Patterns of parental care and chick recognition in the joint-nesting pūkeko (Porphyrio melanotus melanotus)

Patterns of parental care and chick recognition in a joint-nesting rail, Pūkeko (*Porphyrio melanotus melanotus*)

By Courtney Anne Young,

A Thesis Submitted to the School of Graduate Studies in the Partial Fulfillment of the Requirements for the Degree Masters of Science

McMaster University

Masters of Science (2017)

Hamilton, Ontario (Department of Biology)

TITLE: Patterns of parental care and chick recognition in a joint-nesting rail, Pūkeko (*Porphyrio melanotus melanotus*)

AUTHOR: Courtney Anne Young , BSc (McMaster University)

SUPERVISOR: Dr. James S. Quinn

NUMBER OF PAGES: xvii, 76

Lay Abstract

Kin recognition is an essential ability for social species. Knowing whom is kin can help inform decisions on cooperation and conflict. I explored whether the joint-nesting pūkeko use familiarity or phenotype matching to recognise cross-fostered offspring. I experimented to determine if adult pūkeko can recognise the distress vocalizations of chicks in their group. I found no evidence that pūkeko use phenotypic templates to recognise cross-fostered chicks as non-kin. However, adult pūkeko showed a bias in response towards the distress calls of their own versus unfamiliar chicks. Individual chick distress calls, while variable from day-to-day, show group-specific similarities.

Abstract

Group living is a widespread social system among animals. Within these groups, decisions on interactions between individuals can be facilitated through knowledge about individual identity and kinship. Individual identity allows for the recognition of individuals from past interactions and thus, information on likelihood of reciprocity and group-membership can be gained. The benefit for cooperative interactions, specifically, increases with the level of relatedness between the helper and the recipient. Thus, knowing who is kin, is an essential ability among group-living species and remembering individual identity helps to maintain long-term relationships and inform future decisions. Kin recognition can be facilitated through temporal and spatial overlap (i.e. familiarity) or through phenotypic-templates (i.e. phenotype matching). The goal of this thesis was to explore recognition in the joint-nesting pūkeko (*Porphyrio melanotus melanotus*). For the first portion of this thesis (Chapter II), I tested for evidence of phenotype matching in pūkeko using a cross-fostering experiment. Comparing survival and growth between fostered and non-fostered offspring, I provide evidence that pūkeko do not use phenotype matching as their mechanism for kin recognition. In Chapter III, I show that pūkeko chick distress calls may have an individual and group signature. I found variation in the vocal parameters between individual chicks and social groups. I also tested for response of adults towards chick distress calls of their own group. Using a playback-choice experiment, I report a biased response of adult pūkeko towards the distress call of their own group's chicks rather than the call of a distressed chick from a foreign chick.

Acknowledgements

I would first like to extend a huge thank you to my supervisor, Dr. Jim Quinn, for providing the opportunity to further my education and apply my love of bird social behaviour. I am so grateful for the opportunity you have given me, sending me to New Zealand to study pūkeko. I am hugely grateful for the freedom of scope you gave me in my projects, allowing me to further develop my skills in trapping and handling birds, as well as gain new skills in acoustic, behavioural and molecular research.

Next, I would like to thank my committee members, Dr. Ben Bolker and Dr. Sigal Balshine. Both of you have provided invaluable guidance throughout my research. I always left meetings feeling better about my thesis than when I arrived. The critical thinking you both put into my research is a skill I will strive to emulate in the future. Also thank you to Ben for his help with R.

Great appreciation must be given to my field assistants, Julie Galloway and Lauren Snobl. Their help with finding nests, catching chicks and collecting data are vey appreciated, however most appreciated is how well they dealt with so many issues in the field... riding passenger while I learned how to drive our standard field vehicle (Ang), and hanging around Matakana while it went to the shop (several times)... troubleshooting equipment and technical failures... two sprained ankles which left me unable to run after chicks... and just generally dealing with my stressed out self. Without their help and patience during my 2016 field season, I would not have any of the data for this thesis.

I would also like to thank all the people I met at Tawharanui. First and foremost, to Colin Wards and Maurice "Mo" Puckett, and Matt Maitland. Colin and Mo, you were both tremendously helpful helping me navigate the park and troubleshoot (and sometimes save my bacon) when things didn't go right. Thank you to Matt Maitland for your patience and allowing me to conduct pukeko research in the park. I would like to thank all the rangers at Tawharanui as well as the Tawharanui Open Sanctuary Society (TOSSI) volunteers for their help and friendly faces. Thank you especially to Ngaire Wallen and her husband Stewart for being gracious enough to allow us to rent your summer home for both field seasons.

To all of the people who have inhabited the Vol-hole at one time or another; the researchers, student rangers, interns, and contract workers, it was a pleasure meeting you all and having people around to chat to during lunch breaks and after the day was done. It was one of the best parts of the week going to the Rusty Pelican for 2-1 pizzas or $10 burgers or even just staying at the Vol-hole making food and hanging around. It made my life feel normal when it would otherwise be consumed by my research. Specifically, I would like to send my love and gratitude to my German, Jonas Kotlarz. Your friendship during my first field season helped keep me sane and Tawharanui never felt the same without you (no one ever asked me to help them with their laundry).

I would also like to thank my lab mates for their support, guidance and friendship over the last two years. First, to Josh Robertson for being the lab expert and being unwaveringly positive and cheerful. I am forever grateful to you for giving me a ride to the Animal Behaviour Society conference in Scarberia this summer. Next, I would like to thank Sheng Hing for being a beam of sunshine and support.

Your endless patience and willingness to provide codes and lab tutelage have been invaluable. Finally to Meghan Healey. You have been more than a lab mate, but also a room mate, friend, bed mate, confidant, co-conspirator and teacher. Words cannot describe how important you have been to me since I started this journey. Things started out bumpy, but those bumps have molded our relationship into a steadfast and amazing friendship. I have so many great memories with you from that first field season that I cannot listen to a hit song from 2015 without thinking of you. I thoroughly enjoyed living with you both in Tawharanui and Hamilton and still call what is now my office, "Meghan's room". I'm not sure who was the bad influence on whom, but I loved all our shenanigans that resulted in uncontrollable giggling. Besides the emotional support, you taught me how to run down pūkeko chicks, how to call pūkeko to bait (though you will always be the master baiter), how to measure, sample and band birds, and basically how to do any of the basic field techniques I needed for this thesis. I am so thankful to you for being a sounding board during my PCR struggles. I love you so much Meghan and I cannot imagine a world without you in it anymore.

Next, I would like to thank my friends who have been hugely supportive of me in the last two years. Ali, I am so happy that you decided to do your Master's degree at McMaster last year. It was so great to move to Hamilton and have someone from home around. You have always been unconditionally supportive and loving since we became friends almost six years ago. Thank you for everything. Savanna, thank you for proving that "online friending" is a thing. It's been incredibly great to end my weeks spending the night at your place drinking wine, eating cheesy poofs, and getting love from your doggos. Having that time to dish about our

personal and professional lives has been a highlight of living in Hamilton. Finally I would like to thank my best friend and partner, Ryan, you have always been a comforting presence in my life since I met you five years ago, especially this last year since we started dating. Thank you for being ridiculously supportive of me; from adapting to a New Zealand timezone so you could Skype me from Ottawa after I was finished my field work to actually moving to Hamilton to be with me. Thank you for your love and support in every aspect of my life.

In addition, I would like to thank my family for their continued love and support. To my parents for their constant and never ending support for my unconventional career choice of bird chasing. Thank you both for being sympathetic ears when things have gotten hard. I am extremely grateful for everything you have done for me in my life and all my success is a testament to both of you. Also thanks to my sisters, Emily and Kelly for being both friends and sisters. I've loved getting to spend time with you when I've gone home or to Halifax for a break; drinking wine and being too loud. Finally thank you to my extended family, my grandparents, my aunts and uncles, and my cousins. Whether the support was silent, or occasional messages, or hosting me for a dinner or weekend visit, I appreciate everything.

Finally, I would like to thank my birds, the pūkeko at Tawh, without whom this research would not have been possible.

Contents

List of Figures

List of Tables

Declaration of Authorship

I, Courtney Anne YOUNG, declare that this thesis titled, "Patterns of parental care and chick recognition in a joint-nesting rail, Pūkeko (*Porphyrio melanotus melanotus*)" and the work presented in it are my own. I confirm that:

- **Chapter 1: General Introduction**
  - *Author*: Courtney A. Young
- **Chapter 2: Joint-nesting pukeko fail to use direct recognition of kin in a cross-fostering experiment.**
  - *Author*: Courtney A. Young
  - CAY wrote the manuscript and conducted all statistical analyses. Statistical guidance provided by Dr. Ben Bolker. Dr. James Quinn and Dr. Sigal Balshine provided comments and revised the manuscript. Field data collected by CAY, Julie Galloway, and Lauren Snobl.
- **Chapter 3: Adult pukeko use individually variable distress calls to discriminate care between kin and non-kin chicks.**
  - *Author*: Courtney A. Young

– CAY wrote the manuscript and conducted all statistical analyses. Statistical guidance provided by Dr. Ben Bolker. Dr. James Quinn and Dr. Sigal Balshine provided comments and revised the manuscript. Field data collected by CAY, Julie Galloway, and Lauren Snobl. Acoustic analysis conducted by CAY. Scoring of video data by Achini de Silva and Hayley McKee.

"*Alone I wandered between worlds, the objective world of species, natural history and names, and the subjective world of symbols and signs.*"

Eva Saulitis

Chapter 1

General Introduction

1.1 Background

The study of social behaviour is a rich and complicated part of the field of behavioural ecology. It encompasses many aspects of interaction between individuals such as reproduction, dominance, cooperation, aggression, and territoriality, to name a few. Key to the maintenance of these social behaviours, specifically in behaviours that require long-term interactions between the same individuals, is for animals to be able to recognise other individuals to make informed decisions.

Individual recognition occurs when an individual can recognize another based on individually distinctive characteristics (Tibbetts & Dale, 2007). The context in which these characteristics are learned helps to inform future interactions. Contexts of behaviour in which individual recognition is important include territoriality and dominance (Barnard & Burk, 1979; Whitfield, 1986; Karavanich & Atema, 1998; Bee & Gerhardt, 2002), group membership (Clapperton, 1987), parental care (Medvin *et al.*, 1993), and mate identification (Miller, 1979).

Cooperative breeding is a rare social system; an estimated 852 (9%) of 9000 known bird species are shown to exhibit this behaviour (Cockburn, 2006) as well as some mammal (Koenig, 1997; Lukas & Clutton-Brock, 2012), fish (Balshine, 2012; McKaye & McKaye, 1977; Wisenden, 1999) and insect species (Sherman *et al.*, 1995; Peer & Taborsky, 2007). In birds, cooperative breeding is expressed in a variety of ways. The most common form of cooperative breeding in birds is the helper-at-the-nest system. In this system, individuals help a socially monogamous pair by helping to feed offspring. These helpers are typically offspring of one of the dominant pair's previous broods or are other close kin (Crognier *et al.*, 2001).

Alternatively, multiple members of the same sex may contribute to the same nest which is known as joint-nesting. Joint-nesting occurs when multiple reproductive members of the same sex contribute to a portion of the genes represented in a single clutch. This can take the form of communal laying (i.e. joint-female), or cooperative polyandrous (i.e. joint-male) systems (Verhencamp, 2000).

At first glance, cooperative breeding appears maladaptive as the helper's time, energy and resources are being spent to further the genetic lineage of another individual where there is no direct contribution to the helper's fitness. Hamilton's Rule, otherwise known as kin selection theory (Hamilton, 1964) resolves this evolutionary puzzle though the value of inclusive fitness. Kin selection theory states that helping behaviour that results in a direct fitness cost for the acting individual should only be expressed when it increases their inclusive fitness. Thus, cooperative breeding should evolve in situations where the individual gains either direct or indirect fitness benefits through helping others to breed and should most strongly favour closely related individuals (Hatchwell *et al.*, 2014). Such nepotism requires a recognition of closely related kin.

In social systems, the ability to recognise close kin is a valuable tool used by individuals to make decisions on how to interact with another. Kin recognition is the ability to identify relatives, whereas the expression of this ability is what is defined as kin discrimination. Kin discrimination will occur if the benefits of preferential treatment outweigh the costs (Waldman, 1988).

Some researchers argue that alloparental care can, in some part, be due to the inability for an animal to recognize the difference between its own offspring and

another's (Riedman, 1982). Although this may be the case for some species that are likely to experience brood parasitism (Rothstein, 2001), many animals are adept at using various cues to distinguish their own kin and can use this ability to bias their care towards more closely related individuals in the group or nest (Wisenden, 1999).

The two main mechanisms of kin recognition have been categorized as: 1) indirect recognition or familiarity, and 2) direct recognition or phenotype matching, (Hepper, 1986; Waldman, 1988). Indirect recognition is a rule-of-thumb-based recognition which combines contextually reliable circumstantial evidence such as overlapping space and time to determine which individuals are likely to be kin and which are not (Waldman, 1988). This method of recognition is most beneficial and accurate in species where all individuals born to the same social group or brood are likely to be close relatives.

In some species, such as those that participate in intra-specific brood parasitism and communal breeding, we expect selection pressure to be discriminative of young hatched in their nests; therefore, more robust methods of kin recognition should evolve (Beecher, 1982). In these cases, it does not benefit the potential helper to assume relatedness based on spatial and temporal contexts, so a more direct mechanism of kin recognition, is needed. Direct recognition is when individuals use the expression of specific traits to help determine kin status (Waldman, 1988). Examples of situations in which direct recognition is favoured over indirect recognition include species that are subject to brood parasitism non-monogamous mating systems; and systems in which it is advantageous for siblings who have fledged from different broods of the same parents to identify unfamiliar kin in

later years (Waldman, 1988).

It is important to note the distinction between the use of phenotype for individual recognition and kin recognition. A phenotypically expressed trait that signals individuality is learned during the initial encounter and then is used in future encounters to determine individual identity regardless of kinship (Tibbetts & Dale, 2007). On the other hand, phenotype matching for kin recognition uses phenotypically expressed traits that signal genetic relatedness. These traits share features with kin and are learned using themselves or a close relative as a template. Thus, the shared traits can be used to infer kinship upon the initial encounter (Waldman, 1988). Whether kinship is determined through genetically shared phenotypes, or through contextual overlap, individually expressed phenotypes can be used for individual recognition in subsequent encounters. An example of a species that uses familiarity to infer kinship while using individual phenotype to remember individual identity is the cooperatively breeding splendid fairy-wren (*Malurus splendens*). Birds of this species can recognize familiar and non-familiar songs and will react aggressively to songs from wrens from other groups (Payne *et al.*, 1988). In this case, kin recognition is based on familiarity rather than recognising a genetically shared phenotype. The splendid fairy-wren learns the individual phenotypes of familiar birds and uses this for later interactions. Alternatively, phenotype matching for kin recognition can be made without any prior interaction with the individual such as in the case of white-bearded manakins (*Manacus manacus*) in which males preferentially lek with close relatives without familiarity (Shorey *et al.*, 2000). In this species, clutches are small and nest predation is high, suggesting that any adult in the population is unlikely to have any living nest mates. Thus, without

learning their sibling's individual phenotypes at the nest, they must use genetic phenotypes, such as their own or their parents' phenotypes to recognise unfamiliar kin as adults.

In cooperatively breeding, helper-at-the-nest systems, in which breeding is by a genetically monogamous pair and all offspring in the nest are full siblings, the assumption made through indirect recognition is accurate. However, for communal, non-monogamous breeding systems, in which genetic relatedness of offspring or siblings in the nest cannot be accurately assumed, direct methods of recognition must be used. This dichotomy fails to predict more complex social breeding systems, such as groups that are inbred. In highly inbred groups, it may not matter specifically how the dependent young are related to the caring adult. Ultimately though, the ways in which an individual recognizes kin is a product of it's social ecology. This study will investigate this relationship in a joint-nesting bird and determine whether females can recognise and discriminate towards their own offspring.

1.2 Study Species

The pūkeko (*Porphyrio melanotus melanotus*) is a communally breeding, joint-nesting rail native to New Zealand. Social groups of pūkeko consist of 3-12 individuals that maintain a territory and raise chicks cooperatively (Craig, 1980). Pūkeko are polygynandrous breeders. The adults in these groups form a linear dominance hierarchy in which the most dominant individuals of both sexes have the greatest breeding opportunities (Craig, 1980).

