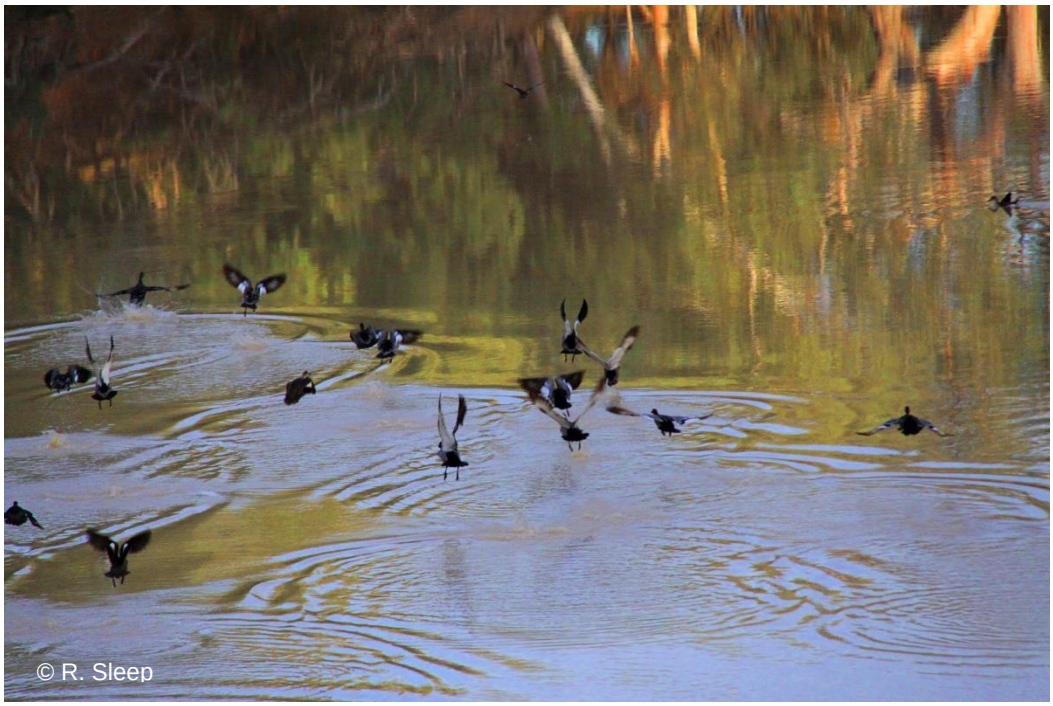
Disease ecology of low pathogenic avian influenza in the Australian environment.



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Abstract

Australia has been fortunate with its history of influenza in animals. The stringent biosecurity restrictions that govern the import and export of animals, and their products, has almost certainly contributed to maintaining the relative isolation from highly pathogenic (HPAIV) viral incursion. However, there are endemic and occasional exotic low pathogenic avian influenza viruses (LPAIV) circulating throughout Australian birdlife, particularly the waterbirds (Anseriformes) and shorebirds (Charadriiformes). HPAIV H7 viruses, which evolved from LPAIV H7 viruses, have been identified in a handful of poultry outbreaks since the 1970's and attributed to the contamination of water sources with low pathogenic influenza viruses by infected wild birds defaecating into the water.

My projects have identified new potential surveillance species, feral pigs, for LPAIV and other influenza A viruses in Australia. The close association of the native waterbird and shorebird species with feral pigs in the harsh environments of arid Australia gives rise to infection opportunities, both from bird to pig and pig to bird. The virus shed by natural hosts is able to persist in the environment, and the abiotic factors affecting viral persistence have been quantified for effect and size and strength in this thesis. Many gaps exist in our understanding of influenza viral ecology in the environment. Understanding more about the ecology of LPAIV in the environment, and the risk host species present can be used to reduce the likelihood of important captive populations becoming infected, and acting as a source of infection for in contact humans. The human public health and veterinary public health domains are inextricably linked when it comes to zoonotic diseases with pandemic potential, such as influenza.

I conclude with a discussion of the direction I believe we should move into, and suggestions of steps to take to improve our knowledge base. In particular, increasing the degree of realism in experimental studies will yield more translatable results to assist the prediction of areas of increased viral persistence, and thereby identify areas for targeted surveillance.

Thesis declaration - Statement

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Signed

Date 27th September 2017

"Something feels funny.
I must be thinking too hard."
Winnie the Pooh

"Unless someone like you cares a whole awful lot,
Nothing is going to get better. It's not."
The Lorax

"Nature doesn't sit still.
Things and individuals are changing,
dying and new things are coming.
They are all stories."
David Attenborough

Acknowledgements

As the child of, and sibling to, academics I have read my fair share of doctorate dedications. I have always found them to be the time the author gets to showcase their personality and be able to express themselves in non-standard terms. Rather than the ‘Oscars acceptance speech’ kind that always seem to have a long list of people to thank, and espouse the great things people have learnt from others during their studentship, this I hope will be more akin to the ‘Rock and Roll Hall of Fame’ story telling.

As my brother has before me, I blame my parents. The commencement and completion of this project has been their fault. From the first days of school to the end of my (many many) years at university, including a reasonable stint in the real world, they have been a strong influence to keep learning whether or not they directly know it. I cannot reasonably lay the decision to work with biological systems at their feet; that is entirely my own doing. Even as a small child I would make sure that my teddy bears had anaesthetics before my mother was allowed to stitch up any holes, the dye was cast at a young age, sorry mum and dad!

