compared with the birds' normal pellet feed supports previous findings by Bruce et al (2003), who found that chickens were highly motivated to access mealworms in comparison with five other food items. Davies et al (2014) also found that anticipation of mealworms resulted in increased heart rates and head movements, as well as a faster latency to reach a food bowl.

2.5.6. Head movements

In this study, the SN cue elicited significantly more alert head movements than all the reward sound cues and the MN treatment. This finding does not support a previous study conducted by Moe et al. (2011) which suggested that head movements are the most salient indicator of anticipatory behaviour in chickens, having found that these movements were attenuated by a dopamine D2 antagonist. Head movements were also found to be a more sensitive measure of arousal than heart-rate during decision-making in chickens (Davies et al. 2014). However, Zimmerman et al (2011) theorized that head movements imply increased vigilance in anticipation of a negative event, after their study revealed that hens increased their head movements prior to being squirted with water.

2.5.6.1 Sound cue effect – context-mediated equivalence?

The fact that the SN treatment elicited the most alert behaviour (head movements) may indicate some context-mediated equivalence (Molet et al. 2012) where cues that share a common context at different times come to be treated as equivalent. This study attempted to use simple sound cues which could be easily differentiated (by humans). However, all sound cues except one indicated a reward, and this contextual information may have been generalised to the sound cue for the SN (no reward) treatment. Moe et al. (2013) experienced a similar result, and suggested that unrewarded blue light (their neutral treatment) may have induced arousal due to context-mediated equivalence, or that hens, to some extent, generalised the stimuli 'light' and responded to the unrewarded cue colour. Zimmerman et al. (2011) investigated whether hens could differentiate between cues signalling positive, negative and neutral events. Their results showed that hens did discriminate, but as a result of the increased attention to one of the cues, the authors suggested that the nature of the cues was paramount; increased attention and head movements were interpreted as resulting from birds attempting to localise the source of the sound. It can be concluded that both sound and light cues seem to have an arousal effect on hens which induces some types of anticipatory behaviour (alert head movements), whether a reward has been signalled or not.

In this study, hens expressed behaviour differentially in response to reward type, both food and non-food, and these differences seemed to reflect the incentive value of the rewards. The frequency and duration of behaviours performed were the defining factors in discriminating between rewards, and therefore behaviour did not appear to be specific to food rewards. When access to a substrate suitable for dustbathing was restricted, and when only slightly food-restricted, chickens appeared to rank a dustbathing substrate as more attractive than food rewards. Mealworms were preferred to normal food, as determined by behavioural responses combined with the latency to access the rewards. These findings suggest that, when chickens are able to see the reward location, and a cue reliably signals impending delivery of a reward, anticipatory behaviour is expressed in attempts to access the reward location as well as a higher frequency of behavioural transitions. This outcome extends the range of behaviour known to be expressed in anticipation of rewards.

2.6 Supplementary Material

Table 2.8 Final model outputs from LMMs for behavioural responses and latency time periods. Table includes model degrees of freedom and AICs.

Model	Estimate	Std. Error	t value	Pr(>|t|)
Stand D ~ Treatment + 1|CG:Bird				
df(7), AIC = 298				
(Intercept) MN	7.493	0.7857		
Dustbath	-4.430	0.9489	-4.67	<0.0001
Mealworm	-0.441	0.9489	-0.47	0.6442
SN	2.425	0.9489	2.56	0.0138
Normal Food	-0.107	0.9489	-0.11	0.9110
Loco D ~ Treatment + 1|CG:Bird				
df(7), AIC = 294				
(Intercept) MN	9.103	0.7484		
Dustbath	1.159	0.9336	1.24	0.2200
Mealworm	-0.148	0.9336	-0.16	0.8750
SN	-0.290	0.9336	-0.31	0.7570
Normal Food	0.666	0.9336	0.71	0.4790
Log10 (Mot D +1)~ Treatment + 1|CG:Bird				
df(7), AIC = 1.5				
(Intercept) MN	0.040	0.0638		
Dustbath	0.953	0.0842	11.32	<0.0001
Mealworm	0.679	0.0842	8.06	<0.0001
SN	0.147	0.0842	1.74	0.0878
Normal Food	0.644	0.0842	7.66	<0.0001
Log10 (Alert D + 1) ~ Treatment + 1|CG:Bird				
df(7), AIC = -14				
(Intercept) MN	0.200	0.0562		
Dustbath	-0.200	0.0738	-2.70	0.0094
Mealworm	-0.094	0.0738	-1.28	0.2082
SN	0.212	0.0738	2.87	0.0061
Normal Food	-0.031	0.0738	-0.41	0.6813
Log10 (Door to Reward + 1) ~ Treatment + 1|CG:Bird				
Intercept (Dustbath)	0.919	0.0650		
Mealworm	0.125	0.0919	1.36	0.1833
Normal Food	0.325	0.0919	3.53	0.0012
Log10 (Enter to Reward + 1) ~ Treatment + 1|CG:Bird				
df(5(, AIC=-14				
Intercept (Dustbath)	0.588	0.0498		
Mealworm	-0.158	0.0704	-2.25	0.0308
Normal Food	0.247	0.0704	3.51	0.0012

CG = cue group

Table 2.9 Final model outputs from GLMMs for behavioural responses and Behavioural Transitions variable. Table includes model degrees of freedom and AICs.

Model	Estimate	Std. Error	z value	Pr(>|z|)
Stand F ~ Treatment + 1|CG:Bird				
df(), AIC = 917				
(Intercept) MN	0.491	0.1160		
Dustbath	-0.431	0.1590	-2.7	0.0069
Mealworm	0.191	0.1350	1.41	0.1584
SN	0.262	0.1330	1.97	0.0485
Normal Food	0.223	0.1340	1.66	0.0963
Loco F ~ Treatment + 1|CG:Bird				
df(), AIC = 1012				
(Intercept) MN	0.7139	0.0938		
Dustbath	0.1918	0.1218	1.57	0.1150
Mealworm	0.2816	0.1194	2.36	0.0180
SN	0.1078	0.1242	0.87	0.3860
Normal Food	0.1436	0.1232	1.17	0.2440
Mot F ~ Treatment + 1|CG:Bird				
df(), AIC = 939				
(Intercept) MN	-3.472	0.7230		
Dustbath	5.055	0.7180	7.04	<0.0001
Mealworm	4.629	0.7190	6.44	<0.0001
SN	2.273	0.7520	3.02	0.0025
Normal Food	4.275	0.7210	5.93	<0.0001
Behavioural Transitions ~ Treatment + Cue Group + Day + 1|CG:Bird				
df(7), AIC = 1523				
(Intercept) MN	2.364	0.1788		
Dustbath	0.622	0.1007	6.18	<0.0001
Mealworm	0.629	0.1007	6.24	<0.0001
SN	0.248	0.1007	2.47	0.0143
Normal Food	0.492	0.1007	4.89	<0.0001
CG2	0.291	0.2185	1.33	0.2082
CG3	0.323	0.2185	1.48	0.1648
CG4	0.239	0.2185	1.09	0.2962
Day 2	0.122	0.1007	1.21	0.2257
Day 3	-0.069	0.1007	-0.69	0.4912
Day 4	-0.055	0.1007	-0.55	0.5838
Day 5	-0.206	0.1007	-2.05	0.0415

CG = cue group

CHAPTER 3

McGrath, N., Dunlop, R., Dwyer, C., Burman, O., Phillips, C.J.C.(2017) Hens vary their vocal repertoire and structure when anticipating different types of reward. *Animal Behaviour* 130: 79-96

Contributor	**Statement of contribution**
McGrath, N	Experimental design (70%) Conducted experiment (100%) Statistical analysis of data (70%) Wrote the paper (100%)
Dunlop, R	Statistical analysis of data (30%) Edited paper (40%)
Burman, O	Experimental design (10%) Edited paper (20%)
Dwyer, C	Experimental design (10%) Edited paper (20%)
Phillips, C	Experimental design (10%) Edited paper (20%)

3.1 Abstract

The vocalisations of non-human animals are considered potential indicators of motivational or internal state. In many species, different call types, and structural variation within call types, encode information about physical characteristics such as age or sex, or about variable traits like motivation. Domestic chickens *Gallus gallus* have an elaborate vocal repertoire, enabling investigation into whether reward-related arousal is encoded within their call type and structure. Twelve hens were given a Pavlovian conditioning paradigm using sound cues to signal the availability of two food rewards (mealworms, normal food), one non-food reward (a container of substrate suitable for dustbathing), and a sound-neutral event, (sound cue, no reward). A muted-neutral treatment (no sound cue, no reward) provided a baseline for vocal behaviour. Sound cues preceded a 15 second anticipation period during which vocalisations were recorded. Hens emitted a 'food' call (previously defined in other studies) in anticipation of all rewards, including the non-food reward. Food calls and 'fast clucks' were more prevalent in anticipation of rewards, and most prevalent following the cue signalling the dustbathing substrate, suggesting that this reward induced the most arousal in hens. The peak frequency of food calls made in anticipation of the dustbathing substrate was 45-75 Hz lower (*P*=0.01) than those made in anticipation of food rewards, potentially reflecting differences in arousal. Vocalisations that reliably indicate hens' motivational state could be used as measures of welfare in on-farm assessment situations. This study is the first to reveal variation in the frequency-related parameters of the food call according to different contexts, and to show prevalence of different call types in reward and non-reward contexts, which may have implications for welfare assessments.

3.2 Introduction

Animal vocalisations contain information encoded within parameters such as frequency (Townsend et al. 2014), amplitude (Gustison & Townsend 2015; Reichard & Anderson 2015), rate of production (Clay et al. 2012) duration (Dentressangle et al. 2012) and energy distribution (Linhart et al. 2015). Some information may be static, relating to age, sex, or body size (Charlton et al. 2009; Briefer & McElligott 2011). However, variation in these acoustic parameters may also provide 'markers' of internal states (Manteuffel et al. 2004; Tallet et al. 2013). Internal states, in this context, refer to states of arousal induced by both internal (e.g. hunger) and external (environmental) stimuli and the interactions between them. Such states exist on a continuum, with arousal levels being in constant flux according to changes in stimuli and internal adjustments to these stimuli (for a detailed discussion see Berridge (2004)).

Flexible traits, such as signaller motivation, can be reflected in vocal frequency, amplitude, duration and rate in both humans (Scherer 1986) and animals (Taylor & Reby 2010; Briefer 2012), as shown in meerkats *Suricata suricatta* (Hollén & Manser 2007) and rats *Rattus norvegicus* (Knutson et al. 2002). The flexible features of vocalisations tend to be subject to certain 'motivation-structural' rules (Morton 1977). According to this concept, vocalisations produced in one motivational context (e.g. a hostile situation) should vary in structure from vocalisations produced in a very different motivational context (e.g. friendly interactions) (Morton 1977). This theory has been tested in many species including domestic dogs *Canis lupus familiaris* (Yin & McCowan 2004), chimpanzees *Pan troglodytes* (Siebert & Parr 2003), coatis *Nasua nasua* (Compton et al. 2001), and elk *Cervus Canadensis* (Feighny et al. 2006). For most of these animals, the call types commonly produced in hostile contexts are long in duration with a low frequency, whereas in fearful or non-aggressive contexts, short, high frequency, tonal calls are produced (see review by Briefer, 2012). In addition to different call types, motivation may also be encoded within a sound's structure. For example, the frequency of particular sounds may be lowered when a signaller is feeling aggressive (Bee & Perrill 1996).

Certain animal vocalisations function referentially, passing information about specific environmental stimuli to receivers (Macedonia & Evans 1993). To fulfil the criteria for functional reference, calls must be elicited by a narrow range of stimuli, and evoke a response in the receivers as if they had experienced the stimuli themselves (Marler et al. 1992; Macedonia & Evans 1993; Evans 1997). For example, some species of primate produce different call types according to particular threats or predators (Zuberbuhler et al. 1997; Murphy et al. 2013), which elicit distinctive adaptive responses in the receivers. Functionally referential vocalisations may also encode motivational information at the same time as being referential (Hollén & Manser 2007). In these instances, the call rate, or structural variation within call types, may encode information relating to arousal, motivation or urgency (Manser 2001; Clay et al. 2012). Townsend & Manser (2013) describe a motivational-referential continuum, giving the example of meerkats which produce calls that refer to nearby predators and also deliver information about the urgency of the threat. The food call of the chicken (Collias 1987; Evans & Marler 1994) has been described as the most rigorous example of food-specific functional reference within terrestrial animals (Clay et al. 2012). This call appears to meet all the criteria of functional reference; there is acoustic specificity between the stimulus (food) and the signal, and playback elicits feeding-related behaviours in receivers (Clay et al. 2012; Townsend & Manser 2013). It is also likely that the food call contains motivational information within its acoustic parameters.

The anticipation of rewards is thought to produce increased arousal in animals induced by changes in motivational state, and this, in turn, may elicit vocalisations. Rats, for example, emit ultrasonic vocalisations at 50-kHz in anticipation of rewards such as the presence of a play partner, during tickling from a familiar human, or in response to a cue signalling food (Knutson et al. 1998; Panksepp & Burgdorf 2000; Burgdorf & Panksepp 2006). By contrast, negative stimuli such as the presence of a predator, or a cue signalling an electric shock, tend to elicit a 22-kHz vocalisation in rats (Knutson et al. 2002). This knowledge, theoretically, allows us to assess whether a rat is in a ‘rewarding’ environment, which has implications when determining their welfare. Clear indicators about whether animals are experiencing rewarding or non-rewarding environments may guide decisions made within management systems. The anticipation of rewards induced by signals has been linked to ‘wanting’ (dopaminergic) activity in the brain, which, if the reward is accessible, leads to consummatory activity, and associating ‘liking’ or pleasure-based feelings (Berridge 1996, 2007). Therefore, while stressful environments are known to have deleterious effects on productivity (Broom 1991), it is reasonable to assume that the reduction of stress through the provision of rewarding environments could positively influence animal health and productivity (Boissy et al. 2007).

The domestic chicken is a good candidate for the study of vocalisations made in anticipation of rewards for three main reasons. Firstly, the behaviour of chickens in anticipation of rewards has already been well documented (Moe et al. 2009; Zimmerman et al. 2011; Moe et al. 2013; Moe et al. 2014; Garland et al. 2015). Secondly, behavioural data suggests that the motivational state of chickens changes according to the type of reward (McGrath et al. 2016), and therefore it may be possible to link anticipatory behaviour with vocalisations produced in anticipation of rewards.

Thirdly, the chicken has a wide and varied vocal repertoire. Between 20 and 25 discrete calls have been documented in various studies (Collias & Joos 1953; Kruijt 1964; Woodgush 1971; Evans 1993; Evans & Evans 1999; Marx et al. 2001), including those classed as referential (Evans & Evans 2007). Notably, it seems that motivational information may be encoded within referential calls. Alarm calls, for example, differentiate between terrestrial threats and aerial predators (Evans et al. 1993) whilst simultaneously encoding the motivational state of the bird (Kokolakis et al. 2010).

Chickens have been shown to modify the rate and numbers of their food calls in response to different types of food reward (Marler et al. 1986; Wauters et al. 1999). These changes appear to reflect variation in motivational state according to food type, which indicates there is an opportunity to test for motivational information within a referential call. The food call of chickens is described by various authors as having a characteristic appearance consisting of trains of pulsatile calls delivered in a regular temporal pattern, emphasizing low frequencies, and at a rate of 4-10 per second (Collias 1987; Evans & Marler 1994). They are given by both males and females, and their structure facilitates location of the sender (Hughes et al. 1982). No studies have investigated vocalisations delivered by chickens in anticipation of other types of reward, or variation in their acoustic parameters. Therefore, one of the goals of this study was to characterise vocalisations made in anticipation of different types of reward (different food types known to be ‘rewarding’ to chickens (Bruce et al. 2003) and a substrate suitable for dustbathing). Another aim of this study was to investigate whether call parameters varied within the call types according to reward type, and according to whether it was a reward or non-reward. To achieve this, anticipation of these rewards was experimentally induced using a Pavlovian conditioning paradigm. The hypothesis that chickens would emit specific call types in anticipation of different rewards was tested, as well as the hypothesis that vocal parameters would differ according to the perceived quality of the reward. These differences would provide information about the motivational state of the signaller, and therefore could be used as indicators of baseline welfare.

3.3 Methods

The vocalisations of chickens were recorded during the experiment described in Chapter 2. Please refer to the methods in this chapter for details of subjects and housing, experimental apparatus and training procedure.

To summarise, Twelve ISA Brown hens, approximately 18 weeks old, were subjected to a Pavlovian conditioning paradigm as used by Zimmerman et al. (2011) and Moe et al. (2009). An initially neutral stimulus (conditioned stimulus, CS) was repeatedly paired with the presentation of one of three different rewards (mealworms, normal food or dustbathing substrate) or a sound neutral (SN) event (an empty compartment) which served as the unconditioned stimuli (US). Sound cues were used for the conditioned stimuli, all of five seconds duration: ‘ring’ (ringing of an old fashioned telephone), ‘beep’ (an alarm-clock style beep) ‘buzz’ (a buzz sound as in a game show) and ‘horn’ (an old-fashioned car horn sound). A muted neutral (MN) treatment (five seconds of ‘nothing’, no CS or US) was used to control for the effect of sound in the other treatments. Each of the four sound cues was used to signal the presence of each type of US. Consequently, four cue groups of three hens (from the same home pen) each experienced different combinations of CS and US.

3.3.1 Test procedure

For testing, a hen was collected from her home pen and put in the experimental pen. The order of testing was determined using an orthogonal latin square design where every single condition followed another on two occasions. Each hen received one test session per day on five consecutive days. Hens were deprived of food for an average of 1.5 hours prior to testing, and deprived of a substrate suitable for foraging and dustbathing in their home pens for all five days of the test period. Each test session consisted of presentation of each of five stimuli; three reward treatments (mealworm, dustbathing substrate, normal food), one SN and one MN trial. At the start of each session a hen was allowed to habituate to the experimental pen for 30 seconds. Then the appropriate CS was given for five seconds, after which behaviour and vocalisations were recorded for 15 seconds before the light was switched on signalling the door was unlocked. There was no CS in the MN trial, but behaviour was recorded for 15 seconds from when the trial started. In the mealworm trial, after the CS and the 15 second anticipation period, the door was unlocked and the hen entered the reward compartment and ate the mealworm. Then the light was switched off and the hen was ushered gently into the waiting compartment by the experimenter who held the swing-door open. In the normal food trial, the same happened except that the hens were allowed one minute to feed before the light was switched off and the hen was returned to the waiting compartment. In the dustbathing substrate trial, the same process was followed except that the hens were allowed to dustbathe or forage (with no food present) for five minutes before the light was switched off and the hen was returned to the waiting compartment. If the hens stopped feeding or foraging / dustbathing and walked away from the stimulus, or engaged in other behaviour in other parts of the pen for a continuous period of 10 seconds, then the trial was ended. In a SN trial, the CS was given and, after a 15 second anticipation period, the light was switched on but the door did not open. In a MN trial, no sound cue was given, and behaviour and vocalisations were recorded for a period of 15 seconds.

In all trials, between the end of the trial and the start of a new waiting period, there was an inter-trial interval of 10, 20 or 30 seconds (balanced between hens), to prevent hens from anticipating the start of the next trial.

During the testing procedure, vocalisations were recorded using a Sennheiser ME66 condenser shotgun microphone connected to a Tascam DR100 MkII DAT recorder. Gain settings were set to High and the rotary dial input gain setting was set at 6. Recordings were made with 24 bit resolution at a sampling rate of 44.1 kHz. The DAT files were transferred to a PC (Dell) to analyse the vocalisations using Raven Pro: Interactive Sound Analysis Software (Version 1.5). Ithaca, NY: The Cornell Laboratory of Ornithology.

3.3.2 Acoustic analysis

Spectrograms of recordings were generated using Raven Pro 1.5 (Cornell Lab of Ornithology 2014) (1792-sample Hann window, 35.4 Hz filter bandwidth, 5.38 Hz frequency resolution (grid spacing), discrete Fourier transform (DFT) size of 8192 samples, and time grid hop size 200 samples (88.8% overlap)). Measurements were made in Raven Pro and values were imported into Excel by syllable. A syllable is defined as a sound that makes a continuous impression in time on the spectrogram. A call is defined as a clearly discernible sequence (both audibly and visually) of syllables grouped together. Recordings with extensive background noise (N=94) were excluded from further analysis (Fig. 3.1). The difference in percentage of calls rejected by treatment was not significant ($\chi^2(8)=0.26$, $P>0.05$). No vocalisations were elicited in 23 out of the 300 treatments, and this number was distributed across contexts as follows: Dustbathing substrate: 5; Mealworm: 6; Normal Food: 2; SN:3; MN: 7.

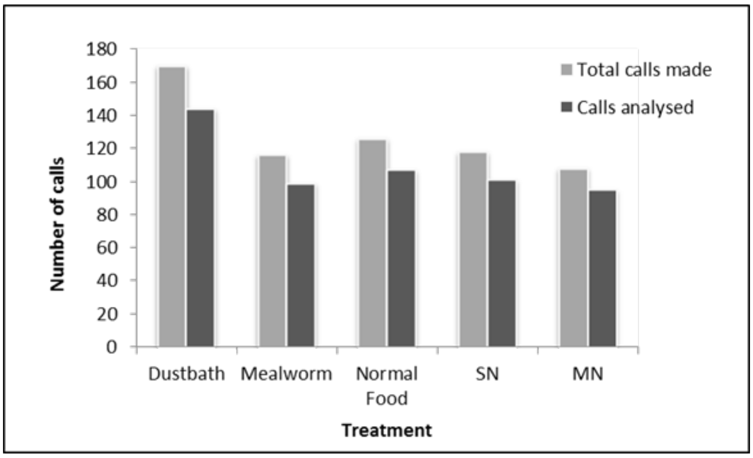

Figure 3.1 Number of calls made and number of calls analysed by treatment

Table 3.1 Description of the measurements used in the quantitative classification of call types.

Measurement	Description
Call duration	Time (in s) from beginning of first syllable to the end of the last syllable in the sequence
Number of syllables	Number of syllables in the call
Average syllable length	Mean length (in s) of syllables in a call
Maximum syllable length	Length of the syllable with the longest duration within the call (in s)
Low Frequency	The lowest frequency bound of the syllable (in Hz.)
High Frequency	The highest frequency bound of the syllable in (in Hz.)
Q1 Frequency	The frequency that divides the selection into two frequency intervals containing 25% and 75% of the energy (in Hz.)
Q3 Frequency	The frequency that divides the selection into two frequency intervals containing 75% and 25% of the energy (in Hz.)
Centre Frequency	The frequency that divides the selection into two frequency intervals of equal energy (in Hz.)
Peak Frequency	The frequency at which the maximum power (dB) occurs within the selection (in Hz.)
Delta Frequency	Difference between the lowest and upper limits of frequency (Hz.)
Bandwidth 90%	Difference between the 5% and 95% frequencies (in Hz.)
Interquartile (IQR) Bandwidth	Difference between the 1st and 3rd Quartile Frequencies (Hz.)

All acoustic measurements are measured on the fundamental frequency component.

3.3.3 Call Classification

The call classification process followed those used in previous studies, including Garland et al. (2015), and Rekdahl et al. (2013). As yet, there are no fully automated classification procedures that do not include a qualitative component. Manual classification is commonly used as a starting point as this method picks up the subtle features of the sounds that quantitative measures will miss. However, because of these subtle differences, there is a need to test the robustness of a manual classification. In this study, the sounds were first classified manually and then subjected to a CART and Random Forest analysis to corroborate this initial classification. A high percentage agreement between the manual and quantitative methods suggests that the manual classification consistently and correctly groups the sounds into types, and therefore is robust and repeatable. A total of 546 calls were manually classified. The data were then grouped by call in Excel and calculations were made for the temporal variables. Frequency variables were averaged across call. All variables measured are detailed in Table 3.1.

Descriptive analyses were performed on call types (Table 3.2). Example spectrograms of these call types were visually compared with already documented chicken calls (Collias, 1987; Evans and Evans, 2007).

Table 3.2 Mean values of acoustic parameters by call type

Description	Peak Frequency (Hz.)		Min Frequency (Hz.)		Max Frequency (Hz.)		Centre Frequency (Hz.)		Q1 Frequency (Hz.)		Q3 Frequency (Hz.)		Delta Frequency (Hz.)		Bandwidth 90% (Hz.)		IQR Bandwidth (Hz.)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Single Cluck	340.6	112.3	261.4	110.2	433.8	121.2	342.7	111.8	324.3	111.2	361.6	112.2	171.8	39.3	97.9	30.4	37.3	13.9
Double Cluck	389.2	122.4	277.2	108.0	516.6	151.3	390.8	122.2	372.0	120.4	410.9	124.7	165.6	33.2	96.4	26.7	38.8	14.3
Fast Cluck	411.6	132.8	244.8	125.8	609.9	194.1	412.2	133.7	393.0	130.4	432.2	136.7	162.9	41.3	95.8	29.0	39.2	13.4
Food call	457.6	84.2	318.5	98.8	620.3	118.5	459.1	84.3	438.5	83.8	479.8	84.2	173.7	30.1	101.7	26.8	41.4	14.4
Mixed	474.6	137.6	287.4	138.0	694.6	184.5	474.9	136.9	452.9	135.2	497.5	138.8	192.4	56.4	108.6	38.4	44.5	21.4
Whine	621.4	137.7	401.4	145.4	869.4	148.1	617.9	128.3	591.4	126.9	641.8	130.7	274.0	78.1	130.0	54.4	50.4	25.9
Singing	644.6	107.6	337.2	108.5	937.3	127.3	638.1	100.0	589.2	95.9	679.3	106.4	366.8	123.8	218.7	94.1	90.2	41.5
Harsh	502.6	96.6	313.7	74.7	733.2	179.0	498.7	93.4	473.4	86.4	521.1	100.4	245.6	99.5	120.5	58.6	47.9	34.0

Hens have distinct call types within their repertoire, but some calls also blend from one type into another on a continuum of calls. Therefore a 'mixed' call category was created to include these calls. The food call given by chickens was identified by comparing our recordings with published spectrograms (Sherry 1977; Evans & Evans 2007). These calls were characterised by a consistent fast rhythm and low pitch (Table 3.2). Out of a total of 60 food calls, 24 were made by one hen (Table 3.3). In order to reduce bias, 16 of this hen's food calls (randomly chosen) were removed from the analysis in order to better balance the number of calls made by individuals.

Table 3.3 Number of call types made by individual birds

Bird	Food call	Fast Cluck	Whine	Harsh	Singing	Mixed	Double Cluck	Single Cluck	Total no. of calls made
1	1	13	0	7	0	19	6	5	51
2	5	4	4	10	2	3	1	6	35
3	1	5	11	1	2	9	8	11	48
4	3	16	0	0	1	11	10	17	58
5	24	3	1	0	0	9	10	11	58
6	4	6	0	10	0	12	5	5	42
7	2	0	16	5	2	4	0	0	29
8	4	5	9	0	0	16	1	1	36
9	0	4	11	1	0	7	12	16	51
10	6	7	3	2	7	9	1	2	37
11	8	6	2	8	1	11	5	6	47
12	2	6	19	9	1	14	1	2	54
Totals	60	75	76	53	16	124	60	82	

In order to validate the manual classification of calls, a non-parametric classification and regression tree analysis (CART) and a Random Forest analysis were used. Applying the CART method, a decision tree was constructed using the rpart package in R, which does not require independence of samples. In a decision tree a set of hierarchical decision rules is created. Each rule can branch into another rule or a terminal category. At each step, the split is made based on the independent variable that results in the largest possible reduction in heterogeneity of the dependent variable; this is called the 'Gini index', which measures impurity or 'goodness of split' (Breiman et al. 1984). The optimal decision tree must contain criteria that classify the data as accurately as possible, without 'over-fitting' the data. Therefore, the tree was pruned to minimise misclassification rate, by choosing a complexity parameter which minimises the cross validated error.

A Random Forest analysis was also conducted using the randomForest package in R (Liaw & Wiener 2002). This classification tool randomly selects a subset of predictor variables and creates a 'forest', or a collection of decision trees (Breiman 2001). The aggregation of trees evaluates the classification uncertainty of each tree [the out of bag (OOB) error] in addition to ranking the importance of each predictor variable (i.e. the call variables measured) (Breiman 2001). As Random Forests estimate error internally, no cross-validation is required (Breiman 2001). Based on the lowest OOB error, the number of predictors randomly selected at a node for splitting was set to three and 1000 trees were grown. Classification success was evaluated using the overall OOB error rate and individual call type errors.

3.3.4 Statistical analyses of call types

All analyses of call types were performed in R (R CoreTeam 2013). The prevalence of call-types used by hens was calculated by treatment. Prevalence of call types was analysed using a generalised linear mixed model (GLMM) within the lme4 package (Bates et al. 2015) with 'treatment' as the predictor variable and each call type as the response variable with subject ID as the random effect. A mixed model was used to account for the within-bird variance associated with repeated measurements. A negative binomial model was used as the most appropriate method to analyse these data, as these models allow for overdispersed data. Incident rate ratios and 95% confidence levels were extracted, and mean predictions were also checked against observed data to ensure they did not deviate, as deviation would indicate a poorly fitting model. The function 'glmmADMB' was used to run all negative binomial models.

To determine whether hens varied their call structure in anticipation of different rewards, the variation of acoustic parameters within call-types was analysed. Only the call types ‘Single clucks’, ‘Double clucks’, ‘Fast clucks’, ‘Food calls’, ‘Whines’ and ‘Gakel calls’ were used in this analysis. ‘Singing’ calls (n=16), were eliminated due to the small number of calls. ‘Mixed’ calls (n=124) were also excluded as their structure was highly variable and therefore they were difficult to classify. Many of the acoustic variables were highly correlated. Therefore, a Factor Analysis (FA) with varimax rotation was conducted using the function ‘factanal’. Acoustic variables were log transformed to better match hearing perception in vertebrates (Cardoso 2013), and plots were examined for linear relationships between variables. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy (MSA) was used to distinguish which variables should be included in the factor analysis. This measure indicates whether a variable belongs to a family of variables psychometrically (Dziuban & Shirkey 1974). Variables with MSA scores below 0.5 indicate that an item does not belong to a group and may be removed from the factor analysis. Following these criteria, the variable ‘Number of Syllables’ (MSA score of 0.35) was removed from the factor analysis.

In a preliminary FA, the model did not converge because variables ‘Centre Frequency’, ‘Q1 frequency’ and ‘Q3 frequency’ had very low levels of uniqueness (less than 0.0000001). These variables were removed, and the models were run on the remaining variables, moving from a one-factor up to a four-factor model. ‘Call duration’ and ‘Average Syllable Length’ loaded on to separate individual factors and were therefore excluded from the FA and tested as separate responses. The overall KMO test output of 0.69 and Bartlett’s test of sphericity (P<0.001) indicated that the FA was justified (McGregor 1992). Using the package nFactors(), the optimal number of factors as determined by both eigenvalues and parallel analysis was two. ‘Bandwidth 90%’, ‘Delta Frequency’ and ‘IQR Bandwidth’ loaded on to Factor 1, with ‘Bandwidth 90%’ loading the highest (0.98) and ‘Peak’, ‘Low’ and ‘High Frequency’ loaded on to Factor 2 with ‘Peak Frequency’ loading the highest (0.96). In order to facilitate interpretation of results, the variables that loaded highest on to each factor – ‘Bandwidth 90%’ (from Factor 1) and ‘Peak Frequency’ (from Factor 2) - were used for the subsequent call structure analysis. ‘Call Duration’ and ‘Average Syllable Length’ were also analysed to detect if hens altered the temporal structure of their calls in anticipation of different rewards.

Variation in acoustic parameters for each call type was analysed using linear mixed effects models (LMMs) from within the lme4 package (Bates et al., 2015). The assumptions of normal distribution, linearity and homoscedasticity of the residuals were checked by visual inspection of residual plots and by Shapiro-Wilks tests. Parameter estimates were computed using the maximum likelihood method, and the significance of predictor variables were tested using maximum likelihood ratio tests, (anova() function in R). Each response variable was modelled separately. For all LMMs, the Chi-squared statistic (χ^2), degrees of freedom and associated *P* values are reported. (Full results are available in Table 3.6). Post hoc analyses were conducted using the lsmeans package (version 2.20-23) in R, applying the Tukey method for individual comparisons of different factor levels within the same model, incorporating an adjustment of *P* values for multiple comparisons. A Bonferroni correction was applied to account for the number of variables being tested using the same data set, and therefore *P* values of below 0.01 were considered significant.

Model selection was carried out using Akaike Information Criterion (AIC) scores, and by performing Chi-square tests to compare models using the anova() function, with values of $P<0.05$ considered significant (Table 3.7). Initial models included ‘Preceding Treatment’ as a predictor variable to establish whether there were any carry-over effects between treatments. As there was no significant effect of preceding treatment, this predictor was left out of the final models. Final models included the following sets of predictor variables: 1) Treatment only, 2) Treatment and Cue Group, 3) Treatment, Cue Group and Day. Model comparison and AIC scores are detailed in Table 3.7. Residual plots were checked by using the plot() function to check for any patterns in the data. All final model outputs are detailed in the supplementary material (Tables 3.8 – 3.14).

3.4 Results

3.4.1 Call classification

Hens produced 8 different call types according to both the manual classification and CART analysis, suggesting a high agreement between the two methods and a high probability this call repertoire is ‘correct’. These ranged from the very short ‘single cluck’, with a call duration of 0.08 s, to a long ‘gakel’ call (Zimmerman and Koene, 1998) (3.1 s) (Table 3.2). Single clucks and ‘Double clucks’ were short sharp clucks with either one or two syllables. Two other ‘cluck’ type calls, the ‘Fast cluck’ and the ‘Food’ call, were structurally similar to the single cluck and double cluck, but consisted of a longer series of short sharp clucks (Fig. 3.6). The fast cluck was not as consistent in rhythm or pitch as the food call.

Hens also produced long, high pitched calls such as a ‘whine’ (Fig 3.7), which was characterised by a wide frequency range (delta frequency = 274 Hz), compared with the ‘cluck’ calls whose frequency range was from 162.9 – 173.7 Hz (Table 3.2). Gakel calls were also identifiable by having a harsh or whiny tone, and a high frequency range (246 Hz). A small number (n=16) of calls identified as ‘singing’ (Collias, 1987) also had a high pitch (644.6 Hz) and frequency range (366.8 Hz). ‘Mixed’ calls could not be categorised into any of the other groups due to their high structural variation.

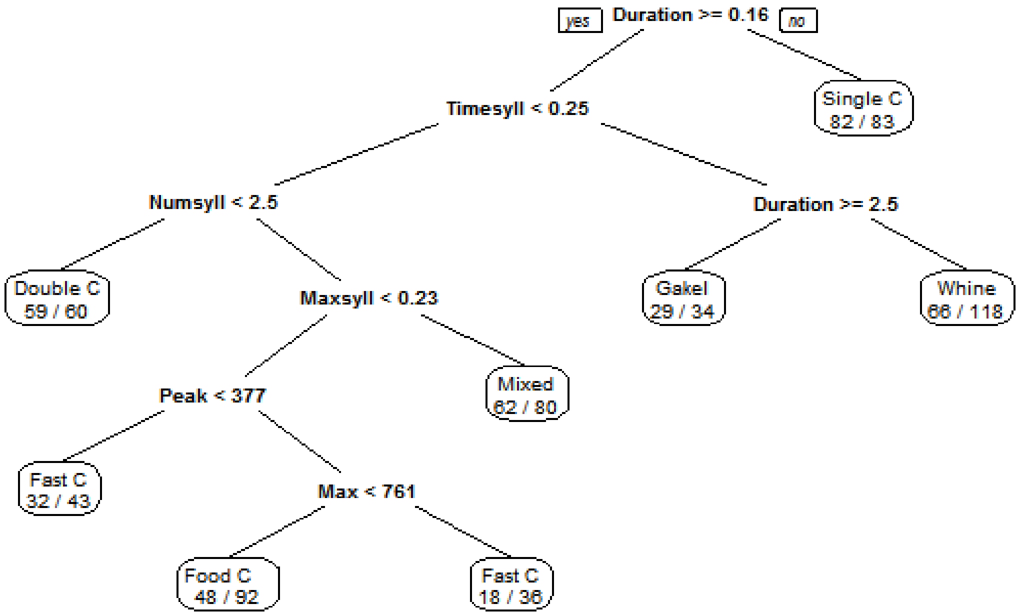

Figure 3.2 Classification and regression tree of vocalisations made by chickens in anticipation of rewards. The tree shows the variables used at each split, with the criteria (<,>,=).Terminal nodes indicate the prediction of call type for that partition and the number of correctly classified call types out of the total in that subgroup. (Abbreviations: ‘Food C’ = Food call, ‘Fast C’ = Fast cluck, ‘Double C’ = Double cluck, ‘Single C’ = Single cluck, ‘Duration’ = Call Duration, ‘Timesyll’ = Average syllable length, ‘Numsyll’ = Number of syllables, ‘Peak’ = Peak Frequency, ‘Max’ = Maximum Frequency)

All variables listed in Table 3.1 were available for construction of the CART decision tree, and the variables CART selected in tree construction were ‘Call duration’, ‘Maximum frequency’, ‘Maximum syllable length’, ‘Number of syllables’, ‘Peak frequency’, and ‘Average syllable length’. The tree correctly classified over 69% of calls (Fig. 3.2). The first branch in the tree was based on call duration, which separated the cluck call types (food calls, fast, single and double clucks) from the gakel calls and whines. Further branching was heavily influenced on the longer calls by duration of the call and maximum frequency, and on the shorter calls by the number of syllables and syllable length, as well as acoustic frequency parameters. The analysis resulted in 8 terminal nodes (call categories), with fast clucks being the most prevalent in 2 terminal nodes.