Typically, a joint-nest includes a clutch of eggs laid by 2-3 females sharing the same nest. Incubation duties are shared by adults in the group, with the both sexes incubating during the day, and males incubating during the night (Craig, 1980). For both sexes, the most dominant individual in the group monopolizes incubation (Jamieson & Craig, 1987). Incubation of the nest begins mid-clutch and lasts a little over three weeks (Craig, 1980).

Despite being able to leave the nest after 2-3 days after hatching, chicks are reliant on adult feedings for up to two months prior to leaving the nest (Craig, 1980). Offspring fledge after 2-3 months (Dey & Jamieson, 2013; Dey *et al.*, 2014). Groups on the North Island have higher rates of inbreeding and relatedness within groups compared to those on the South Island. (Craig & Jamieson, 1988; Jamieson *et al.*, 1994; Jamieson, 1997). Chicks on the North Island, are likely to remain in their natal territory (Craig & Jamieson, 1988).

All pūkeko in a group aggressively maintain a territory (Craig, 1977), although most defensive interactions are by males (Craig, 1979; Craig & Jamieson, 1990); any trespassers on the group's territory are promptly attacked and expelled (Craig, 1980). Identifying a trespassing individual involves the ability to distinguish between group-mates and outsiders and discriminate appropriately. Studies have shown that pūkeko can discriminate based solely on vocalizations of the adults (Clapperton, 1987). This discrimination extends to mobile chicks as young as a week old that accidentally wander into a neighbouring territory. Trespassing chicks are aggressively chased down, held down, and repeatedly pecked by adults belonging to that territory (Young, pers. obs.). There is little doubt that group members

can recognize and discriminate between chicks belonging to their group and foreign chicks from other territories. Whether this recognition is through direct or indirect mechanisms has yet to be determined.

1.3 Thesis Aims

The primary goal of this thesis was to determine if and how joint-nesting pūkeko can use kin recognition to discriminate among kin and non-kin chicks. Previous work has determined that female pūkeko are unable to discriminate their own eggs; however, work has not yet been done to determine the mechanisms involved in chick recognition within a breeding group. The mating system of the pūkeko, in which individuals breed polygynadrously, but exclusively within the group, suggests that they are likely unable to use indirect kin recognition as an accurate measure of kinship. It is, however, possible that female pūkeko have evolved the ability to recognize their own offspring through direct cues to provide them with a higher direct fitness advantage. My first study set out to determine if pūkeko can use direct mechanisms to recognise non-kin chicks hatched in their nest. Using a cross-fostering experiment, I swapped eggs between paired nests and tracked the survival of chicks hatched from these nests. I hypothesised that pūkeko use direct mechanisms of kin recognition due to their non-monogamous, joint-nesting breeding system. Based on personal observations of aggressive interactions of adults towards foreign chicks, I then predicted that foster chicks would have significantly lower survival rates in their foster groups compared to their non-fostered nest mates. My second study's goal was to determine if pūkeko use vocal cues to

recognise and discriminate between group and non-group chicks. Regardless of the mechanism of recognition (i.e. direct versus indirect), there is clearly recognition of chicks belonging to the group. Based on this, I hypothesized that adult pūkeko use vocal cues to recognise and discriminate among group and non-group chicks. From my hypothesis, I predicted that pūkeko chick distress calls have individual acoustic characteristics that can provide aa basis for recognition. To test this, I analysed the distress calls of pūkeko chicks and extracted various acoustic characteristics. I then ran a series of linear discriminant analyses to test whether chick distress calls could be accurately grouped by individual chick as well as social group. I also predicted that adult pūkeko would show a stronger response towards the distress cries of a chick from their group being handled than to that of a distressed chick from a different group. To test this prediction, I designed a playback-choice experiment wherein the calls of a group and non-group chick were played to a group to observe responsiveness of the adults to both calls. The goal of this thesis was to explore the ability of the joint-nesting pūkeko to recognise their own chicks. Through my thesis research on this unusual breeding system, I hope to expand our understanding of cooperative breeding and kin recognition.

1.4 Literature Cited

Barnard, C.J., & Burk, T. 1979. Dominance hierarchies and the evolution of "individual recognition". *J. Theor. Biol.* **81:** 65-73.

Balshine, S. 2012. Patterns of parental care in vertebrates. *The evolution of parental care.* 62-80.

Bee, M. A., & Gerhardt, H.C. 2002. Individual voice recognition in a territorial frog (*Rana catesbeiana*). *Proc. R. Soc. Lond. B.* **269(1499):** 1443-1448.

Beecher, M. D. 1982. Signature systems and kin recognition. *Am. Zool.* **22(3):** 477-490.

Clapperton, BK. 1987. Individual recognition by voice in the pūkeko, *Porphyrio porphyrio melanotus* (Aves: Rallidae). *New Zealand J. of Zool.* **14:** 11-18.

Craig, J.L. 1977. The behaviour of the pūkeko, *Porphyrio porphyrio melanotus. New Zealand J. Zool.* **4(4):** 413-433.

Craig, J.L. 1979. Habitat variation in the social organization of a communal gallinule, the pūkeko, *Porphyrio porphyrio melanotus. Behav. Ecol. Sociobiol.* **5(4):** 331-358.

Craig, J.L. 1980. Pair and group breeding behaviour of a communal gallinule, the Pukeko, *Porphyrio p. melanotus. Anim. Behav.* **28:** 593-603.

Craig, J.L. & Jamieson, I.G. 1988. Incestuous mating in a communal bird: a family affair. *Am. Nat.* **131:** 58-70.

Craig, J.L. & Jamieson, I.G., 1990. Pukeko: different approaches and some different answers. *Cooperative breeding in birds: long-term studies of ecology and behavior.* 385-412.

Crognier, E., Baali, A., & Hilali, M. K. 2001. Do "helpers at the nest" increase their parents' reproductive success? *Am. J. Human. Biol.* **13(3):** 365-373.

Dey, C.J., & Jamieson, I.G. 2013. Pukeko. In Miskelly, CM. (ed.) New Zealand Birds Online. www.nzbirdsonline.org.nz

Dey, C.J., O'Connor, C., & Quinn, J.S. 2014. Hatching order affects offspring growth, survival and adult dominance in the joint-laying Pukeko *Porphyrio melanotus melanotus*. *Ibis.* **156:** 658-667.

Hamilton, WD. 1964. The genetical evolution of social behaviour, I & II. *J. Theor. Biol.* **7:** 1-52.

Hatchwell, BJ., Gullett, PR., & Adams, MJ. 2014. Helping in cooperatively breeding long-tailed tits: a test of Hamilton's rule. *Philos Trans R Soc B.* **369:** 20130565.

Hepper, PG. 1986. Kin recognition: functions and mechanisms, a review. *Bio. Rev.* **61:** 63-93.

Jamieson, I.G., & Craig, J.L. 1987. Dominance and mating in a communal polygynandrous bird: Cooperation or indifference towards mating competitors? *Ethology.* **75:** 317-327.

Jamieson, I. G., Quinn, J. S., Rose, P. A., & White, B. N. 1994. Shared paternity among non-relatives is a result of an egalitarian mating system in a communally breeding bird, the pūkeko. *Proc. R. Soc. Lond. B.* **257(1350):** 271-277.

Jamieson, I. G. 1997. Testing reproductive skew models in a communally breeding bird, the pūkeko, *Porphyrio porphyrio*. *Proc. R. Soc. Lond. B.* **264(1380):**

335-340.

Karavanich, C., & Atema, J. 1998. Individual recognition and memory in lobster dominance. *Anim. Behav.* **56:** 1553-1560.

Koenig, B. 1997. Cooperative Care of Young in Mammals. *Naturwissenschaften.* **84(3):** 95-104.

Lukas, D., & Clutton-Brock, T. 2012. Cooperative breeding and monogamy in mammalian societies. *Proc. R. Soc. B.* **279(1736):** 2151-2156.

McKaye, K.R. & McKaye, N.M. 1977. Communal Care and Kidnapping of Young by Parental Cichlids. *Evolution.* **31(3):** 674-681.

Medvin, M.B., Stoddard, P.K., & Beecher, M.D. 1993. Signals for parent-offspring recognition: a comparative analysis of the begging calls of cliff swallows and barn swallows. *Anim. Behav.* **45(5):** 841-850.

Miller, D.B. 1979. The acoustic basis of mate recognition by female zebra finches (*Taeniopygia guttate*). *Anim. Behav.* **27:** 376-380.

Payne, R. B., Payne, L. L., & Rowley, I. 1988. Kin and social relationships in splendid fairy-wrens: recognition by song in a cooperative bird. Anim. Behav. 36(5): 1341-1351.

Peer, K., & Taborsky, M. 2007. Delayed dispersal as a potential route to cooperative breeding in ambrosia beetles. *Behav. Ecol. Sociobiol.* **61(5):** 729-739.

Riedman, M.L. & B.J. LeBoeuf. 1982. Mother-Pup Separation and Adoption in Northern Elephant Seals. *Behav. Ecol. Sociobiol.* **11:** 203-215.

Rothstein, S.I. 2001. Relic behaviours, co-evolution and the retention versus loos of host defences after episodes of avian brood parasitism. *Anim. Behav.* **61:** 95-107.

Sherman, P. W., Lacey, E. A., Reeve, H. K., & Keller, L. 1995. The eusociality continuum. *Behav. Ecol.* **6(1):** 102-108.

Shorey, L., Piertney, S., Stone, J., & Hoglund, J. 2000. Fine-scale genetic structuring on *Manacus manacus* leks. *Nature.* **408(6810):** 352-353.

Tibbetts, E.A., & Dale, J. 2007. Individual recognition: it is good to be different. *Trends Ecol. & Evol.* **22(10):** 529-537.

Varhrencamp, SL. 2000. Evolutionary routes to joint-female nesting in birds. *Behav. Ecol.* **11(3):** 334-344.

Waldman, B. 1988. The ecology of kin recognition. *Ann. Rev. Ecol. Systems.* **19:** 543-571.

Whitfield, D.P. 1986. Plumage variability and territoriality in breeding turnstone *Arenaria interpres*: status signalling or individual recognition? *Anim. Behav.* **34(5):** 1471-1482

Wisenden, B.D. 1999. Alloparental Care in fishes. *Rev.in Fish Bio. Fisheries.* **9:** 45-70.

Chapter 2

Joint-nesting pukeko fail to use direct recognition of kin in a cross-fostering experiment.

2.1 Abstract

Kin selection is an important driver to explain the evolution of some social behaviours. Kin recognition has been categorized as either indirect recognition (i.e. familiarity), or direct recognition (i.e. phenotype matching). Indirect recognition is beneficial in situations where kinship can be assumed, such as monogamous pair-breeding or helper-at-the-nest systems. In cases where kinship cannot accurately be assumed, for example, communal, polygynandrous systems, direct recognition may be used. My goal was to explain kin recognition mechanisms underlying cooperative breeding in the joint-nesting rail, pūkeko (*Porphyrio melanotus melanotus*). Groups of pūkeko on the North Island of New Zealand are highly inbred and will aggressively defend their territories against other pūkeko, including chicks from other groups. To explore whether pūkeko use familiarity or phenotype matching to determine kinship of young, I created a cross-fostering experiment, swapping eggs between nests and monitoring egg survival to hatching. Upon hatching, both foster and host chicks were monitored and compared for survival to 21 and 30 days as well as for individual growth rates. Our results suggest that pūkeko are unable to use phenotype matching for kin recognition as they show no evidence of discrimination between their own chicks and foster chicks hatched in their nest.

2.2 Introduction

Kin recognition is an important part of sociality, informing many decisions an individual makes on a day-to-day basis. Knowing whom is kin helps determine

whom to mate with, whom to cooperate with, and whom to compete with. This is largely described by Hamilton's rule of inclusive fitness; if kin selection is occurring, helping is expected to be biased towards those of higher kinship to the donor. The degree to which an individual is related to another can change how an interaction affects the actor's inclusive fitness (Hamilton, 1964). Thus, kin recognition is an important attribute in social decision making.

Waldman (1988) described two mechanisms of kin recognition including indirect recognition or familiarity, which relies on rules-of-thumb based on contextual circumstances of shared time and space. Most bird species use indirect recognition to determine whom is and is not kin in their groups and it has been suggested to be an ancestral trait of all birds (Beecher, 1988). Animals may use indirect recognition to direct parental care towards young in their social group, litter or brood if the spatial and temporal overlap reliably predicts genetic relatedness (Hepper, 1986; Waldman,1988). For example, a single-nesting, monogamous pair-breeding female bird can usually assume that a chick born in its nest is its own, and that chick can assume that all its nest mates are its siblings. Thus, most individuals can be certain that being raised in the same nest is a fair rule of thumb for being a sibling or half-sibling (except for in intraspecific brood parasitic species). Furthermore, in cooperative helper-at-the-nest systems, the alloparents are usually safe in assuming they are helping half or full-siblings.

The second mechanism of recognition, direct recognition or phenotype matching, relies on phenotypic cues to determine genetic relatedness; learned either through self-referencing, or based on past encounters with assumed kin (Waldman, 1988). The key difference between familiarity and phenotype matching, is

that phenotype matching is expected when the kin discrimination should be able to occur without any prior interaction between the donor and recipient; while familiarity relies on the donor sharing time and space with the recipient during the early stages of the recipient's life. While not the norm in avian systems, phenotype matching is not uncommon in the animal kingdom and there is evidence of discrimination of unfamiliar kin based on phenotypic cues in birds (Bateson, 1978; Hoglund *et al.*, 1999; Petrie *et al.*, 1999; Piertney *et al.*, 1999; Hauber, 2000; Shorey *et al.*, 2000; Shizuka & Lyon, 2010; McDonald & Wright, 2011). Phenotype matching is most beneficial in breeding systems where temporal and spatial proximity are poor predictors of relatedness. Examples include joint-nesting species in which more than one member of the same sex in a nest is contributing to the parentage of offspring, or in other systems where brood mixing is common such as in colonial or brood parasitic species (Beecher, 1982). In these cases, chicks of mixed parentage overlap in time and space and thus parents must use genetically expressed phenotypic cues to infer relatedness. Many females nesting in a mixed-brood system rely on phenotypic cues to recognise their own egg either by rejecting the "odd" phenotype in a clutch (Lorenzana & Sealy, 2001) or by using her own eggs as a template (Lotem, 1992; Lyon, 1993; Victoria, 1972; Hauber & Sherman, 2001; Lyon, 2003; Dolores *et al.*, 2010).

The polygynandrous, joint-nesting pūkeko (*Porphyrio melanotus melanotus*), is an ideal candidate for further study of kin recognition mechanisms and how they relate to the breeding ecology of the species. On the North Island of New Zealand, pūkeko live in groups of 3-12 individuals. These groups show little natal or adult dispersal and adults may be inbred (Craig & Jamieson, 1988). Although not all

adults in the group may breed, care of young is cooperative and all adults in the group may contribute to caring for chicks (Craig 1980a; Jamieson & Craig, 1987).

Using a cross-fostering experimental design, we tested the ability of adult pūkeko to recognize and discriminate against cross-fostered chicks hatched in their nest. We hypothesized, pūkeko should use direct mechanisms of kin recognition due to their polygynandrous, joint-nesting breeding system. If pūkeko use direct recognition, we predicted that fostered chicks with no adult direct kin in the group should be discriminated against, and have lower survival and growth when compared to their non-fostered nest mates. Alternatively, if pūkeko use indirect recognition, there should be no discrimination and survival and growth rates for fostered and non-fostered chicks should be equal.

2.3 Methods

Methods This study was conducted at Tawharanui Regional Park (36° 22' 10" S, 174° 49' 58" E), a combined open sanctuary and working farm run by the Auckland Regional Council (ARC), located on the North Island of New Zealand. The park is free from exotic terrestrial mammalian predators, protected by a pest-proof fence. In 2015, the Tawharanui pūkeko population was estimated to be about 1035 individuals (Healey, 2017).

Nests were located from early September to late December 2016. Nests were typically found by searching areas of suitable nesting habitat (i.e. *Juncus*, *Carex*, *Typha orientalis*, and *Pennisetum clanestinum*) (Dey et al., 2014), watching for

nesting behaviours, as well as by chance encounter. Upon discovery, eggs in the nest were photographed, measured for length and width and marked with non-toxic markers.