The knowledge that other, apparently normal, people had made it through a PhD, with most of their senses intact was a driver for me, and to my friends at the beginning, middle or end of their projects, hats off! Times have changed since my predecessors went through their post graduate education, but I am mightily glad that they did it, without my biased view of what can be achieved I am not completely sure this would have happened.

Most people try to avoid feral animals, and for that I cannot blame them, especially when the animal in question is a large, moderately homicidal, bristling pig who would think nothing of goring you and eating your remains. They are impressive animals, with an equally impressive list of skills and requirements which have detrimental effects on the ecosystems they have been released into in Australia. Equally impressive are the wonderful people who work to keep numbers of feral animals under control.

It must have seemed like an odd request for a city girl to attend control shoots in the middle of arid Australia, but I was met with amazing hospitality and help. I cannot do them justice in words, but my eternal gratitude is owed in particular to Robbie, Tim, Paul and Erik. They made it possible for me to get my strange project off the ground, who really looks at feral pigs for a bird disease? Oh yes, that would be me. They were only too happy to help me, letting us go up in helicopters, helping us cut open dead things, and getting the all-important samples. The opportunity to spend time in such an incredible part of the world cannot be overlooked either. The landscapes shift as you head north from Adelaide, and though you try, explaining its beauty to those who have not seen it is impossible. If you ever get the chance to ‘go bush’ do it, don’t hesitate, pack up and go.

If going to stay in the outback waiting for blood samples from feral pigs was not odd enough, I upped the ante by asking the general public to help catch ducks on the Torrens. Glutton for punishment, probably, grateful for the eagerness and dedication shown by my volunteers, absolutely. Early, cold, damp mornings, heavy traps and angry coots did not deter them, nor did it stop the wonderful Dr. Rebecca

Boulton, without whom I could not have got the project off the ground. It was a pleasure to work with her, and I am sorry about the large amounts of bird poo that always seemed to end up on us. In relation to that, the people I have shared an office with have made the tougher moments bearable, Sally, Talia and Sarah, thank you for not being too put off by us turning up cold, slightly damp and covered in bird poo.

I have been really fortunate to develop a strong friendship with the staff at Adelaide zoo, in particular the animal health department. They allowed me to spend time with them regularly over the last three and a half years and for that I am eternally indebted. They have taught me a lot (I know I said I would not talk about how much I had learnt, but in this case it is absolutely true), included me in their working family and let me run a project through their department. Their willingness to have an interloper so regularly meant I could also work with the bird department, and Charlie, Emma, Di and Dale gave me free access to their birds and enclosures so I could complete my final project. Trusting someone with the animals you care for every day takes confidence in their abilities and their motives, they were nothing but accommodating to me and for that they deserve a large thank you.

One person has been absent from this so far, my partner in crime, and provider of cups of tea (or stronger stuff) as required. From taking me to the airport more than should be possible, or waiting for a phone call to say that ‘she’s on the ground again’ after days in the helicopter while I was on field work, to the incredible evening we shared in the presence of the incomparable Sir David Attenborough (life goal achieved), he has been interested, patient and supportive. This would have been a

very different journey and prospect without him. I may have started this before we met, but I am certain I would not have got through it with the same ability to still hold a normal conversation, though I am sure I still disturb our friends at dinner.

I am not sure my principal supervisor really knew what he was taking on when we originally met for a coffee and an initial chat, but he can always blame John, and if nothing else it has been an experience! He has allowed me great freedom with my projects, and supported the many odd things I have wanted to do, even roping in his wife to help. I even managed to see him dancing at a conference, an opportunity rarely available, but don't worry Phill, there are no photos. Tom joined as my second supervisor and I could not have asked for a better guide through the complexities of spatial work in R, it still baffles me most of the time but I think we got there.

It is strange to have this part of my life completed, but I am forever grateful to everyone who has had a hand in helping me through the last few years.

Chapter 1.
Introduction

The defining epidemiological feature of a pandemic is the high proportion of the population, which is affected across multiple countries almost simultaneously[1, 2]. To be considered a pandemic influenza, an influenza virus needs to be: (i) novel; (ii) have the ability to cause illness in humans; and (iii) maintain efficient and regular transmissibility between humans[3]. The 'mother of all pandemics', the Spanish flu (influenza A H1N1) resulted in the death of 18 million people at the end of the first world war[4]. Another emergent influenza pandemic is highly probable in the next 10-20 years, particularly with the increased emergence of new viral strains infecting humans over the past decades[5], including H5N1 in Asia[6], pandemic H1N1 worldwide[7], and H10N7 in Australia[8]. This likelihood of a pandemic will also be influenced by the frequency and distribution of circulating animal influenzas[5], although the highly labile nature of the virus means predicting the location and timing of a pandemic may still prove very difficult.

Influenza viruses are divided into three major categories, A, B and C, with the majority of viruses being influenza A viruses (IAV)[9]. All IAVs have an origin in an avian host[10, 11], though they may no longer transmit between, or cause disease in, avian species; having subsequently acquired mutation that allow them to specialise in mammalian species[12, 13]. Influenza A viruses are identified through two proteins found on their surface, haemagglutinin (HA, or H-type) and neuraminidase (NA, or N-type)[9]. Highly labile viruses, influenzas go through two main forms of change, i) the small, incremental antigenic drift and ii) the more definite change caused by antigenic shift, such as occurs when a new HA NA combination occurs. Avian influenza viruses (AIVs) are further classified into highly pathogenic (HPAI) and low pathogenic avian influenza (LPAI), defined by the degree and rate of morbidity and

mortality the virus causes in domesticated poultry[14]. Research suggests that HPAI viruses arise through the acquisition of genetic mutations for cleavage points in the virus through multiple generations[15], as often occurs rapidly in commercial poultry operations.