Table 3.4 Random Forest confusion matrix and classification error for each call type.

Call type	Double Cluck	Fast Cluck	Food Call	Gakel Call	Mixed	Singing	Single Cluck	Whine	Classification error
Double Cluck	59	0	0	0	0	0	1	0	0.017
Fast Cluck	0	49	13	0	13	0	0	0	0.347
Food Call	0	17	33	0	10	0	0	0	0.45
Gakel Call	0	0	0	38	6	1	0	8	0.283
Mixed	0	19	11	3	74	3	0	14	0.403
Singing	0	0	0	0	7	6	0	3	0.625
Single Cluck	0	0	0	0	0	0	82	0	0
Whine	0	0	0	6	10	0	0	60	0.211

The OOB error rate was 26.56%. The first column lists call types from our subjective classification by name, and the following columns shows the number of each call type the initial call types were classified into by Random Forest. The final column shows the classification error rate for each call type.

Table 3.5 Gini index showing importance of predictor variables in the Random Forest analysis.

Variable	Mean decrease in Gini
Call Duration	87.52
Max Syllable Length	61.59
Number of Syllables	72.42
Average syllable length	54.83
Peak Frequency	19.69
Minimum Frequency	22.31
Maximum Frequency	27.06
Centre Frequency	20.22
Q1 Frequency	19.57
Q3 Frequency	20.21
Frequency Range	25.26
Bandwidth	17.56
IQR Bandwidth	16.72

Random Forest correctly classified a high number of calls (out of box estimate error rate was 26.6%; Table 3.4). This represents a high level of agreement in classification with our manual classification of calls. The most important variables used by Random Forest classification were Call duration and Number of syllables (mean decrease in Gini index = 88 and 72 respectively), followed by Maximum syllable length and Average syllable length (mean decrease in Gini index = 62 and 55 respectively; Table 3.5). The majority of misclassifications occurred within the 'singing' category which had a low number of examples, and a high misclassification rate and this increased the measure of error. Fast clucks, food calls and mixed calls also had a moderate misclassification rate when compared with our manual classification. It was expected that mixed calls would be difficult to quantitatively classify, and this was reflected in the misclassification rate. Mixed calls contained a number of calls which represented different call types blending into others; for example food calls may blend into a short whine. Food calls and fast clucks have very similar structures. However the CART analysis correctly classified 80% of food calls. Only calls that demonstrated the regular temporal and acoustic structure described previously, and that were visibly similar to a spectrogram published by Evans & Evans (2007), were selected as food calls, and subjected to further analysis.

3.4.2. Prevalence of call types

The call repertoire of hens varied significantly depending on the type of reward. The prevalence of food calls and fast clucks was significantly greater in anticipation of all rewards compared to both neutral treatments (Figs. 3.3 and 3.4) suggesting that these call types are produced more when hens anticipate a positive event.

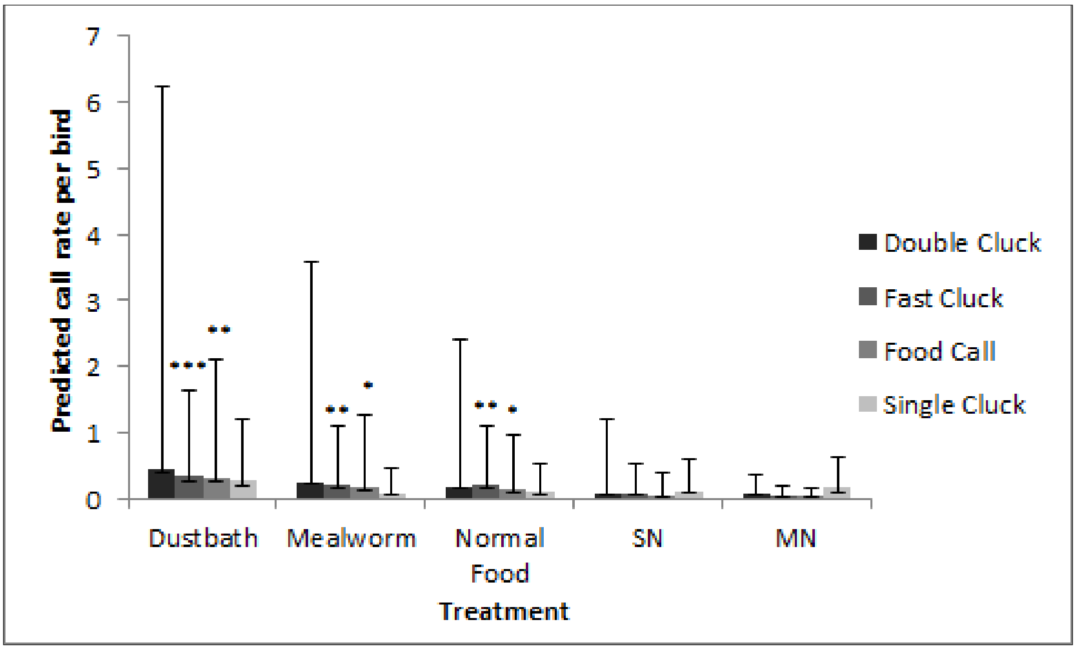

Figure 3.3 Model predictions of call rate per hen for Double clucks, Fast clucks, Food calls and Single clucks in anticipation of five different treatments. Call rates that were significantly higher than the intercept (MN) are represented as $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

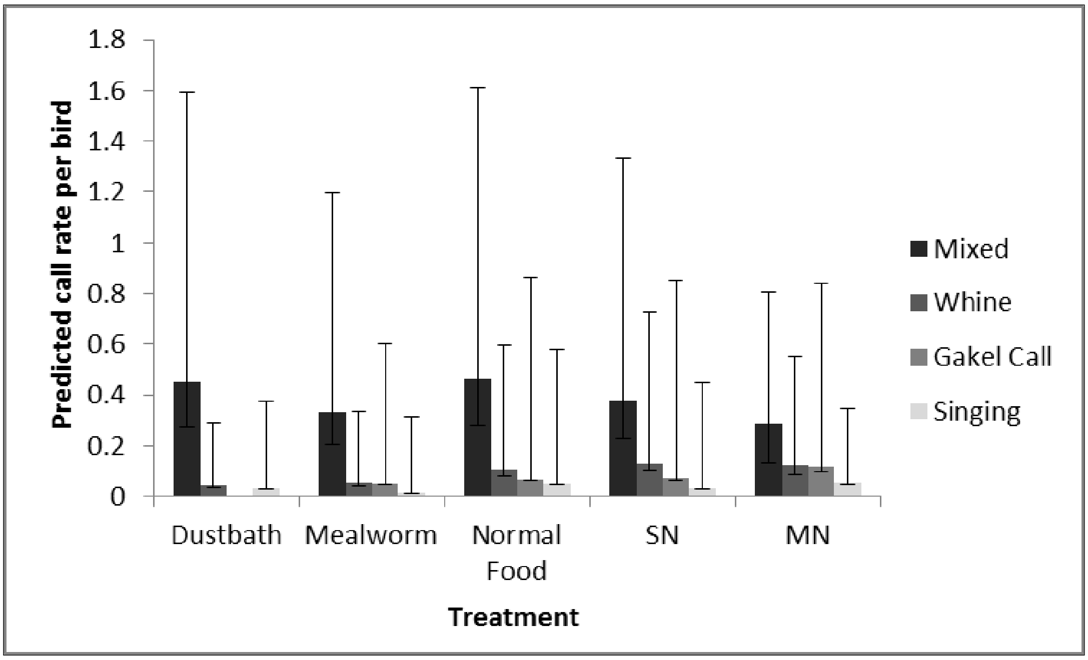

Figure 3.4 Model predictions of call rate per hen for Mixed calls, Whines, Gakel calls and Singing calls in anticipation of five different treatments. Call rates that were significantly higher than the intercept (MN) are represented as *P*<0.05 (*), *P* <0.01 (**) and *P* <0.001 (***).

3.4.3. Differences in call parameters

As food calls were prevalent in reward contexts but scarce in neutral treatments (Fig. 3.3), call parameters were only compared in anticipation of rewards. Only the peak frequency of food calls varied in anticipation of rewards, where they were emitted at significantly lower peak frequencies in anticipation of the dustbathing substrate compared with the mealworm reward (dustbathing substrate 411.85 Hz.^{a}, normal food 467.55 Hz.^{ab}, mealworm 487.16^{b}, $\chi^2(2)=8.78$, *P*=0.01) (Fig. 3.5).

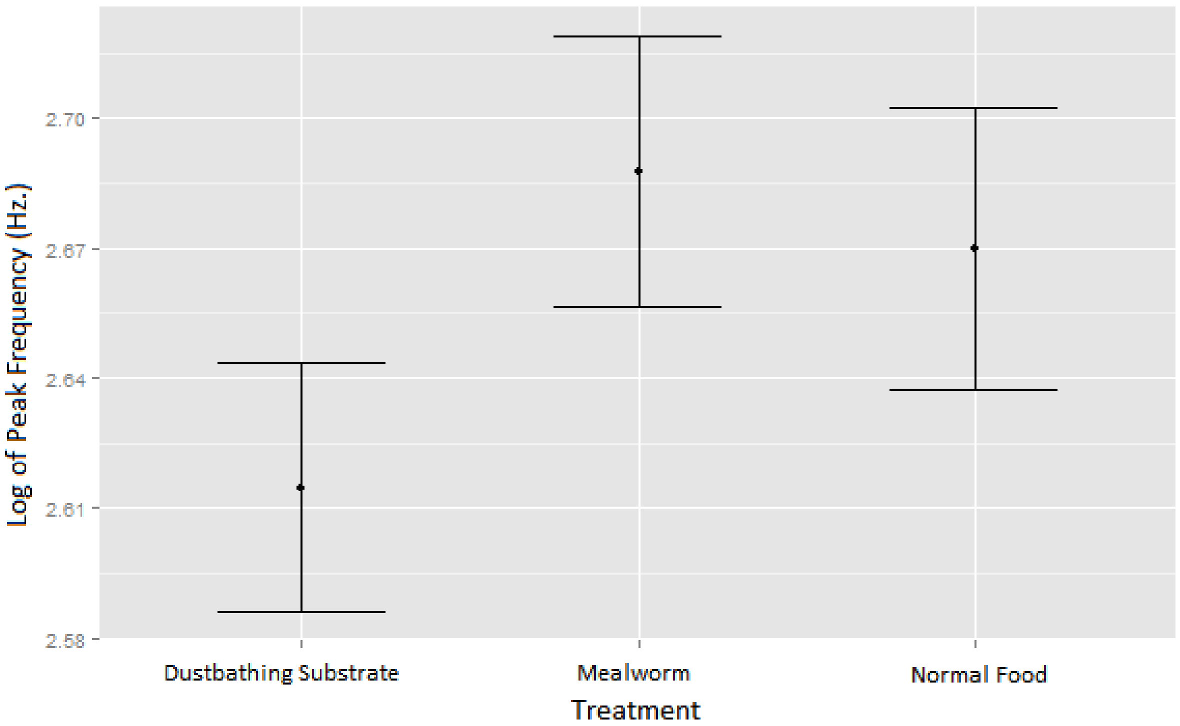

Figure 3.5 Variation in the peak frequency of 'Food calls' by reward. Central dot indicates mean values, and horizontal lines show standard errors.

The 90% Bandwidth of fast clucks was significantly lower during all three reward treatments compared with the muted neutral treatment (SN 86.71 Hz.^{a}, normal food 89.96 Hz.^{a}, dustbathing substrate 90.04 Hz.^{a}, mealworm 98.86^{ab}, MN 126.81 Hz.^{b}, $\chi^2(4)=13.25$, $P=0.01$). Interestingly, fast clucks were also emitted at lower peak frequencies in anticipation of the dustbathing substrate and mealworm rewards, which were presumably high value rewards, compared with the muted neutral treatment, although the results were not significant at $P<=0.01$ (dustbathing substrate 402.66 Hz.^{a}, mealworm 403.12^{a}, normal food 409.19 Hz.^{ab}, SN 456.59 Hz.^{ab}, MN 548.59 Hz.^{b}, $\chi^2(4)=11.30$, $P=0.02$). The above variations suggest that these call types encode information about motivational state according to different types of reward. However, the frequency of double clucks and single clucks did not vary between treatments ($P>0.01$), which indicates that shorter calls do not encode information about hens' internal states (Table 3.7). The acoustic parameters of whines and gakel calls did not vary between treatments ($P>0.01$) (Table 3.6).

Table 3.6 Vocal parameters' lsmeans, 95% confidence limits and significance levels

Treatment		**Dusty Substrate**			**Mealworm**			**Normal**			**SN**			**MN**					
Vocalisation type	**Parameter**	**Lsmean**	**Lower CL**	**Upper CL**	**Lsmean**	**Lower CL**	**Upper CL**	**Lsmean**	**Lower CL**	**Upper CL**	**Lsmean**	**Lower CL**	**Upper CL**	**Lsmean**	**Lower CL**	**Upper CL**	**X^2**	**X^2 *df***	***P***
Food Calls	Peak Frequency	411.85^{a}	(383.86	441.88)	487.16^{b}	(451.36	525.80)	467.55^{ab}	(431.98	506.04)							8.78	2	**0.01**
	Bandwidth 90%	95.24	(90.44	100.29)	96.44	(90.14	103.18)	94.70	(88.15	101.73)							0.04	2	0.98
	Syllable Length	0.10	(0.09	0.11)	0.10	(0.09	0.11)	0.10	(0.09	0.11)							0.22	2	0.89
	Call Duration	0.86	(0.77	0.96)	1.05	(0.92	1.21)	1.02	(0.88	1.18)							2.04	2	0.36
Fast Cluck	Peak Frequency	402.66^{a}	(372.47	435.29)	403.12^{a}	(369.26	440.08)	409.19^{ab}	(374.72	446.84)	456.59^{ab}	(412.44	505.46)	548.59^{b}	(490.62	613.40)	11.30	4	**0.02**
	Bandwidth 90%	90.04^{a}	(85.06	95.32)	98.86^{ab}	(92.08	106.14)	89.96^{a}	(83.74	96.63)	86.71^{a}	(79.33	94.78)	126.81^{b}	(114.72	140.18)	13.25	4	**0.01**
	Syllable Length	0.09	(0.08	0.10)	0.09	(0.09	0.10)	0.10	(0.09	0.11)	0.08	(0.07	0.09)	0.09	(0.08	0.10)	5.34	4	0.25
	Call Duration	0.76	(0.69	0.84)	0.66	(0.59	0.75)	0.73	(0.65	0.83)	0.80	(0.69	0.92)	0.87	(0.74	1.02)	3.51	4	0.48
Whine	Peak Frequency	574.61	(530.93 ,	621.87)	628.35	(578.13 ,	682.94)	630.57	(585.21 ,	679.44)	644.15	(600.14 ,	691.38)	642.80	(597.85 ,	691.12)	4.01	4	0.40
	Bandwidth 90%	128.11	(112.75 ,	145.55)	112.89	(97.99 ,	130.04)	118.50	(105.47 ,	133.14)	119.12	(107.88 ,	131.53)	129.27	(116.26 ,	143.74)	1.25	4	0.87
	Syllable Length	0.42	(0.34 ,	0.51)	0.36	(0.29 ,	0.46)	0.47	(0.39 ,	0.57)	0.47	(0.39 ,	0.56)	0.56	(0.46 ,	0.68)	5.70	4	0.22
	Call Duration	1.38	(1.19 ,	1.60)	1.52	(1.31 ,	1.77)	1.47	(1.29 ,	1.68)	1.58	(1.38 ,	1.80)	1.49	(1.30 ,	1.70)	1.64	4	0.80
Harsh	Peak Frequency		(,	)	481.99	(450.38 ,	515.82)	502.85	(470.60 ,	537.31)	474.48	(444.98 ,	505.95)	484.51	(455.36 ,	515.52)	1.41	3	0.70
	Bandwidth 90%		(,	)	96.74	(82.85 ,	112.95)	113.47	(97.66 ,	131.84)	106.75	(92.64 ,	123.00)	113.82	(99.45 ,	130.27)	1.62	3	0.65
	Syllable Length		(,	)	0.61	(0.49 ,	0.76)	0.65	(0.53 ,	0.81)	0.70	(0.57 ,	0.86)	0.76	(0.63 ,	0.93)	1.61	3	0.66
	Call Duration		(,	)	2.42	(2.03 ,	2.88)	2.52	(2.13 ,	2.99)	3.12	(2.67 ,	3.64)	2.62	(2.26 ,	3.03)	2.36	3	0.50
Double Clucks	Peak Frequency	351.20	(320.54 ,	384.80)	369.98	(333.79 ,	410.10)	456.35	(406.47 ,	512.35)	460.03	(402.85 ,	525.33)	406.20	(350.10 ,	471.29)	10.95	4	**0.03**
	Bandwidth 90%	93.46	(87.60 ,	99.72)	88.15	(81.43 ,	95.42)	95.82	(86.69 ,	105.90)	96.32	(85.09 ,	109.03)	95.29	(82.55 ,	109.98)	0.97	4	0.91
	Syllable Length	0.09	(0.09 ,	0.10)	0.08	(0.07 ,	0.08)	0.09	(0.08 ,	0.11)	0.09	(0.08 ,	0.11)	0.11	(0.09 ,	0.13)	7.04	4	0.13
	Call Duration	0.33	(0.31 ,	0.36)	0.27	(0.25 ,	0.30)	0.32	(0.28 ,	0.36)	0.29	(0.25 ,	0.33)	0.32	(0.27 ,	0.38)	3.62	4	0.46
Single Clucks	Peak Frequency	331.84	(303.89 ,	362.36)	310.08	(277.22 ,	346.83)	350.65	(315.74 ,	389.43)	289.94	(262.10 ,	320.74)	343.98	(311.70 ,	379.60)	4.86	4	0.30
	Bandwidth 90%	89.12	(84.13 ,	94.40)	83.44	(76.11 ,	91.47)	99.37	(91.49 ,	107.92)	89.04	(82.34 ,	96.28)	111.01	(102.84 ,	119.84)	8.78	4	0.07
	Syllable Length	0.09	(0.08 ,	0.10)	0.09	(0.08 ,	0.10)	0.09	(0.08 ,	0.10)	0.07	(0.06 ,	0.08)	0.08	(0.07 ,	0.09)	7.92	4	0.09
	Call Duration	0.09	(0.08 ,	0.10)	0.09	(0.08 ,	0.10)	0.09	(0.08 ,	0.10)	0.07	(0.06 ,	0.08)	0.08	(0.07 ,	0.09)	7.91	4	0.09

Table 3.7 Comparison of the linear mixed-effects models (LMMs) for behavioural responses

Parameter	Call Type	Fixed variables in model	Comparison	*df*	AIC	X^2	X^2 *df*	*P*
Peak Frequency	Fast Cluck	**Treatment**		7	-110			
		Treatment + CG	1 vs 2	10	-109	5.62	3	NS
		Treatment + CG+ Day	2 vs 3	14	-106	4.30	4	NS
Bandwidth 90%	Fast Cluck	**Treatment**		7	-113			
		Treatment + CG	1 vs 2	10	-112	4.60	3	NS
		Treatment + CG+ Day	2 vs 3	14	-106	1.56	4	NS
Average syllable length	Fast Cluck	Treatment		7	-110			
		Treatment + CG	1 vs 2	10	-110	6.46	3	NS
		Treatment + CG+ Day	2 vs 3	14	-116	14.28	4	0.01
Duration	Fast Cluck	**Treatment**		7	-47			
		Treatment + CG	1 vs 2	10	-45	4.12	3	NS
		Treatment + CG+ Day	2 vs 3	14	-42	4.97	4	NS
Peak Frequency	Food Call	**Treatment**		7	-99			
		Treatment + CG	1 vs 2	10	-98	5.71	3	NS
		Treatment + CG+ Day	2 vs 3	14	-93	2.73	4	NS
Bandwidth 90%	Food Call	**Treatment**		7	-74			
		Treatment + CG	1 vs 2	10	-69	1.84	3	NS
		Treatment + CG+ Day	2 vs 3	14	-67	5.87	4	NS
Average syllable length	Food Call	**Treatment**		7	-57			
		Treatment + CG	1 vs 2	10	-53	1.70	3	NS
		Treatment + CG+ Day	2 vs 3	14	-45	0.56	4	NS
Duration	Food Call	**Treatment**		7	-20			
		Treatment + CG	1 vs 2	10	-19	4.47	3	NS
		Treatment + CG+ Day	2 vs 3	14	-11	0.17	4	NS
Peak Frequency	Gakel call	**Treatment**		7	-134			
		Treatment + CG	1 vs 2	10	-129	0.63	3	NS
		Treatment + CG+ Day	2 vs 3	14	-123	2.33	4	NS
Bandwidth 90%	Gakel call	**Treatment**		7	-30			
		Treatment + CG	1 vs 2	10	-27	3.79	3	NS
		Treatment + CG+ Day	2 vs 3	14	-22	2.78	4	NS
Average syllable length	Gakel call	Treatment		7	4			
		Treatment + CG	1 vs 2	10	2	7.98	3	0.05
		Treatment + CG+ Day	2 vs 3	14	5	4.38	4	NS
Duration	Gakel call	**Treatment**		7	-5			
		Treatment + CG	1 vs 2	10	0	1.87	3	NS
		Treatment + CG+ Day	2 vs 3	14	3	4.44	4	NS
Peak Frequency	Whines	**Treatment**		7	-162			
		Treatment + CG	1 vs 2	10	-157	0.65	3	NS
		Treatment + CG+ Day	2 vs 3	14	-157	7.92	4	NS
Bandwidth 90%	Whines	**Treatment**		7	-47			
		Treatment + CG	1 vs 2	10	-42	1.38	3	NS
		Treatment + CG+ Day	2 vs 3	14	-38	3.69	4	NS
Average syllable length	Whines	**Treatment**		7	-1			
		Treatment + CG	1 vs 2	10	1	4.24	3	NS
		Treatment + CG+ Day	2 vs 3	14	1	8.05	4	NS
Duration	Whines	Treatment		7	-81			
		Treatment + CG	1 vs 2	10	-80		3	NS
		Treatment + CG+ Day	2 vs 3	14	-86	13.41	4	0.01
Peak Frequency	Single Cluck	**Treatment**		7	-102			
		Treatment + CG	1 vs 2	10	-96	0.91	3	NS
		Treatment + CG+ Day	2 vs 3	14	-95	6.69	4	NS
Bandwidth 90%	Single Cluck	**Treatment**		7	-104			
		Treatment + CG	1 vs 2	10	-102	4.54	3	NS
		Treatment + CG+ Day	2 vs 3	14	-98	3.27	4	NS
Average syllable length	Single Cluck	Treatment		7	-62			
		Treatment + CG	1 vs 2	10	-58	2.19	3	NS
		Treatment + CG+ Day	2 vs 3	14	-60	9.90	4	0.04
Duration	Single Cluck	Treatment		7	-62			
		Treatment + CG	1 vs 2	10	-58	2.19	3	NS
		Treatment + CG+ Day	2 vs 3	14	-60	9.90	4	0.04
Peak Frequency	Double Cluck	**Treatment**		7	-82			
		Treatment + CG	1 vs 2	10	-83	6.34	3	NS
		Treatment + CG+ Day	2 vs 3	14	-81	6.14	4	NS
Bandwidth 90%	Double Cluck	**Treatment**		7	-80			
		Treatment + CG	1 vs 2	10	-74	0.31	3	NS
		Treatment + CG+ Day	2 vs 3	14	-72	5.69	4	NS
Average syllable length	Double Cluck	Treatment		7	-55			
		Treatment + CG	1 vs 2	10	-58	9.15	3	0.03
		Treatment + CG+ Day	2 vs 3	14	-59	8.82	4	NS
Duration	Double Cluck	**Treatment**		7	-61			
		Treatment + CG	1 vs 2	10	-56	1.22	3	NS
		Treatment + CG+ Day	2 vs 3	14	-54	5.69	4	NS

Data includes model degrees of freedom (*df*), Akaike information criterion (AIC), X^2, X^2 degrees of freedom (X^2 *df*) and *P* values. NS indicates that the model is not significantly improved from the previous model. CG = cue group. Lines in bold indicate the model with the lowest AIC score.

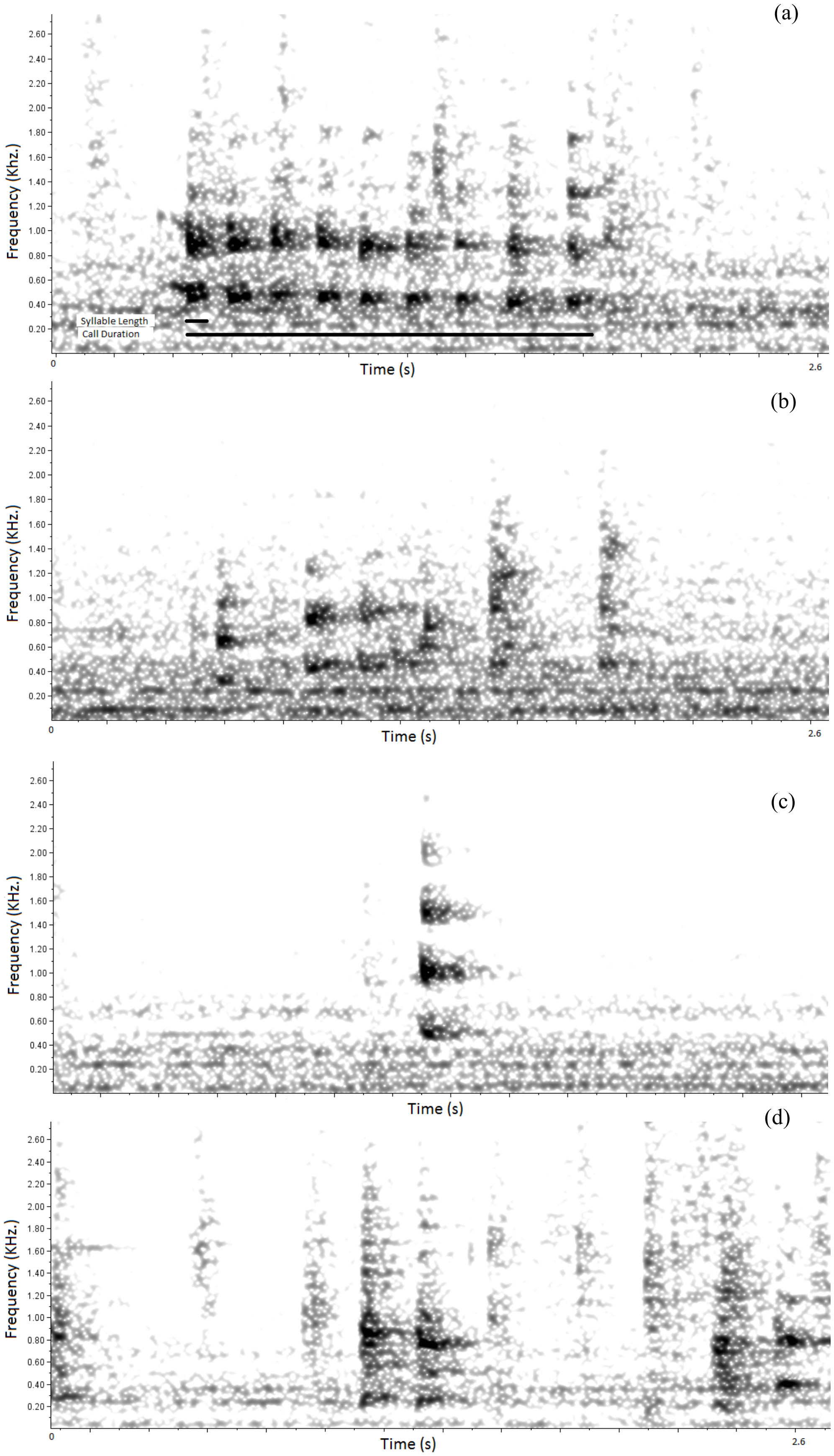

Figure 3.6. Spectrograms of (a) 'Food call', (b) 'Fast cluck', (c) 'Single cluck' and (d) 'Double cluck'. A key within the 'Food call' spectrogram indicates the syllable length and call duration.

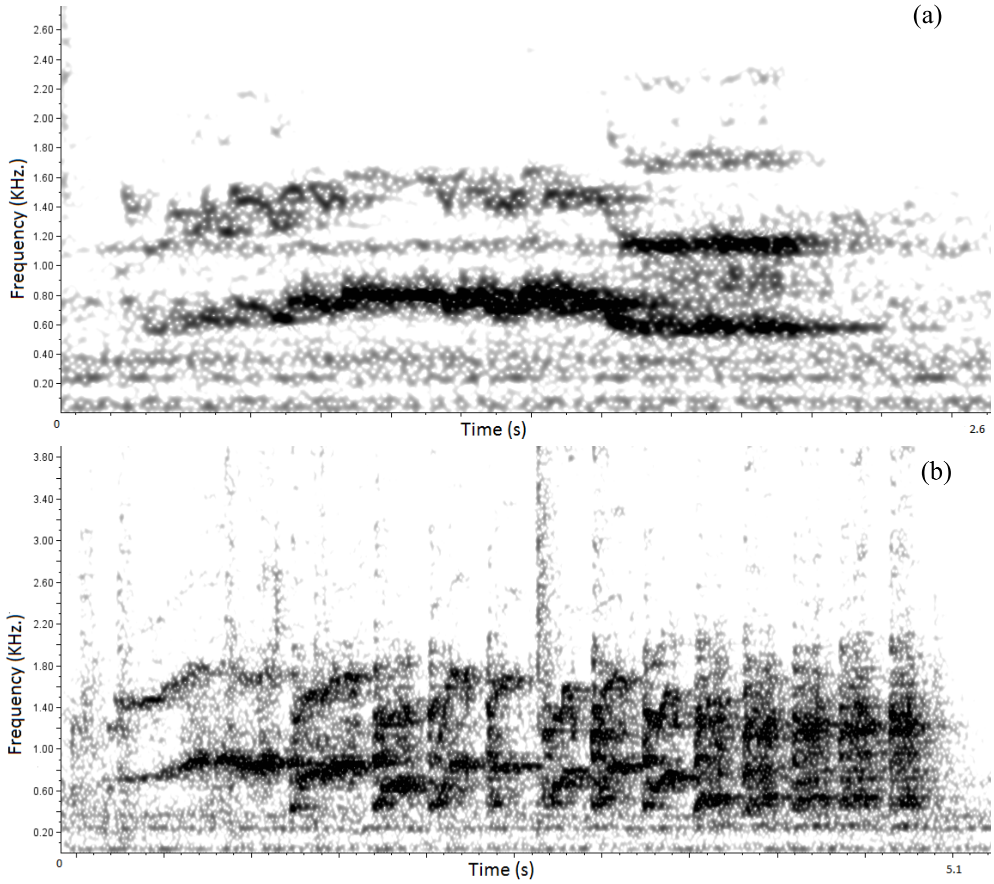

Figure 3.7. Example spectrograms of (a) 'Whine' and (b) 'Gakel call'.

3.5. Discussion

The results of this study show that hens produce 'Food calls', 'Fast clucks', 'Double clucks' and 'Single clucks' when anticipating rewards. Importantly, hens emitted food calls when anticipating a dustbathing substrate, which contradicts the suggestion that food calls made by chickens are functionally referential (Evans & Evans 1999; Clay et al. 2012). The peak frequency of food calls made in anticipation of the dustbathing substrate was 45-75 Hz lower than those made in anticipation of food rewards, potentially reflecting differences in arousal within the same behavioural state.

This study demonstrated that all four call types produced by hens in anticipation of rewards (food calls, fast clucks, double clucks and single clucks) are structurally similar calls, with a fundamental frequency of around 400 Hz and a distinct first harmonic. Calls were mainly differentiated by the number of syllables, with food calls being a series of 3-15 syllables, emitted at a slightly higher peak frequency than fast clucks. During the neutral treatments (i.e. no rewards), hens produced quite different signals. These were long, high frequency, tonal 'whines' and long, distinctive, harsh-sounding 'gakel' calls. Whines produced during this experiment were very similar to the ‘whines of frustration’ given by red jungle-fowl *Gallus gallus* in contexts when food is shown or offered, but subsequently not released to the birds (Collias 1987). Similarly, gakel calls are given by hens in frustrative non-reward contexts (Zimmerman & Koene 1998; Zimmerman et al. 2000; Garland et al. 2015). Hens also produced ‘mixed’ calls, which did not have a regular or specific structure. The anticipatory period in this experiment may have induced conflicting arousal states; a drive to acquire the reward may have existed simultaneously with frustration induced by not having instant access to the reward. The grading of calls, where one signal merges into another, occurs in red jungle-fowl and domestic chickens, particularly under intermediate stimulus conditions (Collias 1987) or in situations of varying intensity (Konishi 1963). Therefore, it appears that hens’ call types indicate the motivational state of the signaller; short, sharp ‘clucks’ and food calls tend to be produced in anticipation of rewards (indicating possible excitement) and longer, higher frequency whines and gakel calls are given in frustrative non-reward contexts. The vocalisations of other animals have been linked to levels of arousal, and in some cases, valence (Briefer 2012). Therefore, these findings present an opportunity to explore how hens’ vocal communication could be used as markers of their welfare in on-farm assessments.

An important finding of this study was that food calls were not solely produced in the context of food, but also given in anticipation of both food and non-food rewards. This result contradicts claims that they are functionally referential (Evans & Evans 1999; Clay et al. 2012). Other studies have indicated that food calling can occur in the absence of food, and either may be associated with stimuli that, in the past, reliably predicted the presence of food, (Moffatt & Hogan 1992; Wauters & Richard-Yris 2002) or may be separation calls (Konishi 1963; Hughes et al. 1982). Certainly, there is evidence to suggest that the majority of food calls made by other species are not food-specific, but rather food-associated, as they don’t fulfil the strict criteria for functional reference with regards to either production or perception (for a review, see Clay et al., 2012). In these cases, food calls may function more as social recruitment calls, to enhance defence, reproductive or social status, or alternatively as a method to reduce competition by announcing resource ownership (Clay et al. 2012). Male chicken food calls appear to be used to attract females by inducing orientation towards the sound source (Evans & Evans 1999). However, food calls made by females may function to recruit others, either to share a resource, or to ensure extra security (vigilance) when engaged in foraging, feeding or dustbathing (Townsend et al. 2011; Clay et al. 2012). Hence, information appears to be contained within the overall structure of hens’ calls, (with hens emitting short sharp cluck sounds when the signaller has located a resource). However, to date, there has been no evidence in the literature which suggests that these calls differentiate between various resources.