Cross-fostering experiment

One day before cross-fostering occurred, every egg in each nest in the study site was measured for progress of development by egg flotation in warm water (36 °C to 38 °C). Eggs were floated in a clear plastic container and assessed for relative stage of development using a method adapted from Hays and LeCroy (1971). Egg angle and position in the water was photographed and recorded. Nests with eggs at similar developmental stages were then paired for swapping.

In total, we assessed 24 nests for progress of development but only used 14 nests for swapping. Nests with < 2 eggs or hatching chicks were excluded from the experiment as well as nests that did not show advanced development of eggs or signs of incubation (Supplementary Figure A.1). We created 5 pairs of nests for swapping leaving the remaining 4 nests as controls, which were manipulated similarly to swap nests but the eggs were returned to their original nest. From these nests, we used 24 eggs in total for this experiment; 6 controls, and 18 eggs for swapping. We selected 1 to 2 eggs for cross-fostering and a similar number for controls at each nest depending on the total clutch size. Sample sizes were kept small to avoid potential conflict with other ongoing experiments with the population.

We took fostered and control eggs from their original nest and placed them in a bucket lined with cloth towels for insulation. Care was taken to ensure that all

eggs involved in the experiment were handled similarly. We kept all study eggs in the bucket away from nests for 30 minutes, which was the longest period it took to get from one end of the study site to the other. After 30 minutes, we placed the foster eggs in their paired nest. Eggs that were used as controls for handling were returned to their original nest. During the time when foster and control eggs were out of the nest, we left the remaining eggs in the nest.

Nest and chick monitoring

After swapping, we checked the nests once a day for warmth, cracks in the eggs, presence in the nest, and clutch size. When signs of hatching were observed in a nest (e.g. piping, peeping, etc.), nest checks increased to twice a day.

Upon hatching, we measured each chick for mass, length from the posterior edge of the shield to the tip of the upper bill, and length of the left tarsus. We also sampled chicks for approximately 30 to 50 uL of blood by basilic venipuncture and capillary tube collection. The blood was stored in 1.8 mL of Queen's Lysis Buffer. Chicks in each nest were marked individuals by specific toenail clipping. If known, chicks were assigned to the egg from which they came. If eggs were not assigned, they were excluded from the experiment.

For the remainder of the field season, study territories were searched 4-7 times a week for chicks. When found, and caught, we measured the chicks and, if large enough, banded them. Upon reaching >30g in mass, we fitted each chick with one colour leg band (approximately 0.2g) above the knee. Chicks that reached >40g in mass received a metal band (approximately 0.4g) on the opposite leg below the knee. Chicks that survived to > 200g were fitted with a unique complement of

four colour bands above the knee (in addition to the metal band). We determined subsequent survival by observing individually banded chicks.

Molecular Sexing

We used a standard phenol-chloroform-isoamyl alcohol (25:24:1) protocol (Sambrook et al., 1989) to extract chick DNA from collected blood samples. DNA samples were diluted to about 50 ng/uL for amplification using Polymerase Chain Reaction (PCR). We sexed pūkeko chicks by amplifying intron 16 in the NIPBL gene using the primers NIPBLi16F (5'-TTGTCAGAGTTGCTGGAGATAC-3') and NIPBLi16R (5'-AATTTGATGGCA- CATAACTGTAG-3') (Suh et al., 2011) to produce amplicons for the Z-chromosome (approximately 1200bp) and W-chromosome (approximately 500bp). PCR amplification was done using a method from Healey *et al.* (2017). Amplicons were observed using gel electrophoresis under trans-UV illumination.

Statistical Analysis

All statistical tests were run in R version 3.1.3 (R Core Team 2017).

To test the effect of fostering on egg survival, we ran a binomial GLMM with logit link function in the '*lme4*' package (Bates *et al.*, 2013). We fitted the models with Laplace approximation as suggested by Bolker *et al.*, (2009). We created a binary score of survival to hatching as the response variable. Eggs were categorized in one of three groups used as a fixed effect: 1) control eggs; 2) fostered eggs; and 3) unmanipulated eggs in the nests of fostered eggs. Control eggs and unmanipulated nest mates were both compared against fostered eggs. The destination nest of the

eggs was a random effect.

Remaining analyses included only manipulated (i.e. control or fostered) eggs that produced chicks. Nests that hatched only non-manipulated eggs were excluded.

To test the effect of fostering on chick survival, we created two binomial GLMMs using the '*blme*' package which allows the use of Bayesian statistics to avoid singularity (Dorie, 2015) using a similar design as for egg survival. We created a binary score of survival to three weeks (21 days) and one month (30 days), based on the date in which the chick was last seen alive, as response variables for the two models. Chicks were categorized in one of three treatment groups as a fixed effect; 1) chicks hatched from control eggs, 2) foster chicks hatched from swapped eggs, and 3) nest-mates of foster chicks. We included sex of the chick as a fixed interaction term. The destination nest of the eggs was a random effect.

The results from the model were then assessed using an informal equivalence test. This test is used in studies to test whether the outcomes of different experimental treatments are similar enough to be considered equivalent (Robinson, & Froese, 2004; Walker & Nowacki, 2011; Welleck & Blettner, 2012; Healey *et al.*, 2017). We tested whether the differences in proportion of surviving chicks between control and fostered chicks and fostered chicks and non-fostered nest mates were small enough to be considered statistically equivalent. To perform this test, we adapted a model from Healey *et al.* (2017), using a (-0.4, 0.4) region of negligible difference (RND) which was based on a log-odds change appropriate for the intermediate probabilities of survival seen in unmanipulated chicks. If the upper

and lower 95% confidence intervals for the treatment effect fell within the RND, we concluded that survival was equivalent between chick categories.

To assess the effect of cross-fostering on chick growth within 60 days, post-hatch, we used a linear mixed-effects model (LMM) using the '*nlme*' package (Pinheiro *et al.*, 2017). We used tarsus length as the response variable, which has been used in previous studies on pūkeko chick growth (Dey *et al.*, 2014; Healey *et al.*,2017). We included treatment (i.e. fostered versus non-fostered nest mates) as the main fixed effect. Chick age (days since hatching) was included as an interaction fixed effect. Due to low sample sizes (Table 2.1) of control chicks, we could not conduct the same comparison between control and fostered chicks.

2.4 Results

Egg Survival

Of the 94 eggs from 14 nests involved in this experiment, 49% survived to hatching. Of these, only 39% (n=7/18) of the fostered eggs survived to hatching compared to 50% (n=14/28) of the non-fostered eggs in the same foster nests (Table 2.1; Figure 2.1). Of the control eggs, 67% (n=4/6) survived to hatching (Table 2.1). The greatest cause of egg failure was destruction of the nest entirely followed by missing and damaged eggs (Table 2.1). The GLMM found no statistical difference between the survival of control and fostered eggs to hatch (estimate = 3.122, 95% CI = [-1.42, 7.66], P = 0.178), nor between fostered eggs and their non-fostered nest mates (estimate = 0.973, 95% CI = [-0.51, 2.46], P = 0.200)

(Table 2.2: Model 1). The final nest the eggs were placed in was found to have a significant effect on survival of eggs (Table 2.3). The results from the informal equivalence test failed to find statistical similarity in survival between control and fostered eggs (95% CI: [-1.42, 7.66], as well as between fostered eggs and their non-fostered nest mates (95% CI: [-0.51, 2.46]) (Figure 2.1, Table 2.2).

Chick Survival

Of the 14 experimental nests, only 7 produced fostered chicks and were thus used in the analysis for chick survival. In total, 50% (n=2/4) of control chicks, 57% (n=4/7) of fostered chicks and 50% (n= 7/14) of non-fostered nest mates survived to 21 days (Figure 2.2). Survival to 30 days was lower for fostered chicks (29%, n=2/7) and their non-fostered nest mates (43%, n=6/14) and there was no decrease in survival of control chicks (Figure 2.3). Results from the GLMM found treatment category of the chick did not significantly influence survival to 21 or 30 days for both comparisons between control and fostered chicks (21 days: estimate = -0.0224, 95% CI = [-2.86, 2.82], $P = 0.998$; 30 days: estimate = 0.5232, 95% CI = [-1.95, 3.00], $P = 0.679$), and fostered chicks and their non-fostered nest mates (21 days: estimate = -0.5935, 95% CI = [-1.51, 2.70], $P = 0.580$; 30 days: estimate = 0.8573, 95% CI = [-1.10, 2.81], $P = 0.390$) (Table 2.2: Model 2 & 3; Figures 2.2 & 2.3). Sex of the chick was not found to have a significant effect on survival to either 21 or 30 days, nor did it have a significant interaction with treatment group (Table 2.2). Hatch nest did not account for any of the variation in the data for either survival to 21 or 30 days (Table 2.3).

Our informal equivalence test failed to find statistical similarity in all comparisons (Table 2.2). We were unable to find statistical evidence to support no difference in survival between control and fostered chicks and fostered and non-fostered chicks to both 21 and 30 days because the 95% confidence intervals for those comparisons exceeded our (-0.4, 0.4) RND (Table 2.2).

Chick Growth

We found no difference in chick size between fostered chicks and their non-fostered nest mates within 60 days of hatching. Age and sex were not found to have any significant effect on chick growth (Table 2.4).

2.5 Discussion

Our results do not support the phenotype matching hypothesis that adult pūkeko can identify their offspring based on phenotypically-expressed genetic cues. These results mirror those of past studies (Dey & O'Connor, 2010; Quinn *et al.*, 2012) that have found no evidence of rejection of foreign eggs from the nests of pūkeko, suggesting pūkeko are unable to recognise their own eggs from foreign additions or, if they do, fail to behave in a way that suggests they can discriminate them. This is also consistent with a recent study on kin discrimination in the joint-nesting greater ani (*Crotophaga major*) which found no discrimination of chicks despite the apparent selective pressures to do so (Riehl & Strong, 2015).

GLMM analyses showed no difference in survival between fostered chicks and their non-fostered nest mates to 21 and 30 days, suggesting that adult pūkeko are

unable to recognise and discriminate between kin and non-kin hatched in their nest. While we did not find statistical difference in probability of survival for different treatment groups in the GLMM, we also cannot claim statistical similarity. The results from the informal equivalence tests could not support the conclusion that the different treatment groups had similar probabilities of survival, however, as the upper and lower 95% CIs of the GLMM fell outside our RND of (-0.4, 0.4) (Table 2.2). This outcome may be due to the low sample sizes following the death of 51% of the eggs (Table 2.1). This experiment was conducted as an exploratory pilot study and to avoid risking the results of other ongoing experiments, the number of nests and eggs involved were kept to a minimum. Higher sample sizes in the future would likely clarify the relationship. A power analysis suggests that to observe a large effect size (> 0.25 for a binomial distribution as defined by Cohen, 1992) at a power of 0.8 and a significance level of 5%, a sample size of 25 to 50 nests should be suitable to detect a significant decrease in survival for fostered chicks (Figure 2.4). To observe medium (0.15; Cohen, 1992) and small (0.05; Cohen 1992) effect sizes, however, we would need over 100 nests (Figure 2.4).

The results of our GLMMs are congruent with most research on birds to date; phenotype matching in birds has been found to be a rare trait with only a handful of species showing evidence of it (Bateson, 1978; Hoglund *et al.*, 1999; Petrie *et al.*, 1999; Piertney *et al.*, 1999; Hauber, 2000; Shorey *et al.*, 2000; Shizuka & Lyon, 2010; McDonald & Wright, 2011). Indirect recognition, which relies on the context in time and space, may be a useful enough tool for pair breeding, genetically monogamous species of birds (Beecher, 1988). Excepting the possibility of extra-pair paternity or brood parasitism, parents may reasonably assume that any chick

in their nest is their own direct offspring. When this assumption cannot be met with certainty, we would expect to see parents use direct recognition, relying on genetically-expressed phenotypic cues to determine relatedness. Pūkeko, while joint-nesting and polygynandrous within their breeding groups, do not appear to show kin recognition through phenotype matching. It is possible that, because pūkeko on the North Island have low dispersal rates coupled with inbreeding (Craig & Jamieson, 1988), the assumption that individuals born within their territory are genetically very similar, is a fair rule-of-thumb.

Beecher (1988) argued that an inability of the parents to recognise their own offspring is required for the evolution of brood-mixing breeding strategies such as brood parasitism, and extra-pair copulations do not favour the evolution of phenotype matching. Phenotype matching should only evolve if it benefits both the signaller (i.e. offspring) as well as the recognizer (i.e. parent). Offspring would need to signal their genetic identity to their parents who, in turn, need to recognise kinship. Under phenotype matching, a chick with no genetic relatives in the nest would not benefit from signalling their genetic identity as parents would bias care towards their own chicks. (Beecher, 1988). This conflict between parents and non-related offspring, in which the parents benefit from recognition, while the offspring resulting from extra-parental breeding is not favourable in mixed-brood systems as unrelated chicks benefit most from not being recognised as non-kin.

Our results also support findings from past studies on pūkeko that suggest females are unable to identify their own eggs. This is unexpected since egg recognition is common in rail species which participate in conspecific brood parasitism and foreign eggs are discriminated against through burial, tossing, banishment, or

desertion of the nest (Sorenson, 1995; Jamieson *et al.*, 2000; Lyon, 2003, Shizuka & Lyon, 2010; McRae, 2011).

Pūkeko eggs have individual patterns that are unique to the female who laid them and these features are consistent over successive nests within a territory (Craig, 1980b). This morphological variation between eggs of different females in a joint-nest is so distinct that it has been found to be an accurate tool for researchers to differentiate maternity (Haselmayer, 2000; Quinn *et al.*, 2012; Dey *et al.*, 2014). This individuality might suggest the presence of female egg recognition; however, previous studies suggest that female pūkeko may not be able to recognise their own eggs (Dey & O'Conner, 2010; Quinn *et al.*, 2012). In pūkeko groups, all breeding individuals, both male and females, incubate indiscriminately, and there does not seem to be any evidence of egg sabotage such as burial, tossing, or destroying. Females who have had their eggs experimentally removed continued to incubate. (Quinn *et al.*, 2012). Furthermore, even when the egg of a heterospecific was found in a pūkeko nest, there was no evidence of a change in parental investment to the nest (Dey & O'Conner, 2010).

Previous studies on pūkeko have shown that in nests in which experimental egg removals occurred, males reduced nocturnal incubation investment in the nest, incubating less frequently and possibly even abandoning the nest altogether (Dey *et al.*, 2013). While there was no evidence of female competition and discrimination during incubation (Quinn *et al.*, 2012), it is possible that the kin discrimination does not occur until after the eggs are hatched. Females may recognise their own eggs, but through limitation by the males' behaviour, forgo discrimination until after the chicks have hatched, however if this is the case, the evidence could not

be discerned by our study.

Alternatively, if pūkeko are unable to recognise their own eggs, it is possible that they can use other cues to identify chicks that are kin. In this way, they could discriminate care after hatching and optimize direct fitness despite the limitation of male incubation effort. Although our study suggests that pūkeko groups are unable to identify fostered chicks, if breeding females are capable of recognising and biasing their care towards their direct offspring, we would expect see differences in survival and growth between chicks with relatives in the group compared with fostered chicks with no relatives.

Dey *et al.* (2014) found that first-hatched chicks were more likely to survive, have faster growth and achieve higher adult dominance compared to their nest mates. If females keep track of the order of egg laying as was suggested by Craig and Jamieson (1985), it is possible that the first-laying dominant females provide preferential care to their first-hatched chicks compared to the later hatched chicks that are more likely offspring of subordinate females. We found no difference in the growth rates between fostered chicks and their non-fostered nest mates, indicating that females may not preferentially feed their own offspring. A higher sample size is recommended for future work comparing growth rates between fostered and non-fostered chicks to see if females preferentially care for their own offspring.

In conclusion, our results do not support the use of direct mechanisms of phenotype matching to recognise pūkeko kin. Based on this evidence, the mechanism of kin recognition within these cooperatively breeding groups is likely based on an indirect model such as familiarity or location-based.

2.6 Acknowledgements

We would like to thank Julie Galloway and Lauren Snobl for their assistance with field work. We also thank Matt Maitland, Maurice Puckett, Colin Wards, the Tawharanui Open Sanctuary Society Incorporated and the Tawharanui Regional Park staff for their logistical help. Ben Bolker provided much appreciated statistical advice. This research was supported by an NSERC Discovery Grant to JSQ and a McMaster Biology Department Travel Scholarship to CAY.