In the northern hemisphere, particularly, there is an influential migratory component to the population dynamics of waterbirds, with many using flyways (Figure 1.1) to travel across the world to reach winter feeding or summer breeding grounds[16]. Annually, mass accumulations of birds, particularly the natural waterfowl (Anseriforme) and shorebird (Charadriiforme) hosts of IAVs, occur at breeding grounds across Europe, Eastern and Central China[17] and North America, facilitating infection of immunologically naïve young and further transmission and mutation of influenza viruses[18].

In the United States in 2015 there were multiple, geographically distant outbreaks of HPAI in turkey farms (Figure 1.2), and throughout 2016 in Europe there were outbreaks of HPAI H5N2 in multiple bird species[19]. These disease outbreaks are believed to have been, at least in part, assisted by the movement of wild birds across these regions[20-22].

Australian Anseriformes, unlike their northern hemisphere counterparts, are largely nomadic, rather than migratory, following sporadic (and often unpredictable) environmental cues for resources[10, 23, 24]. There are Australian species that are considered to be equivalent in their ecological contribution to that of their northern counterparts; for example, Australian resident Pacific Black Ducks (*Anas superciliosa*) are similar ecologically to northern hemisphere Mallards (*Anas*

platyrhynchos)[10]. However, for most Australian birds breeding is associated with stochastic resource availability rather than seasonality, and they display considerable plasticity in the timing of breeding events[23, 25-27].

Australia is host to many migratory shorebirds with 37 species migrating annually to Australia. However, this constitutes the overwintering component of their migratory pattern, and the species do not breed in Australia. In addition to the migratory shorebirds, there are eighteen species of shorebirds that permanently reside within Australia. It was long considered that migratory shorebirds were responsible for introducing new strains of LPAIV to Australian waterbirds. However, recent studies have identified phylogenetically distinct Australasian clades of virus, though northern hemisphere viruses do still occur[28, 29]. Surveillance has also identified that migratory shorebirds are infected after reaching Australia, in the majority of cases, rather than transporting LPAIV from overseas[10].

Perpetuation of viruses that have a component of environmental transmission, such as AIVs, relies on their ability to persist with the prevailing environmental conditions. How a virus persists in the environment for long enough to facilitate transmission, when shed by one host before ingestion/inhalation by the next, is a research question of considerable interest, yet much published information has limited applicability to real world management [10]. Whilst wild waterbirds are believed to be the natural hosts for avian influenza viruses[30, 31], with numerous different strains of virus circulating at any one time[30, 32], the ability of influenza viruses to infect multiple species means that mammals must also be considered potential hosts[33-35].

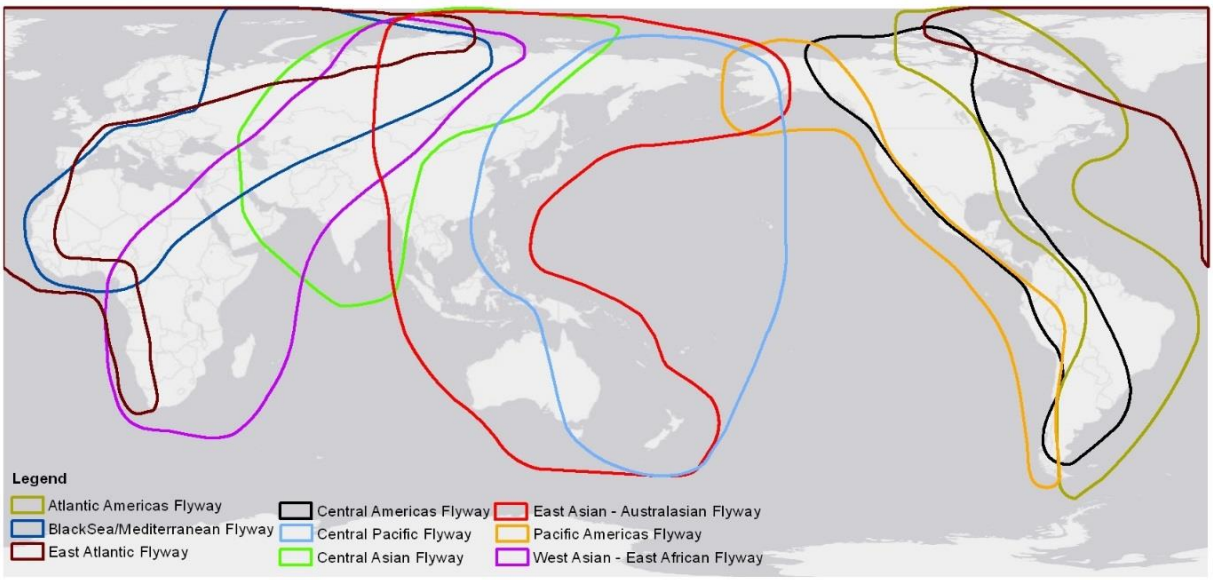

Figure 1.1: The 9 major waterbird flyways of the world. Reproduced from Wetlands.org (http://wpe.wetlands.org/Iwhatfly)