Many species of animal vary the structure of specific call types depending on their motivational state. For example, the call structure of specific food calls in great ape species can vary according to the perceived quality of the food item (e.g. bonobos *Pan paniscus* (Clay & Zuberbühler 2009) and chimpanzees (Slocombe & Zuberbühler 2006; Kalan et al. 2015)). In this study, it was found that hens emitted food calls more often, and at lower peak frequencies, when anticipating the dustbathing substrate compared to the food rewards. Fast clucks were also more prevalent in anticipation of the dustbathing substrate, and emitted at lower frequencies in anticipation of both the dustbath and mealworm rewards compared to the neutral events. This suggests that, in hens, motivational information is contained within the acoustic structure of different call types, as well as within the call type itself. If we group together food calls and fast clucks as call types made in anticipation of rewards, this decrease in frequency may indicate further information relating to motivation, such as the signaller's level of arousal. Behavioural data (McGrath et al. 2016) suggests a hierarchy of arousal state, with increased goal-directed behaviour (pushing and pecking against the door), higher activity levels, and more approach behaviours demonstrated in anticipation of a dustbathing substrate compared with the other rewards, and in anticipation of all rewards compared with neutral treatments. Notably, in animals such as baboons *Papio hamadrayas ursinus* (Rendall 2003), pigs (Puppe et al. 2005), and domestic cats *Felis catus* (Yeon et al. 2011), the acoustic frequency of calls increases in parallel with heightened arousal, and this pattern is in accordance with Morton's motivation-structural rules (Morton 1977). However, when approached by humans, tame silver foxes *Vulpes vulpes* produce calls at lower peak frequencies (based on the fundamental frequency) compared to aggressive (untamed) silver foxes (Gogoleva et al. 2010), suggesting that a lower peak frequency reflects a positively-valenced high arousal state rather than a low-arousal feeling of contentment. Therefore, if we assume that lower acoustic frequency calls reflect a reward-related high arousal state in hens, then call frequency, as well as the call types identified in this study, could be used as a marker of welfare.

One potential issue with this study was that the dustbathing substrate could have been viewed by hens as an opportunity to forage. Hens were deprived of food for 1.5 hours to ensure that they were motivated to eat once they had access to food. As hens dustbathe every two days on average (Vestergaard 1982), the hens in this study were deprived of a dustbathing substrate for a longer (24 hour) period. Chickens tend not to dustbathe during the morning hours, and the fact that some hens dustbathed immediately after accessing the reward chamber suggests that they were highly motivated to do so (testing had ended by 12.30 pm on each day). Based on the behavioural data, it seems that they were motivated to use the dustbathing substrate for exactly this purpose rather than for foraging, although the two potential opportunities are not mutually exclusive. A dustbathing substrate can be a multi-faceted resource for hens, including as a potential nest site in a caged environment (Smith et al. 1993), and scratching, foraging and pecking in litter are not only precursors to dustbathing, but are also behaviours that hens are motivated to perform in their own right (Olsson & Keeling 2005). None of the hens performed nesting behaviour during the trials in this experiment, and, based on published behavioural results (McGrath et al., 2016), anticipation of a dustbathing substrate induced high arousal in hens, indicating they were motivated to dustbathe.

Another limitation of this study is that acoustic variation cannot be attributed to any particular valence or emotional state. Indeed, Briefer (2012) emphasizes that vocal correlates of positive valence are lacking in the literature, often due to a lack of opportunity to record vocalisations in positive situations. Detecting valence is possible if studies use situations of contrasting valence but similar arousal, and studies on dogs (Taylor et al. 2009) squirrel monkeys *Saimiri sciurius* (Fichtel et al. 2001) and goats *Capra hircus* (Briefer et al. 2015) have successfully separated the effects of arousal and valence on vocal parameters. Measuring acoustic parameters such as formants, frequency range and frequency modulation (Briefer 2012; Briefer et al. 2015) in conjunction with behavioural and physiological measurements may provide the most convincing evidence for valence. For example, different ultrasonic call types made by rats have been linked to activation of positive or negative internal states in both signallers and receivers (Brudzynski 2007; Burman et al. 2007). As this study used items that are known to be rewarding to chickens, it can be hypothesized that shifts to a lower peak frequency within food calls and fast clucks in anticipation of rewards reflects an increase in positively-valenced arousal. The function of this affective response, as suggested by Mendl et al. (2010), could be to guide animals towards acquisition of fitness-enhancing rewards. Hence, future investigations could focus on how conspecifics perceive these vocalisations. Furthermore, there is scope for studies to simultaneously investigate vocal, behavioural and physiological indicators of arousal and valence in chickens.

This is the first study to find that hen vocalisations may contain motivational information in call types and call structure. Moreover, the food call was not given exclusively in response to cues signalling food rewards, suggesting this call was not functionally referential but more likely to be a ‘reward’ call. Further work, using other types of reward, will help determine if this is the case. In addition, confirmation of whether males and females produce acoustically different reward calls would help to assess the function of these calls. Linking physiological and behavioural responses of hens with changes in vocal parameters when anticipating rewards will further identify if levels of signaller arousal and valence are encoded within the parameters of their calls. Overall, the results of this study suggest that the vocal behaviour of hens can provide an indicator of whether they are in a rewarding environment. These findings can be developed into a tool which could provide a relatively easy method with which to assess the welfare of hens. In a longer term study, this tool could monitor welfare outcomes as result of environmental improvements that may also enhance health and productivity.

3.6 Supplementary material

Table 3.8 Food call: Final model output from LMM.

Model	Estimate	Std. Error	t value	Pr(>|t|)
Peak Frequency~Treatment+1|CG:Bird				
df(5),AIC -78.6				
(Intercept) Dusty Substrate	2.615	0.0288		
Mealworm	0.073	0.0231	3.16	0.00
Normal Food	0.055	0.0256	2.16	0.04
Bandwidth 90%~Treatment+1|CG:Bird				
df(5),AIC -66.5				
(Intercept) Dusty Substrate	1.979	0.0203		
Mealworm	0.005	0.0340	0.16	0.87
Normal Food	-0.002	0.0351	-0.07	0.94
Syllable Length~Treatment+1|CG:Bird				
df(5),AIC -53.9				
(Intercept) Dusty Substrate	-1.008	0.0299		
Mealworm	0.010	0.0367	0.28	0.79
Normal Food	-0.011	0.0397	-0.27	0.79
Call Duration~Treatment+1|CG:Bird				
df(5),AIC -17.5				
(Intercept) Dusty Substrate	-0.066	0.0433		
Mealworm	0.089	0.0633	1.41	0.17
Normal Food	0.075	0.0670	1.12	0.27

Table include model degrees of freedom and AICs. ‘Estimate’ refers to the estimates of the mean of the dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the estimates in each model being the difference from the intercept. Please note that the estimates refer to the values given using a log transformed dependent variable.

Table 3.9 Fast cluck: Final model output from LMM.

Model	Estimate	Std. Error	t value	Pr(>|t|)
Peak Frequency~Treatment+1|CG:Bird				
df(7),AIC -109.7				
(Intercept) MN	2.739	0.0464		
Dusty Substrate	-0.134	0.0425	-3.16	0.00
Mealworm	-0.134	0.0435	-3.08	0.00
SN	-0.080	0.0490	-1.63	0.11
Normal Food	-0.127	0.0460	-2.77	0.01
Bandwidth 90%~Treatment+1|CG:Bird				
df(7),AIC -113.4				
(Intercept) MN	2.103	0.0413		
Dusty Substrate	-0.149	0.0432	-3.44	0.00
Mealworm	-0.108	0.0452	-2.39	0.02
SN	-0.165	0.0505	-3.27	0.00
Normal Food	-0.149	0.0471	-3.17	0.00
Syllable Length~Treatment+CG+Day+1|CG:Bird				
df(14),AIC -116.2				
(Intercept) MN	-1.050	0.0506		
Dusty Substrate	-0.008	0.0395	-0.20	0.84
Mealworm	0.003	0.0410	0.06	0.95
SN	-0.047	0.0461	-1.02	0.31
Normal Food	0.045	0.0433	1.03	0.31
CG2	0.020	0.0460	0.44	0.67
CG3	0.036	0.0513	0.69	0.50
CG4	0.123	0.0437	2.82	0.02
Day2	-0.032	0.0323	-1.00	0.32
Day3	-0.082	0.0314	-2.61	0.01
Day4	-0.092	0.0374	-2.45	0.02
Day5	0.033	0.0351	0.93	0.36
Call Duration~Treatment+1|CG:Bird				
df(7),AIC -47.1				
(Intercept) MN	-0.059	0.0660		
Dusty Substrate	-0.062	0.0664	-0.93	0.35
Mealworm	-0.118	0.0688	-1.72	0.09
SN	-0.040	0.0771	-0.51	0.61
Normal Food	-0.075	0.0722	-1.04	0.30

Table include model degrees of freedom and AICs. ‘Estimate’ refers to the estimates of the mean of the dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the estimates in each model being the difference from the intercept. Please note that the estimates refer to the values given using a log transformed dependent variable.

Table 3.10 Whine: Final model output from LMM.

Model	Estimate	Std. Error	t value	Pr(>|t|)
Peak Frequency~Treatment+1|CG:Bird				
df(7),AIC -161.93				
(Intercept) MN	2.808	0.0295		
Dusty Substrate	-0.049	0.0265	-1.84	0.07
Mealworm	-0.010	0.0284	-0.35	0.73
SN	0.001	0.0221	0.04	0.97
Normal Food	-0.008	0.0245	-0.34	0.73
Bandwidth 90%~Treatment+1|CG:Bird				
df(7),AIC -47.0				
(Intercept) MN	2.112	0.0424		
Dusty Substrate	-0.004	0.0591	-0.07	0.95
Mealworm	-0.059	0.0646	-0.91	0.37
SN	-0.036	0.0500	-0.71	0.48
Normal Food	-0.038	0.0555	-0.68	0.50
Syllable Length~Treatment+CG+1|CG:Bird				
df(7),AIC -1.2				
(Intercept) MN	-0.252	0.0765		
Dusty Substrate	-0.129	0.0773	-1.67	0.10
Mealworm	-0.185	0.0831	-2.23	0.03
SN	-0.077	0.0646	-1.19	0.24
Normal Food	-0.074	0.0716	-1.03	0.31
Call Duration~Treatment+CG+Day+1|CG:Bird				
df(14),AIC -85.7				
(Intercept) MN	0.270	0.0781		
Dusty Substrate	-0.032	0.0413	-0.78	0.44
Mealworm	0.010	0.0449	0.22	0.83
SN	0.026	0.0363	0.72	0.47
Normal Food	-0.004	0.0392	-0.10	0.92
CG2	-0.156	0.1542	-1.01	0.32
CG3	-0.203	0.0838	-2.43	0.04
CG4	-0.209	0.0881	-2.37	0.04
Day2	0.086	0.0437	1.96	0.05
Day3	0.065	0.0492	1.33	0.19
Day4	-0.021	0.0372	-0.57	0.57
Day5	0.089	0.0412	2.15	0.03

Table include model degrees of freedom and AICs. ‘Estimate’ refers to the estimates of the mean of the dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the estimates in each model being the difference from the intercept. Please note that the estimates refer to the values given using a log transformed dependent variable.

Table 3.11 Gakel call: Final model output from LMM.

Model	Estimate	Std. Error	t value	Pr(>|t|)
Peak Frequency~Treatment+1|CG:Bird				
df(7),AIC -133.9				
(Intercept) MN	2.685	0.0253		
Mealworm	-0.002	0.0215	-0.11	0.92
SN	-0.009	0.0190	-0.48	0.63
Normal Food	0.016	0.0213	0.76	0.45
Bandwidth 90% ~Treatment+1|CG:Bird				
df(7),AIC -29.6				
(Intercept) MN	2.056	0.0547		
Mealworm	-0.071	0.0604	-1.17	0.25
SN	-0.028	0.0534	-0.52	0.60
Normal Food	-0.001	0.0594	-0.02	0.98
Syllable Length~Treatment+1|CG:Bird				
df(10),AIC 1.8				
(Intercept) MN	-0.279	0.0921		
Mealworm	-0.096	0.0793	-1.21	0.23
SN	-0.037	0.0701	-0.52	0.60
Normal Food	-0.066	0.0774	-0.85	0.40
CG2	0.044	0.1511	0.29	0.78
CG3	0.513	0.1460	3.51	0.00
CG4	0.090	0.1166	0.77	0.46
Call Duration~Treatment+1|CG:Bird				
df(7),AIC -4.6				
(Intercept) MN	0.418	0.0590		
Mealworm	-0.034	0.0799	-0.43	0.67
SN	0.076	0.0707	1.08	0.29
Normal Food	-0.016	0.0776	-0.21	0.84

Table include model degrees of freedom and AICs. 'Estimate' refers to the estimates of the mean of the dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the estimates in each model being the difference from the intercept. Please note that the estimates refer to the values given using a log transformed dependent variable.

Table 3.12 Single cluck: Final model output from LMM.

Model	Estimate	Std. Error	t value	Pr(>|t|)
Peak Frequency~Treatment+1|CG:Bird				
df(7),AIC -101.5				
(Intercept) MN	2.537	0.0406		
Dusty Substrate	-0.016	0.0358	-0.44	0.66
Mealworm	-0.045	0.0475	-0.95	0.35
SN	-0.074	0.0417	-1.78	0.08
Normal Food	0.008	0.0450	0.185	0.85
Bandwidth 90% ~Treatment+1|CG:Bird				
df(7),AIC -103.8				
(Intercept) MN	2.045	0.0309		
Dusty Substrate	-0.095	0.0374	-2.55	0.01
Mealworm	-0.124	0.0482	-2.57	0.01
SN	-0.096	0.0431	-2.22	0.03
Normal Food	-0.048	0.0448	-1.074	0.29
Syllable Length~Treatment+CG+Day+1|CG:Bird				
df(14),AIC -59.7				
(Intercept) MN	-1.167	0.0623		
Dusty Substrate	0.053	0.0468	1.13	0.26
Mealworm	0.046	0.0597	0.77	0.44
SN	-0.073	0.0531	-1.38	0.17
Normal Food	0.045	0.0556	0.806	0.42
CG2	0.011	0.0541	0.194	0.85
CG3	-0.009	0.0683	-0.14	0.89
CG4	0.043	0.0666	0.65	0.53
Day2	-0.003	0.0530	-0.06	0.95
Day3	0.175	0.0690	2.531	0.01
Day4	0.025	0.0452	0.543	0.59
Day5	0.104	0.0506	2.06	0.04
Call Duration~Treatment+1|CG:Bird				
df(10),AIC				
(Intercept) MN	-1.167	0.0623		
Dusty Substrate	0.053	0.0468	1.12	0.26
Mealworm	0.046	0.0597	0.77	0.44
SN	-0.073	0.0531	-1.38	0.17
Normal Food	0.045	0.0556	0.805	0.42
CG2	0.011	0.0541	0.195	0.85
CG3	-0.009	0.0683	-0.14	0.89
CG4	0.043	0.0666	0.65	0.52
Day2	-0.003	0.0531	-0.06	0.95
Day3	0.175	0.0691	2.53	0.01
Day4	0.025	0.0453	0.543	0.59
Day5	0.104	0.0506	2.05	0.04

Table include model degrees of freedom and AICs. ‘Estimate’ refers to the estimates of the mean of the dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the estimates in each model being the difference from the intercept. Please note that the estimates refer to the values given using a log transformed dependent variable.

Table 3.13 Double cluck: Final model output from LMM.

Model	Estimate	Std. Error	t value	Pr(>|t|)
Peak Frequency~Treatment+1|CG:Bird				
df(7),AIC -82.4				
(Intercept) MN	2.609	0.0608		
Dusty Substrate	-0.063	0.0562	-1.12	0.27
Mealworm	-0.041	0.0544	-0.75	0.46
SN	0.054	0.0650	0.83	0.41
Normal Food	0.051	0.0636	0.795	0.43
Bandwidth 90% ~Treatment+1|CG:Bird				
df(7),AIC -80.1				
(Intercept) MN	1.979	0.0572		
Dusty Substrate	-0.008	0.0603	-0.14	0.89
Mealworm	-0.034	0.0609	-0.56	0.58
SN	0.005	0.0727	0.06	0.95
Normal Food	0.002	0.0674	0.036	0.97
Syllable Length~Treatment+CG+Day+1|CG:Bird				
df(14),AIC -58.2				
(Intercept) MN	-0.990	0.0666		
Dusty Substrate	-0.075	0.0695	-1.08	0.28
Mealworm	-0.162	0.0721	-2.25	0.03
SN	-0.088	0.0865	-1.02	0.31
Normal Food	-0.070	0.0767	-0.916	0.36
CG2	-0.039	0.0425	-0.927	0.36
CG3	0.136	0.0491	2.77	0.01
CG4	0.041	0.0592	0.69	0.49
Call Duration~Treatment+1|CG:Bird				
df(10),AIC -61.0				
(Intercept) MN	-0.493	0.0676		
Dusty Substrate	0.016	0.0707	0.22	0.83
Mealworm	-0.069	0.0707	-0.98	0.33
SN	-0.051	0.0846	-0.60	0.55
Normal Food	-0.007	0.0792	-0.085	0.93

Table include model degrees of freedom and AICs. 'Estimate' refers to the estimates of the mean of the dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the estimates in each model being the difference from the intercept. Please note that the estimates refer to the values given using a log transformed dependent variable.

Table 3.14 Call-type prevalence model output from GLMM

Call Type	Treatment	Estimate	SE	z value	Pr(>|z|)	Predicted Mean	Incident Rate Ratio	Lower 95% CL	Upper 95% CL
Single Cluck	MN (Intercept)	2.86	0.50			17.41	17.41	6.59	45.99
	Dustbath	0.52	0.40	1.32	0.19	29.42	1.69	0.78	3.67
	Mealworm	-0.62	0.48	-1.29	0.20	9.39	0.54	0.21	1.38
	Normal Food	-0.50	0.48	-1.04	0.30	10.55	0.61	0.24	1.55
	SN	-0.34	0.47	-0.72	0.47	12.44	0.71	0.28	1.79
Double Cluck	MN (Intercept)	1.90	0.77			6.67	6.67	1.48	29.99
	Dustbath	1.87	1.08	1.73	0.08	43.34	6.50	0.78	54.00
	Mealworm	1.32	1.08	1.22	0.22	25.00	3.75	0.45	31.19
	Normal Food	0.92	1.08	0.85	0.40	16.66	2.50	0.30	20.82
	SN	0.22	1.08	0.21	0.84	8.33	1.25	0.15	10.46
Fast Cluck	MN (Intercept)	1.63	0.56			5.10	5.10	1.72	15.11
	Dustbath	1.89	0.53	3.54	**<0.001**	33.74	6.62	2.33	18.85
	Mealworm	1.46	0.54	2.7	**<0.01**	21.86	4.29	1.49	12.34
	Normal Food	1.45	0.55	2.66	**<0.01**	21.76	4.27	1.47	12.43
	SN	0.60	0.60	1	0.32	9.30	1.82	0.56	5.92
Food Call	MN (Intercept)	1.05	0.80			2.85	2.85	0.60	13.61
	Dustbath	2.41	0.76	3.17	**<0.01**	31.67	11.13	2.51	49.41
	Mealworm	1.79	0.79	2.27	**0.02**	16.98	5.97	1.28	27.90
	Normal Food	1.56	0.80	1.94	**0.05**	13.48	4.74	0.98	22.79
	SN	0.14	0.93	0.15	0.88	3.27	1.15	0.18	7.14
Whine	MN (Intercept)	2.50	0.64			12.21	12.21	3.50	42.63
	Dustbath	-1.04	0.56	-1.84	0.07	4.33	0.35	0.12	1.07
	Mealworm	-0.83	0.51	-1.64	0.10	5.34	0.44	0.16	1.18
	Normal Food	-0.14	0.42	-0.33	0.74	10.61	0.87	0.38	1.99
	SN	0.07	0.41	0.18	0.86	13.13	1.08	0.48	2.41
Gakel Call	MN (Intercept)	2.46	0.93			11.69	11.69	1.89	72.14
	Mealworm	-0.84	0.78	-1.08	0.28	5.05	0.43	0.09	1.98
	Normal Food	-0.57	0.87	-0.66	0.51	6.63	0.57	0.10	3.09
	SN	-0.52	0.77	-0.67	0.50	6.96	0.60	0.13	2.71
Singing	MN (Intercept)	1.75	0.82			5.73	5.73	1.14	28.78
	Dustbath	-0.56	0.91	-0.62	0.54	3.27	0.57	0.10	3.41
	Mealworm	-1.48	1.18	-1.26	0.21	1.30	0.23	0.02	2.28
	Normal Food	-0.17	0.84	-0.21	0.84	4.82	0.84	0.16	4.36
	SN	-0.54	0.91	-0.59	0.56	3.35	0.59	0.10	3.48
Mixed	MN (Intercept)	3.35	0.31			28.47	28.47	15.49	52.32
	Dustbath	0.46	0.36	1.27	0.21	44.93	1.58	0.78	3.20
	Mealworm	0.15	0.38	0.4	0.69	33.15	1.16	0.55	2.46
	Normal Food	0.48	0.36	1.33	0.18	46.10	1.62	0.79	3.30
	SN	0.28	0.37	0.77	0.44	37.83	1.33	0.64	2.75

‘Estimate’ refers to the estimates of the mean of the dependent variable in the model. The first estimate given is the mean of the intercept, with the rest of the estimates in each model being the difference from the intercept. Please note that the estimates refer to the values given using a log transformed dependent variable.

CHAPTER 4

Do reward-related calls influence the behaviour of other hens in the flock

Contributor	Statement of contribution
McGrath, N	Experimental design (70%) Conducted experiment (100%) Statistical analysis of data (80%) Wrote the chapter (100%)
Burman, O	Experimental design (10%)
Dwyer, C	Experimental design (10%)
Phillips, C	Experimental design (10%) Edited the chapter (20%)
Dunlop, R	Statistical analysis of data (20%) Edited the chapter (80%)

4.1 Abstract

Referential information, relating to environmental stimuli such as a source of food or the presence of a predator, is sometimes encoded in the type, or structural variation, of animal acoustic signals. This referential information is transmitted by the signaller to receivers. Receivers should then change their behaviour, or make adaptive responses as if they have experienced the stimuli themselves. Domestic chickens *Gallus gallus* produce ‘reward calls’, which are vocalisations elicited by both food and dustbath rewards. These ‘reward’ calls are the same call type, but emitted at a lower frequency in anticipation of a dustbathing substrate compared to a food reward. In this study, the hypothesis that hens respond to reward calls differentially according to reward type was tested. If these calls contain referential information, and hens have the relevant internal motivation, responses to calls made in anticipation of food should result in hens fixating on the ground to locate a food source, and pecking the ground. Responses to calls made in anticipation of a dustbathing substrate should result in hens moving around to investigate a potential dustbathing site, and performing dustbathing-related behaviours. To do this, twelve hens were exposed to a playback paradigm using calls recorded from other hens made when anticipating either a mealworm reward or a dustbathing substrate. A pure tone and a period of silence acted as controls. The behaviour of hens was recorded during a 1 minute test period, which comprised of the sound and an interval of silence looped several times. Hens responded to all sound cues, including the pure tone, with short, sharp ‘alert’ head movements, including turning their head and neck in different directions in order to locate the source of the sound. This implies that all sound signals were audible. Hens made ‘alert’ head movements for longer during the ‘mealworm’ sound treatment compared to the silent treatment. There was no difference in ‘alert’ head movements between sound treatments. Very few hens moved towards the stimulus, and there was no difference in the duration of standing between treatments. The ‘reward calls’ of hens appear to contain no referential information, but seem to induce an increased state of arousal and anticipation in receivers which is expressed in ‘alert’ head movements.

4.2 Introduction

Vocalisations provide an important communication method for animals, enabling transmission of information without any need for visual contact. Animal vocalisations were initially considered by some as purely involuntary expressions of arousal (Darwin 1872). However, many of these vocalisations act as signals which, by definition, influence the behaviour of the receiver (Maynard Smith & Harper 2003; Clay et al. 2012). Information about the signaller, relating to age, sex, or body size, or more flexible information relating to motivational state, may be encoded in animal vocalisations. This information is usually contained within the sound type (Zuberbuhler et al. 1997; Murphy et al. 2013) and/or within parameters such as rate (Clay et al. 2012), frequency (Townsend et al. 2014) duration (Dentressangle et al. 2012) and/or amplitude (Gustison & Townsend 2015; Reichard & Anderson 2015). In other words, animals can produce the same sound type, but change its meaning by adjusting various parameters. In rare cases, animal vocalisations are considered to be functionally referential, meaning they pass information about specific environmental stimuli to receivers. For a vocalisation to be classified as functionally referential, the call must be elicited by a narrow range of stimuli, and receivers must respond as if they had experienced the stimuli themselves (Marler et al. 1992; Macedonia & Evans 1993; Evans 1997). Functionally referential signals have been found in animals such as primates (Zuberbuhler 2003; Zuberbühler 2009), ravens *Corvus corvux* (Bugnyar et al. 2001) noisy miners *Manorina melanocephala* (Cunningham & Magrath 2017), black-capped chickadees *Poecile atricapillus* (Templeton et al. 2005) and meerkats *Suricata suricatta* (Manser 2001; Manser et al. 2001).

There has been significant debate around whether vocalisations are produced intentionally by signallers (Seyfarth & Cheney 2003; Rendall et al. 2009; Wheeler & Fischer 2012; Townsend & Manser 2013; Scarantino & Clay 2015; Sievers & Gruber 2016). Some scientists have contended that signallers do not intend to transmit information in their calls, but receivers are still able to extract information due to the tight link between the context or environment and call production (Seyfarth & Cheney 2003; Wheeler & Fischer 2012). In this sense, calls may be purely emotional, but still provide referential information. However, recent research has suggested that signals can be produced intentionally, and some animals make strategic decisions about whether to produce or withhold a call based on contextual factors (Silk et al. 2016; Seyfarth & Cheney 2018). For example, acoustic variation in the quiet ‘hoos’ of chimpanzees produced in three different contexts could not be explained by emotional arousal, and was instead attributed to social selective pressures, and the need for group cohesion and cooperation (Crockford et al. 2018). In another study, bonobos were observed to challenge individual targets with their ‘contest hoots’ (Genty et al. 2014).

Notwithstanding the possibility of intentionality in signals, the behaviour of receivers indicates whether calls contain referential information (Marler et al. 1992; Macedonia & Evans 1993; Evans 1997). Several studies have looked at receiver responses when the group is under threat from a predator. In these scenarios, the signaller has visual access to a predator, and transmits information about the type of predator to the others in the group (the receivers). This information should enable the receivers to locate the threat and make a strategic choice of escape route. Referential predator signalling has been found to occur in various primate species, e.g. Diana monkeys *Cercopithecus dian*a (Zuberbuhler et al. 1997), blue monkeys *Cercopithecus mitis stuhlmani* (Murphy et al. 2013) and meerkats (Manser 2001; Townsend & Manser 2013), as well as birds such as noisy miners *Manorina melanocephala* (Farrow et al. 2017) and white-tailed ptarmigans *Lagopus leucurus* (Ausmus & Clarke 2014). In these studies, the receivers make adaptive responses relating to predator-type. Food-related signals, however, often induce receivers to approach the signaller (Evans & Evans, 1999), possibly functioning to defend the food source from competing animals (Elgar 1986). The structural variation in food calls is thought to encode different environmental features such as size of food patch (Kalan et al. 2015), quantity of food (Evans & Evans 1999; Bugnyar et al. 2001) or food preferences (Marler et al. 1986; Slocombe & Zuberbühler 2006). In other species, the rate of calling varies according to the number of animals present at the food source (Elgar 1986). For example, wild chimpanzees *Pan troglodytes verus,* vary the acoustic structure of their food calls according to tree or food patch size (Kalan et al., 2015), while ravens vary the delivery rate of their 'Haa' call according to food type (Bugnyar et al., 2000). In house sparrows *Passer domesticus,* the rate of calling varies according to the number of animals present at the food source (Elgar, 1986). In the case of chickens, the male chicken food call appears to encode information about feeding opportunities (Evans & Evans, 1999). However, at times males give food calls when there is no food present, and hens approach the calling male, suggesting that this signal is related to mating opportunities, and could therefore represent deception by the male (Gyger & Marler, 1988). Research on broody hens throws this interpretation into doubt. Moffatt & Hogan (1992) found that chicks have a prefunctional tendency to respond to high quality food calls (relating to high quality food items) rather than to low quality food calls. However, this tendency could be modified by experience; for example, if a low quality food call was paired with a high quality food item, chicks would learn this association and respond more quickly to this call (Moffatt & Hogan, 1992) Hence, if hens learned that a cockerel's produced food calls without food being present, they would stop responding, unless the call was actually an honest signal related to the sexual system (Moffatt & Hogan, 1992).

Experiments have shown that some species produce particular vocalisations when anticipating rewards. Rewards can be considered to be either food-related or non-food-related. Non-food reward-related vocalisations, for example, have been described in rats *Rattus norvegicus* and hens *Gallus gallus* (Knutson et al. 1998; McGrath et al. 2017), for which the rewards were play (rats), sexual activity (rats) and dustbathing (hens). Food-associated vocalisations have been described in species such as tufted capuchins *Cebus apella* (Di Bitetti 2003), chimpanzees *Pan troglodytes* (Slocombe & Zuberbühler 2006; Kalan et al. 2015), cotton topped tamarins *Saguinus oedipus* (Elowson et al. 1991) and gorillas *Gorilla g. gorilla* (Luef et al. 2016). Interestingly, hens do not produce different call types in anticipation of a food and non-food reward. Instead, the peak frequency of a distinctive rhythmic staccato call made by hens, previously described as a 'food' call, varies depending on the reward type, with calls made in anticipation of a dustbathing substrate having a lower peak frequency than those made in anticipation of food rewards (McGrath et al. 2017). This implies that food calls, or more accurately 'reward' calls, are not functionally referential. This is consistent with evidence that the food-associated vocalisations of most animals do not meet the strict criteria for functional reference (Clay et al. 2012). The same call type was found to be produced by male chickens (Evans & Evans 1999) and has been cited as the most rigorous example of a food-specific functionally referential call among terrestrial animals (Clay et al. 2012). Evans and Evans (1999) found that playback of male chicken food calls elicited specific anticipatory feeding movements in hens, with hens fixating downwards towards the ground with their frontal binocular field, and pecking the ground as if inspecting the substrate for food. They suggested that the food calls of male chickens met the criteria for acoustic-specificity (Evans & Marler 1994; Evans & Evans 1999). However, their observations and conclusions may only relate to male chicken calls. Some degree of sexual dimorphism has been observed in food calling in domestic chickens with Konishi (1963) observing that males food call freely whereas females rarely do so except when accompanied by chicks.

Food-related calls can also convey the motivational state of the animal (Marler et al. 1992). The anticipation of rewards, including food, is thought to produce an elevated arousal state. This increase in arousal correlates with increased pleasure-based (dopaminergic) activity in the brain (Berridge 1996), which then translates into changes in signalling behaviour. Levels of arousal, motivation or urgency may be encoded within structural variation or call rate (Clay et al. 2012, Manser, 2001, Townsend & Manser 2013). Domestic chickens have been shown to modify the rate and numbers of their food calls in response to different types of food reward (Marler et al., 1986). These changes appear to reflect a change in motivational state according to food type, in that chickens gave more calls at a faster rate when presented with preferred food items (mealworms or peas) compared with less preferred food items (peanuts or nutshells). It is feasible, then, that some receivers respond to the excitement conveyed by the signaller, and approach more readily when a call communicates high levels of positive arousal (Panksepp & Burgdorf 2003).

If a reward-related call is functionally referential, we would expect to see receivers behave in way that means that information about the reward has been conveyed, such as the specific adaptive predator evasion responses described previously, rather than simply react to the arousal level of the signaller. In the case of rats, various studies examined the response to playback of 50 kHz ultrasonic vocalisations which have been associated with contexts such as play, sexual behaviour, anticipation of social contact and other rewarding events (Barfield et al. 1979; Knutson et al. 1998, 1999; Brudzynski & Pniak 2002). Female rats became more receptive to mating following playback of 50 kHz ultrasonic vocalizations (USVs) (Thomas et al. 1982), and a higher rate of 50 kHz USVs elicited more approaches from other rats, suggesting that these calls may encode information about food or social or mating opportunities (Panksepp & Burgdorf, 2003). In contrast, Burman et al. (2007) did not observe a behavioural response to playback of these 'reward' calls to other rats. Though the results of these experiments imply that the reward-related calls of rats are not likely to be functionally referential, they demonstrate the necessity of measuring receiver responses (in different contexts) to any call suspected to be functionally referential.

Adopting a playback paradigm using the reward calls of domestic hens, and observations of the receivers' behaviour, this study tested if variation in the peak frequency of the reward calls contained referential information about the reward type. The goal was to investigate whether calls made in anticipation of a mealworm reward, or in anticipation of dustbathing substrate reward, elicit different behavioural responses from the receivers. In the previous anticipatory behaviour study (Chapter 2), hens showed a higher intensity of behavioural response in anticipation of the dustbathing substrate compared with the mealworm reward, and this was attributed to increased arousal. Here, it was hypothesised that calls made in anticipation of both rewards would cause the receivers to perform anticipatory behaviour including making sharp head movements as they located the sound source, orienting towards the speaker and then moving towards the speaker. It was expected that differences in arousal level would be observed, with 'dustbathing' calls eliciting a higher intensity of behaviours, such as a faster latency to look or move towards the speaker resulting from a higher arousal level, and increased activity. If the calls were functionally referential, playback of 'mealworm' calls would be likely to induce hens to fixate on the ground in front of them and peck the ground. In contrast, playback of 'dustbathing calls' would be likely to elicit movement to investigate the location of the potential dustbathing site, scratching or pecking the ground, and potentially the full sequence of dustbathing behaviours including bill-raking, erecting feathers, squatting down, wing-shaking, head-rubbing, scratching with one leg, and side-lying or side-rubbing.

4.3 Methods

4.3.1 Subjects and housing

Twelve ISA Brown hens, approximately 52 weeks old, were obtained from the University of Queensland's poultry unit. The required sample size for this experiment was calculated using the Winpepi package using behavioural data from a previous experiment (McGrath et al. 2016). The hens were housed in groups of three birds in cages measuring 100 x 100 x 63 cm (height). Each cage contained a perch and had a deep layer of shredded paper as floor material to enable natural behaviour like nesting, foraging and dustbathing. Food (standard layer pellets) and water were available ad libitum in the home pens. The housing had natural light as well as artificial light (which was on between 06:00 and 18:00 h). There was no temperature control, however all experimental work was conducted between 08:00 and 12.30 to standardise the conditions. Hens were individually identifiable to the experimenter based on plumage colouring, marking and comb size, avoiding the need for individual marking or ringing. The methods used in this study were approved by the University of Queensland Animal Ethics Committee (Ref. CAWE/066/13/VOICELESS)

4.3.2 Experimental apparatus

An experimental pen (200 cm long x 125 cm wide x 60 cm high) was located in a sound-proofed room adjacent to the room in which the birds were housed. Three of the four walls of the experimental pen were made of plywood and one was made of wire-mesh to allow video recording. The floor was covered in a grey tarpaulin to reduce the effect any other stimuli may have on chicken behaviour (e.g. a natural substrate or grass floor could have induced pecking behaviour in the hens which would have confounded the results.). Sounds were presented using a Logitech x623 speaker (frequency response: 35 Hz-20 KHz, output: 200 Watt), positioned 160 cm from the centre of the pen and 90 cm from one of the corners of the experimental pen (opposite the wire mesh wall). The location of the speaker (left or right orientation towards the pen) was changed according to an experimental protocol, and its position precluded visual access by the hens. Amplitude for delivery of playback sounds was set at 70 dB, measured in the centre of the experimental pen using a sound level meter (model Q1362, Dick Smith Electronics). This amplitude reflects natural amplitude levels at the distance from the speaker to the centre of the pen. The microphone (Sennheiser ME66 condenser shotgun) was positioned in a central position opposite the wire mesh wall of the experimental pen, 96 cm from the nearest point and 220 cm from the furthest point in the pen.

4.3.3 Recordings and selection of calls

Playback stimuli were generated using either calls made during reward contexts or a pure tone (artificial sound) for the control treatment. Reward-related calls were taken from recordings made while hens (of the same breed) were anticipating mealworm and dustbathing substrate rewards during the study described in Chapter 2 and 3 (microphone: Sennheiser ME66 condenser shotgun, recorder: Tascam DR100 MkII DAT, accuracy: 24 bit resolution, sampling rate: 44.1 kHz) (see Chapter 2 for methodology). The DAT files were transferred to a PC (Dell) to analyse the vocalisations using Raven Pro: Interactive Sound Analysis Software (Version 1.5). Ithaca, NY: The Cornell Laboratory of Ornithology. The mean peak frequency and mean duration of all calls made during the anticipation periods were calculated for the dustbathing substrate and mealworm rewards, including the Euclidean distance of calls (‘Mealworm’ and ‘Dustbath’) from the mean. Subsequently, the top 12 calls with values closest to the mean of these parameters for both ‘Mealworm’ and ‘Dustbath’ calls were used, having eliminated any signals which were interrupted by background noise. These calls came from 3 birds for ‘Mealworm’ and 7 birds for ‘Dustbath’.