2.7 Literature Cited

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Grothendieck, G., & Green, P. 2017. lme4. R package version 1.1-13. https://cran.r-project.org/web/packages/lme4/lme4.pdf

Bateson, P. 1978. Sexual imprinting and optimal outbreeding. *Nature.* **273:** 659-660.

Beecher, M. D. 1982. Signature systems and kin recognition. *Am. Zool.* **22(3):** 477-490.

Beecher, M.D. 1988. Kin recognition in birds. *Behav. Gen.* **18(4):** 465-481.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., & White, J-S.S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* **24(3):** 127-135.

Cohen, J. 1992. A power primer. *Psych. Bull.* **112:** 115-159.

Craig, J.L. 1980a. Pair and group breeding behaviour of a communal gallinule, the Pukeko, *Porphyrio p. melanotus*. *Anim. Behav.* **28:** 593-603.

Craig, J.L. 1980b. Breeding success of a communal gallinule. *Behav. Ecol. Sociobiol.* **6:** 289-298.

Craig, J.L., & Jamieson, I.G. 1985. The relationship between presumed gamete contribution and parental investment in a communally breeding bird. *Behav. Ecol. Sociobiol.* **17(3):** 207-211.

Craig, J. L., & Jamieson, I. G. 1988. Incestuous mating in a communal bird: a family affair. *Am. Nat.* **131(1):** 58-70.

Dey, C.J. & O'Connor, C.M. 2010. Evidence for a lack of egg recognition in the pūkeko (*Porphyrio porphyrio melanotus*: Rallidae). *Notornis.* **57:** 209-211.

Dey, C.J., O'Connor, C.M., Balshine, S., & Quinn, J.S. 2013. Cooperative males reduce incubation in response to cues of female-female competition. *Ibis.* **156:** 446-451.

Dey, C.J., O'Connor, C., & Quinn, J.S. 2014. Hatching order affects offspring growth, survival and adult dominance in the joint-laying Pukeko *Porphyrio melanotus melanotus*. *Ibis.* **156:** 658-667.

Dolores, M., Lopez-de-Hierro, G., & Moreno-Rueda, G. 2010. Egg-spot pattern rather than egg colour affects conspecific egg rejection in the house sparrow (*Passer domesticus*). *Behav. Ecol. Sociobiol.* **64(3):** 317-324.

Dorie, V. 2015. blme. R package version 1.0-4. https://cran.r-project.org/web/packages/blme

Hamilton, WD. 1964. The genetical evolution of social behaviour, I & II. *J. Theor. Biol.* **7:** 1-52.

Haselmayer, J. 2000. Joint-nesting in the pūkeko (*Porphyrio porphyrio*). M.Sc. thesis. McMaster University.

Hauber, M.E., Sherman, P.W., & Paprika, D. 2000. Self-referenct phenotype matching in a brood parasite: the armpit effect in brown-headed cowbirds (*Molothrus ater*). *Anim. Cognition.* **3(2):** 113-117.

Hauber, M.E., & Sherman, P.W. 2001. Self-referent phenotype matching: theoretical considerations and empirical evidence. *Trends in Neurosci.* **24(10):** 609-616.

Hays, H., & LeCroy, M. 1971. Field criteria for determining incubation stage in eggs of the Common Tern. *The Wilson Bulletin.* **83(4):** 425-429.

Healey, M. 2017. Territoriality of cooperative breeding pūkeko (*Porphyrio melanotus melanotus*) under low and high population density conditions. M.Sc. thesis. McMaster University.

Healey, M.R., Hing, J.S., Dey, C.J., & Quinn, J.S. 2017. A novel PCR-based genetic marker shows that sex of offspring does not account for hatch-order effects on growth, survival and dominance in Pūkeko *Porphyrio melanotus melanotus*. *Ibis.* doi: 10.1111/ibi.12486

Hepper, P.G. 1986. Kin recognition: functions and mechanisms, a review. *Bio. Rev.* **61:** 63-93.

Hoglund, J., Alatalo, RV., Lundberg, A., Rintamaki, P.T., & Lindell, J. 1999. Microsatellite markers reveal the potential for kin selection on black grouse leks. *Proc. R. Soc. Lond. B.* **266:** 813-816.

Jamieson, IG., & Craig, JL. 1987. Dominance and mating in a communal polygynandrous bird: Cooperation or indifference towards mating competitors? *Ethology.* **75:** 317-327.

Jamieson, IG., McRae, SB., Simmons, RE. & Trewby, M., 2000. High rates of conspecific brood parasitism and egg rejection in coots and moorhens in ephemeral wetlands in Namibia. *The Auk.* **117(1):** 250-255.

Lorenzana, J. & Sealy, S.G. 2001. Fitness costs and benefits of cowbird egg ejection by gray catbirds. *Behav. Ecol.* **12:** 325-329.

Lotem, A., Nakamura, H., & Zahavi, A. 1992. Rejection of cuckoo eggs in relation to host age: a possible evolutionary equilibrium. *Behav. Ecol.* **3(2):** 128-132.

Lyon, BE. 1993. Conspecific brood parasitism as a flexible female reproductive tactic in American coots. *Anim. Behav.* **46:** 911-928.

Lyon, BE. 2003. Egg recognition and counting reduce costs of avian conspecific brood parasitism. *Nature.* **422:** 495-499.

McDonald, P.G., & Wright, J. 2011. Bell miner provisioning calls are more similar among relatives and are used by helpers at the nest to bias their effort towards kin. *Proc. R. Soc. B.* **278:** 3403-3411.

McRae, SB. 2011. Conspecific brood parasitism in the tropics: an experimental investigation of host responses in common moorhens and American purple gallinules. *Ecol. Evol.* **1(3):** 317-329.

Petrie, M., Krupa, A., & Burke, T. 1999. Peacocks lek with relatives even in the absence of social and environmental cues. Nature. 401: 155-157.

Piertney, S.B. MacColl, A.D.C., Lambin, X., Moss, R., & Dallas, JF. 1999. Spatial distribution of genetic relatedness in a moorland population of red grouse (*Lagopus lagopus scoticus*). *Biol. J. Linnean Soc.* **68:** 1-2.

Pinheiro J, Bates D, DebRoy S, Sarkar D & R Core Team. 2017. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-131, https://CRAN.R-project.org/package=nlme.

Quinn, JS., Haselmayer, J., Dey, C., & Jamieson, IG. 2012. Tolerance of female co-breeders in a joint-laying pūkeko: the role of egg recognition and peace incentives. *Anim. Behav.* **83:** 1035-1041.

Riehl, C., & Strong. M.J. 2015. Social living without kin discrimination: experimental evidence from a communally breeding bird. *Behav. Ecol. Sociobiol.* **69(8):** 1293-1299.

Robinson, A.P., & Froese, R.E. 2004. Model validation using equivalence tests. *Ecol. Modelling.* **176:** 349-358.

Sambrook, J., Fritsch, E.F., & Maniatis, T. 1989. Molecular Cloning, Vol 2: 14 -19. New York: Cold Spring Harbor Laboratory Press.

Shizuka, D., & Lyon, BE. 2010. Coots use hatch order to learn and recognize and reject conspecific brood parasitic chicks. *Nature.* **463:** 223-228.

Shorey, L., Piertney, S., Stone, J., & Hoglund, J. 2000. Fine-scale genetic structuring on *Manacus manacus* leks. *Nature.* **408(6810):** 352-353.

Sorenson, M.D. 1995. Evidence of conspecific nest parasitism and egg discrimination in the sora. *Condor.* **97(3):** 819-821.

Suh, A., Kriegs, J.O., Brosius, J. & Schmitz, J. 2011. Retroposon insertions and the chronology of avian sex chromosome evolution. *Mol. Biol. Evol.* **28:** 2993-2997.

Victoria, J.K. 1972. Clutch characteristics and egg discriminative ability of the African village weaverbird *Ploceus cucullatus*. *Ibis.* **114:** 367-376.

Waldman, B. 1988. The ecology of kin recognition. *Ann. Rev. Ecol. Systems.* **19:** 543-571.

Walker, E. & Nowacki, A.S. 2011. Understanding equivalence and noninferiority testing. *J. Gen. Int. Med.* **26(2):** 192-196.

Wellek, S. & Blettner, M. 2012. Establishing equivalence or non-inferiority in clinical trials. *Dtsch Arztebl Int.* **109(41):** 674-679.

2.8 Figures and tables

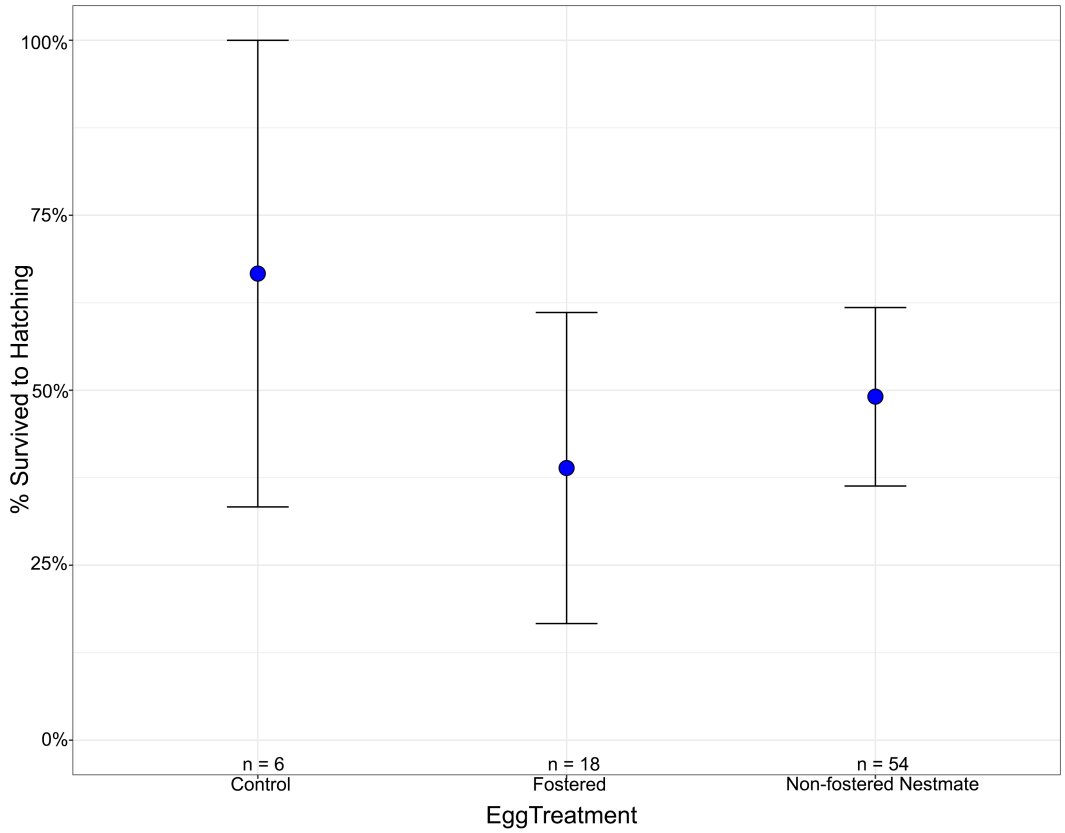

FIGURE 2.1: The percentage of eggs that survived to hatching for each nest treatment. Sample sizes are shown below bars.

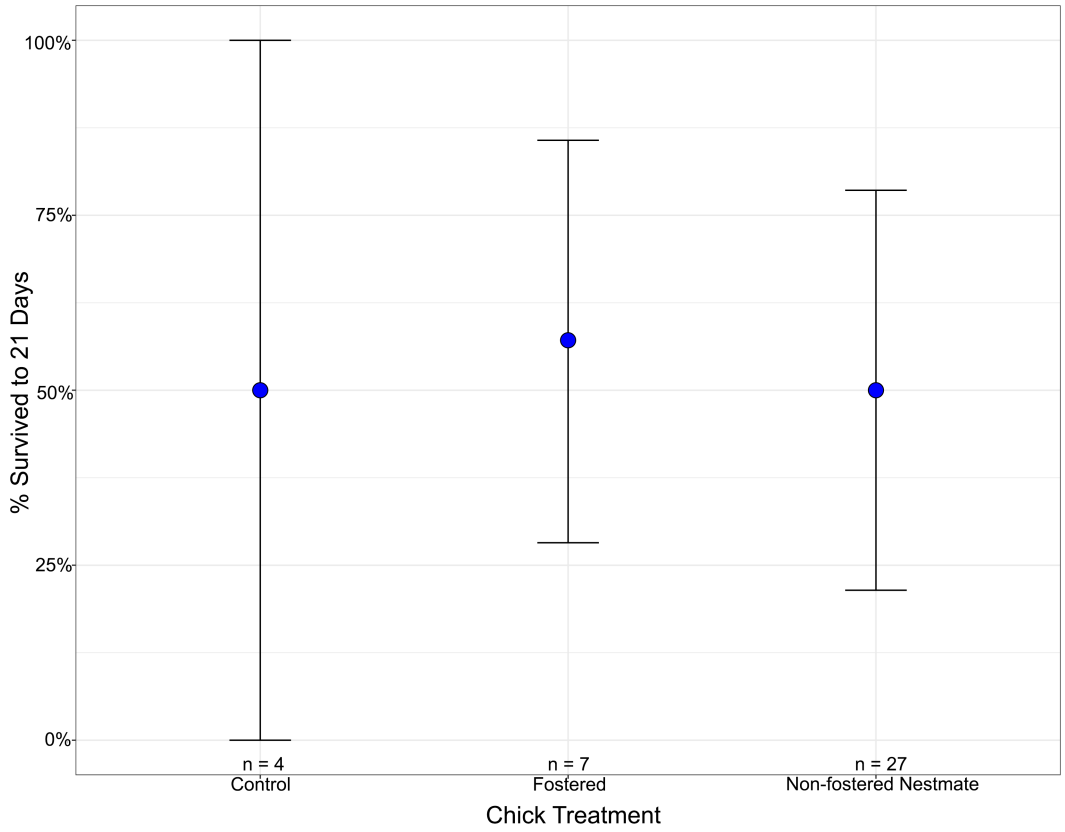

FIGURE 2.2: The percentage of chicks that survived to 21 days post-hatching for each nest treatment. Only groups with at least 1 manipulated egg hatched were included. Sample sizes are shown below bars.

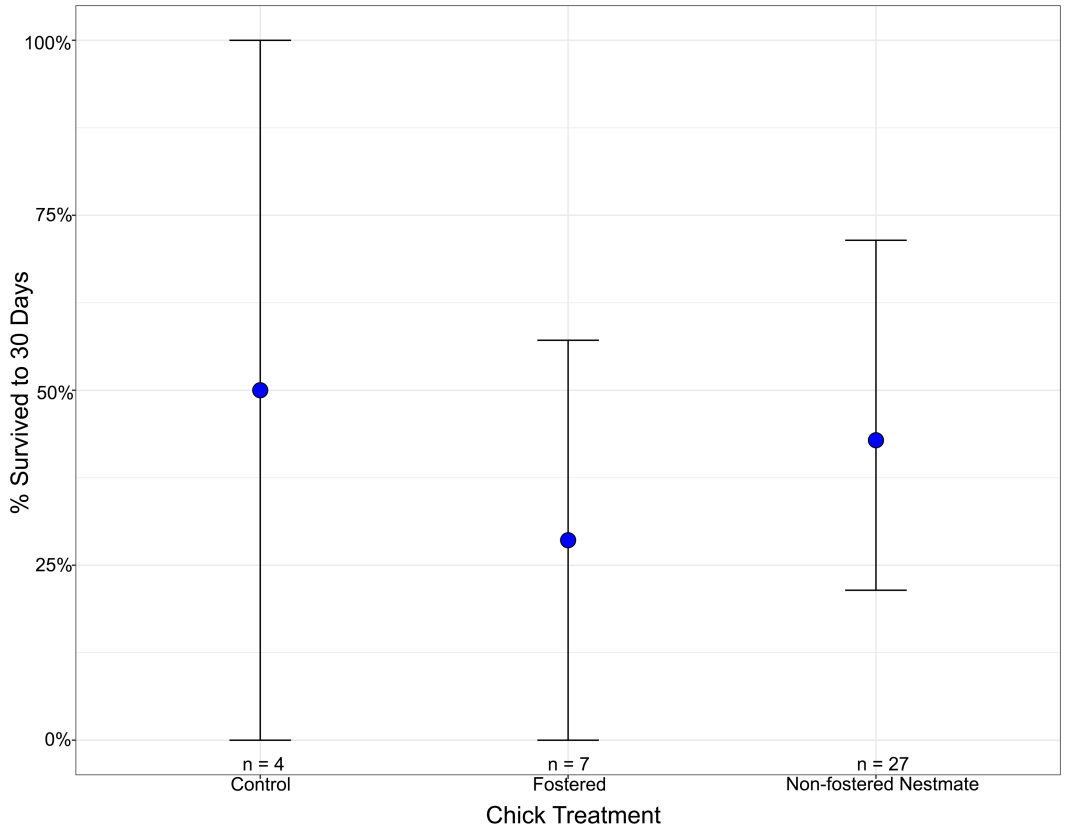

FIGURE 2.3: The percentage of chicks that survived to 30 days post-hatching for each nest treatment. Only groups with at least 1 manipulated egg hatched were considered. Sample sizes are shown below bars.