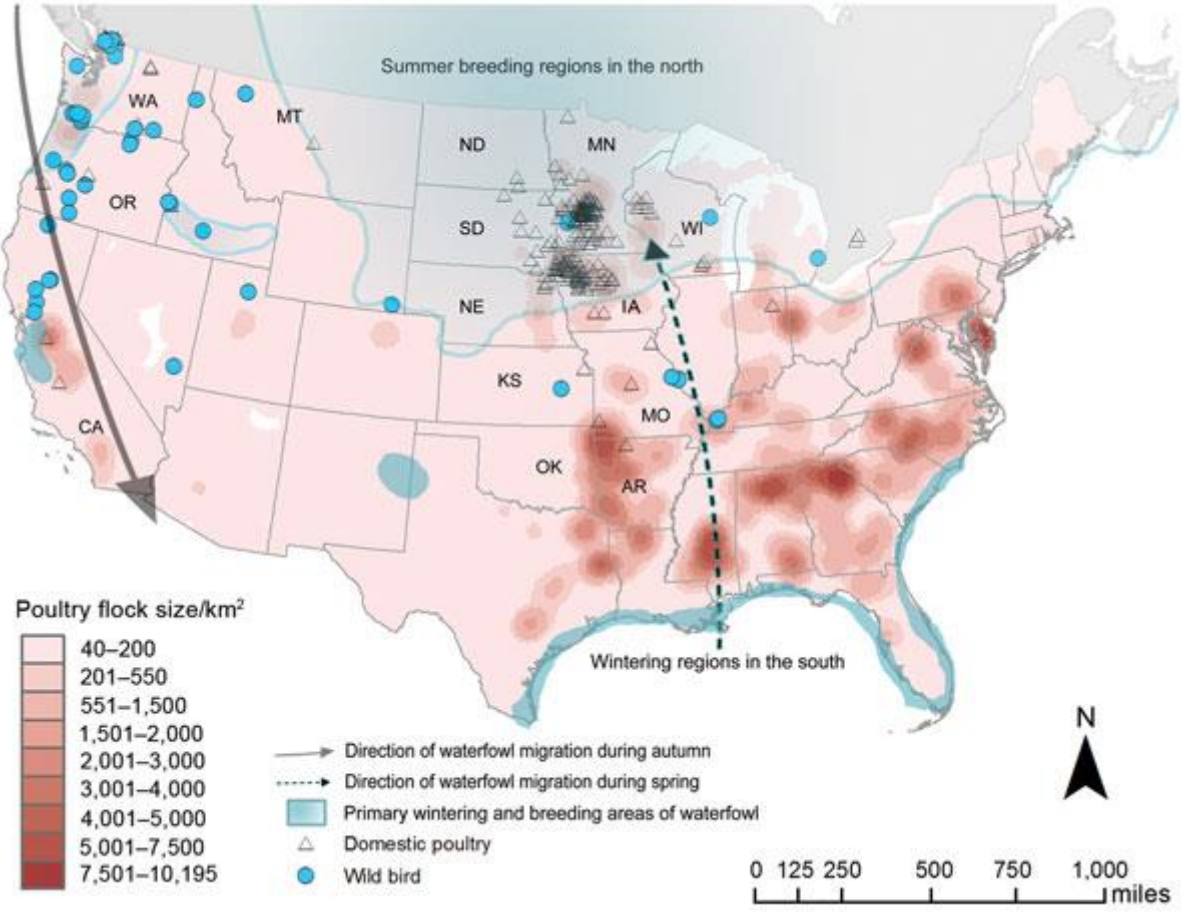

Figure 1.2. Spread of HPAI H5N2 across the United States of America in 2015 (Reproduced from Bui et al 2016)

Environmental contamination with AIVs has been confirmed through the isolation of viruses from waterbodies that support large aggregations of waterbirds and shorebirds in many parts of the world[36-38]. Environmental transmission thus has a potential role to play in the natural history of influenza viruses [39, 40].

The southern hemisphere, and Australia in particular, has a very different natural biogeographic association between flora and fauna. As a continental island, and the World's sixth largest country, Australia covers over 7.6 million square kilometres (www.ga.gov.au) and has a wide array of endemic fauna and environments ranging from lush tropical rainforests, to snow-capped mountains and vast arid deserts. Much of Australia's interior (arid) environment can be very harsh, and often unpredictable, with many large inland water sources ephemeral in nature, only filling when high rainfall events occur in distant regions. Australia is so large that there is both a wet-dry season climate and a four-season temperate climate.

Research into the environmental persistence of LPAIV has been conducted for at least the past 40 years[41], but we are still limited in our understanding of the factors affecting the persistence of the virus in the environment. These limitations also affect our ability to predict the next large influenza outbreak. In particular, we do not have lack information on all of the relevant biotic and abiotic factors that are conducive to persistence in the environment[10, 16]. Influenza viruses are not considered to be very stable outside their hosts. Heat, pH extremes and desiccation can all affect the ability of the virus to remain infectious in the environment[42]. These results are primarily derived from the northern hemisphere studies that used using laboratory-based experimental models[43-48]. In Australia, the natural history of the

primary virus hosts is also likely to affect the persistence of LPAIV[10], however, we do not yet have sufficient information to be able to predict the areas of greatest concern.

Numerous knowledge gaps still exist surrounding the ecology of LPAIV in both the northern and southern hemispheres. Which of the hundreds of Anseriform and Charadriiform species in Australia are most likely to be infected from the environment? How long does infection persist, and what are the immune responses to different strains of virus? Further, what other species, both avian and mammalian, are most likely to become infected? Can we use other species to monitor for virus spillover, where a pathogen moves from the reservoir host into a novel species? Could other species, particularly domestic and feral mammals, be the source of new recombinant viruses? What environmental factors help or hinder the persistence of virus in the environment, and aid environmental transmission[39, 40]? How does diurnal variation affect the persistence of virus in the environment? How does season affect viral persistence, and is it possible to use the southern hemisphere to unpack the seasonal effect without the large aggregations of birds that occur in the northern hemisphere?