All playback stimuli were edited using Adobe Audition CC sound editing software (Adobe Systems 2013). The average ‘Mealworm’ call length was 0.13 seconds while the average ‘Dustbath’ call length was 0.08. In order to create a 1 minute playback stimulus, individual calls were looped, and an interval between these calls was created to mirror a typical temporal calling pattern. Intercall intervals were determined by calculating the interval between the end of each call used as a stimulus and the subsequent call in the recording sequence. For Dustbath, 10 of the calls used as stimuli came from a sequence of calls. The average interval was calculated by adding the intervals between the 10 calls and their subsequent calls together and dividing by 10 to give an average interval of 5.1s. For Mealworm, 8 of the stimulus calls came from a sequence of calls, and therefore the intervals between these calls and their subsequent calls were added together and that figure was divided by 8 to give an average interval of 4.1s. Hence each ‘Mealworm’ stimulus contained a call looped with an intercall interval of 4.1s, and each ‘Dustbath’ stimulus contained a call looped with an intercall interval of 5.1s

For the control stimuli, 12 pure tones were generated. Pure tones were used as they do not, by themselves, elicit any specific response (Mackenzie et al. 1993) whereas white noise has been known to elicit a stress response (Düpjan et al. 2011). Mealworm and dustbathing substrate stimuli (12 of each) were paired, and the average length and average peak frequency for each pair were calculated. Pure tones with a frequency and length equal to the paired results were then created using Adobe Audition CC. As per the vocalisation stimuli, pure tones were looped and an interval of 4.6 seconds was inserted between each tone (equal to the average of the dustbathing substrate and mealworm call intervals), thus creating a similar temporal pattern to the vocalisation stimuli. Hence, the number, duration and amplitude of broadcast sounds were highly comparable. The final set of stimuli used during testing was 1. ‘Dustbath’ call, 2. ‘Mealworm’ call, 3. pure tone, 4. silence.

4.3.4 Design and test procedure

A within-subjects design was used with every hen experiencing every stimulus in separate test sessions. ‘Dustbath’ and ‘Mealworm’ paired stimuli were randomly assigned to each hen. The sequence of testing followed an orthogonal latin square design. To avoid side biases, the position of the speaker was alternated from one corner of the cage to the other between tests.

Hens were allowed to habituate to the test situation for two days prior to the test days. On habituation days, they were placed in the experimental chamber for 15 minutes with no stimulus presented. There was a period of at least 24 hours between each habituation period. During testing, each hen received one test session per day on four consecutive days with a period of at least 24 hours between each test session. At the start of each session individual hens were allowed to habituate to the experimental pen for two minutes. A two minute pre-stimulus observation period followed during which behaviour was recorded. Hens were then exposed to one of the four test conditions; stimuli were broadcast for one minute, or there was one minute’s silence, during which behaviour and vocalisations continued to be recorded. A post-stimulus period of two minutes allowed further recording of behaviour and vocalisations, before the hen was removed from the experimental chamber and returned to its home cage.

During the testing procedure video recordings were made using 2 x K-32HCVF, (Kobi, Taiwan) cameras and recorded onto a K9 XQ H.264 DVR (Kobi, Taiwan). These were then transferred to a PC for analysis using Cowlog: Version 2.11 (Hänninen & Pastell 2009).

4.3.5 Ethogram

Behavioural responses were scored in the 60 second pre-stimulus period and during the 60 second stimulus period. In a previous playback study of male chicken food calls, hens were found to use close binocular fixation at the ground, which, in many bird species, immediately precedes pecking at a food item (Evans & Evans, 1999). Following on from this study, an ethogram of potential behavioural responses to the playback sounds was constructed.

Table 4.1 Ethogram showing a full list of expected behaviours during playback

Behaviour	Description
Walk	Walking in a continuous, fluid manner
Step	Lifting foot and putting it down again
Run	Fast, running movement
Stand	Front of body upwards while neck is stretched upwards
Alert head movements	Short, sharp head movements, including turning head and neck in different directions in order to locate a sound
Look towards speaker	Turn head and fix gaze towards thespeaker
Move towards the speaker	Orient body and move towards the speaker
Look at ground	Fixate on ground with binocular field
Peck ground	Pecks at items (visible or not) on the ground
Scratch ground	Scratching at ground, often followed by one or two steps backwards
Preening neck/chest	Moving beak along feathers of neck and chest
Wing flapping	Lifting wings and flapping them
Feather ruffling	Stretching neck, raising ruff and ruffling feathers and body
Scratching body	Moving foot along feathers
Head flicking	Short, vigorous shaking of the head
Sham dustbathing	Vertical wing shake, bill raking, sidelying and rubbing body on ground, moving wings, and pecking as if dustbathing in substrate

4.3.6 Behavioural recording

The duration of selected behaviours (see ethogram, Table 4.1) was scored from video recordings ensuring that the experimenter was blind to the treatments. Duration was recorded from the beginning of the behavioural sequence until that behaviour ceased. Most behaviours were only infrequently (less than 10 times in 48 tests), or never, observed and therefore were not included in the analysis. For the final analysis, the behaviours ‘Alert’ head movements (duration), Standing (duration) and the latency of chickens to look at or move towards the speaker were measured. In a previous study, different intensities of a behavioural response (pushing and pecking at a door) appeared to reflect the incentive value of the reward (McGrath et al. 2016). Here, the latency of hens to look at, or move towards, the sound source was measured to ascertain if different levels of arousal were reflected in different latency times. The duration of standing behaviour was measured in order to differentiate between investigatory behaviour (which could be movement in any direction or towards the sound source) and potential feeding behaviour. The duration of standing behaviour gives an overall indication of the level of activity (different behaviours) of hens in response to the different treatments, which is an indicator of arousal levels.

4.3.7 Statistical analysis

To test the hypothesis that reward-related vocalisations would elicit an anticipatory and investigatory response as determined by the above behavioural measures, linear mixed effects models (LMM) and generalised linear mixed effects models (GLMM) were constructed. These were performed in R (R Core Team 2017) using the lme4 (Bates et al. 2015) package. Linear mixed effects models and generalised linear mixed effects models were used to account for the within-bird variance associated with repeated measurements. Standard statistical models assume independence of residuals, but when measurements are taken from the same individual they are correlated. Mixed effects models allow us to include individual (bird) identity as a random factor, thus enabling us to separate the total variance in the response variable into a within-subject and between-subject variance component.

Table 4.2 Comparison of the different models for the behavioural responses

Behaviour	**Fixed variables in model**	**Comparison**	***df***	**AIC**	**X^2**	**X^2 *df***	***P*-value**
Alert head movements	Treatment		6	170		6	
	Treatment + Phase	1 vs 2	7	160	12.4	7	<0.001
	Treatment*Phase	2 vs 3	10	158	8.2	10	0.04
Stand	Treatment		5	52.24			
	Treatment + Day	1 vs 2	8	55.08	3.2	3	0.37
	Treatment + Phase	2 vs 3	6	50.84	3.4	1	0.07
	Treatment + Day + Phase	3 vs 4	9	53.13	3.7	3	0.29

Data includes model degrees of freedom (*df*), Akaike information criterion (AIC), X^2, X^2 degrees of freedom (X^2 *df*) and *P* values. Lines in bold indicate the model with the lowest AIC score.

For each behaviour that was observed, an initial model included 'Treatment' ('Dustbath', 'Mealworm', 'Pure Tone', 'Silence'), 'Phase' (Pre-stimulus, Stimulus) and the interaction between 'Treatment' and 'Phase'. If the response variable was continuous and normally distributed a Gaussian distribution was appropriate. If the response variable was not normal, it was transformed using a log(x+1) transformation.

One behaviour (Standing) was constrained in time, and was therefore converted to a proportion out of 60 by dividing the duration of time each bird stood for by 60. Hence, 1 represented a hen that carried out the behaviour for the whole 60 second period, and lower durations of standing behaviour were represented by a value between 0 and 1. A binomial response model was appropriate for this analysis and was conducted using the 'glmer' function in the lme4 package, family = "binomial".

Models including the factors ‘Treatment’, ‘Day’ and ‘Phase’ were compared using Akaike Information Criterion (AIC) scores and the anova() function. The best fitting models, according to AIC scores, are detailed for each response variable in Table 4.2. The final model included ‘Treatment’ (‘Dustbath’, ‘Mealworm’, ‘Pure Tone’, ‘Silence’) and ‘Phase’ (Pre-stimulus, Stimulus). Interaction effects were not used as the models would not converge. The model was tested for overdispersion using the function ‘dispersion_glmer’ (0.52). The assumptions of normal distribution, linearity and homoscedasticity were checked by visual inspection of the residual plots and by a Shapiro-Wilks test. Residuals that deviated from normality were corrected by natural log transformations. Parameter estimates were computed using the maximum likelihood method and the significance of predictor variables were tested using maximum likelihood ratio tests (anova function in R). The Chi-squared statistic (χ^2) and associated *P*-values are reported. Post hoc analyses were conducted using the lsmeans package (version 2.20-23) in R, applying the Tukey method to adjust *P*-values for multiple comparisons.

Hens only approached the speaker in 11 sound treatments out of the total 48 treatments in the stimulus phase which resulted in a sample size that was too low for the statistical modelling procedures used for the other behavioural metrics. Given the lack of occurrences, it was decided to remove this behaviour from any further analysis, as it was unlikely to be a robust metric with which to address the hypotheses. No hens looked towards the speaker during the silent treatment. Hens that looked towards the speaker upon presentation of the sound stimuli, did so almost immediately (within 2.5 seconds), which meant that the data lacked the variance with which to carry out the statistical modelling to test for differences between sound stimuli.

4.4 Results

Hens looked towards the speaker in all the sound treatments and when they did so, they did immediately. This suggests that all played sounds were audible to the hens.

None of the hens performed behaviours relating to searching for food (inspecting, scratching or pecking the ground) in response to the ‘mealworm’ call. Also, no hens responded to the ‘dustbath’ calls by scratching and pecking the ground, or performing the sequence of dustbathing behaviour (see ethogram).

Table 4.3 Lsmeans, 95% confidence limits and significance levels from the final model associated with the effect of sound treatments on the behavioural response ‘Alert head movements’

Behaviour	Dustbath			Mealworm			Pure Tone			Silent					
	Lsmean	Lower CL	Upper CL	Lsmean	Lower CL	Upper CL	Lsmean	Lower CL	Upper CL	Lsmean	Lower CL	Upper CL	χ2	χ2 *df*	*P* value
Alert (Log(x+1) transformed)	5.83^{ab}	4.15	8.05	8.55^{a}	6.21	11.66	6.02^{ab}	4.29	8.30	4.83^{b}	3.40	6.73	22.78	7	0.002

Superscript letters indicate statistically reliable group differences (Tukey’s HSD test; $p \leq 0.05$).

Arousal levels were measured using the duration of ‘alert’ head movements. Hens carried out this behaviour for a significantly longer duration when presented with the food-related sound treatment compared with during the silent treatment (Table 4.3). The other sound treatments also elicited a higher duration of head movements compared to the silent treatment, but the results of the interaction between Treatment and Phase for Dustbath * Stimulus and Pure Tone * Stimulus were not significant at the 0.05 level (Table 4.4).

Table 4.4 Final linear mixed effects model output showing the effects of playback of different sound treatments and a silent treatment on alert head movements in hens

Variable	Categories	Estimate	SE	*t*	Pr(>|t|)
Treatment	*Silent*	Reference			
	Dustbath	-0.187	0.186	-1.0	0.32
	Mealworm	-0.159	0.186	-0.9	0.39
	Pure Tone	-0.177	0.186	-1.0	0.34
Phase	*Pre-stimulus*	Reference			
	Stimulus	-0.087	0.186	-0.5	0.64
Treatment * Phase Interaction	*Silent : Prestimulus*	Reference			
	Dustbath : Stimulus	0.510	0.262	1.9	0.06
	Mealworm : Stimulus	0.747	0.262	2.8	0.01
	Pure Tone : Stimulus	0.514	0.262	2.0	0.054
Intercept		0.809	0.154		

‘Estimate’ refers to the estimates of the mean of the dependent variable in the model. Estimates are the difference from the intercept. The estimates refer to the values given using a log-transformed dependent variable.

Table 4.5 Final mixed-effects, multivariable logistic-regression model of effect of treatment and phase on the standing behaviour of chickens.

Variable	Categories	OR	95% CI	*P*	Wald test *P*
Treatment	*Silent*	Reference			0.62
	Dustbath	5.2	0.34, 126.4	0.22	
	Mealworm	1.98	0.18, 26.4	0.54	
	Pure Tone	1.98	0.18, 26.4	0.54	
Phase	*Pre-stimulus*	Reference			0.07
	Stimulus	26.1	0.74, 50.9		
Intercept		26.1	1.26, 165.2	0.09	

Table includes Odds ratios, 95% confidence intervals, test *P* values and Wald test *P* values

Finally, standing behaviour was used as a measure of non-responsive behaviour. There was no significant difference in the probability that hens would stand still between treatments (Table 4.5). Hens moved in 18 out of the total 48 treatments in the pre-stimulus phase, and hens moved in 20 out of the total 48 treatments in the stimulus phase.

4.5 Discussion

In this study, it was predicted that if reward calls contained referential information about the types of reward, then chickens would show differential behaviour in response to the 'mealworm' and 'dustbath' calls. However, call-specific responses were not observed. Reward calls have been found to differ in their peak frequency according to reward-type (McGrath et al., 2017). This appears to reflect different levels of arousal, with hens producing calls at a lower peak frequency in anticipation of a dustbathing substrate compared with calls made in anticipation of a mealworm reward or their normal food. The only evidence of arousal in this playback study appears to be in the behavioural response 'alert' head movements. Hens made these movements for a significantly longer total duration of time when hearing playback of sounds given in anticipation of a mealworm reward than during the 'silence' treatment, which indicates increased arousal. However, there was no significant difference in hens' head movements between sound treatments which suggests that all these sounds provoked an initial investigatory response of head movements in the hens.

The function of head movements in chickens has been analysed in various studies. In general, birds are not able to move their eyes much. Head movements, therefore, enable them to follow moving objects, thus maintaining their visual field, or to scan their surrounding environment, and obtain a new visual field (Dunlap & Mowrer, 1930). Hens use large, rapid head movements in scenarios when they are approached by conspecifics, or when they are examining novel objects, perhaps to view the same object with different eyes, or with the same eye from different angles

(Dawkins 2002). In one study, when confronted with novel objects, hens did not lessen head movements or fix their gaze on an object, but instead reduced locomotory behaviour while maintaining head movements with large angles (Dawkins, 2002). These large angles appear to allow hens to adjust the direction towards retinal areas not normally used, presumably to capture as much information as possible about a novel object. In this study, head movements may represent hens' attempts to locate the sound source, and any other salient environmental information relating to the sound. However, 'alert' head movements in hens have also been associated with arousal levels and anticipatory behaviour.

Animals exhibit anticipatory behaviour around certain resources, and when the stimuli are positive, such as food items or other rewards, this behaviour appears to be appetitive or sometimes consummatory (Spruijt et al. 2001). Moe et al. (2009) defined the anticipatory behaviour of hens as frequent head movements, either standing still or taking slow steps 'with legs, body and neck stretched upwards'. In a later study, the authors concluded that frequent head movements in any direction were under dopaminergic control (Moe et al., 2011). Davies et al. (2014) used head movements as an indicator of arousal, and found that hens made a greater number of head movements when two bowls of mealworms were presented, rather than one bowl of mealworms, or empty bowls. Although an increase in head movements was generally correlated with an increase in heart-rate when mealworms were presented, there was no difference in heart rate when one or two bowls of mealworms were presented, suggesting that head movements were a more sensitive indicator of arousal during decision-making in this experiment (Davies et al. 2014). Zimmerman et al. (2011) suggested head movements were an indicator of increased attention or vigilance, with hens trying to locate the source of a sound cue, and found that hens performed increased head movements in anticipation of a negative event. In a previous study (Chapter 2), it was found that hens made significantly more head movements in anticipation of the sound-neutral cue which signalled no reward, than all the rewarded sound cues. It was concluded that both sound and light cues induce alert head movements whether a reward has been signalled or not. Hens, in this case, may have been trying to locate the sound source. This theory seems to fit well with the result of this current experiment, in which 'alert' head movements were significantly more prevalent in the 'Mealworm' sound treatment than the 'Silence' treatment but there was no difference in the duration of this behaviour between sound treatments. Hence, it appears that the sound, rather than any associated reward, was the stimulus that induced alert head movements. The function of this response may have been to locate the sound source, and then decide what to do about it - for example, locate other salient environmental indicators related to the context of a call. However, this does not preclude the idea that head movements also represent arousal, as the sound may have produced either a positive arousal effect (due to the association with rewards), or a negative arousal effect (due to possible perception of the pure tone being unpleasant). It is difficult to measure the valence of arousal-related responses without being able to correlate with other responses (e.g. physiological and behavioural) that indicate that the hens are experiencing a positive or a negative event. Rewarding events may induce approach, goal-directed behaviour and consummatory behaviour (McGrath et al., 2016, Zimmerman et al., 2011), whereas negative events may induce hens to try to escape (Zimmerman et al., 2011). The lack of behavioural response in this study makes it impossible to conclude whether hens were experiencing positive or negative arousal.

Evans & Evans (1999) found that when hens heard playback of a male chicken food call they fixated their gaze downwards towards the ground with their frontal binocular field to inspect the substrate. This type of behaviour precedes pecking and feeding in many bird species. This anticipatory behaviour was only related to male ‘food’ calls and not ‘ground predator alarm’ calls which acted as a control. Here, this study was unable to replicate their results. Hens made ‘alert’ head movements, and looked towards the sound source, but only moved in a rare few cases, and performed no other behaviours. This difference in outcome between this study and the study made by Evans & Evans (1999) may relate to the sex of the chicken. In a previous study, it was found that food calls are produced by hens in anticipation of a dustbathing substrate reward (McGrath et al., 2017), and, thus, it was suggested that these calls, produced by female chickens, were in fact reward calls. Although the spectrograms of female reward calls and male food calls appear to be identical, there may be slight acoustic differences between these call types, which affects how they are perceived by hens. Therefore, the lack of referential indicators in this playback study appears to suggest that reward calls contain no referential information about the type of reward eliciting the call.

The alternative explanation for these results is that hens did not consider the playback calls to be realistic, though this seems unlikely as there are numerous other playback studies in which the animals seemed to regard the calls as realistic, and respond as if they are experiencing the call themselves. For example, playback of chicken ‘ground predator’ alarm or ‘aerial predator’ alarm calls respectively elicits vigilance or crouching in receivers (Evans et al., 1993). Diana monkeys respond to playback of other Diana monkey calls given to a leopard, or calls given to an eagle, with leopard or eagle alarm calls respectively (Zuberbuhler et al., 1997). Meerkats crouch, and then run to bolt holes, in response to playback of ‘high aerial predator’ calls, whereas in response to playback of ‘terrestrial predator’ calls, receivers move towards the speaker with frequent scanning activity and gather together (Manser et al., 2001). Notwithstanding these responses, the absence of variation in subsequent calls within the individual stimuli (individual calls were repeated within each stimulus), and the fixed interval between looped calls, do not represent a ‘natural’ sequence of calling, and future studies could investigate whether hens respond to variations in sequences.

One potential issue with this study is the fact that the hens used in this experiment were not very responsive. Evans & Evans (1999) saw an increase in activity after playback of ground alarm calls and food calls. Here, hens barely moved after hearing the sound stimuli, and only moved slightly more during the ‘Silence’ treatment. This lack of response may stem from other factors, such as the fact that the hens were mature in comparison to the hens used in the initial recording experiment (Chapter 2). In addition, specific responses to different rewards may be masked by the general lack of response by all the hens in this study. Hens had been previously housed in cages that lacked stimuli, and they may have become conditioned to this environment. Furthermore, even though habituation time was built into the experimental design, it may be that hens were fearful of the isolation in the test environment, and potentially felt vulnerable in the relatively large space of the test chamber, compared to their home cages, although no overt stress behaviours were observed. It would be prudent, therefore, to re-test these hypotheses using more active hens, habituated to a more stimulating environment. A further limitation to this study is that ‘mealworm’ playback stimuli came from three chickens, which does not avoid pseudoreplication (McGregor 2000).

This study investigated whether playback of hens’ ‘reward calls’ contained referential information, and produced a differential response in hens according to reward-type. Hens responded to the playback of all sounds by making short, sharp head movements, which were most likely attempts to localise the source of the sound. Generally, the hens were not very responsive, and did not attempt to investigate these sound cues by approaching the sound source. This general lack of response may mask any specific response to different reward-related calls. Further investigation is needed to establish whether hens do respond to signals elicited by different types of reward, and whether their responses are specific to reward types. These investigations could also include assessment of physiological changes such as eye temperature or heart rate, to further improve our knowledge of hens’ emotional responses to these sounds. Furthermore, depriving the listening hens of food for up to 2 hours before playback may increase the probability that the hens would respond. In addition, playback of both male and female chicken calls could also help to establish whether there are acoustic differences in these calls that result in different behavioural responses in hens.

CHAPTER 5

Do humans recognise the emotional content of chicken vocalisations?

Contributor	Statement of contribution
McGrath, N	Experimental design (70%) Conducted experiment (100%) Statistical analysis of data (80%) Wrote the chapter (100%)
Burman, O	Experimental design (10%)
Dwyer, C	Experimental design (10%)
Phillips, C	Experimental design (10%) Edited the chapter (20%)
Henning, J	Statistical analysis of data (20%) Edited the chapter (80%)

5.1 Abstract

In the animal world, the ability to detect the emotional content of vocalisations is important for communication, and, in some cases, survival. Humans are able to decode emotional information from the vocal expressions of humans and other animals. This ability has often been attributed to use of basic acoustic cues, although experience or familiarity with the vocalising animal may also be a factor. Few human perceptual studies have investigated how humans judge calls made by birds. Here, it was tested whether humans could identify whether calls made by chickens *Gallus gallus* were made in rewarding or non-rewarding contexts. Participants listened to 16 calls recorded when chickens were anticipating a reward, or when no reward was available. They rated each call according to whether they thought the vocalising chicken was experiencing pleasure or displeasure (valence), and high or low arousal (intensity), using a sliding scale. This study also investigated whether acoustic cues, experience with chickens or demographic variables were associated with participants' ratings of the arousal levels or valence represented by reward or non-reward related calls. Listeners were then asked to identify whether each call was made in a rewarding or non-rewarding context. Experience with chickens did not affect any of these tasks. The findings show that acoustic cues correlated with listener's judgements of valence and arousal, with longer call durations predicting ratings of higher displeasure and higher arousal levels. Older people were less adept at identifying the correct context of call production, and more likely to attribute higher valence ratings (higher pleasure) to the non-reward related calls. This study strengthens evidence that human perception of the emotional expression of animals is not restricted to mammals, and specific acoustic cues may embody a homologous signalling system among vertebrates. Importantly, this study shows that humans are able to identify chicken calls made in rewarding or non-rewarding contexts. This finding could have important ramifications for farm animal welfare assessments.

5.2 Introduction

Emotions are responses to internal or external stimuli that result in physiological changes and activation of the brain. Most emotions are short-term and intense, mobilizing physical responses that enable humans and non-human animals to avoid harm, or to approach resources that will improve their fitness (Rolls 2000; Boissy et al. 2007). A two-dimensional approach is often used to classify emotions in terms of valence (from high displeasure [negative] to high pleasure [positive]) and intensity (or arousal, from low arousal to high arousal). In this respect, emotions may be positively valenced but represent low or high arousal (e.g. contentment vs. joy). Equally, emotions may be negatively valenced but also express either low or high arousal (e.g. depression vs. fear) (Russell 1980; Mendl et al. 2010).

The link between vocalisations and emotional arousal can be explained by physiological changes. In mammal vocal production, airflow from the lungs passes into the larynx, where vibrations in the vocal folds convert this airflow into a sound. These vibrations determine the fundamental frequency (pitch) and harmonics of the sound (Fant 1960; Titze 1994; Taylor & Reby 2010). The vocal tract then acts as a 'filter', honing the sound by softening some frequencies and amplifying other frequencies which become 'formants' (Titze et al. 2015). In birds, the syrinx generates the sound, which is then modified by the suprasyringeal vocal tract (Gaunt et al. 1976; Nowicki 1987). When an animal vocalises, the acoustic parameters of the sound may be modulated by the animal's level of emotional arousal, and these acoustic cues may be perceived by others.

Some of the emotional information in human communication is decoded from acoustic cues. 'Affective prosody' refers to the melodic or rhythmic aspects of human vocalisations, including the changes in pitch, tone, timbre, pauses and loudness that give human speech and non-verbal expressions their emotional quality (Murray & Arnott 1993; Banse & Scherer 1996; Ilie & Thompson 2006). Speakers across different cultures convey emotion using similar prosodic cues (Bachorowski & Owren 1995; Calder et al. 2001) and people of different languages and cultures have interpreted the same emotions from these paralinguistic vocal cues (Scherer et al. 2001; Sauter et al. 2010b). Cross-taxa studies have attempted to pin down the biological roots of this capacity in humans to establish whether emotional prosody is related to specialised areas of the human brain, or whether precursors in other mammals can be traced (Filippi et al. 2017a).

Recent studies have demonstrated that humans are able to decode the emotional content of calls produced by various animals (Nicastro & Owren 2003; Pongrácz et al. 2005; Pongrácz et al. 2006; Tallet et al. 2010; Pongrácz et al. 2011; Faragó et al. 2014; Maruščáková et al. 2015; Faragó et al. 2017; Filippi et al. 2017a; Filippi et al. 2017b). This capacity may stem from homologous vocal expression and perception systems across vertebrates (Filippi et al. 2017a). From an evolutionary perspective, the ability to perceive the emotional content of vocalisations is crucial when in situations of impending danger or threat (Nesse 1990). Some animals have a hierarchical structure in their auditory systems, optimised for conspecific sound processing (Newman & Wollberg 1973; Mello et al. 1992; Hauber et al. 2013; Theunissen & Elie 2014). However, 'eavesdropping' occurs across a wide range of species, with birds eavesdropping on other birds and mammals, and mammals eavesdropping on other mammals, birds or lizards (Magrath et al. 2015). Some animals may assimilate information extracted from heterospecific vocalisations and conspecific behaviour to determine their own response to potential threats (Filippi et al. 2017b). For example, one species' alarm call may alert another species to an impending threat, whether the call is acoustically similar (Aubin 1991), or not (Templeton et al. 2005).

A growing body of evidence suggests that humans use basic acoustic rules to decode the emotional content of human and other species' vocalisations. Some animal vocalisations follow Morton's 'motivation-structural' rules, which dictate that a call's frequency (pitch) is determined by the context it is produced in. Within this framework, high frequency calls are produced in fearful or appeasing contexts, whereas low frequency calls represent aggressive contexts. In two studies, the success of participants in correctly classifying and describing emotionality in pig *Sus scrofa* and dog *Canis familiaris* calls, respectively, was attributed to use of these rules (Pongrácz et al. 2006; Tallet et al. 2010). Acoustic cues appear to accurately predict human ratings of the emotional content of calls in many animals (Pongrácz et al. 2005; Pongrácz et al. 2006; Molnár et al. 2010; Tallet et al. 2010; Pongrácz et al. 2011; Faragó et al. 2014; Maruščáková et al. 2015; Filippi et al. 2017a; Filippi et al. 2017b). Furthermore, it has been hypothesised that human preference for vocalisations with a specific acoustic structure may have been a selective pressure in the development of dog bark production (Pongrácz et al. 2010). Similarly, cat *Felis catus* 'miaows' may have evolved to provoke a nervous-system and affective response in humans (Nicastro & Owren 2003). Cats have been found to introduce a high-frequency component into their purr when soliciting food from humans. Adult humans rated these purrs as more 'urgent', and both cat owners and non-owners were highly sensitive to them (McComb et al. 2009). This sensitivity to acoustic cues is not restricted to other mammals. Neuroimaging studies of human brains have revealed that human brains tune into heterospecific sounds, but not to non-vocal environmental sounds (Andics et al. 2014). Humans appear to use the same frequency-related parameters to identify levels of arousal in the vocalisations of non-mammalian vertebrates including amphibians, reptiles and birds (Filippi et al. 2017a).

The most common vocal correlates of arousal in non-human animals are peak frequency, amplitude contour, energy distribution, harmonic to noise ratio, fundamental frequency (*F0*) contour, *F0* range, formant contour, vocalisation / element duration and rate, and decrease in inter-vocalisation interval, with other potential measures being jitter, spectral noise, and time of peak frequency (Briefer 2012; Linhart et al. 2015). Increases in arousal generally produce vocalisations that are harsher, louder, faster and longer, with a higher frequency (*F0*) and a wider frequency range. Acoustic links to valence are less easy to define, though positive contexts mainly elicit shorter call durations (Brudzynski 2007; Taylor et al. 2009; McGrath et al. 2017). However, while acoustic cues frequently predict how humans rate arousal (e.g. Filippi et al. 2017), and (less often) valence (e.g. Farago et al. 2014; Maruščáková et al. 2015), experience with the vocalising animal is often associated with correct identification of the context calls are made in. Experience improves the correct classification of pig (Tallet et al. 2010), cat (Nicastro & Owren 2003) and dog vocalisations (Faragó et al. 2017). Furthermore, a cross-taxa study, which asked humans to identify the emotional content and context of human infant, chimpanzee *Pan troglodytes*, dog, and tree shrew *Tupaia glis* vocalisations concluded that cognitive-experience based mechanisms predicted correct classification and identification (Scheumann et al. 2014).

Research on how humans perceive non-human animal vocalisations has thus provided interesting insights on the mechanisms used to identify emotion in vocalisations. It has also provided a potential link to the evolutionary roots of affective prosody. However, the major body of research has focussed on mammals, and there is little evidence on birds, with no studies examining how humans perceive the calls of chickens. Humans have co-existed with chickens for around 8-10,000 years, although there is no evidence of an inter-species communication system in the literature as there appears to be in dogs (Albuquerque et al. 2016). However, chickens are an extremely vocal species, and our ability to detect the emotional content of their vocalisations could have important implications for their welfare.

To address this gap in perceptual studies, this study investigated whether humans could correctly identify whether chicken vocalisations were made in rewarding or non-rewarding contexts. It also tested whether experience, or other demographic factors, influenced the correct identification of context. Factors that could predict how humans perceived the valence and arousal level of the calls were examined. It was hypothesized that the ability of humans to correctly identify the context of calls would be modified by experience with chickens, with those having more experience with chickens making more correct identifications. It was also hypothesized that arousal and valence ratings would be affected by the acoustic parameters of the calls. Specifically, it was expected that longer call durations would predict lower valence ratings, and higher arousal ratings would be predicted by higher frequency parameters.

5.3 Methods

5.3.1 Experimental study to record chicken calls

5.3.1.1 Study design

Playback stimuli were generated using calls recorded during the experiment described in Chapters 2 and 3. These vocalisations were elicited by sound cues signalling rewards, or during two non-reward (control) treatments ('sound cue – no reward' and 'no sound – no reward').

Twelve ISA Brown hens, approximately 18 weeks old, were subjected to a Pavlovian conditioning paradigm. An initially neutral stimulus (conditioned stimuli, CS) was repeatedly paired with the presentation of one of three different rewards (mealworms, normal food or a dustbathing substrate), or a sound-neutral event (an empty compartment) which served as unconditioned stimuli. Hens were placed individually in the first chamber of an experimental pen with two chambers connected by a swing door. Vocalisations were recorded during a 15s period after a sound cue was played to signal the availability of a dustbathing substrate, mealworms or normal food in a second chamber. After the 15 s recording period, a light was switched on to signal the door had been unlocked and hens were able to push through the swing door to access the rewards.

Non-reward vocalisations were recorded during the sound-neutral event (CS paired with an empty compartment) and a 'muted-neutral' treatment (no sound cue, empty compartment).

5.3.1.2 Sound recordings

All vocalisations were recorded using the following: (microphone: Sennheiser ME66 condenser shotgun, recorder: Tascam DR100 MkII DAT, accuracy: 24 bit resolution, sampling rate: 44.1 kHz) (see Chapter 3 for details). The DAT files were transferred to a PC (Dell) to analyse the vocalisations using Raven Pro: Interactive Sound Analysis Software (Version 1.5). Ithaca, NY: The Cornell Laboratory of Ornithology.

During recording, hens produced different call types, and four of those call types were used as stimuli for the survey. Hens produced two main call types in anticipation of rewards, the 'Food' call and the 'Fast cluck', and two other call types in non-reward contexts, the 'Whine' and the

'Gakel' call. In total, sixteen recordings were selected, one of each call type, from four hens. Eight of these calls were made in a rewarding context, and 8 were made in a non-rewarding context. Selection of recordings for use in the cross-sectional study was based on the quality of the recordings available, minimising background noise as much as possible. All playback stimuli were edited using Adobe Audition CC sound editing software (Adobe Systems 2013). In order to create a standardised 6s playback stimulus, the shorter calls (Food calls and Fast clucks) were looped. The average call lengths for each call type were as follows: Food call 0.89s; Fast cluck 0.88s; Whine 1.76s; Gakel call 3.77s. Samples were downsampled to 22.5 Khz, normalised to -26dB RMS, and saved as wav files, before being converted to mp3 files for use in the survey.

5.3.1.3 Acoustic analyses

Spectrograms of recordings were generated using Raven Pro 1.5 (Cornell Laboratory of Ornithology; 1792-sample Hann window, 35.4Hz filter bandwidth, 5.38 Hz frequency resolution (grid spacing), discrete Fourier transform (DFT) size of 8192 samples and time grid hop size 200 samples (88.8% overlap)). The peak frequency (Hz), call duration (s) and 90% Bandwidth (Hz) was measured for each call used in the survey, by creating a box around each syllable (defined as a continuous impression in time on the spectrogram). A call is defined as a clearly discernible sequence (both audibly and visibly) of syllables grouped together, and measurements were averaged across calls. Peak Frequency represents the frequency (Hz) at which the maximum power occurs within the fundamental frequency (*F0*) component. Call duration is a measure of time (s) from the beginning of the first syllable in a sequence to the end of the last syllable in a sequence. 90% Bandwidth is the difference between the 5% and the 95% frequencies (Hz). All three of these parameters have been demonstrated to vary according to differences in arousal or valence in some animals (Briefer, 2012).

5.3.2 Cross-sectional study on human recognition of chicken calls

5.3.2.1 Study design and participant selection

Participants in this study were recruited using a 'virtual snowballing' technique which involved requesting personal and professional contacts of the research team (by email, or through Twitter or Facebook) to complete an online questionnaire. The initial message (email, tweet and facebook post) asked contacts to forward a link to the online questionnaire to their personal and professional contacts. A direct email was sent to 24 personal contacts. A link to the online questionnaire was also posted in an article in the December 2017 issue of eChook, run by PoultryHub. Those who volunteered to participate clicked on a link in the message, tweet, or post, and were then redirected to the online questionnaire. The cross-sectional study was conducted between October 1st 2017 and February 1st 2018.

Sample size was calculated to estimate the proportion of participants that correctly identify the context in which a call was made. As the expected sample proportion was unknown, it was set to 50% to maximize the sample size. Using a 95% confidence interval, a precision of 7% and an estimated population of 1,000 of people viewing the survey, a sample size of 165 participants would have been sufficient.

The study, including the consent procedure, was approved by the University of Queensland Human Ethics Committee (#2016001225).

5.3.2.2 Questionnaire

The online questionnaire was accessed through the SurveyGizmo platform. On the first page of the questionnaire, participants were informed that they needed to be 18 years or older, that participation was voluntary, and that all responses would remain anonymous.

Before listening to any sounds, participants were informed that the study investigated how people perceive information contained within animal vocalisations. They were then told that they would hear a number of calls made by chickens. The questionnaire specified that it should be completed on a computer and not a smartphone, using headphones or speakers. Participants were asked to test their volume settings before beginning.