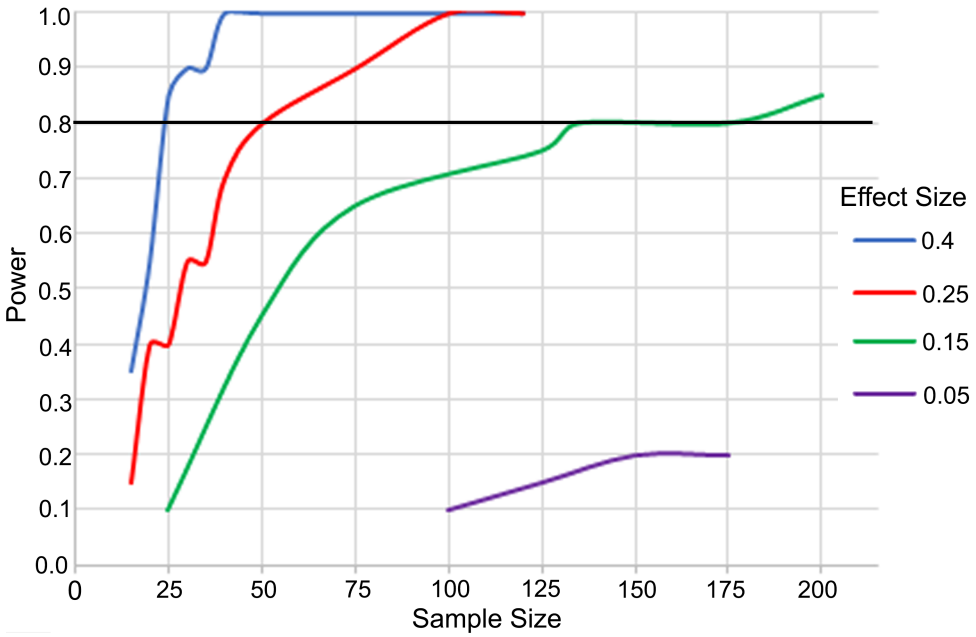

FIGURE 2.4: A simulated binomial GLMM showing the expected statistical power for a statistically significant decrease in survival for fostered chicks compared to their non-fostered nest mates at four effect sizes for different nest samples sizes. Horizontal black line indicates a power of 0.8.

TABLE 2.1: Final fate of eggs involved in the egg swapping experiment categorized by nest treatment type and whether the egg was manipulated (i.e. control or fostered) or not (i.e. nest mates). Eggs that did not hatch were further categorized as 1) nest destroyed, 2) missing (not found in or around nest), 3) damaged (cracks or punches in shell), 4) ejected (found out of the nest and cold), and 5) rotten.

Egg fate	Control (n = 6)	Fostered (n = 18)	Non-fostered nest mates (n = 54)	Total (n = 93)
Hatched	4	7	27	46
Nest destroyed	2	5	15	26
Missing	0	2	7	12
Damaged	0	2	1	3
Ejected	0	1	3	4
Rotten	0	1	1	2

TABLE 2.2: Binomial family GLMMs looking at the effect of treatment on survival of eggs to hatching (model 1), and chicks to 21 days (model 2) and 30 days (model 3). Survival was coded as a binary response variable. The effect of treatment is reported for fostered individuals relative to control individuals and non-fostered nest mates of the fostered individuals. The final nest for each offspring was included as a random intercept. The model estimates and 95% confidence interval (CI) of the estimates are shown. Sample sizes for model 1 are from $n = 4$ control and $n = 14$ foster nests. Sample sizes for models 2 and 3 are from $n = 3$ control and $n = 5$ foster nests.

Fixed Effect	**Estimate**	***z*-value**	**95% CI**	***P*-value**
{Model 1: Egg survival to hatch}				
Intercept	-1.1664	-1.07	-3.29, 0.96	0.283
Foster vs. control	3.1223	1.35	-1.42, 7.66	0.178
Foster vs. nest mate	0.9732	1.28	-0.51, 2.46	0.200
{Model 2: Chick survival to 21 days post-hatch}				
Intercept	0.7159	0.164	-1.92, 2.28	0.870
Foster vs. control	-0.0224	-0.015	-2.86, 2.82	0.988
Foster vs. nest mate	0.5935	0.554	-1.51, 2.70	0.580
Sex	0.2218	0.201	-1.94, 2.39	0.841
Control*Sex	-0.4991	-0.311	-3.65, 2.65	0.756
Nestmate*Sex	-1.2946	-1.013	-3.80, 1.21	0.311
{Model 3: Chick survival to 30 days post-hatch}				
Intercept	-0.7793	-0.887	-2.50, 0.94	0.375
Foster vs. control	0.5232	0.414	-1.95, 3.00	0.679
Foster vs. nest mate	0.8573	0.860	-1.10, 2.81	0.390
Sex	0.2456	0.241	-1.75, 2.24	0.809
Control*Sex	-0.1022	-0.069	-3.07, 2.81	0.945
Nestmate*Sex	-1.1367	-0.933	-3.52, 1.25	0.351

TABLE 2.3: The random effects of final nest on survival of eggs to hatching (model 1), and chicks to 21 (model 2) and 30 days (model 3) post-hatch. *P*-values are derived from likelihood ratio tests.

Random Effect	**Variance**	***P*-value**
Model 1: Egg survival to hatch		
Final Nest	6.619	2.50e-10
Model 2: Chick survival to 21 days post-hatch		
Hatch Nest	3.08	0.50
Model 3: Chick survival to 30 days post-hatch		
Hatch Nest	0.6645	0.50

TABLE 2.4: Results of a linear mixed model looking at the effect of treatment on chick growth. Tarsus length was coded as the normally distributed response variable. The effect of treatment is reported for fostered individuals relative to their non-fostered nest. Age of the chick was included as a fixed interaction term. The final nest for each offspring was included as a random intercept. The model estimates and 95% confidence interval (CI) of the estimates are shown. Sample sizes are from $n = 10$ chicks (n *foster* $= 4$, n *nestmate* $= 6$).

Fixed Effect	Estimate	*t*-value	95% CI	*P*-value
Intercept	19.872	1.681	-17.76, 57.51	0.192
Age	0.842	2.488	-0.23, 1.91	0.089
Sex	1.914	0.161	-35.93, 39.76	0.882
Treatment	-42.397	-1.424	-137.12, 52.32	0.250
Age*Treatment	0.788	1.525	-1.12, 0.78	0.225
Sex*Treatment	31.79	1.311	-34.53, 98.11	0.281

Chapter 3

Adult pukeko use individually variable distress calls to discriminate care between kin and non-kin chicks.

3.1 Abstract

Individual recognition is important for social species that need to identify and remember individuals such as mates, neighbours, enemies and kin. In a variety of contexts Kin recognition, whether learned through contextual cues or through phenotypic cues, may be essential in parent-offspring interactions; helping to inform parents about the direction of care. The pūkeko (*Porphyrio melanotus melanotus*), is a cooperatively breeding, joint-nesting rail. Adults in a group defend a territory together and have been seen to expel foreign chicks as young as two weeks old. Here, we test the hypothesis that adult pūkeko can discriminate between kin and non-kin chicks through vocal cues. To do so, we recorded the distress calls of young chicks and analysed the calls using a linear discriminant analysis to test for variation among chicks and groups. Responses of adults to group versus non-group chicks were assessed through a playback-choice experiment. Our results show distinct differences in distress call structure between chick recording sessions, as well as between groups. We also show that adult pūkeko respond more strongly to calls from their own group's chicks than to those of a foreign chick.

3.2 Introduction

In many interactions among conspecifics, the knowledge of whom you are interacting with is valuable and can inform one's actions. In cooperatively breeding groups, the use of individual recognition may be important in various contexts such as kin recognition, and territory defense. For a trait to be used for individual

recognition, there must be negative frequency dependent selection, such that the trait has high, multimodal variance of expression (Tibbets & Dale, 2007). In other words, traits must be selected to be variable enough to be individually distinct to be a reliable signal for recognition.

Many birds use vocal signatures to identify individuals and make decisions on how they will interact with the signalling individual (Beer, 1970; Lambrechts & Dhondt, 1995). In terms of complexity, bird songs often are highly variable and thus offer the opportunity for individual recognition (Emlen, 1972; Lambrechts & Dhondt, 1995). Even simple calls can provide sufficient cues for recognition (Chaiken, 1992; Jouventin & Aubin, 2002).

Vocal signatures can be used in the context of kin recognition (Chaiken, 1992). Recognition of kin through vocalizations can be either learned (Payne *et al.*, 1988; Hatchwell *et al.*, 2001; Sharp *et al.*, 2005), or recognised based on a phenotypic template, (Beecher, 1988; Medvin *et al.*, 1992; Jouventin & Aubin, 2002). Many bird species use the vocalisation of their offspring to discriminate their own chicks from foreign chicks they may encounter (Buckley & Buckley, 1972; Jouventin & Aubin, 2002; McDonald & Wright, 2011). This is often seen in species where broods are likely to mix such as colonial nesters (Buckley & Buckley, 1972; Jouventin & Aubin, 2002). Cooperatively breeding bell miner (*Manorina melanophrys*) helpers, for example, use chick calls to bias care towards more closely related chicks (McDonald & Wright, 2011). Recognition is also more developed in ground-nesting species where broods are mobile relatively early (Burger, 1974).

Screaming when being handled by a perceived predator is a behaviour that is

seen in many species of birds; both adults and chicks alike (Rowher *et al.*, 1976). Within a species, the frequency of distress screaming can vary from individual to individual (Rowher *et al.*, 1976). This variation also exists from species to species. In kin groups of high relatedness, these screams may elicit a mobbing or anti-predator response from the group in defense of the individual under attack (Rowher *et al.*, 1976). Distress screams in offspring are thought to function as a call for help from parents and, to date, the ability of a parent to recognise their offspring based on distress calling has been found only in the semi-colonial European starling (*Sturnus vulgaris*) (Chaiken, 1992).

The pūkeko (*Porphyrio melanotus melanotus*) is a cooperatively breeding, joint-nesting bird native to New Zealand (Craig, 1980a). Territories are maintained primarily by the males in the group and trespassers are aggressively ejected (Craig, 1980a, Young, pers. obs.). Identifying a trespassing individual involves the ability to distinguish between group-members and outsiders and discriminate appropriately. This discrimination extends to mobile chicks as young as a week old that may accidentally wander into a neighbouring territory. Trespassing chicks are aggressively chased down, held down, and repeatedly pecked by adults belonging to that territory (C. Young, pers. obs.).

Adult pūkeko have complex, individual vocalizations used in many contexts such as aggression, contact, mating and chick care (Clapperton & Jenkins, 1984; Clapperton & Jenkins, 1987). Clapperton (1987) found that individuals in in a group can distinguish between familiar and unfamiliar adult males. As with recognition of adults, it is likely that individuals use vocal cues to recognise chicks within their territory and preferentially direct care accordingly. Pūkeko chicks have

a wide repertoire of sounds as documented by Clapperton and Jenkins (1984), and when handled, pūkeko chicks give harsh, squawking calls (C. Young, pers. obs.), which are likely analogous to a distress call in response to a predator.

In Chapter 2, I provided evidence that recognition of kin is likely not based on direct mechanisms; adults consider any chick hatched in their nest to be worthy of parental care. The question then becomes; how do adults recognise chicks hatched in their territory? What cues does a chick provide to inform adults of their membership in the group? The breeding system of the pūkeko provides an opportunity to study mechanisms of kin recognition and discrimination. Using a playback choice experiment, I tested the hypothesis that these distress calls are individually unique and that adults can discriminate between calls made by chicks in their group versus calls made from chicks from other groups. This predicts 1) chick distress vocalizations are highly variable with both individual and group signatures, and 2) when faced with a choice between a group and non-group distress call, a pūkeko will respond more strongly to their own chick in distress. This is the first study to document pūkeko chick distress calls and assess the response of adults to them.

3.3 Methods

Creating Stimuli

Sound recordings, video recordings of trials and associated data were collected between October 31, 2016 and December 13, 2016 at Tawharanui Regional Park

(36° 22' 10" S, 174° 49' 58" E), a combined open sanctuary and working farm run by the Auckland Regional Council (ARC), located on the North Island of New Zealand. The park is free from exotic terrestrial mammalian predators, protected by a pest-proof fence.

Chicks were collected from their nest within one week of hatching and taken off their natal territory to be recorded. Vocal calls of the chicks were recorded with a Marantz PMD660 recording unit (sampling rate = 44.1 kHz, bit depth = 16 bits) equipped with a Sennheiser ME67 microphone capsule which operated using a Sennheiser K6 power module. Calls were recorded 15 cm from the microphone. To ensure there was no distortion in the sound, headphones were worn and the recording level of the unit was manually adjusted for each recording so that it did not exceed 0dB. We recorded for 1.5 to 2 minutes to ensure enough audio data to retrieve usable calls. Care was made during the recording process to minimize background noise such as other pūkeko, heterospecific birds, human noise, and wind. When possible, chicks were brought to the shelter of a car for noise reduction.

Spectrograms were produced using Syrinx version 2.6f (John Burt, www.syrinxpc.com). For each playback stimulus, one call out of the recording was isolated and copied so that it was repeated 4 times, with 150 ms between each repeat. Stimuli were then high-pass filtered at 0.8 Hz. Playbacks were further filtered using the noise reduction filter in Goldwave v6.24 and amplitudes were standardized using the maximize amplitude function.

Playback Experiments

Playback-choice experiments were conducted near the center of the group's territory. The location of the experiments was baited daily up to one week prior to experimentation with approximately 50 of whole maize. At the time of experimentation, two speakers were set up at 3m on either side of the bait pile. Speakers used were a remotely triggered FoxPro Scorpion X1-B and a FoxPro NX4 that was triggered by a mobile device attached by an auxiliary cable. Speaker volumes were set at 60-70dB SPL at 15cm which is within the natural range of a chick distress call's amplitude. A blind and Sony Handycam HDR-CX160 were set up 9.5m from the bait station where the observer could trigger the sounds to play when most the adults in the group arrived at the bait station. (Figure 3.1).

Experiments for each group tested consisted of three experimental trials. During the trial, the distress call stimulus from the group chick was played against that of a non-group chick. Trials for each group were conducted once a day to once every other day until three successful trials were completed. Playback stimuli were paired for age and mass of chicks when they were recorded. Different chick stimuli were created for every playback trial conducted. When triggered, the sound was left to play 5 times in succession. The speaker from which either chick stimulus came was switched from trial to trial to avoid individual effects of the speaker. Effort was made to reduce the length of time between recording and playback trials, however due to lack of participation by the birds, trials occurred between 2 to 15 days after recordings with 8 out of 20 trials exceeding 7 days.