The likelihood of an outbreak of HPAI in Australia remains high, and the ongoing surveillance of wild birds is conducted on a regular basis at multiple sites throughout Australia, coordinated by the National Avian Influenza Wild Bird working group (NAIWB)[49, 50]. Maintaining vigilance and increasing the number of known and isolated virus strains within Australia, is vital to enable any outbreaks to be traced to source and highlight areas of particular risk.

Australia has its own endemic avian influenza viruses, and an excellent history of combating HPAIV outbreaks in poultry. However, the current knowledge about the ecology of the virus in the southern hemisphere, particularly in Australia, is limited.

If we accept that the proposal that a pandemic will occur again, and that they generally occur through antigenic shift often by jumping species or acquiring greater pathogenicity for the host, then understanding the ecology of influenza virus must be a priority. Where the virus is able to persist, the species it is capable of multiplying in and the mixing of new potential hosts become key factors that influence the next pandemic. This thesis aims to move the field of influenza pandemic prediction and preparedness forward by identifying knowledge gaps, looking for interspecies interactions leading to infection, and providing a means of assessing the risks that face captive populations.

The key objectives for this thesis are to (i) review the available information and identify the current knowledge gaps with regards to persistence of LPAIV in water; (ii) investigate the exposure of a widespread feral species to influenza A virus across Australia, (iii) provide a system to target surveillance of wild birds to areas of the highest potential risk, and iv) assess the risk of transfer of LPAIV from wild to captive birds, using a zoo as a case study.

A brief summary of the main objectives and chapters to follow, is provided here:

- Chapter 2: Persistence of low pathogenic influenza A virus in water: a systematic review and quantitative meta-analysis.

Influenza viruses are highly labile are often shed into water by the natural host. The main objectives of this chapter is to identify the current state of knowledge around persistence of influenza virus in water, and to determine which factors are the most influential for viral persistence in water. In this Chapter I have collated, assimilated, and analysed all the available information, in the published literature, regarding the persistence of low pathogenic influenza A viruses in water. Persistence of LPAIV in water is of importance as it is where the avian hosts are likely to shed virus and counteracts one of the most important limiting factors to persistence, desiccation. I identify the abiotic factors that have been investigated, to date, and quantify their contribution to the degradation of LPAIV in water.

- Chapter 3: Spatial prediction of low pathogenic avian influenza A (LPAIV) persistence risk and surveillance across continental Australian waters

Surveillance for emerging and exotic diseases is an essential component of early detection and subsequent disease prevention. Environmental persistence of LPAIV constitutes part of the faecal-oral transmission cycle between avian hosts. Virus is shed into waterbodies by the natural hosts in their faeces. The objective of this chapter is using the information from the meta-analysis detailed in Chapter 2, as well as spatial data on water availability and the distribution of waterbirds and shorebirds, to predict the locations most likely to meet the abiotic and biotic requirements for the persistence of LPAIV, and subsequent transmission between hosts.

- Chapter 4: Sympatric feral omnivorous species as a secondary host of LPAIV in Australia

Pigs (*Sus scrofa*) have the receptors for both human and avian adapted influenza viruses, a key feature that has identified them as a potential 'mixing vessel' for the emergence of new influenza viruses, potentially with greater transmissibility or pathogenicity in humans. In Australia the feral pig population is estimated to be anywhere between 5 and 20 million individuals, and feral pigs are subject to extensive nationwide control programs due to the damage they cause to native vegetation, disturbance to water sources, and predation of native animals. Pigs are also controlled because of their potential to carry diseases of economic or public health importance, such as brucellosis, foot and mouth disease, leptospirosis and Q fever. The main aim was to investigate the exposure of feral pigs to influenza A virus, possibly of avian origin. I established and coordinated a national project to obtain serum samples and nasal swabs, to investigate exposure to influenza A virus, from feral pigs culled as part of control programs in Australia.

- Chapter 5: Semi-quantitative disease risk assessment for LPAIV transmission from free-ranging wild birds

In this chapter I present a framework for the semi-quantitative assessment of a single pathogen across multiple species in a large captive-holding institution. Captive exotic and native birds in zoological institutions are often exposed to free-ranging wild birds. The risk that these interactions pose to captive birds can be mitigated through various husbandry actions and facility designs, however, they can never be completely eliminated. Disease risk assessments traditionally consider a single host species and attempt to identify all exposure hazards in a particular management activity or environmental setting. It is not commonly possible to conduct a quantitative risk assessment for situations involving wild animals, or

species, which have not been exhaustively studied. Often qualitative assessments based on expert opinion have been the most effective option available. Some pathogens can have effects over multiple species, be zoonotic and therefore be of public health concern.

The final chapter (Chapter 6) is a general discussion of the low pathogenic avian influenza disease ecology in Australia. I provide an executive summary of the major findings and identify knowledge gaps that can and should be subsequently closed, suggesting how surveillance can be improved and some review of the steps that can be taken to view avian influenza from a One Health perspective.

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Overall percentage (%)	90
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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ii. permission is granted for the candidate in include the publication in the thesis; and

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Chapter 2.