Following this, there was a short explanation about the scales that the participants would need to use to rate the calls by. The two scales used were an emotional scale representing the valence of the emotion (from high displeasure (negative) to high pleasure (positive)) and an arousal level (intensity) scale that represented how intensely hens were experiencing the emotion in terms of arousal (from low arousal to high arousal). The mid points of the scale represented a neutral rating (valence or arousal). The final introductory page asked participants to take 10-15 minutes to complete the survey, and that by continuing to the next page, they were consenting to take part in the survey, they could withdraw at any time, and that their response was anonymous and would be treated as confidential. By clicking a link on this page, participants could also access further information about the survey and the ethics clearance and contact.

The subsequent section in the questionnaire asked participants to listen to 16 individual sounds that were presented in a random order using the ‘Randomize Pages’ tool in SurveyGizmo. Participants had to rate each sound according to the level of valence and arousal they thought the chickens were experiencing, using a sliding scale from 0-100 (represented in the questionnaire by low to high). Participants were also asked whether the calls were made in a rewarding context or a ‘no-reward’ context.

The final section featured questions to gain information about participants’ experience with chickens, and demographic information, including age (18-24 yrs, 25-34 yrs, 35-44 yrs, 45-54 yrs, 55-64 yrs, 65 + yrs), gender (male, female), nationality (Europe, North America, South America, Australasia, Africa, Asia), education level (primary, secondary, certificate, diploma, undergraduate, postgraduate) and where participants currently lived (urban, suburban or rural). Participants were asked whether they had had experience with chickens in the following five categories a) working in the industry, b) scientific research with live chickens, b) keeping chickens at home, c) interacting with chickens outside home or the workplace, or d) any other experience with chickens. Subsequently, participants were asked how many years of experience they had in each category (none, up to 1 year, 2-5 years, 6-10 years, more than 10 years), and about their current rate of contact with chickens (none, once or twice a year, once or twice a month, once or twice a week, or several times a week). Finally, they were asked whether they currently owned a pet, and whether they had owned a pet during their childhood.

5.3.2.3 Participants

A total of 351 participants accessed the online survey. Those who did not complete the survey were excluded and 194 complete responses were analysed. Each participant evaluated 16 chicken calls. Thirty seven percent of participants were aged between 18 and 34 years old (n=72), with 46% aged between 35 and 54 years (n=89), and 17% aged over 55 (n=33). Seventy six percent of participants were female, and 52% percent originated from Europe, with 23% from Australasia, 13% from North America, 4.1% from South America and 2.1% from Africa. Thirty percent of participants lived in urban and rural environments, with 40% living in suburban areas. Thirty eight participants had worked in the chicken industry, with 37 participants involved now, or in the past, in scientific research with chickens. There were 111 participants who keep, or had kept, chickens, and a total of 102 participants had interacted with chickens outside their home or workplace.

5.3.2.4 Statistical analyses

Data from the arousal and valence scales were transformed into numeric values from 1 to 100 and the correlations between these mean valence and arousal ratings were explored for the four call types. The correlation between the acoustic variables 'Peak Frequency', 'Call duration', '90% Bandwidth' and the valence and arousal ratings made by participants was examined. As the residuals for acoustic variables were not normally distributed, the non-parametric Kendall's tau correlation coefficient was used to describe this relationship.

Subsequently, the associations between levels of experience with chickens and demographic variables of participants on the valence and arousal ratings, as well as assignment of calls to the correct context, were analysed. The demographic variables 'Age', 'Education', as well as respondents' length of experience within each category of experience with chickens, were reclassified. Age categories were condensed into three age groups: 18-34 yrs, 35-54 yrs and 55+ yrs. Education levels were condensed into pre-university, undergraduate and postgraduate, and levels of experience with chickens were collapsed into 'none', 'up to 1 yr', '2-5 yrs' and 'over 5 yrs'.

Linear mixed models (LMM) were developed to assess the strength of associations between demographic factors, or level of experience with chickens, and the valence and arousal ratings provided by participants. These analyses were repeated to examine the effects of these factors on the assignment of calls to the correct context (rewarding or non-rewarding) using a generalized linear mixed model (GLMM). Correct context assignments were denoted by a 1, and incorrect by a 0, and the logit link function was used to model the probability of rating the calls in the correct context. As both valence and arousal ratings were continuous and normally distributed variables, a Gaussian distribution was appropriate. Maximum likelihood estimation was used for the linear mixed models, while the Gauss–Hermite quadrature approximation was utilized for the mixed-effects logistic regression. All models were constructed using the lme4 package (Bates et al. 2015) in R (R Core Team 2017).

To account for the clustering of measurements within participants, a participant identity number was included as a random effect in the models. The correlation of measurements within participants was described by the intraclass-correlation coefficient. The identity of the four different chickens was included as a fixed effect in all models.

Predictors showing a significant effect (*P*<0.05) on each of the three dependent variables (Valence rating, Arousal rating, Correct context assignment) were included in the final models. Models were compared using Akaike Information Criterion (AIC) scores and likelihood ratio tests to detect if any models fitted significantly better than the others. Wald tests were used to evaluate the overall significance of variables with more than two levels. Coefficients of the mixed-effects logistic regression were converted into odds ratios and presented with their 95% confidence intervals.

Table 5.1 Comparison of the different mixed effects models for Valence, Arousal and Correct Context

Parameter	Fixed variables in model	Comparison	*df*	AIC	χ2	χ2 *df*	*P*
Valence	Bird ID + Call*Age*Origin		41	28097			
	Bird ID + Call+Age+Origin	1 vs 2	13	28103	61.338	28	<0.001
	Bird ID + Call*Age	2 vs 3	17	**28090**	20.494	4	<0.001
	Bird ID + Call+Age	3 vs 4	11	28100	21.711	6	0.001
Arousal							
	Bird ID + Call*Age*Other		29	27547			
	Bird ID + Call+Age+Other	1 vs 2	12	27586	73.09	17	<0.001
	Bird ID + Call*Age	2 vs 3	17	**27543**	52.44	5	<0.001
	Bird ID + Call+Age	3 vs 4	11	27589	58.20	6	<0.001
Correct Context							
	Bird ID + Call*Age*Education		40	3739			
	Bird ID + Call+Age+Education	1 vs 2	12	**3709**	26.25	28	NS
	Bird ID + Call*Age	2 vs 3	16	3713	3.97	4	NS
	Bird ID + Call+Age	3 vs 4	10	3711	9.56	6	NS

Data includes model degrees of freedom (*df*), Akaike information criterion (AIC), χ2, χ2 degrees of freedom (χ2 *df*) and *P* values. NS indicates that the model is not significantly improved from the previous model. Lines in bold indicate the model with the lowest AIC score.

To establish whether the random effect was required in the model, I tested whether the random effect ‘participant identification’ was significant. Two intercept-only models were created, with and without the random effect and fitted with maximum likelihood. These were compared using AIC scores and likelihood ratio tests. The assumptions of normal distribution, linearity and homoscedasticity were checked by visual inspection of the residual plots (Petrie & Sabin 2013). Mean predictions were also checked against observed data to ensure they did not deviate, as deviation would indicate a poorly fitting model. Models were tested for overdispersion using the function ‘dispersion_glmer’ (1.07) in R (R Core Team 2017). The best fitting models, according to AIC scores, are detailed for each response variable in Table 5.1.

The residuals and random effects looked smooth and normal. Caterpillar plots were used to visualize the random effect estimates for 'participants 'on Valence / Arousal / Correct context with 95% confidence intervals, and are shown in the supplementary material (Figures 5.6 – 5.8).

5.4 Results

5.4.1 Valence and arousal ratings

Participants rated reward-related calls to be of high valence (high pleasure) but low arousal (low intensity). Gakel calls (a non-reward call) were rated as representing low valence and high arousal, while the Whine (the other non-reward call) was judged as representing neutral valence and arousal levels. Correlations between valence and arousal ratings are shown in Figure 5.1.

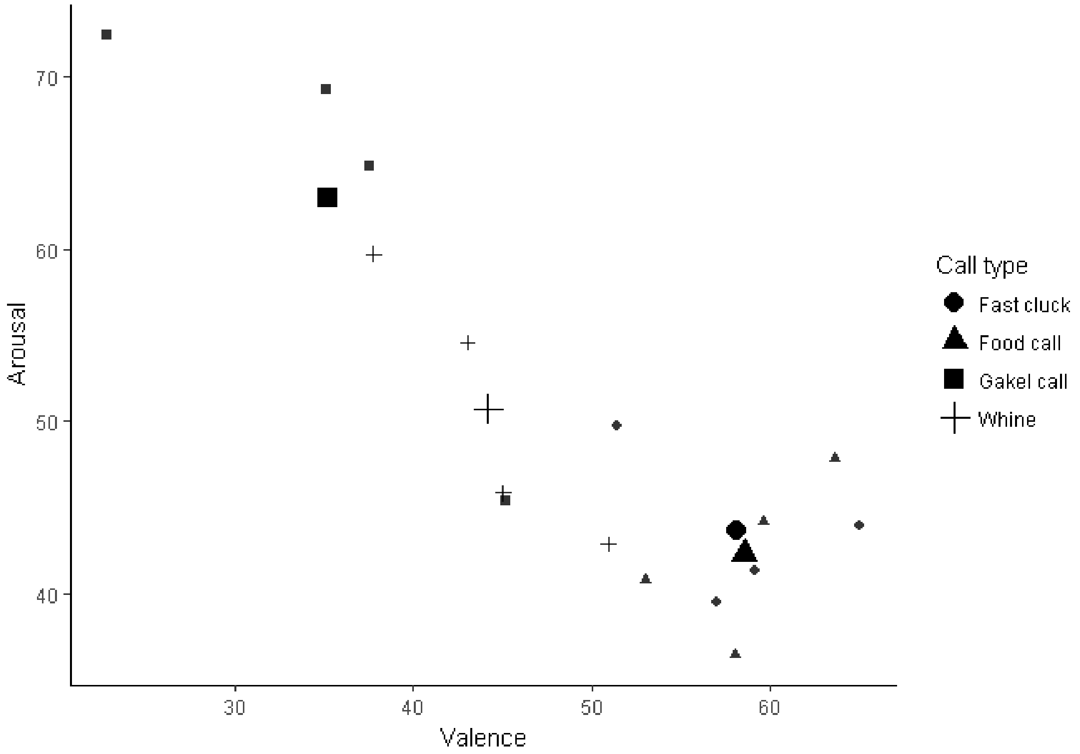

Figure 5.1 Valence and arousal ratings provided by participants for 16 chicken calls (smaller shapes). Larger shapes indicate the mean rating for each call type. Food calls and Fast clucks are reward-related calls. Gakel calls and and Whines are non-reward calls

5.4.2 Effect of acoustic variables on valence and intensity ratings

The acoustic structure of calls influenced how participants judged what chickens were experiencing when they produced the calls. Shorter calls were rated as representing higher pleasure and lower arousal (Figure 5.2; see Table 5.2 for correlation coefficients).

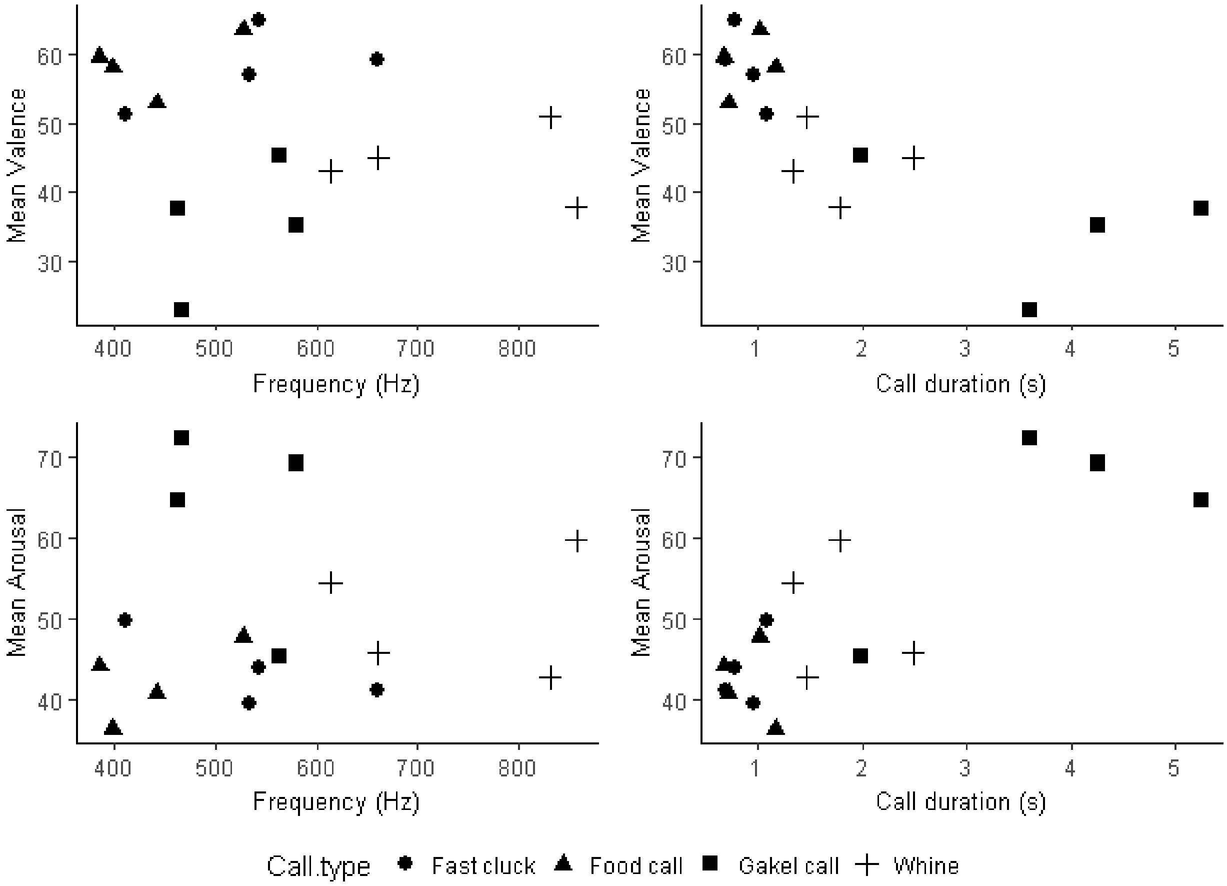

Figure 5.2 Correlations between peak frequency / call duration and mean valence and arousal ratings for four call types

The peak frequency of calls also affected the valence and arousal ratings, with lower frequency calls rated as higher valence and lower arousal, but these ratings did not differentiate between reward and non-reward related calls. Whines were of a higher frequency than the other call types, but were rated as neutral (around the mid points of about 50 on the 0-100 scale) in terms of arousal and valence (Fig. 5.1 and Fig. 5.2). The bandwidth of calls affected arousal ratings with calls, with a wider bandwidth being rated as representing a lower arousal level than other calls (Table 5.2).

Table 5.2 Kendall's tau-b results between acoustic variables and valence and arousal ratings

		Peak Frequency		Call Duration		90% Bandwidth	
		tau-b	*P* value	tau-b	*P* value	tau-b	*P* value
Valence rating	Food call	0.03	0.23	0.02	0.53	**0.15**	**<0.001**
	Fast cluck	**0.12**	**<0.001**	**-0.12**	**<0.001**	**-0.06**	**0.04**
	Whine	-0.03	0.23	-0.02	0.36	-0.05	0.07
	Gakel	**0.06**	**0.04**	-0.05	0.05	**0.13**	**<0.001**
Arousalrating	Food call	**0.06**	**0.02**	**-0.06**	**0.02**	0.05	0.06
	Fast cluck	**-0.08**	**0.005**	**0.08**	**<0.01**	**-0.07**	**0.01**
	Whine	0.04	0.10	-0.03	0.22	**0.11**	**<0.001**
	Gakel	-0.04	0.12	**0.19**	**<0.001**	**-0.23**	**<0.001**

Correlation coefficients with associated *P* values are reported. Figures in bold represent significant correlations.

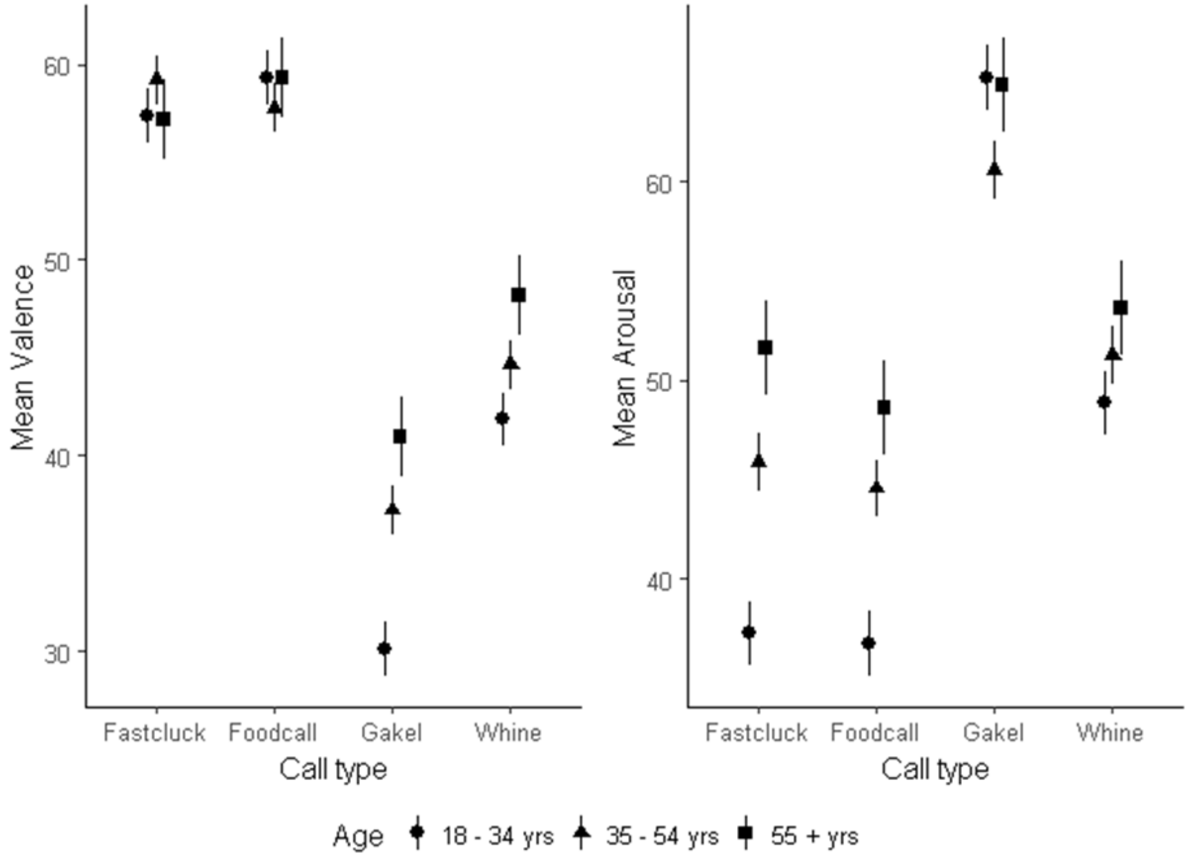

Figure 5.3 Mean valence and arousal ratings with 95% confidence limits for each call type by age group.

5.4.3 Effect of participant characteristics on valence and arousal ratings

Age significantly influenced the evaluations of valence and intensity of the calls, depending on the call type. Older participants (55 years plus) rated the valence of the non-reward calls (Whines & Gakel calls) as representing higher pleasure than people in the 18-34 year age group did, while the valence rating for reward calls (Food calls and Fast clucks) was similar across age groups (Fig.5.3, Table 5.3). In contrast, participants over 55 years old rated the arousal levels of the reward-related calls (Food calls and Fast clucks) as representing higher arousal than people aged between 18-34 years did, while the arousal ratings for non-reward calls were similar across age groups (Fig.5.3 Table 5.4).

Table 5.3 Final mixed-effects, multivariable linear model of participant factors associated with valence ratings of chicken calls

Variable	Categories	Estimate	SE	*t*	Pr(>|t|)
Call type	*Fastcluck*	Reference			
	Foodcall	1.934	1.824	1.1	0.29
	Gakel	-27.215	1.824	-14.9	<0.001
	Whine	-15.500	1.824	-8.5	<0.001
Age	*18-34 years*	Reference			
	35-54 years	1.851	1.858	1.0	0.32
	>55 years	-0.159	2.464	-0.1	0.95
Call * Age Interaction	*Fast cluck: Age 18 - 34 yrs*	Reference			
	Food call: Age 35 - 54 yrs	-3.384	2.453	-1.4	0.17
	Gakel: Age 35 - 54 yrs	5.221	2.453	2.1	0.03
	Whine: Age 35 - 54 yrs	0.955	2.453	0.4	0.70
	Food call: Age 55 + yrs	0.187	3.254	0.1	0.95
	Gakel: Age 55 + yrs	10.996	3.254	3.4	<0.001
	Whine: Age 55 + yrs	6.508	3.254	2.0	0.05
Bird ID	*Bird 1*	Reference			
	Bird 2	4.829	1.111	4.3	<0.001
	Bird 3	4.530	1.111	4.1	<0.001
	Bird 4	-1.215	1.111	-1.1	0.27
Intercept		55.305	1.540	35.9	<0.001

Random effect	Variance	SE	95% CI
Participant	17.55	4.89	10.17, 30.29
Residual ICC	0.04	0.01	0.02, 0.06

P values for Tukey multiple comparisons of Call*Age

	Foodcall			Gakel			Whine		
Fastcluck	18-34	34-54	55+	18-34	34-54	55+	18-34	34-54	55+
18-34	0.996	1	0.9997	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.01**
34-54		0.9993	1		**<0.001**	**<0.001**		**<0.001**	**<0.01**
55+			0.9998			**<0.001**			**0.04**

'Estimate' refers to the estimates of the mean of the dependent variable in the model. Estimates show the difference from the intercept. ICC = intraclass correlation coefficient. SE = Standard error. CI = Confidence interval

Table 5.4 Final mixed-effects, multivariable linear model of participant factors associated with arousal ratings of chicken calls

Variable	Categories	Estimate	SE	*t*	Pr(>|t|)
Call type	*Fastcluck*	Reference			
	Foodcall	-0.50	1.611	-0.31	0.75
	Gakel	27.98	1.611	17.37	<0.001
	Whine	11.61	1.611	7.21	<0.001
Age	*18-34 years*	Reference			
	35-54 years	8.65	2.173	3.98	<0.001
	>55 years	14.38	2.882	4.99	<0.001
Call * Age Interaction	*Fast cluck: Age 18 - 34 yrs*	Reference			
	Food call: Age 35 - 54 yrs	-0.84	2.167	-0.39	0.70
	Gakel: Age 35 - 54 yrs	-13.34	2.167	-6.16	<0.001
	Whine: Age 35 - 54 yrs	-6.24	2.167	-2.88	0.004
	Food call: Age 55 + yrs	-2.52	2.874	-0.88	0.38
	Gakel: Age 55 + yrs	-14.76	2.874	-5.14	<0.001
	Whine: Age 55 + yrs	-9.61	2.874	-3.34	<0.001
Bird ID	*Bird 1*	Reference			
	Bird 2	-6.96	0.981	-7.10	<0.001
	Bird 3	-14.52	0.981	-14.79	<0.001
	Bird 4	-5.47	0.981	-5.58	<0.001
Intercept		43.97	1.724	25.51	<0.001

Random effect	Variance	SE	95% CI
Participant	92.78	11.99	72.02, 119.53
Residual ICC	0.19	0.02	0.15, 0.23

P values for Tukey multiple comparisons of Call*Age

	Foodcall			Gakel			Whine		
Fastcluck	18-34	34-54	55+	18-34	34-54	55+	18-34	34-54	55+
18-34	1	0.040	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
34-54		0.999	0.998		**<0.001**	**<0.001**		**0.012**	0.197
55+			0.983			**<0.001**			**1.00**

'Estimate' refers to the estimates of the mean of the dependent variable in the model. Estimates show the difference from the intercept. ICC = intraclass correlation coefficient. SE = Standard error. CI = Confidence interval

Table 5.5: Final mixed-effects, multivariable logistic-regression model of participant factors associated with correctly identifying if chicken calls were made in a rewarding or non-rewarding context

Fixed effects	Categories	OR	95% CI	*P*	Wald test *P*
Call type	*Fastcluck*	Reference			<0.001
	Foodcall	1.27	1.01, 1.59	0.042	
	Gakel	1.24	0.99, 1.56	0.063	
	Whine	0.69	0.55, 085	<0.001	
Age	*18-34 years*	Reference			0.055
	35-54 years	0.90	0.71, 1.15	0.397	
	>55 years	0.68	0.50, 0.93	0.016	
Education	*Up to High School*	Reference			0.061
	Undergraduate	0.93	0.69, 1.25	0.622	
	Postgraduate	1.24	0.96, 1.61	0.105	
Bird ID	*Bird 1*	Reference			<0.001
	Bird 2	1.38	1.09, 1.73	0.006	
	Bird 3	0.57	0.46, 0.71	<0.001	
	Bird 4	1.36	1.08, 1.71	0.009	
Intercept		2.35	1.70, 3.27	<0.001	

Random effect	Variance	SE	95% CI
Participant	0.26	0.06	0.16, 0.41
Residual ICC	0.07	0.02	0.05, 0.11

Table includes odds ratios (OR), 95% confidence intervals (CI), test *P* values and Wald test *P* values. ICC = intraclass correlation coefficient. SE = Standard error. CI = Confidence interval

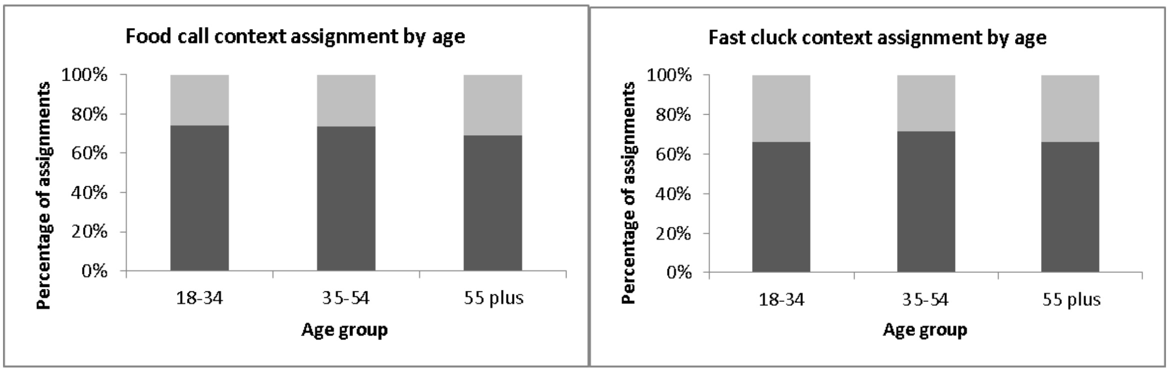

Figure 5.4 Food call and fast cluck context assignments by age group. Correct assignments are denoted by dark grey shading, and incorrect assignments by light grey shading.

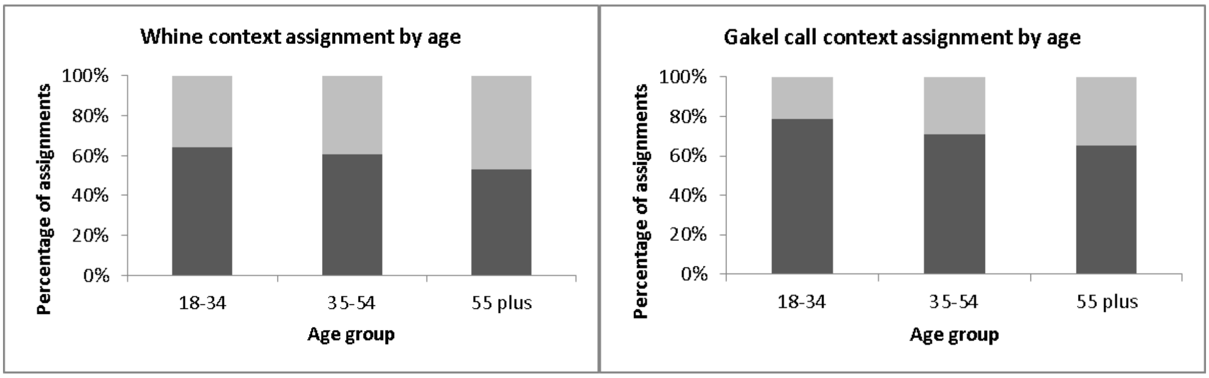

Figure 5.5 Whine and Gakel call context assignments by age group. Correct assignments are denoted by dark grey shading, and incorrect assignments by light grey shading.

5.4.4 Effect of demographic variables on context assignment

Participants were able to assign 69% of calls to their correct recording context. Participants performed better at categorising reward-related calls, with 71% of reward calls classified correctly, compared with 67% of non-reward calls. The probability that calls were correctly assigned to the correct context decreased with age (Figs.5.4 and 5.5), while education level was an important confounder in the final multivariable model (Table 5.5). Experience with chickens, and other demographic variables (as detailed in section 5.3.2.2), did not have an effect on assignment of calls to the correct context ($P \geq 0.10$).

5.5 Discussion

This study reveals that humans are able to identify whether chicken vocalisations were produced in rewarding or non-rewarding contexts. In addition, the findings demonstrate that listeners rely on basic acoustic cues to rate the valence and arousal levels of vocalisations. Call duration most accurately predicted participants' ability to identify the valence of the call, with shorter calls rated as representing chickens experiencing higher pleasure, and longer calls being perceived as conveying displeasure. Frequency parameters (peak frequency and bandwidth 90%) did not affect valence and arousal judgements as predicted, and, notably, ratings did not follow Morton's motivation-structural rules (Morton, 1977) with participants rating high frequency calls as representing neutral arousal levels. Older people were less adept at identifying the correct context, and more likely to attribute higher valence ratings to non-reward calls. Participants' experience with chickens had no effect on correct identification of context, or ratings of valence and arousal.

Investigations into whether humans can recognise semantic or emotional information in the vocalisations of other animals have revealed that humans are able to identify the contexts calls are made in by mammals such as dogs (Pongrácz et al. 2005; Molnár et al. 2010; Pongrácz et al. 2011;

Faragó et al. 2017), cats (Nicastro & Owren 2003) and pigs (Tallet et al. 2010; Maruščáková et al. 2015). This study adds to this growing field of research by showing that humans can identify the context chicken calls are recorded in, and perceive the emotional content of chicken calls. Significantly, it also reinforces the finding made by Filippi et al. (2017a) that the human ability to perceive the emotional content of vocalisations is not restricted to mammals, but extends to other taxa. Evidence from studies on humans and other mammals indicates that mammals share mechanisms for perception of emotion in the vocalisations of conspecifics and heterospecifics (Andics et al. 2014; Lingle & Riede 2014; Magrath et al. 2015; Albuquerque et al. 2016). In addition, humans are able to recognise emotional information contained in calls made by amphibians, reptiles, and birds, including hour-glass treefrogs *Dendropsophus ebraccatus*, alligators *Alligator mississippiensis*, black-capped chickadees *Poecile atricapillus*, ravens *Corvus corax*, giant pandas *Ailuropoda melanoleuca*, elephants *Loxodonta Africana*, Barbary macaques *Macaca Sylvanus* (Filippi et al. 2017a) and now domestic chickens. This finding has potentially important ramifications for the welfare of farmed chickens. Reward and non-reward related vocalisation could be considered reliable 'markers' of internal states, and automated detection of these could improve assessments of compromised or good welfare states within poultry management systems. One recent study found that affective computing-based acoustic feature sets (used to capture emotional information in human vocalisations) could be used to correctly classify either the context or the valence of dog barks (Cummins et al. 2017). As perception of the emotional content of calls appears to be shared across vertebrates, there is an opportunity to extend this system to poultry.

This study corroborates previous findings that call duration predicts the human ability to identify valence in heterospecific vocalisations. The results show that longer calls made by domestic chickens were perceived as more negative than shorter calls. This finding is consistent with other findings on the human perception of dog (Faragó et al. 2014) and pig (Maruščáková et al. 2015) vocalisations. A relationship between call duration and emotional valence has been found in dogs (Taylor et al. 2009), rats *Rattus norvegicus* (Brudzynski 2007), and pigs (Tallet et al. 2013), with longer calls associated with negative contexts. In other perceptual studies, different temporal measures, such as call rate, have been found to predict valence ratings, with a faster rate of dog growls (Taylor et al. 2009), and of human speech (Laukka et al. 2005) being associated with higher valence ratings. Similarly, faster music is often linked to happiness, with slower music associated with sadness (Juslin & Laukka 2003).

This study also shows that listeners rated longer calls as representing higher arousal levels. This result aligns with listeners' ratings of pig vocalisations (Maruščáková et al. 2015), although the effect of call duration could not be disentangled from that of intercall interval. In contrast, Faragó et al.(2014) found that longer dog vocalisations were rated as less intense than shorter ones. Evidence on vocal correlates of arousal delivers contrasting results in different species. While longer calls often express high arousal levels, as in three species of arboreal monkeys (Lemasson et al. 2012), kittens (Scheumann et al. 2012), bats *Megaderma lyra* (Bastian & Schmidt 2008) and infant African elephants (Stoeger et al. 2011), some species' calls do not follow this pattern. For example, arousal levels did not affect call duration of pig grunts or screams (Linhart et al. 2015). In addition, chimpanzee screams were found to be shorter during presumed high-arousal, aggressive contexts, compared with neutral contexts (Siebert & Parr 2003). However, longer calls cannot be construed as the only predictors of high arousal ratings of chicken calls. It is probable that listeners used a combination of acoustic cues, including call duration, to determine their judgements of arousal level, although testing listeners' ratings of more species' vocalisations will determine whether call duration is a reliable indicator of arousal for some animals.

Notably, this study shows that listeners were able to identify high negative arousal in chicken calls, but rated positively-valenced high arousal calls as expressing low arousal. Calls made by chickens in anticipation of rewards are assumed to reflect high, positively-valenced arousal, and analysis on concurrent behaviour supports this assumption (Chapter 2). This result bears a similarity to a recent study which found that humans identified assumed low arousal calls as expressing high arousal (Filippi et al. 2017b). The authors suggested that positively-valenced calls may be emitted in contexts which are not crucial to survival, whereas the ability to identify negative high-arousal calls may be adaptive for humans (and other animals) to be able to identify threats. An alternative suggestion is that the assumed levels of arousal in this study were incorrect, and participants correctly identified the level of arousal in the chickens.

It was found that peak frequency did not predict listeners' ratings of the arousal levels of chicken calls. Listeners rated high frequency calls (whines) as neutral for arousal level. High frequency calls are often associated with increased arousal (Briefer, 2012) and humans appear to rely on increases in fundamental frequency to rate calls as representing increased arousal in humans and other animals (Juslin & Laukka 2003; Pongrácz et al. 2006; Sauter et al. 2010a; Faragó et al. 2014; Maruščáková et al. 2015). In the case of chickens, however, a decrease in the peak frequency of a reward-related call was associated with an increase in arousal in anticipation of a highly prized reward (Chapter 3). This result is not replicated in other birds, although evidence on acoustic links to arousal in birds is poor. High aggression in blackbirds *Turdus merula* is correlated with an increase in the frequency of their twitter (Ripmeester et al. 2007) and increased stress in zebra finches *Taeniopygia guttata* elicits higher pitched calls (Perez et al. 2012). These results follow Morton's motivation structural rules (Morton, 1977). However, although frequency is a common predictor of arousal ratings in perceptual studies, other parameters are also relied upon. In Filippi's (2017a) perceptual study on different taxa, humans used the spectral centre of gravity ratio to identify increased arousal in calls of the common raven, African bush elephant, giant panda, pig and Barbary macaque. Other predictors of ratings of increased arousal were harmonic to noise ratio, ratio of duration, and fundamental frequency ratio (Filippi et al. 2017a).

In this study, older people's ratings of the arousal levels of reward-related calls were higher than younger people's ratings. These ratings, however, were still low to mid arousal, while the assumed arousal level of the chickens, when these calls were produced, was high. In addition, older people rated the valence of non-reward calls as representing higher pleasure compared with ratings made by younger people. Crucially, older people were less adept at identifying the correct context calls were made in, although this result is more marked in relation to the non-reward-related whine made by chickens. This difference in age groups may be due to hearing ability. Whines are wavering, high frequency tonal calls, which may not be perceived as easily as the harsh Gakel calls. In another study, younger listeners were better at identifying the context of human infant vocalisations correctly, but this was a weak effect (Lindová et al. 2015). Age improved participants' ability to interpret macaque *Macaca arctoides* vocalisations, although this related to differences between young children and older children / adults, with children aged 9-10 performing as well as adults (Linnankoski et al. 1994).