Analysing the behaviour

Video footage of adult Pukeko response to sound was assessed by two observers whom were blind to the type of sounds played back and to the side from which the foreign chick call was played. They both watched and scored all videos independently. Videos were compiled and edited into 1 to 2min clips containing the stimuli. Videos were muted to remove bias between sounds and instead a visual cue was given during the period when the stimuli were playing. The observers watched the videos and scored the reactions of all adults present at the bait station at the time of stimulus. Behavioural responses of adults to the stimuli were ranked on a score of 0-5, using a modified ethogram from Clapperton, (1987; Table 1). Due to the observers being blind to which speaker played which sound, responses towards the left speaker were given a positive value, and responses towards the right a negative value. Scores for reaction of each adult towards each speaker were averaged. Adults in this study were not individually identifiable so averaging was done to account for individual variation within a group. Each of the individual reaction scores was weighted by the proportion of the group that the particular adult represented. For example, if five adults were present during the trial and two of the three adults responded in the direction of the speaker playing a group chick while three responded towards the foreign chick, each adult bird's response was multiplied by one fifth and then these responses were averaged for each speaker to create one average direction-of-response score (see equation 3.3 for this example below). These two average scores were then used in statistical analyses.

$$AvgScore_{group} = [(Score_1) * (1/5) + (Score_2) * (1/5)]/2$$

$$AvgScore_{non-group} = [(Score_3) * (1/5) + (Score_4) * (1/5) + (Score_4) * (1/5)]/3$$

Analysing vocalizations

To analyse call structure, we randomly isolated 38 individual calls from recordings and high-pass filtered at 0.8 kHz using Syrinx. Each call was then analysed in Sound Analysis Pro 2011 (SAP2011) to extract acoustic parameters (Tchernichovski *et al.*, 2000).

The features that we measured were call duration (ms), mean amplitude (dB), mean, minimum, maximum and standard deviation of pitch (Hz), mean frequency (Hz) and peak frequency (Hz), goodness of pitch (unitless), mean, minimum, maximum and standard deviation of frequency (FM, degrees) and amplitude modulation (AM, 1/t), mean Weiner entropy (unitless) and, mean, minimum, maximum and standard deviation of spectral continuity (Hz) and temporal continuity (ms). These values, as defined by Tchernichovski et al. (2000) and Feher et al. (2009), were measured in SAP2011. Amplitude is defined as the intensity of the sound. Mean frequency assesses the central tendency of power distribution across frequencies. Peak frequency is the frequency of maximum power. Pitch is defined as a combination of peak and mean frequency estimates and describes the perceived tone of the sound. Goodness of pitch is a value without units that measures harmonic stack. Wiener entropy, also a measurement without units, measures the width and uniformity of the power spectrum and informs whether the sound is

pure toned or noisy. FM estimates the absolute slope of frequency traces with respect to the horizontal line. AM measures changes in amplitude over time. Continuity over time and frequency uses the zero crossings of the spectral derivatives and their durations. Spectral continuity measures the mean frequency range across the frequency contours. Temporal continuity measures the mean duration across the time contours. These features have been used in other bird vocal studies to measure variability in vocalizations (Baker & Logue, 2003; Grieves *et al.*, 2015).

Statistical Analysis

All statistical analyses were conducted using R version 3.1.3 (R Core Team 2017).

To test the individuality in vocalizations, we used the extracted parameters from the distress calls of 31 chicks. To account for day to day variation, we included four chicks where we had two recordings of the same chicks but on different days (within a week of each other). In total, we ended up with 36 recording sessions from 32 chicks (10 calls/recording session).

Using these data, we ran a linear discriminant analysis (LDA) with recording session of individual chick as the grouping variable and the parameters extracted in SAP2011 as the independent variables. The goal of this analysis was to quantify acoustic differences between chicks as well as to determine which vocal parameters contributed most to variability between distress calls. The parameters were run through a forward stepwise selection analysis using the '*klar*' package (Roever *et al.*, 2015) set at a threshold of 0.001 to avoid over-parameterization. The output gave us 19 of the initial 23 parameters put into the model of which the top 8 were

chosen for the LDA, which was run using the '*MASS*' package (Ripley *et al.*, 2017). This number of variables was used for the LDA model as it is recommended that LDAs are run with 2 fewer parameters than there are samples for each group. The model was then run through a prediction function to determine how accurately the parameters of the call could be used to categorize the call into the correct chick. This function gives us the prediction accuracy as well as which calls were classified to which chick.

To test for a group signature, another LDA and prediction was performed, using social group as the grouping variable. We used 3 social groups, each with 5 chicks and took the average parameter value of 10 calls in a single chick's recording such that each chick was one data point. Due to there being 5 chicks from each group we limited this analysis to the 3 strongest predictor variables from the previous analysis; entropy, goodness and FM.

Playback responses were analysed using the '*lme4*' package (Bates *et al.*, 2017). Using average score as the response variable, data were fitted to a nested, repeated measures ANOVA design, with stimulus type as the mixed effect with the weight of the chick and time between group chick recording and trial as interaction terms. We added group ID as a random effect to account for any non-independence within groups.

3.4 Results

Distress calls were found to be distinct and variable between recording sessions (Figure 3.2), however, although calls were consistent within individual chick recordings, we were unable to find evidence of day-to-day consistency for individual chick distress calls (Figure 3.3). Distress calls had on average, 387.5 ±114.2 ms in length and have an average frequency of 2849.6 ±351.8 Hz (see Table 3.1 for other values). Calls varied in structure but typically consisted of two components, a pure whistle composed of 2-4 harmonic frequencies, and a harsher croak. Calls varied in terms of order, number and length of these two components.

The eight parameters determined most important in the stepwise selection were entropy, variance of pitch, mean frequency of the call, FM, duration, minimum pitch, goodness, and maximum frequency (Table 3.1). The first three functions explained 80.9% of the total variance (LD1: 60.2%; LD2: 13.2%; LD3: 9.7%) with the remaining five functions explaining the remaining 16.9%. The first linear discriminant function had high positive coefficient for entropy (5.79), while the highest negative coefficients were goodness (-0.018) and FM (-0.038). The second linear discriminant function had high negative coefficient for entropy (-2.69) as well as for FM (-0.088), while goodness had a lower, positive coefficient (0.011) (Table 3.2). When a predictive model was run, the LDA classified 91.1% of the calls to the correct chick/session recording, significantly exceeding chance-level classification (Figure 3.4). Of the four chicks with more than one recording sessions included in the model, accuracy of calls to recording session ranged from 70% to 100% with only one chick (Chick D) showing misclassification between its own recording

sessions (Supplementary Figure A.2).

Using entropy, goodness and FM, two linear functions were created explaining the variation in the calls. The first linear function explained 89.2% of the variation and had a high negative coefficient for entropy (-7.96), as well as lower positive coefficients for goodness (0.073) and FM (0.16) (Table 3.3). When a predictive model was run, the LDA classified 87.5% of chicks to the correct group, significantly exceeding chance-level classification (Figure 3.5).

Playback results showed a statistically significant difference in strength of response of adults to group versus foreign chicks with a stronger response towards the group chick (t = -2.93, 95% CI = [-3.28, -0.65], P = 0.006; Table 3.4; Figure 3.6). Furthermore, responses scoring > 2 (i.e. those in which an adult approached the speaker) were solely directed towards the group chick. We also found a significant interaction between the date the group chick's distress call was recorded and the date of the trial (t = 0.20, 95% CI = [0.04, 0.36], P = 0.01; Table 3.4). On their own, neither the weight of the chick nor the time between recording and trials significantly influenced adult response. We found no significant interaction between weight of the chick and stimulus type (Table 3.4).

3.5 Discussion

Pūkeko chick distress calls appear to have sufficient individuality that adults may be able to discriminate chicks. Chick distress vocalizations are variable for individual chicks on a day-to-day basis. Although we found no evidence of a

consistent distress call signature for individual chicks (Figure 3.3), our LDA had a high accuracy rate for assigning calls to the correct chick recording (Figure 3.4, Supplementary Figure B1). This high accuracy shows that although chick distress calls may not have day-to-day consistency, there is still very little overlap in the call from one chick to another. Whether the parameters of the chick's call vary with age, recording conditions, or other unknown variables, is unknown. The lack of day-to-day could be due to vocal changes with age as the number of days between call recording and playback was found to have a significant effect. The number of chicks in our study that had more than one usable recording in the same week was low and future studies should aim for greater sample sizes.

While the variability in individual chick distress calling does not necessarily show a vocal signature, results from our playbacks suggest that adults can learn these vocal cues as they showed a statistically significant preference for the speaker playing distress calls from their own group over those of a foreign chick (Figure 6). Although we could not deduce whether adults are learning individual chick signatures, or simply a familiar sounding voice, our results showed a statistical bias of adults towards their own chicks. This may suggest that to recognise individual chicks with day-to-day variation in their calls, adults are to learn a variety of calls from chicks in their groups to bias their care accordingly.

We also found evidence of a group-specific vocalizations made by chicks which may further inform adults about group membership (Figure 3.5). These within-group similarities may be either genetically determined or learned via association and if adults are unable to discriminate between individual chicks, it is possible that this group signature is sufficient to inform decisions on chick care.

Within-group similarity of vocalizations may be useful for social birds who need ways to identify and locate group members (Baker, 2004). Indeed, other species of social birds have been found to have group vocal signatures such as black-capped chickadees (*Poecile atricapillus*) (Mammen & Nowicki, 1981), corvids (Brown, 1985; Hopp *et al.*, 2001), budgerigars (*Melopsittacus undulatus*) (Wright, 1996), yellow-rumped cacique (*Cacicus cela*) (Feekes, 1982), bobwhite quail (*Colinus virginianus*) (Bailey & Baker, 1982), green woodhoopoe (*Phoeniculus purpureus*) (Radford, 2005), Australian magpie (*Cracticus tibicen*) (Brown *et al.*, 1988), and laughing kookaburra (*Dacelo novaeguineae*) (Baker, 2004). Of these species, however, all studies have focused on adult songs and calls; none have shown evidence of group specific chick vocalizations.

A study by McDonald and Wright (2011) showed that adult bell miners (*Manorina melanophrys*), a cooperatively breeding helper-at-the-nest species, use the "mew" calls of nestlings to discriminate care towards unfamiliar kin in a group of both kin and non-kin, suggesting a kin-based vocal signature but not necessarily a group signature as not all nestlings in the group are related. Pūkeko show evidence of group-level similarities in calls between chicks. These similarities may be kin-based due to the high inbreeding on the North Island resulting in closely related groups. If these similarities are learned, however, the group signature may still be possible without a genetic signature.

The earlier study by Clapperton and Jenkins (1987) which reported the vocal repertoire of pūkeko did well to document many contextual calls however it was entirely descriptive and function of these calls were not tested. Furthermore, they

did not record distress calls or the calls of chicks that were being handled. Although many contextual calls were documented such as contact calls, attention calls, feeding calls, the researchers failed to document any calls by chicks in the context of predatory danger. Our results add an additional call in the repertoire of pūkeko chicks; the distress call.

My study is one of only a couple that examined parent responses to chick distress calls. Chicks of the European starling (*Sturnus vulgaris*) were found to have individual variation in acoustic parameters and parents demonstrated a strong anti-predator response, favouring the screams of their chicks compared to the screams of neighbouring chicks (Chaiken, 1992). The study also used a playback experiment, however it was not a choice experiment and half the parents had one trial with their own chick's calls while the other half had one trial with a foreign chick's call. Chaiken (1992) found parents were more likely to dive at the speaker playing their own chick's screams. The only other study examining parent responses to chick distress calls, examined the response of California towhee (*Melozone crissalis*) parents to chick distress call playbacks and found no difference in parental response towards calls their own offspring and those of foreign chicks (Benedict, 2007).

The chicks used in my playback experiment were between 1 to 5 days old at the time of recording. Errors made by adults responding to a foreign chick over their own could be due to the chicks being recorded at an age where recognition is less important. Recognition of offspring in birds often starts at the time when using nest location as a cue becomes less reliable. Species that have free-roaming nidifugous chicks, like pūkeko, may need to recognise their individual chicks at an earlier age as the chances of brood-mixing becomes heightened with chick mobility.

On the other hand, species that are not nidifugous, and have chicks that stay in the nest until fledging, would lack the need for early chick recognition since brood-mixing is relatively reduced. In tree swallows (*Tachycineta bicolor*) and barn swallows (*(Hirundo rustica*), for example, chicks are non-nidifugous and parents do not recognise their own chicks until fledging (Burtt, 1977). Conversely, the nidifugous ring-billed gull (*Larus delawarensis*) can identify their own chicks after just 7 days of hatching (Miller & Emlen, 1975). The chicks used in my study were 1 to 5 days old post-hatching at the time of recording and while chicks between 2 to 4 days old leave the nest while feeding in the vicinity of the nest, (Craig, 1980a; Dey & Jamieson, 2013), it is possible that the period in recognition is more important at an older age when chicks are more mobile and feed further from the central nest location. Thus, we may expect stronger discrimination in chick calls recorded from older chicks. Due to difficulties in catching older chicks compared to younger chicks, this comparison was not possible in my study but could be assessed in future studies.

The chances of brood mixing may reflect whether the species is a solitary breeder or not. For example, in the family Hirundinidae, colonial bank swallows (*Riparia riparia*) can discriminate against nearly or completely fledged offspring which become misplaced in their burrow, and can identify their own offspring outside the burrow (Hoagland & Sherman, 1976). This recognition was only found for chicks that were old enough to leave the nest burrow and adults accepted foster chicks until fledging (Hoagland & Sherman, 1976). Alternatively, the noncolonial rough-winged swallows (*Stelgidopteryx serripennis*) were unable to discriminate between

kin and non-kin chicks at a near to complete stage of fledging (Hoagland & Sherman, 1976). Within joint-nesting groups, pūkeko females are subject to clutch-mixing as soon as eggs are laid. After hatching, chicks have very little opportunity to integrate into another breeding group's brood. Territories are large (Healey, 2017), and adults attend to, feed and defend chicks within the first 4-6 weeks after hatching (Craig & Jamieson, 1985). Chicks do not spend time alone from adults until they are nearly two months old (Craig, 1980a). On the occasion that they do stray into a neighbouring territory, they are aggressively expelled from the foreign territory by the adult territory holders (Young, pers. obs.).

The rejection of unfamiliar pūkeko chicks by territory holders, could reduce the chance of rearing non-kin chicks. Chicks entering the territory from another territory may be perceived to be intruders and are attacked and driven off. This is a tactic often seen in Laridae species and is thought to prevent accidental adoption (Ashmole, 1963; Beer, 1965; Quinn *et al.*, 1994). More studies looking at adult pūkeko response to familiar and unfamiliar chicks could further investigate this hypothesis.

In conclusion, my study is the first to document pūkeko chick distress vocalizations and experimentally test the response of adults to pūkeko chick vocalizations. My results suggest vocalizations are variable at both the individual and social group level. Finally, I showed that when faced with the distress vocalisations of chicks, adult pūkeko show a statistical biased their response towards their own group's chicks.

3.6 Acknowledgements

We would like to thank Julie Galloway and Lauren Snobl for their assistance with field work. Also, we would like to thank Achini de Silva and Hayley McKee for their assistance in video analysis. We also thank Matt Maitland, Maurice Puckett, Colin Wards, the Tawharanui Open Sanctuary Society and the Tawharanui Regional Park staff for their logistical help. This research was supported by an NSERC Discovery Grant to JSQ and travel support was provided by a McMaster Biology Department Travel Scholarship to CAY.

3.7 Literature Cited

Ashmole, N.P. The biology of the wideawake or sooty tern *Sterna fuscata* on Ascension Island. *Ibis*. **103(3):** 297-351.

Bailey, E.D., & Baker, J.A. 1982. Recognition characteristics in covey dialects of bobwhite quail. *Condor*. **84:** 317-320

Baker, M.C., & Logue, D.M. 2003. Population differentiation in a complex bird sound: a comparison of three bioacoustical analysis procedures. *Ethology*. **109:** 223-242.

Baker, M.C. 2004. The chorus song of cooperatively breeding laughing kookaburras (Coraciiformes, Halcyonidae: *Dacelo novaeuineae*): Characterization and comparison among groups. *Ethology*. **110:** 21-35.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Grothendieck, G., & Green, P., lme4. R package version 1.1-13. https://cran.r-project.org/web/packages/lme4/lme4.pdf

Beer, C.G. 1965. Adaptations to nesting habitat in the reproductive behaviour of the black-billed gull *Larus bulleri*. *Ibis*. **108(3):** 394-410

Beer, C.G. 1970. On the responses of laughing gull chicks (*Larus atricilla*) to the calls of adults I. Recognition of the voices of the parents. *Anim. Behav.* **18:** 652-660.

Benedict, L. 2007. Offspring discrimination without recognition: California towhee responses to chick distress calls. *Condor.* **109:** 79-87.

Brown, E.D. 1985. The role of song and vocal imitation among common crows (*Corvus bracyrhynchos*). *Ethology.* **68(2):** 115-136.

Brown, E.D., Farabaugh, S.M., & Veltman, C.J. 1988. Song sharing in a group-living songbird, the Australian magpie, *Gymnorhina tibicen.* Part 1. Vocal sharing within and among social groups. *Behaviour.* **104:** 1-28.