Persistence of low pathogenic influenza A virus in water: A systematic review and quantitative meta-analysis

Prepared for publication and published in *PLoS ONE*

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Abstract

Avian influenza viruses are able to persist in the environment, in-between the transmission of the virus among its natural hosts. Quantifying the environmental factors that affect the persistence of avian influenza virus is important for determining our ability to predict future outbreaks, and target surveillance and control methods. I conducted a systematic review and quantitative meta-analysis of the environmental factors that affect the decay of low pathogenic avian influenza virus (LPAIV) in water. Abiotic factors affecting the persistence of LPAIV have been investigated for nearly 40 years, yet published data were produced by only 26 quantitative studies. These studies have been conducted by a small number of principal authors (n = 17) and have investigated a narrow range of environmental conditions, all of which were based in laboratories with limited reflection of natural environmental conditions. The use of quantitative meta-analytic techniques allowed me to assess persistence across a greater range of conditions than each individual study can achieve; i.e., through the estimation of mean effect-sizes and relationships among multiple variables. Temperature was the most influential variable, for both the strength and magnitude of the effect-size; with higher temperatures reducing the persistence time of the virus. Moderator variables explained a large proportion of the heterogeneity among effect-sizes. Salinity and pH were important factors, although future work is required to broaden the range of abiotic factors examined, as well as including further diurnal variation and greater environmental realism generally. We were unable to extract a quantitative effect-size estimate for approximately half (50.4%) of the reported experimental outcomes and we strongly recommend a minimum set of quantitative reporting to be included

in all subsequent studies, which will allow robust assimilation and analysis of future findings. In addition we suggest possible means of increasing the applicability of future studies to the natural environment, and evaluating the biological content of natural waterbodies.

Introduction

An organism's persistence depends on it being capable of surviving the extremes of the prevailing environmental conditions[1]. Viruses are often capable of naturally persisting in a wide variety of environments, including water[2], and can remain infective for varying lengths of time. Viruses may then transmit to new hosts, including new taxa, when the opportunity and circumstances arise[2]. A notable example is the megavirus *Pithovirus sibericum*, which was recently isolated from the Siberian permafrost, dated to being more than 30,000 years old, and still infectious on thawing[3]. The environmental conditions that are conducive to viral persistence and transmission vary, depending on the type of virus and the protection they possess[4]. Physical protection of a virus is provided by its capsid protein coat, present in all virions (viral particles)[4, 5]. Non-enveloped viruses (in particular) are more resistant to environmental degradation than enveloped viruses[6].

Influenza viruses (*Orthomyxoviridae*) are enveloped, single stranded, negative sense RNA viruses and are divided into four types: influenza A, which infect both avian and mammalian hosts; influenza B, which circulate in humans, and have been isolated from seals[7]; influenza C, found in humans, pigs and marine mammals[8], and influenza D, recently found in cattle and pigs[9]. Influenza A is the largest group

of influenza viruses, with recognised differentiation of individual types through the two glycoproteins located on their surface[10, 11]; haemagglutinin (HA, or H-type) and neuraminidase (NA, or N-type). To date, there are sixteen HAs and nine NAs that have been identified from their natural waterbird hosts (predominantly Anseriformes; although Charadriiformes are also known hosts[12, 13]), with multiple strains arising within each HA and NA combination[8].

Outbreaks of influenza in live bird markets[14], zoonotic infections of humans[15, 16], and pandemic influenza events in the last twenty years have each highlighted the wide range of species susceptible to influenza A viruses[17-19]. Considerable research effort has focused on low pathogenic avian influenza viruses (LPAIV), which naturally occur in wild birds, contrasted with highly pathogenic avian influenzas (HPAIV), which have high mortality rates in poultry[20]. LPAIVs circulate within their wild waterbird hosts, whereas HPAIVs are believed to mostly arise after multiple passages through domesticated poultry[21, 22]. LPAIVs are found in both the respiratory and gastrointestinal system of their natural hosts. Waterbirds spend considerable time on or around water, eating, preening and defaecating; with a single duck producing 7.5-10kg of faeces per year[23]. LPAIVs are most commonly shed into the aquatic environment in large volumes via waterbird faeces[24, 25].

Viruses are transmitted either directly by host-to-host contact or indirectly by air, fomites, or environmental contamination[1, 2]. Transmission of LPAIVs includes an environmental component[26, 27], which enables indirect transfer of virus between hosts[28]. The length of time LPAI virus can remain infective in the environment, the specific conditions of the environment that are conducive to persistence, and the infective dose required for transmission[5], have all been the subject of nearly 40

years investigation. As a notifiable disease to both WHO (World Health Organisation) and FAO (Food and Agriculture Organisation of the United Nations), avian influenza is of global importance[29]. Control of LPAIV, and the prevention of disease outbreaks, requires an accurate understanding of: (i) the spread and transmission of the virus both among waterbirds, and between waterbirds and other potential reservoir species (e.g., shorebirds, poultry, pigs, horses, cats and humans); and (ii) the survival and persistence of the virus within the environment, prior to, and facilitating, novel transmission.

Webster et al. (1978) provided the first published quantitative information on the survival of influenza A virus in water, and showed that the virus persisted for up to 30 days, three times longer than in faeces[30]. Their pioneering work has been followed by multiple studies, and thirty years after Webster's initial study Brown et al. (2009) noted that the majority of previous investigations had concentrated on laboratory-based investigations, using distilled water in most cases. This subsequently highlighted the need for broader testing of the properties that affect the survival/persistence of the virus. Laboratory-based studies have provided information regarding persistence of twelve out of sixteen identified HA types (see below) in varying simulated environmental conditions. In the past decade, environmental water samples have been more regularly used in laboratory based studies, including samples from water bodies with known populations of waterbirds, and known circulating LPAIVs in the hosts[31-35]. Two reviews, which synthesised the available information on LPAIV persistence, were previously published by Irwin[36] and Stallknecht[17]. In the most part they agreed with the observed findings from previous individual studies, although notably Irwin did not find temperature to

be an important moderator of virus half-life in water, possibly due to the final sample size (7 studies, 127 data points) included for analysis. Meta-analytic review techniques and statistical packages are evolving rapidly and it is now possible to examine quantitative relationships, in the persistence of LPAIV across studies (controlling for replicate observations), and the contributions of these variables in explaining persistence of LPAIV.