The results show that there was a large variability in the ratings of whines. This may reflect participants' difficulty in categorising low arousal calls. Whines are wavering, high frequency tonal calls, with a wide frequency range, and are said to express mild disturbance or frustration in hens (Collias 1987). They are markedly different in acoustic structure from Gakel calls, which express high arousal in frustration or non-reward contexts (Zimmerman & Koene 1998; Zimmerman et al. 2000; McGrath et al. 2017). High arousal appears to be easier to decode by humans. In perceptual studies on humans, portrayals of emotions with strong intensity (arousal levels) were decoded with significantly higher accuracy than portrayals with weak intensity (Juslin & Laukka 2001). In addition, conscious evaluations of the calls of non-human animals do not always match activation of human brain areas in response to these calls. Belin et al. (2008) found that humans were unable to recognise the valence of animal vocalisations using a behavioural rating task. However, concurrent fMRI results showed that neural mechanisms within participants' brains responded to the valence of animal vocalisations at an unconscious level, and showed different responses according to whether calls were positively or negatively valenced. The authors interpreted this activation as an evolutionary retained mechanism, differentiating the valence of human and animal vocalisations at an unconscious level, which was obscured by cognitive processes when rating vocalisations.

Notably, experience did not have any effect on valence and arousal ratings or classification of calls into context. Scheumann et al. (2014) concluded that humans rely more on experience-dependent cognitive mechanisms than induced emotional state or acoustic cues to recognise the emotional content of vocalisations. However, some perceptual studies provide contrasting evidence (Linnankoski et al. 1994; Molnár et al. 2010; Pongrácz et al. 2011). This study's findings strengthen the evidence that acoustic cues are salient predictors of human recognition of the emotional content of non-human animal vocalisations. One limitation of this study is that only three acoustic measures were used, and two of these did not accurately predict arousal ratings. Future studies should expand on this study by testing the effect of other acoustic cues, especially spectral centre of gravity, and harmonic to noise ratio, as well as other frequency and formant-related parameters (Briefer, 2012; Filippi et al., 2017a).

A further limitation of this study is that the introduction to the online survey named some emotions that chickens may experience (e.g fear, depression, excitement, frustration). This could have led some participants to have a preconceived idea of how to rate or categorise the calls they heard. Future studies could avoid naming potential emotional states to see whether this has an impact on participants' interpretation of the calls.

This study provides evidence that humans decode the emotional content of chicken vocalisations using acoustic cues. This research has potential implications for identification of welfare states of chickens in farmed situations. Future studies should examine the effect of more acoustic measures on perception of arousal and valence in chicken calls.

5.6 Supplementary material

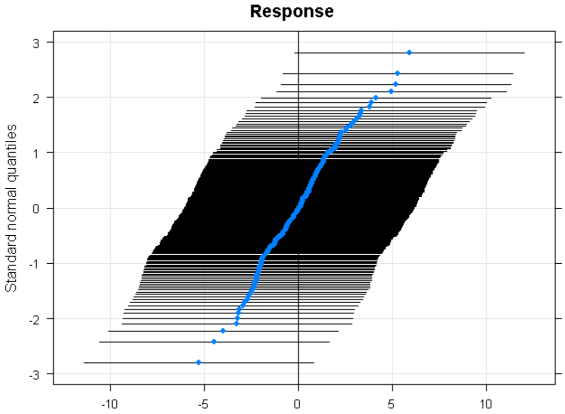

Figure 5.6 Caterpillar plot of random-effects of ‘participant’ on Valence

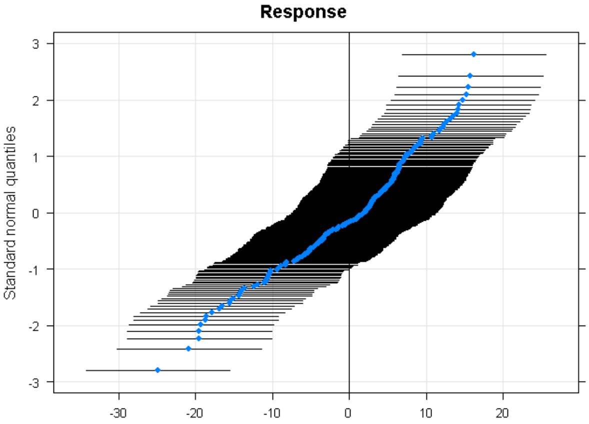

Figure 5.7 Caterpillar plot of random-effects of ‘participant’ on Arousal

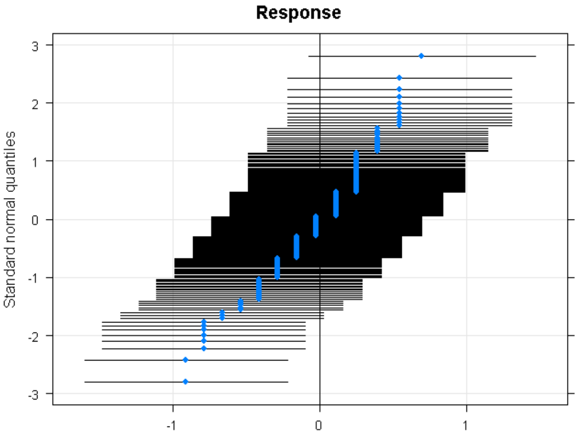

Figure 5.8 Caterpillar plot of random-effects of ‘participant’ on Correct context.

Questions used in online questionnaire

Do humans recognise the emotional content of chicken vocalisations?

Introduction

Hello and welcome to this survey! We are studying how people perceive information contained within animal vocalisations, and as part of this survey you will hear a number of calls made by chickens. Please ensure that you complete this survey on a computer (not a smartphone) using either headphones or external speakers.

Please click on this sound to test your volume settings

The study focuses on the emotional properties of chicken vocalisations. We ask you to rate calls on two scales:

1. Calls can be rated on a scale of valence.
   Negative valence (the chicken is experiencing negative emotions, such as frustration, fear or depression).
   Positive valence (the chicken is feeling positive emotions such as excitement or contentment).

2. Calls can also be rated on a scale of intensity.
   Intensity is a reflection of arousal in an animal. Arousal can be either negative (fear = high negative arousal, depression = low negative arousal or positive (excitement = high positive arousal, content = low positive arousal)

   High intensity - a chicken is experiencing high arousal. (e.g. excitement or fear)
   Low intensity - a chicken is experiencing low arousal (e.g. content or depressed)
   NB: Sadness and happiness are examples of human emotions that haven't necessarily been proven to exist in animals.

About the survey

Please ensure you are not disturbed during the 10-15 minutes it will take to complete this survey. Clicking next at the bottom of this page will confirm the following:

I understand that by continuing with this survey:

- I consent to take part in the survey
- I am aware that I am free to withdraw from the survey at any time
- I understand that my response will remain anonymous
- I am aware that all information provided by me will be treated as confidential by the research team

Your task is to rate every sound you hear for how you believe the chicken is feeling when it makes the call. To rate a sound, simply click on the screen with the mouse, and drag the marker along the scale to where you think best describes the call you have heard. Don't think too long, decide as fast as you can!

Call evaluation questions (repeated for call recordings 1-16)

Please listen to the first call and use the sliding scales below to rate this call

1. Please rate how you think the chicken feels when making the above call.
2. Please rate the intensity of the call you hear.
3. Please click on the option that best describes the context you think this call is made in.

Reward - the chicken is anticipating a reward
No reward - the chicken is experiencing frustration

Demographics

In the final part of the questionnaire, we would like you to complete some details about you and your experience with chickens.

1. **What is your age?**
    - 18-24 yrs
    - 25-34 yrs
    - 35-44 yrs
    - 45-54 yrs
    - 55-64 yrs
    - 65 + yrs
2. **Are you male or female?**
    - Female
    - Male
3. **Where do you originate from**?
    - Europe
    - North America
    - South America
    - Africa
    - Australasia
    - Asia
4. **What type of environment do you live in (spend the majority of time in)**
    - Urban
    - Suburban
    - Rural

5. **What is the highest level of education you have completed?**

- Primary
- Secondary
- Certificate
- Diploma
- Undergraduate
- Postgraduate

6. **How much interaction do you have, or have you had, with live chickens?**

- Never
- Once or twice in my life
- Once or twice a year
- Every month
- Almost every day (several times a week)

7. **How would you classify yourself in terms of your contact with live chickens and hens**

Type of experience/Years of that experience	Never	Up to 1 year	2-5	6-10	>10	Comments/Details
I worked/work in the poultry industry						
I am/was involved in scientific research with chickens						
I keep/kept chickens at home						
I have/had only interacted with chickens in places outside my home or workplace						
Other (specify)						

8. **What is your current level of contact with live chickens?**

- Several times a week
- Once or twice a week
- Once or twice a month
- Once or twice a year
- None

9. **Do you have a pet (dog, cat, horse etc.)?**

- Yes
- No

10. **Did you keep a pet during childhood?**

- Yes
- No

Thank you for taking our survey. Your response is very important to us.

Participant information sheet

Study: Human perception of the vocalisations of laying hens

The purpose of the study
The purpose of this study is to examine human perceptions of the vocalisations of laying hens. This study is being conducted by a PhD. student (Nicky McGrath) from the University of Queensland, under the supervision of Professor Clive Phillips in the School of Veterinary Science.

Participation and withdrawal
Participation in this study is completely voluntary and you are free to withdraw from this study at any time without prejudice or penalty. If you wish to withdraw, you may simply withdraw from the questionnaire. If you do withdraw from the study, the materials that you have completed to that point will be deleted and will not be included in the study.

What is involved
You will be asked to listen to hen calls, and indicate how you rate the valence of each call (from positive to negative). You will also be asked to rate the intensity of each call (from low to high). Subsequently you will be asked to answer questions about your previous experience with chickens. The questionnaire will also ask you for your rating of how much you like hens.
The proposed duration of participation should be no longer than 15 minutes.

Risks
Participation in this study should involve no physical or mental discomfort, and no risks beyond those of everyday living. If, however, you should find any question or procedure to be invasive or offensive, you are free to omit answering or participating in that aspect of the study.

Confidentiality and security of data
All data collected in this study will be confidential. Specifically, you will not be asked to provide your name or any other data that could identify you. The data will be seen only by the chief investigator and the research team and will be stored on a laptop that is not accessible to any individual but the chief investigator and her research team. The data from this study will only be used for research purposes.

Ethics Clearance and Contacts
This study has been cleared in accordance with the ethical review processes of the University of Queensland and within the guidelines of the National Statement on Ethical Conduct in Human Research. You are, of course, free to discuss your participation with project staff. You may contact Nicky McGrath directly on (0487) 748867, or by email at nicmcgrath@hotmail.com or Clive Phillips on (7) 5460 1158, or by email at c.phillips@uq.edu.au If you would like to speak to an officer of the University not involved in the study, you may contact the University of Queensland Ethics Officer, Dr Ruth Delaforce on (7) 3365 3924, e-mail: humanethics@research.uq.edu.au

If you would like to learn the outcome of the study in which you are participating, you can contact Nicky McGrath at the email above after December 2017, and she will send you an Abstract of the study and findings.

Thank you for your participation in this study.

Nicky McGrath

Chapter 6: General discussion and future directions

6.1 Discussion

The vocalisations of non-human animals encode information in their acoustic structure, and this information may be used by both conspecifics and heterospecifics to determine their response. The emotional content of human speech and non-verbal expressions has been widely studied (Bachorowski, 1999; Banse and Scherer, 1996; Juslin and Laukka, 2001; Laukka et al., 2005; Scherer et al., 2001), and a growing body of research is decoding how non-human animals express their emotional states vocally (e.g. Gogoleva et al., 2010; Soltis et al., 2011; Tallet et al., 2013; Yeon et al., 2011; Yin and McCowan, 2004). From a welfare perspective, then, animal vocalisations may serve as 'markers' of their motivational or emotional state. This project has explored the possibility that chickens convey how they experience rewarding environments acoustically (vocally), and that humans are able to decode these acoustic cues.

The anticipation of rewards is known to activate pleasure-based areas of the brain. Animals have long been used as models for how brains process reward stimuli, and findings suggest that various areas of the brain are activated in anticipation of rewards. These include the striatum, orbitofrontal cortex (Schultz et al., 2000), thalamus (Komura et al., 2001) and anterior cingulate (Bussey et al., 1997). This brain activation is apparently 'motivation-dependent', and stronger activation of reward-processing mechanisms is shown in response to rewards with high incentive values (Elliott et al., 2003; Kirsch et al., 2003). Behaviours associated with the anticipation of rewards have been studied across a variety of animals, including rats *Rattus norvegicus* (van den Bos et al., 2003), horses *Equus caballus* (Peters et al., 2012), dolphins *Tursiops truncatus* (Clegg and Delfour, 2018) and chickens (Moe et al., 2014; Zimmerman et al., 2011). In farmed animals, these behaviours could be used as part of a welfare assessment, to establish whether animals under human management are experiencing rewarding environments. However, identifying or recording reward-related vocalisations may also offer a convenient method to assess welfare, or to corroborate other behavioural observations (Manteuffel et al., 2004).

In order to identify and characterise reward-related vocalisations made by chickens, the first phase of this research investigated the behaviour of chickens in anticipation of rewards, and in situations when a lack of reward was signalled, or there was no signal and no reward (Chapter 2). The behavioural responses documented in this study provided the basis for comparing vocalisations elicited in anticipation of rewards. Characterising the vocalisations made in anticipation of rewards formed the second study (Chapter 3). This part of the project also investigated differences in acoustic structure produced by different reward types. If there were acoustic differences, they may convey referential information about the different rewards to others in the flock. The third study (Chapter 4) explored this possibility using a playback paradigm to investigate whether ‘reward’ calls, produced in the context of a food reward and a dustbath reward, elicited differential behavioural responses in conspecifics. The culmination of this project was an investigation into whether humans could identify whether chicken vocalisations were made in a rewarding or non-rewarding context, and how they perceived the valence and arousal levels represented in these vocalisations (Chapter 5). This provided an insight into whether reward-related vocalisations produced by chickens could indeed be used as ‘markers’ of welfare.

In order to validate the hypothesis that specific vocalisations represent a certain motivational state, it is essential to correlate vocalisations with other behavioural responses (Manteuffel et al., 2004). Chapter 2 details the results of the study examining the behaviour of chickens in anticipation of different reward types. Although the behaviour of chickens in anticipation of rewards has been documented in several previous studies (Kostal et al., 1992; Moe et al., 2014; Moe et al., 2013; Moe et al., 2011; Moe et al., 2009; Zimmerman et al., 2011), no study has examined anticipatory behaviour in the context of both food and non-food rewards. This study revealed that hens showed an increase in goal-directed activity, and transitions between behaviours, in anticipation of all reward types. This finding contradicts the outcomes of a previous study, which concluded that head movements were the most salient indicator of anticipatory behaviour in hens (Moe et al., 2011). The increase in activity also contrasts with the lack of activity shown in other studies (Moe et al., 2013; Moe et al., 2011; Moe et al., 2009). However, these differences appear to lie in the different experimental procedures. Hens showed goal-directed behaviour when they had been trained that a sound cue signalled a reward in a location they could see, and had to actively push against a door to reach. In contrast, hens showed increased head movements in response to a light signalling delivery of a reward into their home pen (Moe et al., 2013; Moe et al., 2011). In this case, hens were unable to see the location of the reward prior to delivery.

Hens also revealed how they ranked rewards through differential anticipatory behaviour in Chapter 2. Importantly, this study extends our knowledge of anticipatory behaviour in chickens. Cues signalling different rewards elicit quantitative, but not qualitative, differences in anticipatory behaviour, and therefore goal-directed activity is a general response to rewards. In addition, this finding emphasized that access to a dustbathing substrate is rewarding to chickens, and the cue signalling this reward appeared to elicit an increase in arousal. Behavioural responses indicated that hens ranked the dustbathing substrate as more attractive than the food rewards. This has clear implications for their welfare, and suggests that hens in barren environments with no access to a dustbathing substrate may experience compromised welfare. Future studies could expand on these findings, by inducing anticipation of other non-food rewards, or by using preference or motivation testing such as choice or demand tests. This would allow a comparison of how hens rank rewards such as perches, nest boxes, or items that enrich the environment of hens, such as toys or mirrors. This ranking could aid in prioritising environmental resources in managed poultry systems.

A primary aim of this project was to characterise vocalisations produced in anticipation of rewards. To date, no study has investigated whether chickens produce specific reward calls associated with both food and non-food rewards. As reward-related behaviour (including vocalisations) represents pleasure-based activation in the brain (Berridge, 1996), reward calls could be considered 'markers' of internal states (Boissy et al., 2007; Manteuffel et al., 2004). The experimental outcomes outlined in Chapter 3 reveal that hens produce a reward call when anticipating both food and non-food rewards. This call has previously been described as a 'food' call (Evans and Evans, 1999), and has been cited as a rigorous example of functional reference (Clay et al., 2012). However, the results in Chapter 3 contradict this claim by demonstrating that this call is made in the context of non-food rewards, and therefore may be more accurately described as a reward call. A lack of reward, both signalled and not signalled, also elicited consistent call types, which were acoustically very different from reward-related calls. 'Whines' and 'gakel' calls were produced in non-rewarding contexts, and appear to represent frustration in hens. This finding may have significant practical applications in terms of animal welfare. Vocalisations are increasingly being considered as convenient methods of assessing the welfare of animals (Meen et al., 2015; Schon et al., 2004; Vandermeulen et al., 2015; Zala et al., 2017). In addition, the emotional content of animal vocalisations may be detected using automated systems (Cummins et al., 2017). Detection of reward calls may contribute to welfare assessments in poultry management systems. Further work, using other types of rewards, will help to verify the functionality of reward calls in this context. Furthermore, there is scope for studies to simultaneously investigate vocal, behavioural and physiological indicators of arousal and valence in chickens. Multiple measures would help validate how anticipation of rewards affects the internal states of chickens.

This is the first study to show that hens' vocalisations may contain motivational information encoded in call types and call structure. Chapter 3 demonstrates that the peak frequency of the reward call made by hens varies according to the reward type. Specifically, reward calls made in anticipation of a dustbathing substrate have a lower peak frequency than those produced in anticipation of food rewards. Hence, reward calls appear to encode information about hens' motivational states. This result also corroborates findings from Chapter 2, which suggested that hens expressed differential behaviour according to the assumed incentive value of the rewards. Both the vocalisation and behavioural analyses found differences between the arousal states of the hens according to whether the reward was a dustbath or a food reward. Surprisingly, an increase in arousal produces calls with a lower peak frequency. The literature on vocal correlates of arousal suggests that increased arousal usually results in higher frequency vocalisations in mammals and birds (Briefer, 2012; Morton, 1977; Perez et al., 2012; Ripmeester et al., 2007). Hens' reward calls appear not to follow this pattern, and so future studies could elucidate whether a lower peak frequency is a predictor of increased arousal in hens across different call types. It is also possible that the hens' actual arousal states were different from those assumed following vocal and behavioural analysis. Future studies should therefore assess physiological indicators of arousal, such as heart rate, in concurrence with behavioural and vocal measures.

To establish whether referential information was contained in hens' reward calls, other hens were exposed to reward calls using a playback paradigm. Playback studies are used to substantiate hypotheses that calls are functionally referential. If calls do contain referential information, receivers should behave as if they have experienced the stimuli themselves (Evans, 1997; Macedonia and Evans, 1993; Marler et al., 1992). Hens were played calls produced in anticipation of mealworms or a dustbathing substrate. The results presented in Chapter 4 suggest that reward calls do not contain referential information about different types of reward. However, this outcome is in contrast with the results of a previous study, which found that hens fixated on, and pecked at, the ground in response to playback of 'mealworm' calls (Evans and Evans, 1999). This response was used as evidence that the food calls of chickens are functionally referential. In that study, however, the food calls used were those of male chickens. There may be slight acoustic differences between the calls made by cockerels and those made by hens, which have not been elucidated in previous studies on chicken vocalisations. This may explain the different responses in the playback study described in this thesis compared to the one conducted by Evans and Evans (1999). Male food calls function to attract females to the food source, and, as a result, this increases mating opportunities for males (Evans and Evans, 1999). Although the function of female food (or reward) calls is ostensibly to attract others to the food source (Clay et al., 2012), hens also give reward calls in isolation (Chapter 3). The differences in function and behaviour may result in acoustically different calls. In addition, hens used in the playback study showed a general lack of response, which may have masked any specific response to different reward-related calls. Hens used in the playback study were mature hens who had been housed in barren cages since birth. Their lack of response could have been conditioned as a result of experiencing an unstimulating environment over a long period of time. Therefore, the response of hens to playback of reward-related calls warrants further exploration. Future studies should investigate differences between the acoustic structure of, and responses to, male food calls and female reward calls. In addition, the use of younger hens from a more stimulating environment would help to validate whether referential information relating to reward-type is contained within reward calls. Furthermore, other methods to assess hens’ responses should be considered; for example, assessment of eye temperature changes, and heart rate variation may provide further information on hens’ emotional responses to playback of these calls.

The final study in this project explored whether humans can identify chicken calls made in rewarding or non-rewarding contexts, and whether they can decode the emotional content of the calls. Humans are able to correctly classify calls made by pigs (Maruščáková et al., 2015; Tallet et al., 2010), cats (Nicastro and Owren, 2003) and dogs (Faragó et al., 2017; Molnár et al., 2010; Pongrácz et al., 2005) according to their context of production. At times, experience or familiarity with the vocalising animal may contribute to this ability (Nicastro and Owren, 2003; Tallet et al., 2010). Here, it was hypothesised that participants with more experience with chickens would be more successful at recognising the context of chicken calls. Four different call types were used: the acoustically similar reward calls and ‘fast clucks’ which are produced in rewarding contexts, and the whine and gakel call, which are produced in non-rewarding contexts. Notably, the results presented in Chapter 5 show that humans are able to identify whether chicken calls are made in rewarding or non-rewarding contexts. Experience with chickens did not influence this ability. Age was a modifying factor, with people aged over 55 significantly less likely to place a call in its correct context than listeners aged between 18 and 34. Significantly, this outcome reinforces the finding made by Filippi et al. (2017a), that the human ability to perceive the emotional content of vocalisations is not restricted to mammals, but extends to other taxa. Research shows that humans are able to perceive the context of, or emotional content of, calls made by mammals (e.g. Faragó et al., 2017; Linnankoski et al., 1994; Tallet et al., 2010). However, this finding has recently been extended to other animals such as amphibians, reptiles, and birds (Filippi et al., 2017a). This study has shown that this finding also applies to the vocalisations of domestic chickens. The ramifications of this outcome are also significant in terms of animal welfare. This project has demonstrated that chickens produced reward calls in anticipation of different types of reward. In addition, it has found that humans are able to recognise calls made in rewarding contexts. Applying these results to practical on-farm situations may mean that reward-related vocalisations could be used as ‘markers’ of welfare. Furthermore, the assessment of hen vocalisations may strengthen the pool of non-invasive techniques available in farm animal welfare assessments.

To establish whether humans could detect the emotional content of vocalisations, this study asked participants to rate the valence of calls on a sliding scale from high displeasure to high pleasure. Participants were also asked to rate the calls on a scale of intensity, which relates to the arousal levels the chickens were experiencing when producing the calls. It was hypothesised that acoustic cues would predict the judgements of arousal and valence. The outcomes presented in Chapter 5 demonstrate that acoustic cues correlated with listener's judgements of valence and arousal, with longer call durations predicting ratings of higher displeasure and higher arousal levels. Acoustic correlates of valence are poorly documented in the literature, and the only measure that appears to consistently change with differences in valence is call duration. Therefore, the results presented here strengthen this scant knowledge base.

Surprisingly, the peak frequency of calls did not predict judgements of arousal level. Studies have found that arousal, in contrast to valence, is reliably correlated with frequency-related acoustic parameters (Briefer, 2012; Faragó et al., 2014; Maruščáková et al., 2015; Morton, 1977). Peak frequency is also cited as one of the most common vocal correlates of arousal (Briefer, 2012). Furthermore, in the majority of human perception studies on the acoustic correlates of arousal, listeners mainly rely on increases in fundamental frequency to rate both human and heterospecific vocalisations as expressing heightened levels of arousal (Filippi et al., 2017a). However, although frequency is a common predictor of arousal ratings in perceptual studies, other parameters are also relied upon by listeners. These include spectral centre of gravity ratio, harmonic to noise ratio, ratio of duration and fundamental frequency ratio (Filippi et al., 2017a). Future studies on the human perception of the chicken vocalisations should, therefore, include the above acoustic measures as possible predictors of arousal ratings.

This study also highlighted the difficulty human listeners experience in identifying the arousal level of assumed positively valenced calls. Participants rated positively-valenced high arousal calls, produced in anticipation of prized rewards, as expressing low arousal. Similarly, in another study, humans identified assumed positive low arousal calls as expressing high arousal. One explanation could be that this is because positively valenced calls may be emitted in contexts that do not impact survival (Filippi et al., 2017b). The ability to perceive the arousal levels in negatively valenced calls may therefore be adaptive. In addition, whines, which are associated with mild disturbance and frustration in hens (Collias, 1987), received extremely variable ratings, with most participants rating the calls as neutral in terms of arousal level. This finding corresponds with findings made in perceptual studies of human vocal expressions. These revealed that portrayals of intense emotions were decoded with significantly higher accuracy than portrayals with weak intensity (Juslin & Laukka 2001).

Crucially, this study demonstrated that experience with chickens does not influence the human ability to decode the emotional content of chicken vocalisations, or to recognise their context of production. This is an important finding from a welfare perspective, as it means that decoding chicken vocalisations does not need to rely on potentially subjective assessments. Instead, after validating acoustic correlates of valence and arousal, vocalisations could be detected and decoded using automated systems that accurately detect acoustic cues. Further studies should invest in exploring the most salient acoustic correlates of arousal and valence across the specific call types that most accurately represent different welfare states in chickens.

6.2 Summary of main findings

1. Hens produce a 'reward' call in anticipation of both food and non-food rewards.
2. Variation in the acoustic structure of this call appears to relate to arousal levels.
3. Behavioural responses suggest that hens rank a dustbathing substrate as more desirable than food, under certain food deprivation conditions.
4. Augmented arousal in anticipation of a dustbathing substrate is reflected in increased frequency and duration of goal-directed behaviour, and also in a lower peak frequency of reward calls.
5. Hens respond to all sound cues with 'head movements'.
6. Reward calls don't appear to contain referential information, although further investigation is warranted.
7. Human listeners are able to identify whether calls are made in rewarding or non-rewarding contexts.
8. Humans use basic acoustic cues to rate the emotional content (valence and arousal levels) of chicken vocalisations.

6.3 Future Directions

Chickens are extremely vocal, and this is notable in large poultry systems. This provides an opportunity to test for associations between vocal output and welfare outcomes of hens. For example, Fontana et al. (2015) found that vocalisations provided an indicator of growth in broilers, while Moura et al. (2008) found that variation in acoustic parameters of chick calls correlated with thermal comfort. Victims of feather pecking produce more gakel calls and alarm calls when they experience frustration as a result of being unable to access a food reward (Rodenburg et al., 2005). Another study investigated the calling rates and acoustic parameters in feather pecking and non-feather pecking laying hen flocks, to establish whether there was a difference in the vocalisations of these two groups (Bright, 2008). The results of that study suggested that the rate of 'squawk' and total vocalisations was an indicator of feather pecking in farms. However, more work needs to be done to investigate the types of vocalisations associated with different welfare outcomes in poultry systems. Various contexts need to be tested, and investigations also need to examine the results in different strains of hens.

As demonstrated in this project, hens produce specific vocalisations in anticipation of rewards. This provides an opportunity to ask whether hens are experiencing their environments as rewarding, rather than focussing on whether they are suffering from compromised welfare. One tool that may help improve welfare is the automated detection of vocalisations. Different tools have successfully detected and classified the calls of elephants (Clemins et al., 2005), zebra finches *Taeniopygia guttata*, indigo buntings *Passerina cyanea* (Anderson et al., 1996) killer whales *Orcinus orca* (Brown and Miller, 2007), and pigs (Ferrari et al., 2008), among other animals. However, use of bioacoustic detection has to take into account the fact that individual animals may not be as vocal as others, and certain states, such as lethargy, induced by poor health, may inhibit vocal production (Manteuffel et al., 2004). In addition, detection systems need to reliably classify vocalisations and any variations in the vocalisations as a result of changes in the environment. The cost of such systems would need to be considered, as well as training of users such as farm owners and managers. Furthermore, reward vocalisations must be clearly defined in terms of acoustic parameters, so that detection systems can identify them amongst the background noise. Nevertheless, the assessment of welfare using vocalisations appears to be a promising tool, and automated systems to do this could improve the efficiency of welfare assessments.

List of references

Albuquerque, N, Guo, K, Wilkinson, A, Savalli, C, Otta, E & Mills, D 2016, 'Dogs recognize dog and human emotions', *Biology Letters*, vol. 12, no. 1

Altenmüller, E, Schmidt, S & Zimmermann, E 2013, *Evolution of emotional communication: From sounds in nonhuman mammals to speech and music in man*, Oxford University Press, Oxford.

Amsel, A 1992, *Frustration theory: An analysis of dispositional learning and memory*, Cambridge University Press, Cambridge, UK.

Anderson, C, Yngvesson, J, Boissy, A, Uvnäs-Moberg, K & Lidfors, L 2015, 'Behavioural expression of positive anticipation for food or opportunity to play in lambs', *Behavioural Processes*, vol. 113, no. Journal Article, pp. 152-158.

Anderson, David J & Adolphs, R 2014, 'A framework for studying emotions across species', *Cell*, vol. 157, no. 1, pp. 187-200.

Anderson, SE, Dave, AS & Margoliash, D 1996, 'Template-based automatic recognition of birdsong syllables from continuous recordings', *The Journal of the Acoustical Society of America* 100, pp. 1209-1219.

Andics, A, Gácsi, M, Faragó, T, Kis, A & Miklósi, Á 2014, 'Voice-sensitive regions in the dog and human brain are revealed by comparative fMRI', *Current Biology*, vol. 24, no. 5, pp. 574-578.

Andics, A, McQueen, JM, Petersson, KM, Gál, V, Rudas, G & Vidnyánszky, Z 2010, 'Neural mechanisms for voice recognition', *Neuroimage*, vol. 52, no. 4, pp. 1528-1540.

Aubin, T 1991, 'Why do distress calls evoke interspecific responses? An experimental study applied to some species of birds', *Behavioural Processes*, vol. 23, no. 2, pp. 103-111.

Ausmus, DM & Clarke, JA 2014, 'Mother knows best: functionally referential alarm calling in white-tailed ptarmigan', *Animal Cognition*, vol. 17, no. 3, pp. 671-679.

Bachorowski, J-A & Owren, MJ 1995, 'Vocal expression of emotion: Acoustic properties of speech are associated with emotional intensity and context', *Psychological Science*, vol. 6, no. 4, pp. 219-224.

Bachorowski, J-A 1999, 'Vocal expression and perception of emotion', *Current Directions in Psychological Science*, vol. 8, no. 2, pp. 53-57.

Balsam, PD & Gallistel, CR 2009, 'Temporal maps and informativeness in associative learning', *Trends in Neurosciences*, vol. 32, no. 2, pp. 73-78.

Banse, R & Scherer, KR 1996, 'Acoustic profiles in vocal emotion expression', *Journal of Personality and Social Psychology*, vol. 70, no. 3, pp. 614-636.

Bänziger, T & Scherer, KR 2005, 'The role of intonation in emotional expressions', *Speech Communication*, vol. 46, no. 3-4, pp. 252-267.

Barfield, RJ, Auerbach, P, Geyer, LA & McIntosh, TK 1979, 'Ultrasonic vocalizations in rat sexual behavior', *American Zoologist*, vol. 19, no. 2, pp. 469-480.

Bastian, A & Schmidt, S 2008, 'Affect cues in vocalizations of the bat, *Megaderma lyra*, during agonistic interactions', *The Journal of the Acoustical Society of America*, vol. 124, no. 1, pp. 598-608.

Bates, D, Maechler, M, Bolker, B, Walker, S, Christensen, RHB, Singmann, H, Dai, B & Eigen, C 2015, *Package 'lme4'*.

Bayly, KL & Evans, CS 2003, 'Dynamic changes in alarm call structure: A strategy for reducing conspicuousness to avian predators?', *Behaviour*, vol. 140, no. 3, pp. 353-369.

Bee, MA & Perrill, SA 1996, 'Responses to conspecific advertisement calls in the green frog (*Rana clamitans*) and their role in male-male communication', *Behaviour*, vol. 133, no. 3, pp. 283-301.

Bekoff, M 2007, *The emotional lives of animals*, New World Library, Novato, US.

Belin, P, Fecteau, S, Charest, I, Nicastro, N, Hauser, MD & Armony, JL 2008, 'Human cerebral response to animal affective vocalizations', *Proceedings of the Royal Society B-Biological Sciences*, vol. 275, no. 1634, pp. 473-481.

Berridge, KC 1996, 'Food reward: Brain substrates of wanting and liking', *Neuroscience & Biobehavioral Reviews*, vol. 20, no. 1, pp. 1-25.

Berridge, KC 2004, 'Motivation concepts in behavioral neuroscience', *Physiology & Behavior*, vol. 81, no. 2, pp. 179-209.

Berridge, KC 2007, 'The debate over dopamine's role in reward: the case for incentive salience', *Psychopharmacology*, vol. 191, no. 3, pp. 391-431.

Berridge, KC & Kringelbach, ML 2008, 'Affective neuroscience of pleasure: reward in humans and animals', *Psychopharmacology*, vol. 199, no. 3, pp. 457-480.

Boissy, A, Arnould, C, Chaillou, E, Desire, L, Duvaux-Ponter, C, Greiveldinger, L, Leterrier, C, Richard, S, Roussel, S, Saint-Dizier, H, Meunier-Salaun, M, Valance, D & Veissier, I 2007, 'Emotions and cognition: a new approach to animal welfare', *Animal Welfare*, vol. 16, Supplement 1, pp. 37-43.

Boissy, A, Manteuffel, G, Jensen, M, Moe, R, Spruijt, B, Keeling, L, Winckler, C, Forkman, B, Dimitrov, I & Langbein, J 2007, 'Assessment of positive emotions in animals to improve their welfare', *Physiology & Behavior*, vol. 92, no. 3, pp. 375-397.

Bradbury, JW & Vehrencamp, SL 2011, *Principles of Animal Communication*, 2nd edn, Sinauer Associates Inc., Sunderland, MA.

Breiman, L 2001, 'Random forests', *Machine learning*, vol. 45, no. 1, pp. 5-32.

Breiman, L, Friedman, J, Stone, CJ & Olshen, RA 1984, *Classification and regression trees*, Chapman and Hall, London, UK.

Briefer, E & McElligott, AG 2011a, 'Indicators of age, body size and sex in goat kid calls revealed using the source–filter theory', *Applied Animal Behaviour Science*, vol. 133, no. 3, pp. 175-185.

Briefer, E & McElligott, AG 2011b, 'Mutual mother–offspring vocal recognition in an ungulate hider species (*Capra hircus*)', *Animal cognition*, vol. 14, no. 4, pp. 585-598.

Briefer, E, Mandel, R, Maigrot, A-L, Freymond, S, Bachmann, I & Hillmann, E 2017, 'Perception of emotional valence in horse whinnies', *Frontiers in Zoology*, vol. 14.

Briefer, EF 2012, 'Vocal expression of emotions in mammals: mechanisms of production and evidence', *Journal of Zoology*, vol. 288, no. 1, pp. 1-20.

Briefer, EF 2018, 'Vocal contagion of emotions in non-human animals', *Proceedings of the Royal Society B*, vol. 285, no. 1873.

Briefer, EF, Tettamanti, F & McElligott, AG 2015, 'Emotions in goats: mapping physiological, behavioural and vocal profiles', *Animal Behaviour*, vol. 99, no. Journal Article, pp. 131-143.

Bright, A 2008, 'Vocalisations and acoustic parameters of flock noise from feather pecking and non-feather pecking laying flocks', *British Poultry Science*, vol. 49, no. 3, pp. 241-249.