Brown, S.D., Dooling, R.J., & O'Grady, K. 1988. Perceptual organization of acoustic stimuli by budgerigars (*Melopsittacus undulatus*): III. Contact calls. *J. Comp. Psychol.* **102(3):** 236-247.

Buckley, P.A. & Buckley, FG. 1972. Individual egg and chick recognition by adult royal terns (*Sterna maxima maxima*). *Anim. Behav.* **20(3):** 457-IN1.

Bunin, J.S., & Jamieson, IG. 1996. Responses to a model predator of New Zealand's endangered takahe and its closest relative, the pukeko. *Cons. Biol.* **10(5):** 1463-1466.

Burger, J. 1974. Breeding adaptations of Franklin's Gull (*Larus pipixcan*) to a marsh habitat. *Anim. Behav.* **22:** 521-567.

Burtt, E.H. 1977. Some factors in the timing of parent-chick recognition in swallows. *Anim. Behav.* **25:** 231-239.

Chaiken, M. 1992. Individual recognition of nestling distress screams by European Starlings (*Sturnus vulgaris*). *Behaviour.* **120:** 139-150.

Clapperton, BK., & Jenkins, PF. 1984. Vocal repertoire of the pukeko (Aves: Rallidae). *New Zealand J of Zool.* **11:** 71-84.

Clapperton, B.K. 1987. Individual recognition by voice in the pukeko, *Porphyrio porphyrio melanotus* (Aves: Rallidae). *New Zealand J. of Zool.* **14:** 11-18.

Clapperton, B.K., & Jenkins, PF. 1987. Individuality in contact calls of the pukeko (Aves: Rallidae). *New Zealand J of Zool.* **14:** 19-28.

Cockburn, A. 2006. Prevalence of different modes of parental care in birds. *Proc. R. Soc. B.* **273:** DOI: 10.1098/rspb.2005.3458

Craig, J.L. 1980a. Pair and group breeding behaviour of a communal Gallinule, the pukeko, *Porphyrio p. melanotus. Anim. Behav.* **28:** 593-603.

Craig, J.L. 1980b. Breeding success of a communal gallinule. *Behav. Ecol. Sociobiol.* **6:** 289-298.

Craig J.L., & Jamieson, I.G. 1985. The relationship between presumed gamete contribution and parental investment in a communally breeding bird. *Behav. Ecol. Sociobiol.* **17(3):** 207-211.

Cullen, E. 1957. Adaptations in the kittwake to cliff-nesting. *Ibis.* **99(2):** 275-302.

Davies, S.J.J.F., & Carrick, R. 1962. On the ability of crested terns, *Sterna bergii*, to recognize their own chicks. *Australian J. Zool.* **10(2):** 171-177.

Dey, C.J., & Jamieson, I.G. 2013. Pukeko. In Miskelly, CM. (ed.) *New Zealand Birds Online.* www.nzbirdsonline.org.nz

Emlen, S.T. 1972. An experimental analysis of the parameters of bird song eliciting species recognition. *Behaviour.* **41(1):** 130-171.

Evans, R.M. 1970. Parental recognition and the "mew call" in black-billed gulls (*Larus bulleri*). *The Auk.* **87(3):** 503-513.

Feekes, F. 1982. Song mimesis within colonies of *Cacicus c. cela* (Icteridae, Aves). A colonial password? *Ethology.* 58(2): 119-152.

Gouzoules, H. & Gouzoules, S. 1990. Matrilineal signatures in the recruitment screams of pigtail macaques, *Macaca nemestrina. Behaviour.* **115(3):** 327-347.

Grieves, L.A., Logue, D.M., & Quinn, J.S. 2015. Vocal repertoire of cooperatively breeding Smooth-billed Anis. *J. Field. Ornithol.* **86(2):** 130-143.

Hatchwell, BJ., Ross, DJ., Fowlie, MK., & McGowan, A. 2001. Kin discrimination in cooperatively breeding long-tailed tits. *Proc. R. Soc. Lond. B.* **268:** 885-890.

Hoagland, J. L., & Sherman, P. W. (1976). Advantages and disadvantages of bank swallow (*Riparia riparia*) coloniality. *Ecol. Monographs.* **46(1):** 33-58.

Hopp, S.L., Jablonski, P., & Brown, J.L. 2001. Recognition of group membership by voice in Mexican jays, *Aphelocoma ultramarina. Anim. Behav.* **62:** 297-303.

Jouventin, P., & Aubin, T. 2002. Acoustic systems are adapted to breeding ecologies: individual recognition in nesting penguins. *Anim. Behav.* **64:** 747—757.

Lambrechts, M.M., & Dhondt, A.A. 1995. "Individual voice discrimination in birds." *Current Ornithology.* Springer US. pp 115-139.

Lefevre, K., Montgomerie, R., & Gaston, A.J. 1988. Parent-offspring recognition in thick-billed murres (Aves: Alcidae). *Anim. Behav.* **55:** 925-938.

Lenth, R. 2017. lsmeans. R package version 2.25-5. https://cran.r-project.org/web/packages/

Mammen, D.L., & Nowicki, S. 1981. Individual differences and within-flock convergence in chickadee calls. *Behav. Ecol. Sociobiol.* **9(3):** 179-186.

McDonald, P.G., & Wright, J. 2011. Bell miner provisioning calls are more similar among relatives and are used by helpers at the nest to bias their effort towards kin. *Proc. R. Soc. B.* **278:** 3403-3411.

Medvin, M.B., Stoddard, P.K., & Beecher, MD. 1992. Signals for parent-offspring recognition: strong sib-sib call similarity in cliff swallows but not barn swallows. *Ethology.* **90:** 17-28.

Miller, D.E., & Emlen, J.T. 1975. Individual chick recognition and family integrity in the ring-billed gull. *Behaviour.* **52:** 124-144.

Payne, R.B., Payne, L.L., & Rowley, I. 1988. Kin and social relationships in splendid fairy-wrens: recognition by song in a cooperative bird. *Anim. Behav.* **36:** 1341-1351.

Quinn, J.S., L. A. Whittingham & R.D. Morris. 1994. Infanticide in skimmers and terns: generalised territorial attacks or intergenerational conflict? *Anim. Behav.* **47:** 363-367.

Radford, A.N. 2005. Group-specific vocal signatures and neighbour-stranger discrimination in the cooperatively breeding green woodhoopoe. *Anim. Behav.* **70:** 1227-1234.

Ripley, B., Venables, B., Bates, D.M., Hornik, K., Gebhardt, A., & Firth, D. 2017. MASS. R package version 7.3-47. https://cran.r-project.org/web/packages/MASS/MASS.

Roever, C., Raabe, N., Luebke, K., Ligges, U., Szepannek, G., & Zentgraf, M. klar. R package version 0.6-12. https://cran.r-project.org/web/packages/klaR/klaR.pdf

Rowher, S., Fretwell, SD., & Tuckfield, RC. 1976. Distress screams as a measure of kinship in birds. *The American Midland Naturalist.* **96(2):** 418-430.

Sharp, SP., McGowan, A., Wood, MJ., & Hatchwell, BJ. 2005. Learned kin recognition cues in a social bird. *Nature.* **434:** 1127-1130.

Tibbetts E.A., & Dale, J. 2007. Individual recognition: is it good to be different? *Trends Ecol. Evol.* **22(10):** 529-537.

Tchernichovski, O., Nottebohm, F., Ho, C.E., Pesaran, B., & Mitra, P.P. 2000. A procedure for an automated measurement of song similarity. *Anim. Behav.* **59:** 1167-1176.

Wright, T.F. 1996. Regional dialects in the contact call of a parrot. *Proc. R. Soc. Lond. B.* **263:** 867-872.

3.8 Figures and tables

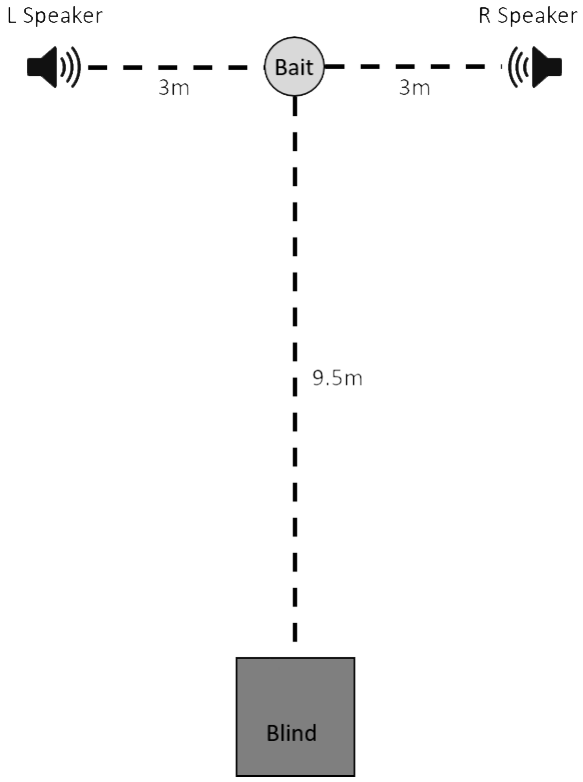

FIGURE 3.1: Experimental set up of the playback-choice experiment. A video camera, not shown, was placed with the blind.

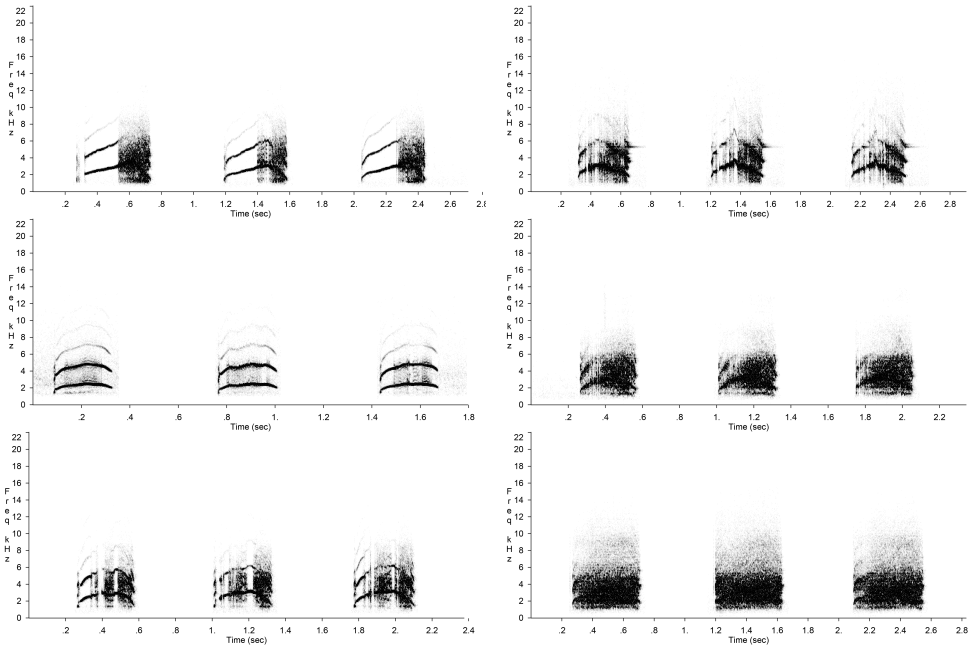

FIGURE 3.2: Spectrograms showing three distress calls of six different chicks. Each call is separated by 400ms of silence and time between calls is not representative of actual calling frequency. Note the difference in the time scale on the x-axis for duration.

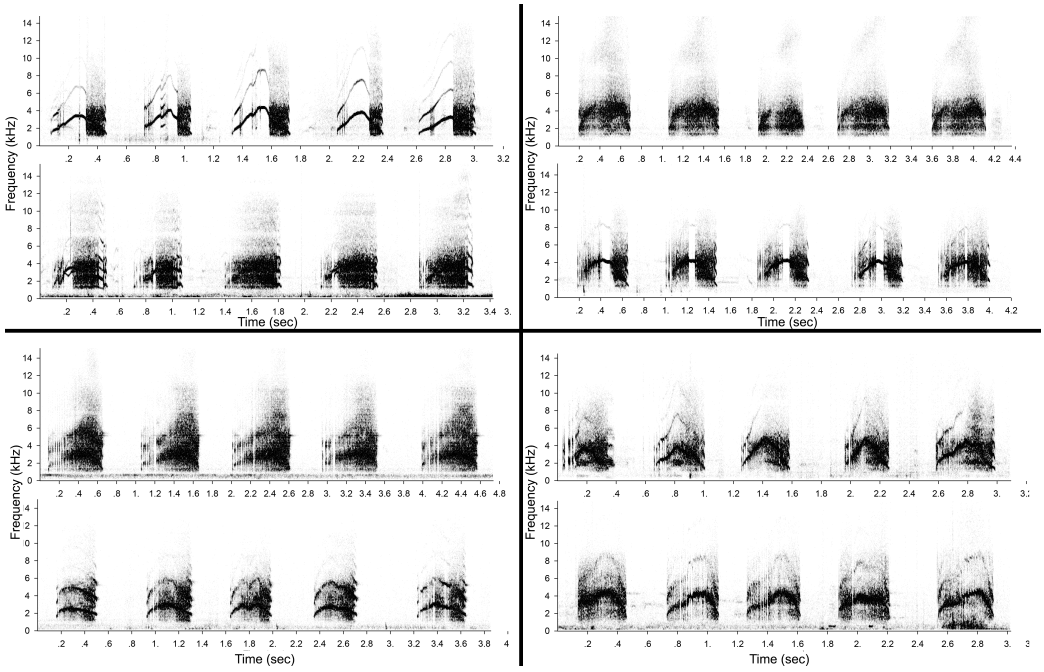

FIGURE 3.3: Spectrograms showing the distress calls of four different chicks recorded on two separate days. Five calls are represented for each recording. Time between calls is not representative of actual calling frequency. Note the difference in the time scale on the x-axis for duration.

TABLE 3.1: Average and standard deviation values for the 23 acoustic parameters extracted from SAP2011 and put into the stepwise selection. Asterisks denote which variables were determined to be most important (*) and which variables were ultimately used in the LDA (**).

Parameter	Mean Value	(+/-)
Duration (ms) **	387.5	114.2
Amplitude (dB) *	55.9	3.04
Pitch (Hz) *	2436.1	656.6
Min. pitch (Hz) **	697.7	234.2
Max. pitch (Hz)	3902.6	602.7
Var. pitch (Hz) **	2457.7	444.8
Mean frequency (Hz) **	2849.6	351.8 *
Min. frequency (Hz) *	1618.3	536.0
Max. frequency (Hz) **	3976.5	591.1
Var. frequency (Hz) *	196847.5	112747.5
Peak frequency (Hz) *	2798.9	395.1
Min. peak (Hz)	1252.7	491.5
Max. peak (Hz)	4506.1	615.7
Var. peak (Hz) *	637.6	153.2
Goodness of pitch **	113.1	36.1
Min. goodness *	18.4	8.2
Max. goodness *	593.9	245.5
Var. goodness	81.5	35.6
FM (degrees) **	25.5	11.9
AM (1/t)	-0.022	0.81
Weiner entropy **	-4.75	0.99
Temporal continuity (ms) *	35.8	54.4
Spectral contiuity (Hz) *	300.5	113.2

TABLE 3.2: Results of a discriminant function analysis that categorizes individual chicks based on eight acoustic parameters showing scaling for each parameter in eight discriminant functions.