How the virus interacts with, and is affected by, the environment is a crucial component to understanding its circulation and transmission, particularly if we wish to improve targeting of surveillance and future disease control. Information regarding the environmental persistence of LPAIV is spread across the primary scientific literature. In this study we conducted the first quantitative meta-analysis of the environmental factors that influence the persistence of LPAIV in water. We surveyed and assimilated all of the available literature, in order to provide a comprehensive analysis of the environmental variables previously investigated, and to draw robust conclusions and inferences regarding the persistence of LPAIV in water. We specifically investigated the survival of the virus in water (c.f.[36]), as the natural hosts are intimately associated with, and shed the virus into, a wide range of natural water bodies[37], and LPAIV has been previously isolated from open water [38]. The persistence of the virus in water can be difficult to measure, and so in most studies has been quantified as infectivity to hosts. This is predicated on the virus occurring at an adequate concentration to infect a host, and therefore having a biological effect.

The objectives of my study were threefold. First, I have identified which of the most commonly studied environmental variables (i.e., temperature, pH, salinity and water

type) have a consistent influence on persistence of LPAIV in water. By summarising quantitative results across a broad range of environmental information, and from all known studies, I was able to conduct my analyses for an increasingly realistic range of values. Second, I have investigated the size (and influence) of the effect that these environmental variables have on persistence of LPAIV in water, expanding on the previous reviews and allowing for a greater understanding of the effect in different water-body types. This allows for a more robust translation (and prediction) of the effects of persistence in novel environments, as well as under the potentially altered conditions of climate change[39]. Finally, I have highlighted obvious knowledge gaps among the previously investigated environmental variables, and discussed future priorities for research, including some of my own recommendations for conducting trials that more closely mimic the natural environment.

Methods and materials

Literature search

I used the systematic review framework, PRISMA[40], to conduct a quantitative meta-analysis of published studies on the environmental factors affecting the persistence of LPAIV in water. I searched four databases (Web of Science, which itself encompasses multiple databases; Aquatic Sciences and Fisheries Abstracts; PubMed; and Google Scholar), for primary scientific studies of the environmental factors that have been studied in relation to persistence of LPAIV. The search terms were chosen to be as broad as possible, whilst keeping the specific search objectives within reasonable bounds. I used the following search terms: (infl* OR orthomyx*) AND (avian OR bird) AND (surviv* OR persis*). I included studies

referred to in two previously published literature surveys[17, 36], if they were not already identified in the search, and completed a forwards (citations within a relevant paper) and backwards (citations of a relevant paper) search, including the reference lists of any papers that met my inclusion criteria (see Figure 2.1 and details below).