Broom, DM 1991, 'Animal welfare: concepts and measurement', *Journal of Animal Science*, vol. 69, no. 10, pp. 4167-4175.

Broom, DM 1991, 'Assessing welfare and suffering', *Behavioural Processes*, vol. 25, no. 2–3, pp. 117-123.

Brown, JC & Miller, PJ 2007. ‘Automatic classification of killer whale vocalizations using dynamic time warping’, *The Journal of the Acoustical Society of America* 122, pp. 1201-1207.

Bruce, EH, Prescott, NB & Wathes, CM 2003, 'Preferred food rewards for laying hens in behavioural experiments', *British Poultry Science*, vol. 44, no. 3, pp. 345-349.

Brudzynski, SM & Pniak, A 2002, 'Social contacts and production of 50-kHz short ultrasonic calls in adult rats', *Journal of Comparative Psychology*, vol. 116, no. 1, p. 73.

Brudzynski, SM 2007, 'Ultrasonic calls of rats as indicator variables of negative or positive states: acetylcholine–dopamine interaction and acoustic coding', *Behavioural Brain Research*, vol. 182, no. 2, pp. 261-273.

Brumm, H & Todt, D 2004, 'Male–male vocal interactions and the adjustment of song amplitude in a territorial bird', *Animal Behaviour*, vol. 67, no. 2, pp. 281-286.

Brumm, H, Schmidt, R & Schrader, L 2009, 'Noise-dependent vocal plasticity in domestic fowl', *Animal Behaviour*, vol. 78, no. 3, pp. 741-746.

Bryant, G 2013, 'Animal signals and emotion in music: coordinating affect across groups', *Frontiers in Psychology*, vol. 4, no. 990.

Bugnyar, T, Kijne, M & Kotrschal, K 2001, 'Food calling in ravens: are yells referential signals?', *Animal Behaviour*, vol. 61, no. 5, pp. 949-958.

Buijs, S, Zimmermann, P & Keeling, L 2006, 'Anticipation as a tool to explore positive emotions in laying hens', in *Proceedings of the 40th International Congress of the ISAE*, p. 50.

Burgdorf, J & Panksepp, J 2006, 'The neurobiology of positive emotions', *Neuroscience and Biobehavioral Reviews*, vol. 30, no. 2, pp. 173-187.

Burman, OHP, Ilyat, A, Jones, G & Mendl, M 2007, 'Ultrasonic vocalizations as indicators of welfare for laboratory rats (*Rattus norvegicus*)', *Applied Animal Behaviour Science*, vol. 104, no. 1–2, pp. 116-129.

Bussey, TJ, Everitt, BJ, & Robbins, TW 1997. 'Dissociable effects of cingulate and medial frontal cortex lesions on stimulus-reward learning using a novel Pavlovian autoshaping procedure for the rat: implications for the neurobiology of emotion'. *Behavioral Neuroscience* 111, no.6, pp. 908-919

Calder, AJ, Burton, AM, Miller, P, Young, AW & Akamatsu, S 2001, 'A principal component analysis of facial expressions', *Vision Research*, vol. 41, no. 9, pp. 1179-1208.

Cardoso, GC 2013, 'Using frequency ratios to study vocal communication', *Animal Behaviour*, vol. 85, no. 6, pp. 1529-1532.

Charlton, BD, Zhihe, Z & Snyder, RJ 2009, 'The information content of giant panda, *Ailuropoda melanoleuca*, bleats: acoustic cues to sex, age and size', *Animal Behaviour*, vol. 78, no. 4, pp. 893-898.

Cheney, DL & Seyfarth, RM 1985, 'Vervet monkey alarm calls: Manipulation through shared information?', *Behaviour*, vol. 94, no. 1, pp. 150-166.

Cheney, DL & Seyfarth, RM 1988, 'Assessment of meaning and the detection of unreliable signals by vervet monkeys', *Animal Behaviour*, vol. 36, no. 2, pp. 477-486.

Clay, Z & Zuberbühler, K 2009, 'Food-associated calling sequences in bonobos', *Animal Behaviour*, vol. 77, no. 6, pp. 1387-1396.

Clay, Z, Smith, CL & Blumstein, DT 2012, 'Food-associated vocalizations in mammals and birds: what do these calls really mean?', *Animal Behaviour*, vol. 83, no. 2, pp. 323-330.

Clegg, ILK & Delfour, F 2018. 'Cognitive judgement bias is associated with frequency of anticipatory behavior in bottlenose dolphins', *Zoo Biology* 37, pp. 67-73.

Clemins, PJ, Johnson, MT, Leong, KM & Savage, A 2005. 'Automatic classification and speaker identification of African elephant (*Loxodonta africana*) vocalizations', *The Journal of the Acoustical Society of America* 117, pp. 956-963.

Collias, N & Joos, M 1953, 'The spectrographic analysis of sound signals of the domestic fowl', *Behaviour*, vol. 5, no. 3, pp. 175-188.

Collias, NE 1987, 'The vocal repertoire of the red junglefowl: A spectrographic classification and the code of communication', *The Condor*, vol. 89, no. 3, pp. 510-524.

Compton, L, Clarke, J, Seidensticker, J & Ingrisano, D 2001, 'Acoustic characteristics of white-nosed coati vocalizations: a test of motivation-structural rules', *Journal of Mammalogy*, vol. 82, no. 4, pp. 1054-1058.

Cooper, JJ & Appleby, MC 1996, 'Demand for nest boxes in laying hens', *Behavioural Processes*, vol. 36, no. 2, pp. 171-182.

Cooper, JJ & Appleby, MC 2003, 'The value of environmental resources to domestic hens: a comparison of the work-rate for food and for nests as a function of time', *Animal Welfare*, vol. 12, no. 1, pp. 39-52.

Cummins, N, Hantke, S, Schnieder, S, Krajewski, J & Schuller, B 2017, 'Classifying the context and emotion of dog barks: A comparison of acoustic feature representations', paper presented to *Proceedings of the Pre-Conference on Affective Computing 2017 SAS Annual Conference*, Boston.

Cunningham, S & Magrath, RD 2017, 'Functionally referential alarm calls in noisy miners communicate about predator behaviour', *Animal Behaviour*, vol. 129, pp. 171-179.

Custance, D & Mayer, J 2012, 'Empathic-like responding by domestic dogs (*Canis familiaris*) to distress in humans: an exploratory study', *Animal Cognition*, vol. 15, no. 5, pp. 851-859.

Dabelsteen, T 2004, 'Strategies that facilitate or counter eavesdropping on vocal interactions in songbirds', *Anais da Academia Brasileira de Ciencias*, vol. 76, pp. 274-278.

Damasio, AR 1998, 'Emotion in the perspective of an integrated nervous system', *Brain Research Reviews*, vol. 26, pp. 83-86.

Dantzer, R, Gonyou, HW, Curtis, SE & Kelley, KW 1987, 'Changes in serum cortisol reveal functional differences in frustration-induced chain chewing in pigs', *Physiology & Behavior*, vol. 39, no. 6, pp. 775-777.

Darwin, C 1872, *The expression of the emotions in man and animals*, 3rd edn, Oxford University Press, Oxford.

Davies, A, Radford, A & Nicol, C 2014, 'Behavioural and physiological expression of arousal during decision-making in laying hens', *Physiology & Behavior*, vol. 123, pp. 93-99.

Dawkins, M 2002, 'What are birds looking at? Head movements and eye use in chickens', *Animal Behaviour*, vol. 63, no. 5, pp. 991-998.

Dawkins, MS 1983, 'Battery hens name their price: Consumer demand theory and the measurement of ethological 'needs'', *Animal Behaviour*, vol. 31, no. 4, pp. 195-205.

Dawkins, MS 1990, 'From an animal's point of view: Motivation, fitness, and animal welfare', *Behavioral and Brain Sciences*, vol. 13, no. 1, pp. 1-9.

Dawkins, MS 2012, *Why animals matter: Animal consciousness, animal welfare and human well-being*, 1st edn, Oxford University Press, Oxford.

Dawkins, R & Krebs, J 1978, 'Animal signals: information or manipulation', in J Krebs & N Davies (eds), *Behavioural ecology: an evolutionary approach*, pp. 282-309.

de Waal, FBM 2008, 'Putting the altruism back into altruism: The evolution of empathy', *Annual Review of Psychology*, vol. 59, no. 1, pp. 279-300.

Dentressangle, F, Aubin, T & Mathevon, N 2012, 'Males use time whereas females prefer harmony: individual call recognition in the dimorphic blue-footed booby', *Animal Behaviour*, vol. 84, no. 2, pp. 413-420.

Di Bitetti, MS 2003, 'Food-associated calls of tufted capuchin monkeys (Cebus apella nigritus) are functionally referential signals', *Behaviour*, vol. 140, no. 5, pp. 565-592.

Dickinson, A & Balleine, B 1995, 'Motivational control of instrumental action', *Current Directions in Psychological Science*, vol. 4, no. 5, pp. 162-167.

Dudink, S, Simonse, H, Marks, I, de Jonge, FH & Spruijt, BM 2006, 'Announcing the arrival of enrichment increases play behaviour and reduces weaning-stress-induced behaviours of piglets directly after weaning', *Applied Animal Behaviour Science*, vol. 101, no. 1–2, pp. 86-101.

Duncan, IJH & Woodgush, DG 1972, 'Analysis of displacement preening in domestic fowl', *Animal Behaviour*, vol. 20, no.1 pp. 68-71.

Duncan, IJH & Wood-Gush, DGM 1972, 'Thwarting of feeding behaviour in the domestic fowl', *Animal Behaviour*, vol. 20, no. 3, pp. 444-451.

Düpjan, S, Tuchscherer, A, Langbein, J, Schön, P-C, Manteuffel, G & Puppe, B 2011, 'Behavioural and cardiac responses towards conspecific distress calls in domestic pigs (*Sus scrofa*)', *Physiology & Behavior*, vol. 103, no. 5, pp. 445-452.

Dziuban, CD & Shirkey, EC 1974, 'When is a correlation matrix appropriate for factor analysis? Some decision rules', *Psychological Bulletin*, vol. 81, no. 6, pp. 358-361.

Edgar, JL, Lowe, JC, Paul, ES & Nicol, CJ 2011, 'Avian maternal response to chick distress', *Proceedings of the Royal Society B: Biological Sciences*, vol. 278, no. 1721, pp. 3129-3134.

Edgar, JL, Nicol, CJ, Clark, CCA & Paul, ES 2012, 'Measuring empathic responses in animals', *Applied Animal Behaviour Science*, vol. 138, pp. 182-193.

Ekman, P 1999, 'Basic Emotions', in T Dalgleish & M Power (eds), *Handbook of Cognition and Emotion*, Wiley & Sons, Ltd., Sussex.

Elemans, CPH, Zaccarelli, R & Herzel, H 2008, 'Biomechanics and control of vocalization in a non-songbird', *Journal of the Royal Society*, Interface, vol. 5, no. 24, p. 691.

Elgar, MA 1986, 'House sparrows establish foraging flocks by giving chirrup calls if the resources are divisible', *Animal Behaviour*, vol. 34, Part 1, pp. 169-174.

Elliott, R, Newman, JL, Longe, OA, & Deakin, JW 2003. ‘Differential response patterns in the striatum and orbitofrontal cortex to financial reward in humans: a parametric functional magnetic resonance imaging study’, *Journal of Neuroscience* 23, pp. 303-307.

Elowson, AM, Tannenbaum, PL & Snowdon, CT 1991, 'Food-associated calls correlate with food preferences in cotton-top tamarins', *Animal Behaviour*, vol. 42, no. 6, pp. 931-937.

Etienne, AS 1984, 'The meaning of object permanence at different zoological levels', *Human Development*, vol. 27, no. 5-6, pp. 309-320.

Evans, C.S., 1997. ‘Referential signals’, *Communication*, Springer, pp. 99-143.

Evans, CS & Evans, L 1999, 'Chicken food calls are functionally referential', *Animal Behaviour*, vol. 58, pp. 307-319.

Evans, CS & Evans, L 2007, 'Representational signalling in birds', *Biology Letters*, vol. 3, no. 1, pp. 8-11.

Evans, CS & Marler, P 1994, 'Food calling and audience effects in male chickens, *Gallus gallus*: their relationships to food availability, courtship and social facilitation', *Animal Behaviour*, vol. 47, no. 5, pp. 1159-1170.

Evans, CS 1993, 'Recognition of contentment call spectral characteristics by mallard ducklings - evidence for a consistent perceptual process', *Animal Behaviour*, vol. 45, no. 6, pp. 1071-1082.

Evans, CS 1997, 'Referential signals', in *Communication*, Springer, pp. 99-143.

Evans, CS, Evans, L & Marler, P 1993, 'On the meaning of alarm calls - functional reference in an avian vocal system', *Animal Behaviour*, vol. 46, no. 1, pp. 23-38.

Fallow, PM, Gardner, JL & Magrath, RD 2011, 'Sound familiar? Acoustic similarity provokes responses to unfamiliar heterospecific alarm calls', *Behavioral Ecology*, vol. 22, no. 2, pp. 401-410.

Fant, G 1960, *Acoustic theory of speech production*, Mouton, The Hague.

Faragó, T, Andics, A, Devecseri, V, Kis, A, Gácsi, M & Miklósi, Á 2014, 'Humans rely on the same rules to assess emotional valence and intensity in conspecific and dog vocalizations', *Biology Letters*, vol. 10, no. 1.

Faragó, T, Takács, N, Miklósi, Á & Pongrácz, P 2017, 'Dog growls express various contextual and affective content for human listeners', *Royal Society Open Science*, vol. 4, no. 5.

Farrow, LF, Doohan, SJ & McDonald, PG 2017, 'Alarm calls of a cooperative bird are referential and elicit context-specific antipredator behavior', *Behavioral Ecology*, vol. 28, no. 3, pp. 724-731.

Faure, JM 1994, 'Choice tests for space in groups of laying hens', *Applied Animal Behaviour Science*, vol. 39, no. 1, pp. 89-94.

Favaro, L, Gamba, M, Alfieri, C, Pessani, D & McElligott, AG 2015, 'Vocal individuality cues in the African penguin (*Spheniscus demersus*): a source-filter theory approach', *Scientific Reports*, vol. 5.

Feighny, J, Williamson, K & Clarke, J 2006, 'North American elk bugle vocalizations: male and female bugle call structure and context', *Journal of Mammalogy*, vol. 87, no. 6, pp. 1072-1077.

Ferrari, S, Silva, M, Guarino, M, Aerts, JM & Berckmans, D 2008, ‘Cough sound analysis to identify respiratory infection in pigs’, *Computers and Electronics in Agriculture* 64, pp.318-325.

Fichtel, C, Hammerschmidt, K & Jürgens, U 2001, 'On the vocal expression of emotion. A multi-parametric analysis of different states of aversion in the squirrel monkey', *Behaviour*, vol. 138, no. 1, pp. 97-116.

Field, SE, Rickard, NS, Toukhsati, SR & Gibbs, ME 2007, 'Maternal hen calls modulate memory formation in the day-old chick: The role of noradrenaline', *Neurobiology of Learning and Memory*, vol. 88, no. 3, pp. 321-330.

Filippi, P, Congdon, JV, Hoang, J, Bowling, DL, Reber, SA, Pašukonis, A, Hoeschele, M, Ocklenburg, S, de Boer, B & Sturdy, CB 2017a, 'Humans recognize emotional arousal in vocalizations across all classes of terrestrial vertebrates: evidence for acoustic universals', *Proceedings of the Royal Society B*, vol. 284, no. 1859.

Filippi, P, Gogoleva, SS, Volodina, EV, Volodin, IA & de Boer, B 2017b, 'Humans identify negative (but not positive) arousal in silver fox vocalizations: implications for the adaptive value of interspecific eavesdropping', *Current Zoology*, vol. 63, no. 4, pp. 445-456.

Fitch, WT & Suthers, RA 2016, 'Vertebrate vocal production: An introductory overview', in RA Suthers, WT Fitch, RR Fay & AN Popper (eds), *Vertebrate sound production and acoustic communication*, Springer International Publishing, Cham, pp. 1-18.

Font, E & Carazo, P 2010, 'Animals in translation: why there is meaning (but probably no message) in animal communication', *Animal Behaviour*, vol. 80, no. 2, pp. E1-E6.

Forkman, B 2000, 'Domestic hens have declarative representations', *Animal Cognition*, vol. 3, no. 3, pp. 135-137.

Forkman, B, Coerse, NCA & Haskell, MJ 2012, 'Incentive value learning in domestic hens', *Ethology*, vol. 118, no. 5, pp. 489-493.

Fraser, D & Duncan, IJH 1998, ‘Pleasures, pains and animal welfare: Toward a natural history of affect', *Animal Welfare*, vol. 7, no. 4, pp. 383-396.

Fraser, D 1993, 'Assessing animal well-being: common sense, uncommon science. Food animal well-being', in In Purdue University, Office of Agricultural Research Programs (Ed.), pp. 37-54.

Freire, R, Appleby, MC & Hughes, BO 1996, 'Effects of nest quality and other cues for exploration on pre-laying behaviour', *Applied Animal Behaviour Science*, vol. 48, no. 1-2, pp. 37-46.

Garland, EC, Castellote, M & Berchok, CL 2015, 'Beluga whale (*Delphinapterus leucas*) vocalizations and call classification from the eastern Beaufort Sea population', *The Journal of the Acoustical Society of America*, vol. 137, no. 6, pp. 3054-3067.

Gaunt, AS, Gaunt, SLL & Hector, DH 1976, 'Mechanics of the syrinx in *Gallus gallus*. A comparison of pressure events in chickens to those in oscines', *The Condor*, vol. 78, no. 2, pp. 208-223.

Genty, E, Clay, Z, Hobaiter, C & Zuberbühler, K 2014, 'Multi-Modal Use of a Socially Directed Call in Bonobos', *PloS ONE*, vol. 9, no. 1, e84738.

Gergely, A, Faragó, T, Galambos, Á & Topál, J 2017, 'Differential effects of speech situations on mothers’ and fathers’ infant-directed and dog-directed speech: An acoustic analysis', *Scientific Reports*, vol. 7, no. 1.

Gogoleva, SS, Volodin, IA, Volodina, EV, Kharlamova, AV & Trut, LN 2010, 'Sign and strength of emotional arousal: vocal correlates of positive and negative attitudes to humans in silver foxes (*Vulpes vulpes*)', *Behaviour*, vol. 147, no. 13, pp. 1713-1736.

Goodall, J 1985, *The chimpanzees of Gombe*, Harvard University Press, Cambridge, MA.

Goudbeek, M & Scherer, K 2010, 'Beyond arousal: Valence and potency/control cues in the vocal expression of emotion', *The Journal of the Acoustical Society of America*, vol. 128, no. 3, pp. 1322-1336.

Grafen, A 1990, 'Biological signals as handicaps', *Journal of Theoretical Biology*, vol. 144, no. 4, pp. 517-546.

Grandjean, D, Sander, D, Pourtois, G, Schwartz, S, Seghier, ML, Scherer, KR & Vuilleumier, P 2005, 'The voices of wrath: brain responses to angry prosody in meaningless speech', *Nature Neuroscience*, vol. 8, no. 2, pp. 145-146.

Greiveldinger, L, Veissier, I & Boissy, A 2011, 'The ability of lambs to form expectations and the emotional consequences of a discrepancy from their expectations', *Psychoneuroendocrinology*, vol. 36, no. 6, pp. 806-815.

Gustison, ML & Townsend, SW 2015, 'A survey of the context and structure of high- and low-amplitude calls in mammals', *Animal Behaviour*, vol. 105, pp. 281-288.

Guyomarc’h, J-C 1974, 'Le rôle de l’expérience sur la sémantique du cri d’offrande alimentaire chez le poussin', *Rev. Comp. Animal*, vol. 9, pp. 219-236.

Gyger, M & Marler, P 1988, 'Food calling in the domestic fowl, Gallus gallus: the role of external referents and deception', *Animal Behaviour*, vol. 36, no. 2, pp. 358-365.

Gyger, M, Marler, P & Pickert, R 1987, 'Semantics of an avian alarm call system: The male domestic fowl, Gallus domesticus', *Behaviour*, vol. 102, no. 1, pp. 15-40.

Hacker, PM 1999, 'The conceptual framework for the investigation of emotions', *International Review of Psychiatry*, vol. 16, no. 3, pp. 199-208.

Hahn, AH, Hoeschele, M, Guillette, LM, Hoang, J, McMillan, N, Congdon, JV, Campbell, KA, Mennill, DJ, Otter, KA, Grava, T, Ratcliffe, LM & Sturdy, CB 2016, 'Black-capped chickadees categorize songs based on features that vary geographically', *Animal Behaviour*, vol. 112, pp. 93-104.

Hammerschmidt, K & Jürgens, U 2007, 'Acoustical correlates of affective prosody', *Journal of Voice*, vol. 21, no. 5, pp. 531-540.

Hänninen, L & Pastell, M 2009, 'CowLog: Open source software for coding behaviors from digital video', *Behavior Research Methods*, vol. 41, no. 2, pp. 472-476.

Hauber, ME, Woolley, SM, Cassey, P & Theunissen, FE 2013, 'Experience dependence of neural responses to different classes of male songs in the primary auditory forebrain of female songbirds', *Behavioural Brain Research*, vol. 243, pp. 184-190.

Hauser, MD 1988, 'How infant vervet monkeys learn to recognize starling alarm calls: the role of experience', *Behaviour*, vol. 105, no. 3-4, pp. 187-201.

Herzog, M & Hopf, S 1984, 'Behavioral responses to species-specific warning calls in infant squirrel monkeys reared in social isolation', *American Journal of Primatology*, vol. 7, no. 2, pp. 99-106.

Hirsh-Pasek, K & Treiman, R 1982, 'Doggerel: Motherese in a new context', *Journal of Child Language*, vol. 9, no. 1, pp. 229-237.

Hofer, MA 1984, 'Relationships as regulators - A psychobiologic perspective on bereavement', *Psychosomatic Medicine*, vol. 46, no. 3, pp. 183-197.

Hofer, MA 2006, 'Psychobiological roots of early attachment', *Current Directions in Psychological Science*, vol. 15, no. 2, pp. 84-88.

Holland, PC 1980, 'CS–US interval as a determinant of the form of Pavlovian appetitive conditioned responses', Journal of Experimental Psychology: Animal Behavior Processes, vol. 6, no. 2, pp. 155-174.

Hollén, LI & Manser, MB 2007, 'Motivation before meaning: Motivational information encoded in meerkat alarm calls develops earlier than referential information', *The American Naturalist*, vol. 169, no. 6, pp. 758-767.

Holmstrom, LA, Eeuwes, LB, Roberts, PD & Portfors, CV 2010, 'Efficient encoding of vocalizations in the auditory midbrain', *Journal of Neuroscience*, vol. 30, no. 3, pp. 802-819.

Hughes, BO & Duncan, IJH 1988, 'The notion of ethological need: models of motivation and animal welfare', *Animal Behaviour*, vol. 36, pp. 1696-1707.

Hughes, MK, Hughes, AL & Covalt-Dunning, D 1982, 'Stimuli eliciting foodcalling in domestic chickens', *Applied Animal Ethology*, vol. 8, no. 6, pp. 543-550.

Ilie, G & Thompson, WF 2006, 'A comparison of acoustic cues in music and speech for three dimensions of affect', *Music Perception: An Interdisciplinary Journal*, vol. 23, no. 4, pp. 319-330.

Imfeld-Mueller, S & Hillmann, E 2012, 'Anticipation of a food ball increases short-term activity levels in growing pigs', *Applied Animal Behaviour Science*, vol. 137, no. 1-2, pp. 23-9.

Inglis, IR, Forkman, B & Lazarus, J 1997, 'Free food or earned food? A review and fuzzy model of contrafreeloading', *Animal Behaviour*, vol. 53, no. 6, pp. 1171-1191.

Juslin, PN & Laukka, P 2001. ‘Impact of intended emotion intensity on cue utilization and decoding accuracy in vocal expression of emotion’. *Emotion* 1, no.4, pp. 381-412.

Juslin, PN & Laukka, P 2003, 'Communication of emotions in vocal expression and music performance: Different channels, same code?', *Psychological Bulletin*, vol. 129, no. 5, pp. 770-814.

Kalan, AK, Mundry, R & Boesch, C 2015, 'Wild chimpanzees modify food call structure with respect to tree size for a particular fruit species', *Animal Behaviour*, vol. 101, pp. 1-9.

Kent, JP 1989, 'On the acoustic basis of recognition of the mother hen by the chick in the domestic fowl (*Gallus gallus*)', *Behaviour*, vol. 108, no. 1, pp. 1-9.

King, A & Sueur, C 2011, 'Where next? Group coordination and collective decision making by primates', *International Journal of Primatology*, vol. 32, no. 6, pp. 1245-1267.

Kirkden, RD & Pajor, EA 2006, 'Using preference, motivation and aversion tests to ask scientific questions about animals’ feelings', *Applied Animal Behaviour Science*, vol. 100, no. 1–2, pp. 29-47.

Kirsch, P, Schienle, A, Stark, R, Sammer, G, Blecker, C, Walter, B, Ott, U, Burkart, J & Vaitl, D 2003. ‘Anticipation of reward in a nonaversive differential conditioning paradigm and the brain reward system: an event-related fMRI study’. *Neuroimage* 20, pp. 1086-1095.

Klinnert, MD 1984, 'The regulation of infant behavior by maternal facial expression', *Infant Behavior and Development*, vol. 7, no. 4, pp. 447-465.

Knutson, B, Burgdorf, J & Panksepp, J 1998, 'Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats', *Journal of Comparative Psychology*, vol. 112, no. 1, pp. 65-73.

Knutson, B, Burgdorf, J & Panksepp, J 1999, 'High-frequency ultrasonic vocalizations index conditioned pharmacological reward in rats', *Physiology & Behavior*, vol. 66, no. 4, pp. 639-643.

Knutson, B, Burgdorf, J & Panksepp, J 2002, 'Ultrasonic vocalizations as indices of affective states in rats', *Psychological Bulletin*, vol. 128, no. 6, pp. 961-977.

Kokolakis, A, Smith, CL & Evans, CS 2010, 'Aerial alarm calling by male fowl (*Gallus gallus*) reveals subtle new mechanisms of risk management', *Animal Behaviour*, vol. 79, no. 6, pp. 1373-1380.

Komura, Y, Tamura, R, Uwano, T, Nishijo, H, Kaga, K & Ono, T 2001. ‘Retrospective and prospective coding for predicted reward in the sensory thalamus’, *Nature* 412, pp. 546-549

Konishi, M 1963, 'The role of auditory feedback in the vocal behavior of the domestic fowl', *Zeitschrift für Tierpsychologie*, vol. 20, no. 3, pp. 349-367.

Kostal, L, Savory, CJ & Hughes, BO 1992, 'Diurnal and individual variation in behavior of retricted-fed broiler breeders', *Applied Animal Behaviour Science*, vol. 32, no. 4, pp. 361-374.

Kringelbach, ML & Berridge, KC 2017, 'The affective core of emotion: linking pleasure, subjective well-being, and optimal metastability in the brain', *Emotion Review*, vol. 9, no. 3, pp. 191-199.

Kruijt, JP 1964, 'Ontogeny of social behaviour in burmese red junglefowl (*Gallus gallus spadiceus*)', *Behaviour.* Supplement no. 12, pp. 1-201.

Lang, PJ, Bradley, MM & Cuthbert, BN 1992, 'A motivational analysis of emotion: Reflex-cortex connections', *Psychological Science*, vol. 3, no. 1, pp. 44-49.

Lang, PJ, Bradley, MM & Cuthbert, BN 1998, 'Emotion, motivation, and anxiety: brain mechanisms and psychophysiology', *Biological Psychiatry*, vol. 44, no. 12, pp. 1248-1263.

Laukka, P, Juslin, P & Bresin, R 2005, 'A dimensional approach to vocal expression of emotion', *Cognition & Emotion*, vol. 19, no. 5, pp. 633-653.

Leighty, KA, Soltis, J, Wesolek, CM & Savage, A 2008, 'Rumble vocalizations mediate interpartner distance in African elephants, *Loxodonta africana*', *Animal Behaviour*, vol. 76, no. 5, pp. 1601-1608.

Lemasson, A, Remeuf, K, Rossard, A & Zimmermann, E 2012, 'Cross-taxa similarities in affect-induced changes of vocal behavior and voice in arboreal monkeys', *PloS ONE*, vol. 7, no. 9, e45106.

Liaw, A & Wiener, M 2002, 'Classification and regression by randomForest', *R news*, vol. 2, no. 3, pp. 18-22.

Lindová, J, Špinka, M & Nováková, L 2015, 'Decoding of baby calls: Can adult humans identify the eliciting situation from emotional vocalizations of preverbal infants?', *PloS ONE*, vol. 10, no. 4, e0124317.

Lingle, S & Riede, T 2014, 'Deer mothers are sensitive to infant distress vocalizations of diverse mammalian species', *The American Naturalist*, vol. 184, no. 4, pp. 510-522.

Linhart, P, Ratcliffe, VF, Reby, D & Špinka, M 2015, 'Expression of emotional arousal in two different piglet call types', *PloS ONE*, vol. 10, no. 8, e0135414.

Linnankoski, I, Laakso, M, Aulanko, R & Leinonen, L 1994, 'Recognition of emotions in macaque vocalizations by children and adults', *Language & Communication*, vol. 14, no. 2, pp. 183-192.

Löw, A, Lang, PJ, Smith, JC & Bradley, MM 2008, 'Both predator and prey: emotional arousal in threat and reward', *Psychological Science*, vol. 19, no. 9, p. 865.

Luef, EM, Breuer, T & Pika, S 2016, 'Food-associated calling in Gorillas (*Gorilla g. gorilla*) in the Wild', *PloS ONE*, vol. 11, no. 2, e0144197.

Macedonia, JM & Evans, CS 1993, 'Essay on contemporary issues in ethology: Variation among mammalian alarm call systems and the problem of meaning in animal signals', *Ethology*, vol. 93, no. 3, pp. 177-197.

Mackenzie, JG, Foster, TM & Temple, W 1993, 'Sound avoidance by hens', *Behavioural Processes*, vol. 30, no. 2, pp. 143-156.

Magrath, RD, Haff, TM, Fallow, PM & Radford, AN 2015, 'Eavesdropping on heterospecific alarm calls: from mechanisms to consequences', *Biological Reviews*, vol. 90, no. 2, pp. 560-586.

Manser, MB 2001, 'The acoustic structure of suricates' alarm calls varies with predator type and the level of response urgency', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, vol. 268, no. 1483, pp. 2315-2324.

Manser, MB, Bell, MB & Fletcher, LB 2001, 'The information that receivers extract from alarm calls in suricates', *Proceedings of the Royal Society of London B: Biological Sciences*, vol. 268, no. 1484, pp. 2485-2491.

Manteuffel, G, Puppe, B & Schon, PC 2004, 'Vocalization of farm animals as a measure of welfare', *Applied Animal Behaviour Science*, vol. 88, no. 1-2, pp. 163-182.

Marler, P, Dufty, A & Pickert, R 1986a, 'Vocal communication in the domestic chicken - Does a sender communicate information about the quality of a food referent to a receiver', *Animal Behaviour*, vol. 34, pp. 188-193.

Marler, P, Dufty, A & Pickert, R 1986b, 'Vocal communication in the domestic chicken: II. Is a sender sensitive to the presence and nature of a receiver?', *Animal Behaviour*, vol. 34, Part 1, pp. 194-198.

Marler, P, Evans, CS & Hauser, MD 1992, 'Animal signals: motivational, referential, or both', *Nonverbal vocal communication: Comparative and Developmental Approaches*, pp. 66-86.

Martin, GB & Clark, RD 1982, 'Distress crying in neonates: Species and peer specificity', *Developmental psychology*, vol. 18, no. 1, pp. 3-9.

Maruščáková, IL, Linhart, P, Ratcliffe, VF, Tallet, C, Reby, D & Špinka, M 2015, 'Humans (*Homo sapiens*) judge the emotional content of piglet (*Sus scrofa domestica*) calls based on simple acoustic parameters, not personality, empathy, nor attitude toward animals', *Journal of Comparative Psychology*, vol. 129, no. 2, pp. 121-131.

Marx, G, Leppelt, J & Ellendorff, F 2001, 'Vocalisation in chicks (*Gallus gallus dom.*) during stepwise social isolation', *Applied Animal Behaviour Science*, vol. 75, no. 1, pp. 61-74.

Mason, G, McFarland, D & Garner, J 1998, 'A demanding task: assessing the needs of captive animals', *Animal Behaviour*, vol. 55, pp. 1071-1075.

Matthews, L, Temple, W, Foster, TM & McAdie, TM 1994, 'Quantifying the environmental requirements of layer hens by behavioural demand functions', *Applied Animal Behaviour Science*, vol. 40, no. 1, p. 91.

Matthews, L, Temple, W, Foster, T, Walker, J & McAdie, T 1995, 'Comparison of the demand for dustbathing substrates by layer hens', in *Proceedings of the 29th International Congress of the International Society for Applied Ethology*, vol. 3, p. 11.

Matthews, L, Walker, J, Foster, T & Temple, W 1998, 'Influence of reward magnitude on elasticity of demand for dustbathing in hens', in *Proceedings of the 32nd Congress of the International Society for Applied Ethology*, p. 86.

Matthews, K, Wilkinson, LS & Robbins, TW 1996, 'Repeated maternal separation of preweanling rats attenuates behavioral responses to primary and conditioned incentives in adulthood', *Physiology & Behavior*, vol. 59, no. 1, pp. 99-107.

Maynard Smith, J & Harper, D 2003, *Animal Signals*, Oxford University Press, Oxford.

McComb, K, Taylor, AM, Wilson, C & Charlton, BD 2009, 'The cry embedded within the purr', *Current Biology*, vol. 19, no. 13, pp. R507-R508.

McConnell, PB 1991, 'Lessons from animal trainers: the effect of acoustic structure on an animal's response', *Perspectives in Ethology* (USA).

McGrath, N, Burman, O, Dwyer, C & Phillips, CJ 2016, 'Does the anticipatory behaviour of chickens communicate reward quality?', *Applied Animal Behaviour Science*, vol. 184, pp. 80-90.

McGrath, N, Dunlop, R, Dwyer, C, Burman, O & Phillips, CJ 2017, 'Hens vary their vocal repertoire and structure when anticipating different types of reward', *Animal Behaviour*, vol. 130, pp. 79-96.

McGregor, PK 1992, 'Quantifying responses to playback: One, many, or composite multivariate measures?', in PK McGregor (ed.), *Playback and Studies of Animal Communication*, Springer US, Boston, MA, pp. 79-96.

McGregor, PK 2000, 'Playback experiments: design and analysis', *Acta Ethologica*, vol. 3, no. 1, pp. 3-8.

Meen, GH, Schellekens, MA, Slegers, MHM, Leenders, NLG, van Erp-van der Kooij, E & Noldus, LPJJ 2015. 'Sound analysis in dairy cattle vocalisation as a potential welfare monitor'. *Computers and Electronics in Agriculture* 118, pp. 111-115.

Meijsser, FM & Hughes, BO 1989, 'Comparative analysis of pre-laying behavior in battery cages and in 3 alternative systems', *British Poultry Science*, vol. 30, no. 4, pp. 747-760.

Mello, CV, Vicario, DS & Clayton, DF 1992, 'Song presentation induces gene expression in the songbird forebrain', *Proceedings of the National Academy of Sciences*, vol. 89, no. 15, pp. 6818-6822.

Mendl, M, Burman, OHP & Paul, ES 2010, 'An integrative and functional framework for the study of animal emotion and mood', *Proceedings of the Royal Society B*, vol. 277, no. 1696, pp. 2895-2904.

Merola, I, Prato-Previde, E & Marshall-Pescini, S 2012, 'Social referencing in dog-owner dyads?', *Animal Cognition*, vol. 15, no. 2, pp. 175-185.

Miklosi, A, Polgárdi, R, Topál, J & Csányi, V 2000, 'Intentional behaviour in dog-human communication: an experimental analysis of "showing" behaviour in the dog', *Animal Cognition*, vol. 3, no. 3, pp. 159-166.

Moe, RO, Nordgreen, J, Janczak, AM, Bakken, M, Spruijt, BM & Jensen, P 2014. 'Anticipatory and foraging behaviors in response to palatable food reward in chickens: Effects of dopamine D2 receptor blockade and domestication', *Physiology & Behavior* 133, pp. 170-177.