Parameter	LD1	LD2	LD3	LD4	LD5	LD6	LD7	LD8
Duration	2.06E-03	-1.72E-03	6.28E-03	1.17E-03	1.64E-02	-4.78E-03	6.33E-03	3.99E-03
MeanF	-1.66E-03	6.55E-03	-6.91E-03	7.45E-03	2.28E-03	-2.63E-03	2.04E-03	-7.98E-03
Goodness	1.82E-02	1.20E-02	9.66E-05	-2.34E-02	3.58E-02	5.24E-02	-4.49E-02	3.34E-02
FM	1.49E-02	-5.90E-02	-2.25E-01	-1.41E-01	5.01E-02	-1.55E-01	4.79E-02	-6.68E-02
Entropy	-5.47E+00	-2.71E+00	2.87E+00	1.65E+00	-9.66E-01	1.34E+00	-7.01E-01	5.80E-01
MinPitch	5.20E-03	-5.81E-03	3.46E-03	1.74E-03	-5.12E-04	-1.27E-03	-7.13E-03	-6.70E-03
MaxF	6.17E-04	-1.96E-04	-3.90E-04	7.18E-04	-8.04E-04	-2.54E-03	-1.06E-03	2.53E-03
STDPitch	1.14E-03	-8.90E-03	3.62E-03	-4.00E-03	-5.60E-04	9.01E-03	-6.86E-04	7.52E-03

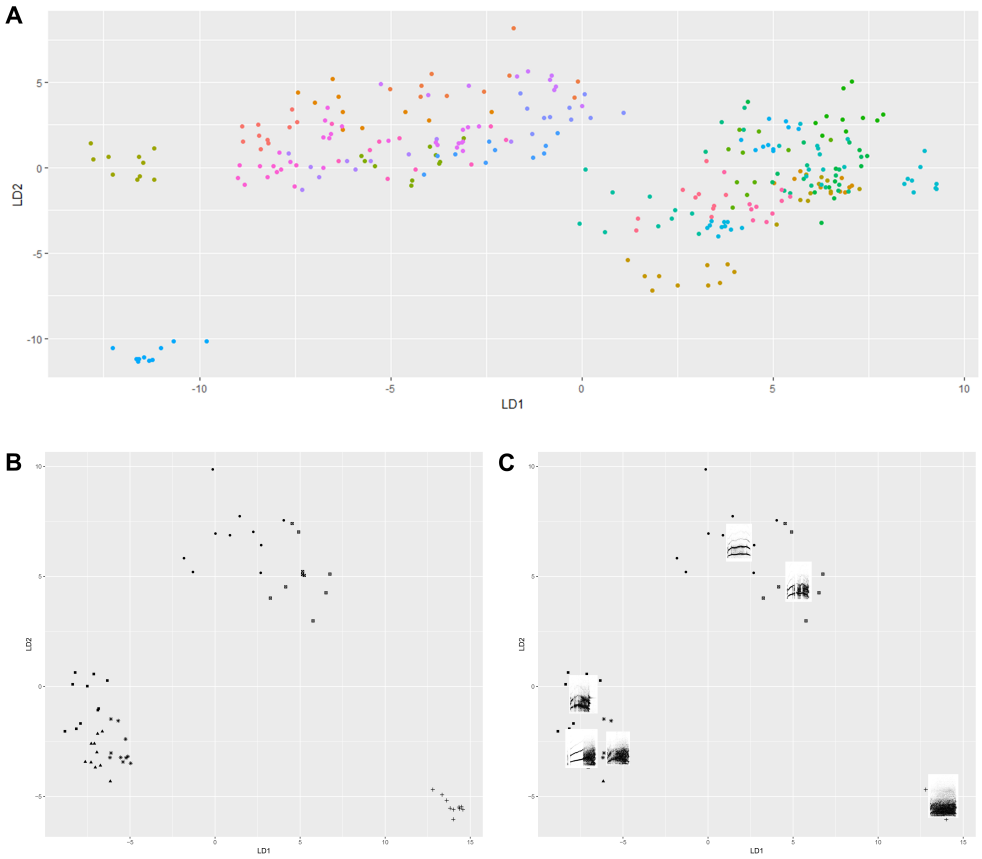

FIGURE 3.4: Results of a discriminant function analysis that categorizes individual chicks based on eight acoustic parameters. A) Scatter plot of discriminant function scores for all chicks involved in the analysis. Colour different colours indicate different chicks. B) Subset of the data using six chicks from Figure 1. C) Group centroids for each chick from Figure 1 are plotted as spectrograms.

TABLE 3.3: Results of a discriminant function analysis that categorizes chicks into group based on three acoustic parameters showing scaling for each parameter in two discriminant functions.

Parameter	LD1	LD2
Goodness	0.073	0.012
FM	0.156	-0.108
Entropy	-7.962	-0.232

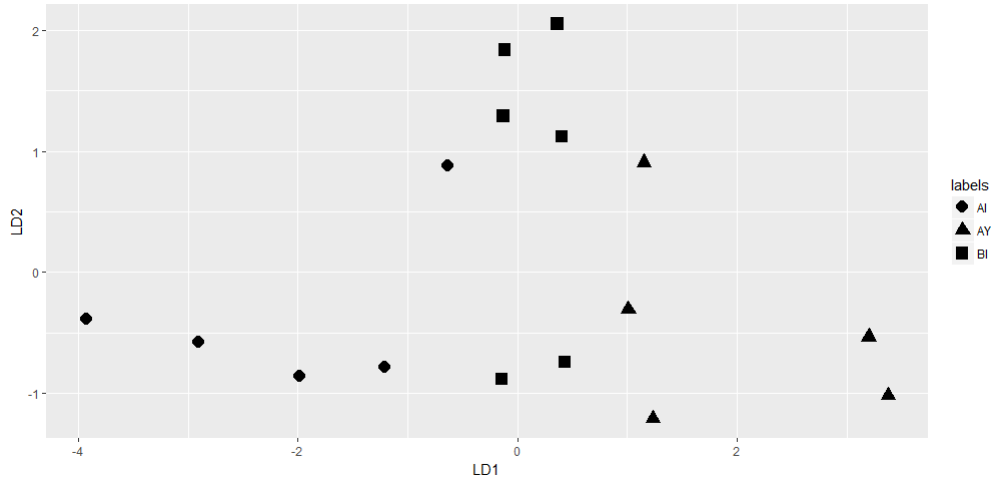

FIGURE 3.5: Results of a discriminant function analysis that categorizes individual chicks based on eight acoustic parameters. Scatter plot of discriminant function scores for the 9 groups involved in the analysis. Colour different colours indicate different groups. Each point indicates one call of one chick.

TABLE 3.4: Results of a repeated measures LMM looking at the difference in adult pukeko response to group and non-group chick distress calls. Average group response score to group or non-group stimulus was coded as the continuous response variable. The effect of stimulus is reported for group chick distress calls versus those of a foreign chick as the main fixed effect. Days between initial call recording and behavioural trial as well as weight of the chick in grams are included as fixed effects and interaction terms with stimulus type. The final nest for each individual was included as a random intercept . The model estimates and 95% confidence interval (CI) are shown. Sample sizes are from 21 trials from 7 groups.

Fixed Effect	Estimate	t-value	95% CI	p-value
Intercept	2.13	3.85	1.04, 3.21	>0.001*
Stimulus	-1.96	-2.93	-3.28, -0.65	0.006*
Days (Trial-Rec)	-0.073	-1.42	-0.17, 0.03	0.165
Weight (g)	-0.004	-0.21	-0.03, 0.03	0.837
Stimulus * Days (Trial-Rec)	0.201	2.54	0.05, 0.36	0.015*
Stimulus * Weight	-0.0003	-0.02	-0.04, 0.04	0.988

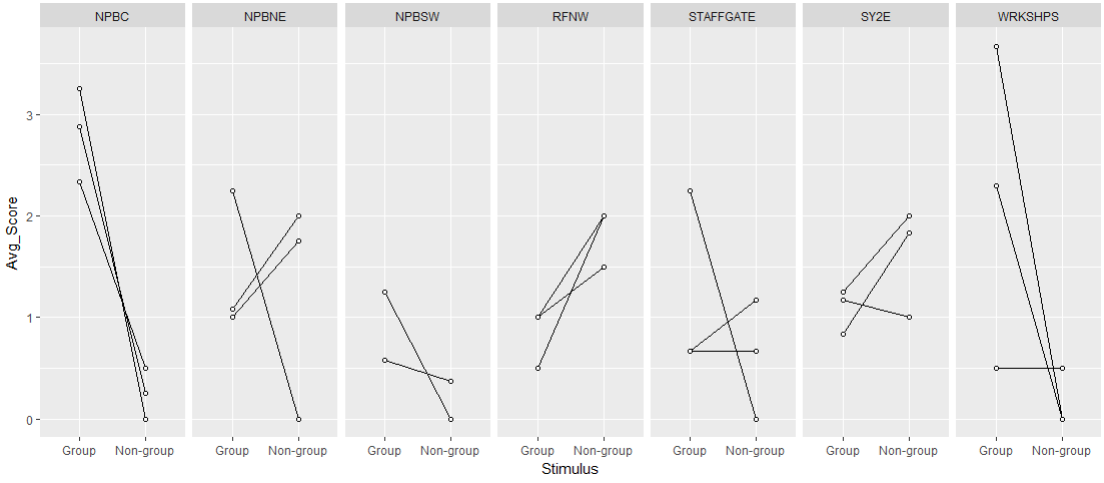

FIGURE 3.6: Average response to either group or non-group stimulus over three separate trials for each group. Each data point represents the proportional response of pukeko in the group during one trial. Data points paired by a line represents one trial. Group NPBSW only has two trials shown due to technical issues.

Appendix A

Supplementary Data

TABLE A.1: A list of chicks hatched from fostered eggs showing band information, original territory, foster territory, and whether the chick was confirmed dead at the end of the study.

Chick ID	**Band combination**	**Band #**	Original Territory	Foster Territory	Confirmed dead
AI_TN2	YG/RY-M	84014	NPBC	SY2E	N
AI_TN3	M-NB/RY	83957	NPBC	SY2E	N
AR_TN1	Y/-M	83971	RFNW	SY1CE	N
AR_TN3	W/-	N/A	RFNW	SY1CE	N
AL_TN6	N/A	N/A	SY1CE	RFNW	Y
F_TN3	N/A	N/A	NPBCW	NPBSW	Y
F_TN4	N/A	N/A	NPBCW	NPBSW	Y

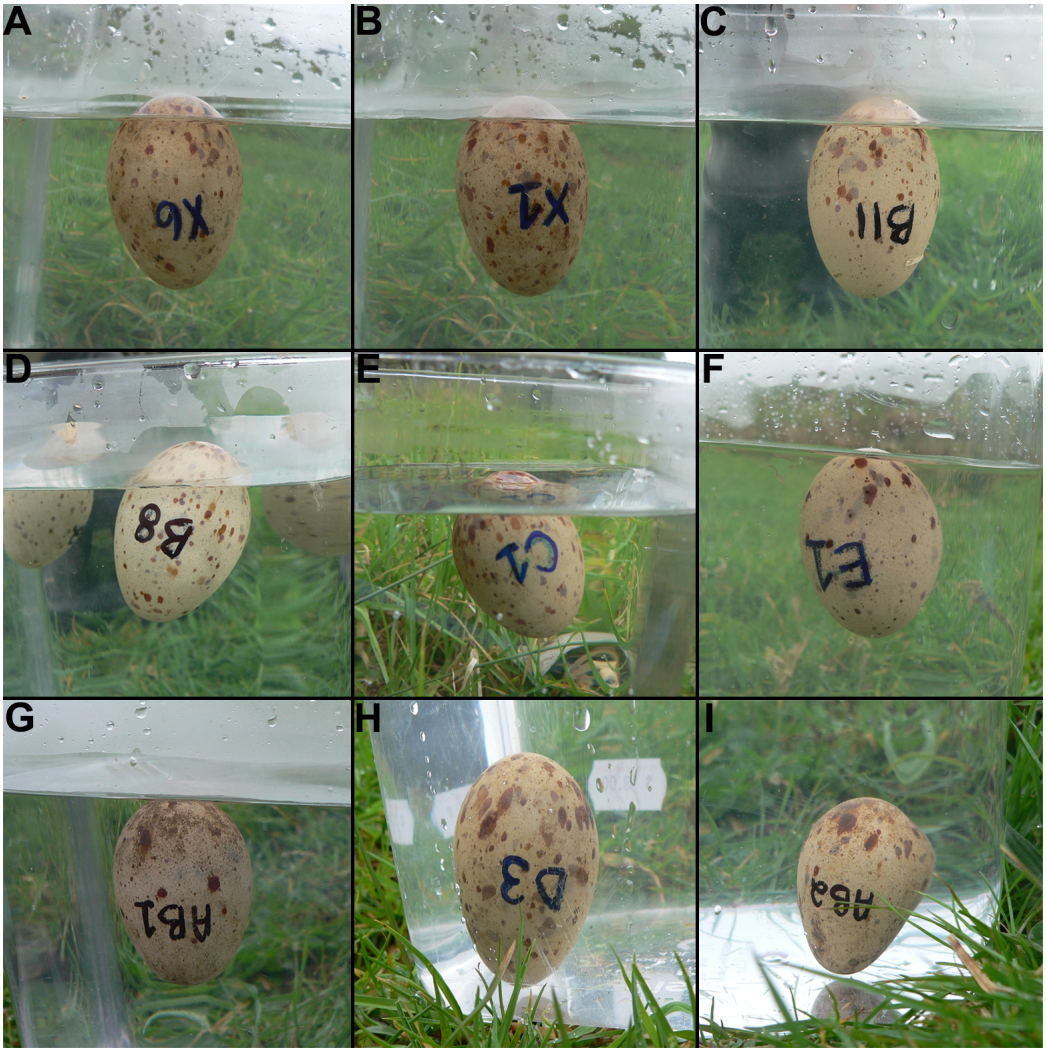

FIGURE A.1: Eggs floated in water showing development from most developed (A) to least (I). A-C show eggs floating at a 90° angle to the water surface. D-E show eggs floating at approximately 45° to the surface of the water. F-G show eggs floating just beneath the surface of the water not. H-I show eggs sunk to the bottom indicating no advanced development of embryo.

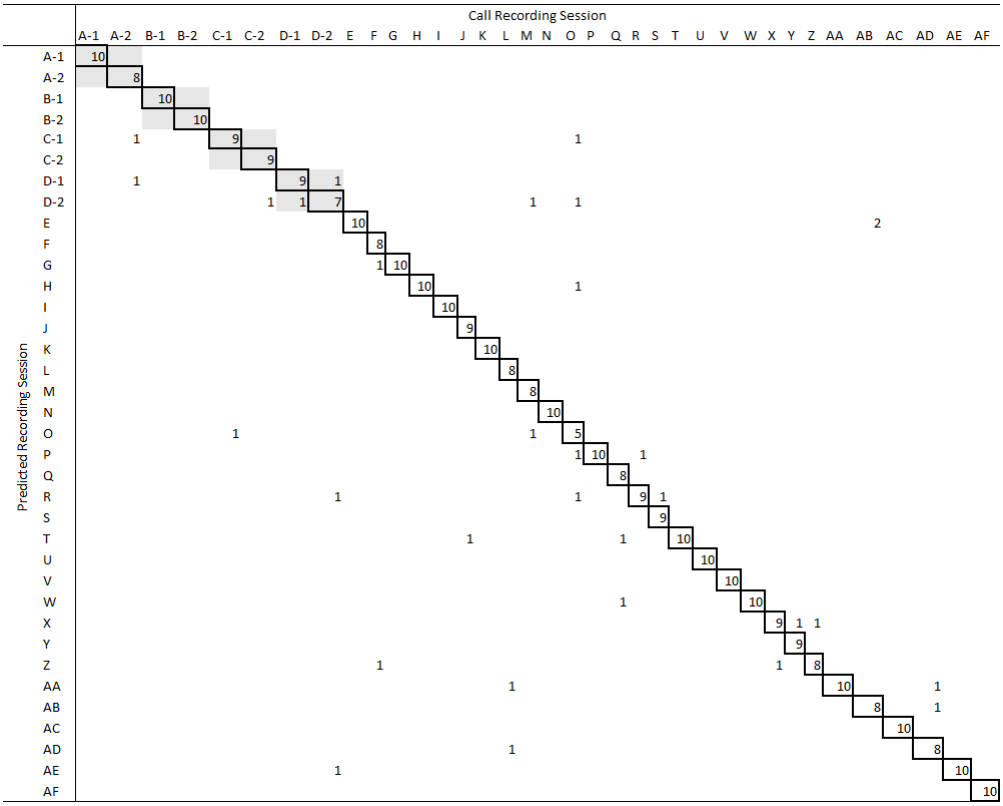

FIGURE A.2: Results of a predictive model using a linear discriminant function analysis that categorizes individual chick recording sessions based on duration (ms), mean frequency (Hz), goodness of fit, FM, entropy, minimum pitch (Hz), maximum frequency (Hz) and standard variation of pitch (Hz). Each letter represents a single chick. Letters paired with 1 or 2 indicate a chick with more than one recording session. Columns show which recording session the call was originally from while rows indicate which recording session the call was categorized into. Outlined black boxes indicate predictions where the recording session was accurately predicted. Light grey boxes indicate predictions where a single chick had more than one recording session.