Moe, RO, Nordgreen, J, Janczak, AM, Spruijt, BM & Bakken, M 2013. 'Effects of signalled reward type, food status and a μ-opioid receptor antagonist on cue-induced anticipatory behaviour in laying hens (*Gallus domesticus*)', *Applied Animal Behaviour Science* 148, pp. 46-53.

Moe, RO, Nordgreen, J, Janczak, AM, Spruijt, BM, Kostal, L, Skjerve, E, Zanella, AJ & Bakken, M 2011. 'Effects of haloperidol, a dopamine D2-like receptor antagonist, on reward-related behaviors in laying hens', *Physiology & Behavior* 102, pp. 400-405.

Moe, RO, Nordgreen, J, Janczak, AM, Spruijt, BM, Zanella, AJ & Bakken, M., 2009. 'Trace classical conditioning as an approach to the study of reward-related behaviour in laying hens: A methodological study', *Applied Animal Behaviour Science* 121, pp. 171-178.

Moe, RO, Bakken, M, Kittilsen, S, Kingsley-Smith, H & Spruijt, BM 2006, 'A note on reward-related behaviour and emotional expressions in farmed silver foxes (*Vulpes vulpes*) - Basis for a novel tool to study animal welfare', *Applied Animal Behaviour Science*, vol. 101, no. 3-4, pp. 362-368.

Moffatt, CA & Hogan, JA 1992, 'Ontogeny of chick responses to maternal food calls in the Burmese red junglefowl (*Gallus gallus spadiceus*)', *Journal of Comparative Psychology*, vol. 106, no. 1, pp. 92-96.

Molet, M, Miller, H & Zentall, T 2012, 'Acquired equivalence of cues by presentation in a common context in rats', *Animal Cognition*, vol. 15, no. 1, pp. 143-147.

Molnár, C, Pongrácz, P & Miklósi, Á 2010, 'Seeing with ears: Sightless humans' perception of dog bark provides a test for structural rules in vocal communication', *Quarterly Journal of Experimental Psychology*, vol. 63, no. 5, pp. 1004-1013.

Morton, ES 1977, 'On the occurrence and significance of motivation-structural rules in some bird and mammal sounds', *The American Naturalist*, vol. 111, no. 981, pp. 855-869.

Murphy, D, Lea, SEG & Zuberbühler, K 2013, 'Male blue monkey alarm calls encode predator type and distance', *Animal Behaviour*, vol. 85, no. 1, pp. 119-125.

Murray, IR & Arnott, JL 1993, 'Toward the simulation of emotion in synthetic speech: A review of the literature on human vocal emotion', *The Journal of the Acoustical Society of America*, vol. 93, no. 2, pp. 1097-1108.

Musolf, K, Hoffmann, F & Penn, DJ 2010, 'Ultrasonic courtship vocalizations in wild house mice, Mus musculus musculus', *Animal Behaviour*, vol. 79, no. 3, pp. 757-764.

Nelson, DGK, Hirsh-Pasek, K, Jusczyk, PW & Cassidy, KW 1989, 'How the prosodic cues in motherese might assist language learning', *Journal of Child Language*, vol. 16, no. 1, pp. 55-68.

Nesse, RM 1990, 'Evolutionary explanations of emotions', *Human Nature*, vol. 1, no. 3, pp. 261-289.

Newman, JD & Wollberg, Z 1973, 'Multiple coding of species-specific vocalizations in the auditory cortex of squirrel monkeys', *Brain Research*, vol. 54, pp. 287-304.

Nicastro, N & Owren, MJ 2003, 'Classification of domestic cat (*Felis catus*) vocalizations by naive and experienced human listeners', *Journal of Comparative Psychology*, vol. 117, no. 1, pp. 44-52.

Nicol, CJ, Caplen, G, Edgar, J & Browne, WJ 2009, 'Associations between welfare indicators and environmental choice in laying hens', *Animal Behaviour*, vol. 78, no. 2, pp. 413-424.

Nowicki, S 1987, 'Vocal tract resonances in oscine bird sound production: evidence from birdsongs in a helium atmosphere', *Nature*, vol. 325, no. 6099, pp. 53-55.

Ohms, VR, Snelderwaard, PC, Cate, CJT & Beckers, GJL 2010, 'Vocal tract articulation in zebra finches', *PloS ONE*, vol. 5, no.7, e11923.

Olsson, IAS & Keeling, LJ 2000, 'Night-time roosting in laying hens and the effect of thwarting access to perches', *Applied Animal Behaviour Science*, vol. 68, no. 3, pp. 243-256.

Olsson, IAS & Keeling, LJ 2005, 'Why in earth? Dustbathing behaviour in jungle and domestic fowl reviewed from a Tinbergian and animal welfare perspective', *Applied Animal Behaviour Science*, vol. 93, no. 3–4, pp. 259-282.

Olsson, IAS, Keeling, LJ & McAdie, TM 2002, 'The push-door for measuring motivation in hens: An adaptation and a critical discussion of the method', *Animal Welfare*, vol. 11, no. 1, pp. 1-10.

Owings, DH & Morton, ES 1998, *Animal vocal communication: A new approach*, Cambridge University Press, Cambridge, UK.

Panksepp, J & Burgdorf, J 2000, '50-kHz chirping (laughter?) in response to conditioned and unconditioned tickle-induced reward in rats: effects of social housing and genetic variables', *Behavioural Brain Research*, vol. 115, no. 1, pp. 25-38.

Panksepp, J & Burgdorf, J 2003, '“Laughing” rats and the evolutionary antecedents of human joy?', *Physiology & Behavior*, vol. 79, no. 3, pp. 533-547.

Panksepp, J 1998, *Affective Neuroscience: the foundations of human and animal emotions*, Oxford University Press, Oxford, UK.

Panksepp, J 2005, 'Affective consciousness: Core emotional feelings in animals and humans', *Consciousness and Cognition*, vol. 14, no. 1, pp. 30-80.

Panksepp, J 2010, 'Emotional causes and consequences of social-affective vocalization', in *Handbook of Behavioral Neuroscience*, Elsevier, vol. 19, pp. 201-208.

Panksepp, J 2011, 'Toward a cross-species neuroscientific understanding of the affective mind: Do animals have emotional feelings?', *American Journal of Primatology*, vol. 73, no. 6, pp. 545-561.

Perez, EC, Elie, JE, Soulage, CO, Soula, HA, Mathevon, N & Vignal, C 2012, 'The acoustic expression of stress in a songbird: Does corticosterone drive isolation-induced modifications of zebra finch calls?', *Hormones and Behavior*, vol. 61, no. 4, pp. 573-581.

Peters, G 2012, ‘Purring and similar vocalizations in mammals’, Mammal Review, vol. 32, pp. 245-271.

Peters, SM, Bleijenberg, EH, van Dierendonck, MC, van der Harst, JE & Spruijt, BM 2012, 'Characterization of anticipatory behaviour in domesticated horses (*Equus caballus*)', *Applied Animal Behaviour Science*, vol. 138, no. 1-2, pp. 60-69.

Petherick, JC, Seawright, E & Waddington, D 1993, 'Influence of motivational state on choice of food or a dustbathing/foraging substrate by domestic hens', *Behavioural Processes*, vol. 28, no. 3, pp. 209-220.

Petrie, A & Sabin, C 2013, *Medical statistics at a glance*, John Wiley & Sons.

Pollak, GD 2013, 'The dominant role of inhibition in creating response selectivities for communication calls in the brainstem auditory system', *Hearing Research*, vol. 305, pp. 86-101.

Pongrácz, P, Molnár, C & Miklósi, Á 2006, 'Acoustic parameters of dog barks carry emotional information for humans', *Applied Animal Behaviour Science*, vol. 100, no. 3–4, pp. 228-240.

Pongrácz, P, Molnár, C & Miklósi, Á 2010, 'Barking in family dogs: An ethological approach', *The Veterinary Journal*, vol. 183, no. 2, pp. 141-147.

Pongrácz, P, Molnár, C, Dóka, A & Miklósi, Á 2011, 'Do children understand man's best friend? Classification of dog barks by pre-adolescents and adults', *Applied Animal Behaviour Science*, vol. 135, no. 1, pp. 95-102.

Pongrácz, P, Molnar, C, Miklosi, A & Csanyi, V 2005, 'Human listeners are able to classify dog (*Canis familiaris*) barks recorded in different situations', *Journal of Comparative Psychology*, vol. 119, no. 2, pp. 136-144.

Preston, SD & de Waal, FBM 2002, 'Empathy: Its ultimate and proximate bases', *Behavioral and Brain Sciences*, vol. 25, no. 1, pp. 1-20.

Puppe, B, Schön, PC, Tuchscherer, A & Manteuffel, G 2005, 'Castration-induced vocalisation in domestic piglets, *Sus scrofa*: complex and specific alterations of the vocal quality', *Applied Animal Behaviour Science*, vol. 95, no. 1, pp. 67-78.

Quervel-Chaumette M, Faerber V, Faragó T, Marshall-Pescini S, Range F 2016, 'Investigating empathy-like responding to conspecifics' distress in pet dogs', PLoS ONE vol. 11, e0152920

R Core Team 2017, *R: A language and environment for statistical computing.*, R Foundation for Statistical Computing, Vienna, Austria,

Reichard, DG & Anderson, RC 2015, 'Why signal softly? The structure, function and evolutionary significance of low-amplitude signals', *Animal Behaviour*, vol. 105, pp. 253-265.

Reimert, I, Bolhuis, JE, Kemp, B & Rodenburg, TB 2013, 'Indicators of positive and negative emotions and emotional contagion in pigs', *Physiology & Behavior*, vol. 109, no. 0, pp. 42-50.

Rekdahl, ML, Dunlop, RA, Noad, MJ & Goldizen, AW 2013, 'Temporal stability and change in the social call repertoire of migrating humpback whales', *The Journal of the Acoustical Society of America*, vol. 133, no. 3, pp. 1785-1795.

Rendall, D & Owren, MJ 2002, 'Animal vocal communication: Say what?', in M Bekoff, C Allen & G Burghardt (eds), *The cognitive animal. Empirical and theoretical perspectives on animal cognition*, MIT Press, Cambridge, MA, pp. 307-313.

Rendall, D 2003, 'Acoustic correlates of caller identity and affect intensity in the vowel-like grunt vocalizations of baboons', *The Journal of the Acoustical Society of America*, vol. 113, no. 6, pp. 3390-3402.

Rendall, D, Owren, MJ & Ryan, MJ 2009, 'What do animal signals mean?', *Animal Behaviour*, vol. 78, no. 2, pp. 233-240.

Ridley, AR, Wiley, EM & Thompson, AM 2014, 'The ecological benefits of interceptive eavesdropping', *Functional Ecology*, vol. 28, no. 1, pp. 197-205.

Riede, T, Mitchell, BR, Tokuda, I & Owren, MJ 2005, 'Characterizing noise in nonhuman vocalizations: Acoustic analysis and human perception of barks by coyotes and dogs', *Journal of the Acoustical Society of America*, vol. 118, no. 1, pp. 514-522.

Riesch, R & Deecke, V 2011, 'Whistle communication in mammal-eating killer whales (Orcinus orca): further evidence for acoustic divergence between ecotypes', *Behavioral Ecology and Sociobiolog*y, vol. 65, no. 7, pp. 1377-1387.

Ripmeester, EAP, De Vries, AM & Slabbekoorn, H 2007, 'Do blackbirds signal motivation to fight with their song?', *Ethology*, vol. 113, no. 11, pp. 1021-1028.

Rodenburg, TB, Koene, P, Bokkers, EAM, Bos, MEH, Uitdehaag, KA & Spruijt, BM, 2005. ‘Can short-term frustration facilitate feather pecking in laying hens?’, *Applied Animal Behaviour Science* 91, pp. 85-101.

Rolls, ET 2000, 'Precis of *The Brain and Emotion*', *Behavioural and Brain Sciences*, vol. 23, no. 2, pp. 177-234.

Rolls, ET 2005, *Emotion explained*, Oxford University Press.

Russell, CL, Bard, KA & Adamson, LB 1996, 'Social referencing by young chimpanzees', *Journal of Comparative Psychology*, vol. 111, no.2, pp. 185-193

Russell, JA 1980, 'A circumplex model of affect', *Journal of Personality and Social Psychology*, vol. 39, no. 6, pp. 1161-1178

Sander, K & Scheich, H 2001, 'Auditory perception of laughing and crying activates human amygdala regardless of attentional state', *Cognitive Brain Research*, vol. 12, no. 2, pp. 181-198.

Sauter, DA & Eimer, M 2009, 'Rapid detection of emotion from human vocalizations', *Journal of Cognitive Neuroscience*, vol. 22, no. 3, pp. 474-481.

Sauter, DA, Eisner, F, Calder, AJ & Scott, SK 2010a, 'Perceptual cues in nonverbal vocal expressions of emotion', *The quarterly journal of experimental psychology*, vol. 63, no. 11, pp. 2251-2272.

Sauter, DA, Eisner, F, Ekman, P & Scott, SK 2010b, 'Cross-cultural recognition of basic emotions through nonverbal emotional vocalizations', *Proceedings of the National Academy of Sciences*, vol. 107, no. 6, pp. 2408-2412.

Scarantino, A & Clay, Z 2015, 'Contextually variable signals can be functionally referential', *Animal Behaviour*, vol. 100, pp. e1-e8.

Scherer, KR, Banse, R & Wallbott, HG 2001, ‘Emotion inferences from vocal expression correlate across languages and cultures’, *Journal of Cross-Cultural Psychology* 32, pp. 76-92.

Scherer, KR 1984, 'On the nature and function of emotion: A component process approach', in KR Scherer & P Ekman (eds), *Approaches to Emotion,* Psychology Press, New York, NY pp. 293-317

Scherer, KR 1986, 'Vocal affect expression: A review and a model for future research', *Psychological Bulletin*, vol. 99, no. 2, pp. 143-165.

Scherer, KR 2003, 'Vocal communication of emotion: A review of research paradigms', *Speech Communication*, vol. 40, no. 1–2, pp. 227-256.

Scheumann, M, Hasting, A, Kotz, S & Zimmermann, E 2014, 'The voice of emotion across species: How do human listeners recognize animals' affective states?', *PloS ONE*, vol. 9, no. 3, e91192.

Scheumann, M, Hasting, AS, Zimmermann, E & Kotz, SA 2017, 'Human novelty response to emotional animal vocalizations: Effects of phylogeny and familiarity', *Frontiers in Behavioral Neuroscience*, vol. 11, no.204.

Scheumann, M, Roser, A-E, Konerding, W, Bleich, E, Hedrich, H-J & Zimmermann, E 2012, 'Vocal correlates of sender-identity and arousal in the isolation calls of domestic kitten (*Felis silvestris catus*)', *Frontiers in Zoology*, vol. 9, no. 36.

Scheumann, M, Zimmermann, E & Deichsel, G 2007, 'Context-specific calls signal infants' needs in a strepsirrhine primate, the gray mouse lemur (*Microcebus murinus*)', *Developmental Psychobiology*, vol. 49, no. 7, pp. 708-718.

Schmidt, KL & Cohn, JF 2001, 'Human facial expressions as adaptations: Evolutionary questions in facial expression research', *American Journal of Physical Anthropology*, vol. 116, no. 33, pp. 3-24.

Schon, P, Puppe, B & Manteuffel, G 2004. 'Automated recording of stress vocalisations as a tool to document impaired welfare in pigs', *Animal Welfare* 13, pp. 105-110.

Schultz, W, Tremblay, L & Hollerman, JR 2000. 'Reward processing in primate orbitofrontal cortex and basal ganglia', *Cerebral Cortex* 10, pp. 272-283.

Scott-Phillips, TC 2008, 'Defining biological communication', *Journal of Evolutionary Biology*, vol. 21, no. 2, pp. 387-395.

Searcy, WA & Nowicki, S 2005, *The evolution of animal communication: reliability and deception in signalling systems*, Princeton University Press, Princeton.

Seay, B, Hansen, E & Harlow, HF 1962, 'Mother-infant separation in monkeys', *Journal of Child Psychology and Psychiatry and Allied Disciplines*, vol. 3, no. 3-4, pp. 123-132.

Seyfarth, RM & Cheney, DL 2003a, 'Meaning and emotion in animal vocalizations', *Annals of the New York Academy of Sciences*, vol. 1000, pp. 32-55.

Seyfarth, RM & Cheney, DL 2003b, 'Signalers and receivers in animal communication', *Annual Review of Psychology*, vol. 54, pp. 145-173.

Seyfarth, RM & Cheney, DL 2010, 'Production, usage, and comprehension in animal vocalizations', *Brain & Language*, vol. 115, pp. 92-100.

Seyfarth, R & Cheney, D 2018, 'Pragmatic flexibility in primate vocal production', *Current Opinion in Behavioral Sciences*, vol. 21, pp. 56-61.

Seyfarth, RM, Cheney, DL & Marler, P 1980, 'Vervet monkey alarm calls: Semantic communication in a free-ranging primate', *Animal Behaviour*, vol. 28, no. 4, pp. 1070-1094.

Seyfarth, RM, Cheney, DL, Bergman, T, Fischer, J, Zuberbühler, K & Hammerschmidt, K 2010, 'The central importance of information in studies of animal communication', *Animal Behaviour*, vol. 80, no. 1, pp. 3-8.

Sherry, DF 1977, 'Parental food-calling and the role of the young in the Burmese red junglefowl (*Gallus gallus spadiceus*)', *Animal Behaviour*, vol. 25, no. 3, pp. 594-601.

Shettleworth, SJ 2010, 'Clever animals and killjoy explanations in comparative psychology', *Trends in Cognitive Sciences*, vol. 14, no. 11, pp. 477-481.

Siebert, ER & Parr, LA 2003, 'A structural and contextual analysis of chimpanzee screams', *Annals of the New York Academy of Sciences*, vol. 1000, no. 1, pp. 104-109.

Siebert, K, Langbein, J, Schoen, P-C, Tuchscherer, A & Puppe, B 2011, 'Degree of social isolation affects behavioural and vocal response patterns in dwarf goats (*Capra hircus*)', *Applied Animal Behaviour Science*, vol. 131, no. 1-2, pp. 53-62.

Sievers, C & Gruber, T 2016, 'Reference in human and non-human primate communication: What does it take to refer?', *Animal Cognition*, vol. 19, no. 4, pp. 759-768.

Silk, JB, Seyfarth, RM & Cheney, DL 2016, 'Strategic use of affiliative vocalizations by wild female baboons', *PloS ONE*, vol. 11, no. 10, e0163978.

Skaug, H, Fournier, D, Nielsen, A, Magnusson, A & Bolker, B 2013, 'glmmADMB: Generalized linear mixed models using AD Model Builder', *R package version*, vol. 0.7.5.

Slocombe, KE & Zuberbühler, K 2006, 'Food-associated calls in chimpanzees: responses to food types or food preferences?', *Animal Behaviour*, vol. 72, no. 5, pp. 989-999.

Smith, JM 1994, 'Must reliable signals always be costly?', *Animal Behaviour*, vol. 47, no. 5, pp. 1115-1120.

Smith, SF, Appleby, MC & Hughes, BO 1993, 'Nesting and dust bathing by hens in cages: Matching and mis-matching between behaviour and environment', *British Poultry Science*, vol. 34, no. 1, pp. 21-33.

Soltis, J, Blowers, TE & Savage, A 2011, ‘Measuring positive and negative affect in the voiced sounds of African elephants (*Loxodonta africana*)’, *Journal of the Acoustical Society of America* 129, pp. 1059-1066.

Song, X, Osmanski, M, S. , Guo, Y & Wang, X 2016, 'Complex pitch perception mechanisms are shared by humans and a New World monkey', *Proceedings of the National Academy of Sciences*, vol. 113, no. 3, pp. 781-786

Spruijt, BM, van den Bos, R & Pijlman, FTA 2001, 'A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems', *Applied Animal Behaviour Science*, vol. 72, no. 2, pp. 145-171.

Stoeger, AS, Charlton, BD, Kratochvil, H & Fitch, WT 2011, 'Vocal cues indicate level of arousal in infant African elephant roars', *The Journal of the Acoustical Society of America*, vol. 130, no. 3, pp. 1700-1710.

Šuta, D, Popelář, J, Burianová, J & Syka, J 2013, 'Cortical representation of species-specific vocalizations in Guinea pig', *PloS ONE*, vol. 8, no. 6, e65432.

Taglialatela, JP, Russell, JL, Schaeffer, JA & Hopkins, WD 2008, 'Visualizing vocal perception in the chimpanzee brain', *Cerebral Cortex*, vol. 19, no. 5, pp. 1151-1157.

Tallet, C, Linhart, P, Policht, R, Hammerschmidt, K, Simecek, P, Kratinova, P & Spinka, M 2013, 'Encoding of situations in the vocal repertoire of piglets (*Sus scrofa*): A comparison of discrete and graded classifications', *PloS ONE*, vol. 8, no. 8, e71841.

Tallet, C, Spinka, M, Maruscakova, I & Simecek, P 2010, 'Human perception of vocalizations of domestic piglets and modulation by experience with domestic pigs (*Sus scrofa*)', *Journal of Comparative Psychology*, vol. 124, no. 1, pp. 81-91.

Taylor, AM & Reby, D 2010, 'The contribution of source–filter theory to mammal vocal communication research', *Journal of Zoology*, vol. 280, no. 3, pp. 221-236.

Taylor, AM, Reby, D & McComb, K 2009, 'Context-related variation in the vocal growling behaviour of the domestic dog (*Canis familiaris*)', *Ethology*, vol. 115, no. 10, pp. 905-915.

Templeton, CN, Greene, E & Davis, K 2005, 'Allometry of alarm calls: black-capped chickadees encode information about predator size', *Science*, vol. 308, no. 5730, pp. 1934-1937.

Theunissen, FE & Elie, JE 2014, 'Neural processing of natural sounds', *Nature Reviews Neuroscience*, vol. 15, pp. 355-366

Thomas, DA, Howard, SB & Barfield, RJ 1982, 'Male-produced ultrasonic vocalizations and mating patterns in female rats', *Journal of Comparative and Physiological Psychology*, vol. 96, no. 5, pp. 807-815

Titze, IR 1994, *Principles of voice production*, Englewood Cliffs, N.J. Prentice Hall.

Titze, IR, Baken, RJ, Bozeman, KW, Granqvist, S, Henrich, N, Herbst, CT, Howard, DM, Hunter, EJ, Kaelin, D & Kent, RD 2015, 'Toward a consensus on symbolic notation of harmonics, resonances, and formants in vocalization', *The Journal of the Acoustical Society of America*, vol. 137, no. 5, pp. 3005-3007.

Topal, J, Gacsi, M, Miklosi, A, Viranyi, Z, Kubinyi, E & Csanyi, V 2005, 'Attachment to humans: a comparative study on hand-reared wolves and differently socialized dog puppies', *Animal Behaviour*, vol. 70, pp. 1367-1375.

Townsend, SW & Manser, MB 2013, 'Functionally referential communication in mammals: the past, present and the future', *Ethology*, vol. 119, no. 1, pp. 1-11.

Townsend, SW, Charlton, BD & Manser, MB 2014, 'Acoustic cues to identity and predator context in meerkat barks', *Animal Behaviour*, vol. 94, pp. 143-149.

Townsend, SW, Zöttl, M & Manser, MB 2011, 'All clear? Meerkats attend to contextual information in close calls to coordinate vigilance', *Behavioral Ecology and Sociobiology*, vol. 65, no. 10, pp. 1927-1934.

Ungerfeld, R, Hotzel, MJ, Scarsi, A & Quintans, G 2011, 'Behavioral and physiological changes in early-weaned multiparous and primiparous beef cows', *Anim*al, vol. 5, no. 8, pp. 1270-1275.

Vaish, A & Striano, T 2004, 'Is visual reference necessary? Contributions of facial versus vocal cues in 12-month-olds' social referencing behavior', *Developmental Science*, vol. 7, no. 3, pp. 261-269.

van den Bos, R, Meijer, MK, van Renselaar, JP, van der Harst, JE & Spruijt, BM 2003, 'Anticipation is differently expressed in rats (*Rattus norvegicus*) and domestic cats (*Felis silvestris catus*) in the same Pavlovian conditioning paradigm', *Behavioural Brain Research*, vol. 141, no. 1, pp. 83-89.

van der Harst, JE & Spruijt, BM 2007, 'Tools to measure and improve animal welfare: reward-related behaviour', *Animal Welfare*, vol. 16, no. Supplement 1, pp. 67-73.

van der Harst, JE, Baars, AM & Spruijt, BM 2003, 'Standard housed rats are more sensitive to rewards than enriched housed rats as reflected by their anticipatory behaviour', *Behavioural Brain Research*, vol. 142, no. 1-2, pp. 151-156.

van der Harst, JE, Baars, AM & Spruijt, BM 2005, 'Announced rewards counteract the impairment of anticipatory behaviour in socially stressed rats', *Behavioural Brain Research*, vol. 161, no. 2, pp. 183-189.

Vandermeulen, J, Bahr, C, Tullo, E, Fontana, I, Ott, S, Kashiha, M, Guarino, M, Moons, CPH, Tuyttens, FAM, Niewold, TA & Berckmans, D 2015, 'Discerning pig screams in production environments', *PloS ONE*, vol. 10, no. 4, e0123111.

Vannoni, E & McElligott, AG 2007, 'Individual acoustic variation in fallow deer (Dama dama) common and harsh groans: A source-filter theory perspective', *Ethology*, vol. 113, no. 3, pp. 223-234.

Vasconcellos, AdS, Harumi Adania, C & Ades, C 2012, 'Contrafreeloading in maned wolves: Implications for their management and welfare', *Applied Animal Behaviour Science*, vol. 140, no. 1–2, pp. 85-91.

Vestergaard, K 1980, 'The regulation of dustbathing and other behaviour patterns in the laying hen: a Lorenzian approach', *Current Topics in Veterinary Medicine and Animal Science*, Hague, vol. 8, pp. 101-113.

Vestergaard, K 1982, 'Dust-bathing in the domestic fowl—diurnal rhythm and dust deprivation', *Applied Animal Ethology*, vol. 8, no. 5, pp. 487-495.

Vestergaard, KS, Skadhauge, E & Lawson, LG 1997, 'The stress of not being able to perform dustbathing in laying hens', *Physiology & Behavior*, vol. 62, no. 2, pp. 413-419.

Vinke, CM, Houx, BB, van den Bos, R & Spruijt, BM 2006, 'Anticipatory behaviour and stereotypical behaviour in farmed mink (*Mustela vison*) in the presence, absence and after the removal of swimming water', *Applied Animal Behaviour Science*, vol. 96, pp. 129-142

Waitt, C & Buchanan-Smith, HM 2001, 'What time is feeding?: How delays and anticipation of feeding schedules affect stump-tailed macaque behavior', *Applied Animal Behaviour Science*, vol. 75, no. 1, pp. 75-85.

Waran, N 2001, 'The social behaviour of horses', in L Keeling & HW Gonyou (eds), *Social Behaviour in Farm Animals*, CABI Publishing, Wallingford, UK, pp. 247-274.

Watts, JM, Stookey, JM, Schmutz, SM & Waltz, CS 2001, 'Variability in vocal and behavioural responses to visual isolation between full-sibling families of beef calves', *Applied Animal Behaviour Science*, vol. 70, no. 4, pp. 255-273.

Waugh, CE & Gotlib, IH 2008, 'Motivation for reward as a function of required effort: Dissociating the 'liking' from the 'wanting' system in humans', *Motivation and Emotion*, vol. 32, no. 4, pp. 323-330.

Wauters, A, Richard-Yris, M, Pierre, J, Lunel, C & Richard, J 1999, 'Influence of chicks and food quality on food calling in broody domestic hens', *Behaviour*, vol. 136, no. 7, pp. 919-933.

Wauters, AM & Richard-Yris, MA 2002, 'Mutual influence of the maternal hen's food calling and feeding behavior on the behavior of her chicks', *Developmental Psychobiology*, vol. 41, no. 1, pp. 25-36.

Weary, DM & Fraser, D 1995, 'Calling by domestic piglets: reliable signals of need?', *Animal Behaviour*, vol. 50, no. 4, pp. 1047-1055.

Weary, DM & Fraser, D 1997, 'Vocal response of piglets to weaning: effect of piglet age', *Applied Animal Behaviour Science*, vol. 54, no. 2-3, pp. 153-160.

Weary, DM, Appleby, MC & Fraser, D 1999, 'Responses of piglets to early separation from the sow', *Applied Animal Behaviour Science*, vol. 63, no. 4, pp. 289-300.

Weeks, CA & Nicol, CJ 2006, 'Behavioural needs, priorities and preferences of laying hens', *Worlds Poultry Science Journal*, vol. 62, no. 2, pp. 296-307.

Wheeler, BC & Fischer, J 2012, 'Functionally referential signals: A promising paradigm whose time has passed', *Evolutionary Anthropology: Issues, News, and Reviews*, vol. 21, no. 5, pp. 195-205.

Wichman, A & Keeling, LJ 2008, 'Hens are motivated to dustbathe in peat irrespective of being reared with or without a suitable dustbathing substrate', *Animal Behaviour*, vol. 75, no. 4, pp. 1525-1533.

Wichman, A, Keeling, LJ & Forkman, B 2012, 'Cognitive bias and anticipatory behaviour of laying hens housed in basic and enriched pens', *Applied Animal Behaviour Science*, vol. 140, no. 1–2, pp. 62-69.

Widowski, TM & Duncan, IJH 2000, 'Working for a dustbath: are hens increasing pleasure rather than reducing suffering?', *Applied Animal Behaviour Science*, vol. 68, no. 1, pp. 39-53.

Wit, S & Dickinson, A 2009, 'Associative theories of goal-directed behaviour: a case for animal–human translational models', *Psychological Research*, vol. 73, no. 4, pp. 463-476.

Wood-Gush, D & Guiton, P 1967, 'Studies on thwarting in the domestic fowl', *Rev. Comp. Anim*, vol. 5, pp. 1-23.

Woodgush, DG 1971, *The behaviour of the domestic fowl*, Heinemann Educational Books Ltd, London.

Wood-Gush, DGM & Gilbert, AB 1964, 'The control of the nesting behaviour of the domestic hen. II. The role of the ovary', *Animal Behaviour*, vol. 12, no. 4, pp. 451-453.

Wunder, MB 2014, 'Western burrowing owls (Athene cunicularia hypugaea) eavesdrop on alarm calls of black-tailed prairie dogs (Cynomys ludovicianus)', Ethology, vol. 120, pp. 180-188.

Wundt, WM 1905, *Grundzüge der physiologischen Psychologie*, Leipzig: W. Engelmann.

Yeon, SC, Kim, YK, Park, SJ, Lee, SS, Lee, SY, Suh, EH, Houpt, KA, Chang, HH, Lee, HC, Yang, BG & Lee, HJ 2011, 'Differences between vocalization evoked by social stimuli in feral cats and house cats', *Behavioural Processes*, vol. 87, no. 2, pp. 183-189.

Yin, S & McCowan, B 2004, 'Barking in domestic dogs: context specificity and individual identification', *Animal Behaviour*, vol. 68, no. 2, pp. 343-355.

Yong, MH & Ruffman, T 2014, 'Emotional contagion: Dogs and humans show a similar physiological response to human infant crying', *Behavioural Processes*, vol. 108, pp. 155-65.

Zahavi, A & Zahavi, A 1997, *The handicap principle: a missing piece of Darwin's puzzle*, Oxford University Press, New York.

Zala, SM, Reitschmidt, D, Noll, A, Balazs, P & Penn, DJ, 2017, ‘Automatic mouse ultrasound detector (A-MUD): A new tool for processing rodent vocalizations’, *PloS ONE,* vol. 12, no.7, e0181200.

Zimmerman, PH & Koene, P 1998, 'The effect of frustrative nonreward on vocalisations and behaviour in the laying hen, *Gallus gallus domesticus*', *Behavioural Processes*, vol. 44, no. 1, pp. 73-79.

Zimmerman, PH, Buijs, SAF, Bolhuis, JE & Keeling, LJ 2011, 'Behaviour of domestic fowl in anticipation of positive and negative stimuli', *Animal Behaviour*, vol. 81, no. 3, pp. 569-577.

Zimmerman, PH, Koene, P & van Hooff, J 2000, 'Thwarting of behaviour in different contexts and the gakel-call in the laying hen', *Applied Animal Behaviour Science*, vol. 69, no. 4, pp. 255-264.

Zimmerman, PH, Lundberg, A, Keeling, LJ & Koene, P 2003, 'The effect of an audience on the gakel-call and other frustration behaviours in the laying hen (*Gallus gallus domesticus*)', *Animal Welfare*, vol. 12, no. 3, pp. 315-326.

Zuberbühler, K 2000, 'Interspecific semantic communication in two forest monkeys', *Proceedings of the Royal Society B*, vol. 267, pp. 713-718.

Zuberbuhler, K 2003, 'Referential signalling in non-human primates: cognitive precursors and limitations for the evolution of language', *Advances in the Study of Behavior*, vol. 33, pp. 265-308.

Zuberbühler, K 2009, 'Survivor signals: the biology and psychology of animal alarm calling', *Advances in the Study of Behavior*, vol. 40, pp. 277-322.

Zuberbuhler, K, Noe, R & Seyfarth, RM 1997, 'Diana monkey long-distance calls: messages for conspecifics and predators', *Animal Behaviour*, vol. 53, no. 3, pp. 589-604.

UQ Research and Innovation
Director, Research Management Office
Nicole Thompson

ANIMAL ETHICS APPROVAL CERTIFICATE

20-Jul-2012

Activity Details

Chief Investigator:	Professor Clive Phillips, Animal Welfare and Ethics
Title:	Vocalisations of Domestic Fowl (Gallus gallus) in anticipation of, during and after Rewarding Situations
AEC Approval Number:	SVS/314/12
Previous AEC Number:	
Approval Duration:	01-Aug-2012 to 01-Aug-2013
Funding Body:	Internal Funds - UQ
Group:	Production and Companion Animal
Other Staff/Students:	Nicola McGrath
Location(s):	Centre for Advanced Animal Science (CAAS)

Summary

Subspecies	Strain	Class	Gender	Source	Approved	Remaining
Poultry		Adults	Female	Privately owned	12	12

Permit(s):

Proviso(s):

Approval Details

Description	Amount	Balance
Poultry (Female, Adults, Privately owned)		
18 Jul 2012 Initial Approval	12	12

THE UNIVERSITY OF QUEENSLAND

UQ Research and Innovation
Director, Research Management Office
Nicole Thompson

ANIMAL ETHICS APPROVAL CERTIFICATE

22-Apr-2013

Activity Details

Chief Investigator:	Professor Clive Phillips, Animal Welfare and Ethics
Title:	Vocalisations of domestic fowl
AEC Approval Number:	CAWE/066/13/VOICELESS
Previous AEC Number:	SVS/314/12/
Approval Duration:	23-Apr-2013 to 23-Apr-2016
Funding Body:	Voiceless
Group:	Production and Companion Animal
Other Staff/Students:	Nicky McGrath
Location(s):	Gatton Bldg 360 - CAAS

Summary

Subspecies	Strain	Class	Gender	Source	Approved	Remaining
Poultry	Brown ISA	Adults	Female	UQ	24	24

Permit(s):

Proviso(s):

Approval Details

Description	Amount	Balance
Poultry (Brown ISA, Female, Adults, UQ)		
17 Apr 2013 Initial Approval	24	24

THE UNIVERSITY OF QUEENSLAND

Institutional Human Research Ethics Approval

Project Title: Human Perception of the Vocalisations of Laying Hens

Chief Investigator: Ms Nicola McGrath

Supervisor: Prof Clive Phillips

Co-Investigator(s): None

School(s): School of Veterinary Science

Approval Number: 2016001225

Granting Agency/Degree: PhD

Duration: 31st January 2017

Comments/Conditions:

Expedited review - Low Risk

Note: if this approval is for amendments to an already approved protocol for which a UQ Clinical Trials Protection/Insurance Form was originally submitted, then the researchers must directly notify the UQ Insurance Office of any changes to that Form and Participant Information Sheets & Consent Forms as a result of the amendments, before action.

Name of responsible Committee:
University of Queensland Human Research Ethics Committee A
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:
Dr. Frederick Khafagi
Chairperson
University of Queensland Human Research Ethics Committee
Registration: EC00457

02/11/2016

Signature __________________________ Date ______________________