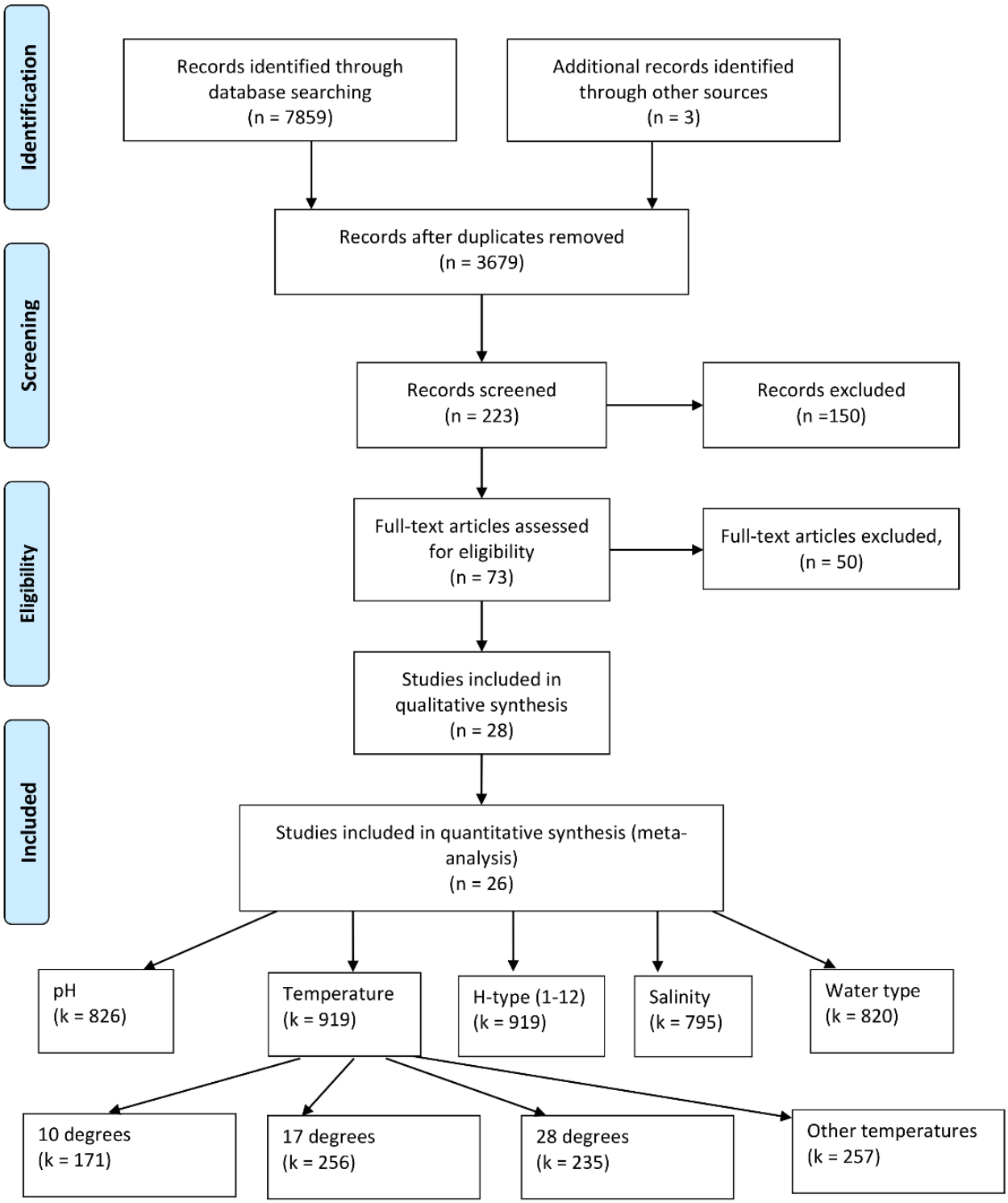

Figure 2.1. Systematic review PRISMA chart[40]. The chart illustrates the inclusion/exclusion process of reviewing studies and the numbers of papers identified at each stage (n). The forward and backward reference search was conducted at the eligibility stage. The number of samples (k) are provided for the five key variables: temperature, pH, water type, H-type and salinity. An example of the temperatures and number of observations is also given

My broad search results were narrowed by excluding all studies concerned with HPAIV, human, swine or equine influenza, vaccines, or outbreaks in poultry (Figure 2.1). In order to be included in our analysis a study had to quantitatively assess the persistence of an LPAIV virus strain in water, and provide at least one environmental moderator variable of interest (see below).

Quantitative studies either measured the 50% tissue culture infective dose ($TCID_{50}$) or 50% egg infective dose (EID_{50}), determined by the dose of virus that causes a cytopathic effect in 50% of the inoculated tissue or eggs. A single study presented information as plaque forming units (PFU), for which I converted the values using a standard conversion of 1 $TCID_{50}$ = 0.69 PFU[41]. $TCID_{50}$, or EID_{50}, was used to calculate the log-scale reduction in infective dose, Rt. Specifically, Rt is the time taken to achieve a 1-unit log-scale reduction in the $TCID_{50}$ (or EID_{50}) and is usually provided in days (although two studies presented Rt in minutes or months, and were subsequently converted to days), thus providing a measure of the degradation rate of the virus strain.

The initial search terms returned 7862 records (see Figure 2.1). I removed 4183 duplicates, leaving 3679 studies. A further 3456 studies were excluded at the first level of screening because they did not meet the inclusion criteria (see above), and a further 150 studies, which involved poultry, human, swine or other species, or were related to vaccination, immunology or treatment, were removed at the second screening. I assessed 73 full-text articles for eligibility, and excluded 45 studies because they did not specifically examine LPAIV in wildlife, LPAIV in the environment, or shedding of the virus. From this final set I excluded 25 studies because they were observational studies only with no quantitative information on

persistence of the virus. The remaining 28 studies were included in the final systematic review, with two studies excluded from the quantitative synthesis as they did not contain empirical relationships that could be meaningfully extracted.

Data collection

I found (and included) 26 studies conducted between 1978 and 2014, which contained 1824 experimental outcomes. Due to a large amount of missing information, I was only able to estimate effect sizes (see below) for 919 (50.4%) individual outcomes of persistence (Table 2.1). For each individual outcome of persistence I extracted the following moderator variables:

i) *temperature*; reported in degrees celsius, (n = 919 data points, range -30 to 55°C);

ii) *pH*; reported in standard units, (n = 836 data points, range 4.2 – 9.4);

iii) *salinity*; converted to parts per million (ppm) across all studies (n = 795 data points, range 0 – 42477ppm). Where salinity was not reported directly, but reference was made to distilled water, salinity was assumed to be 0ppm. The distribution of salinities showed a clear tri-modal pattern (Figure 2.2a) and for further analyses they were grouped into three categories, salinity group 0 (0ppm; n = 163 data points), salinity group 1 (1 to 1000ppm; n = 307 data points) and salinity group 2 (>1000 ppm; n = 255 data points);

iv) *water type*; categorised as sterilised, distilled, filtered or unfiltered, based on descriptions within the text of each study. Water was classified as distilled when expressly described so in the study (n = 211 data points).

Unfiltered water was assumed when no information was given for the nature of water, or when the water sample was expressly reported as being unfiltered (n = 104 data points). Filtered water was assigned to any experiments that described a filtration technique (n = 481 data points). Sterilised water refers to any mention of the use of an autoclave, regardless of whether or not it was filtered prior to sterilisation (n = 34 data points);

v) *H-type*; every study detailed the H and N type of the strain used (n = 919 data points; H1 = 13, H2, = 26, H3 = 201, H4 = 220, H5 = 107, H6 = 111, H7 = 48, H8 = 127, H9 = 36, H10 = 8, H11 = 15, H12 = 7).

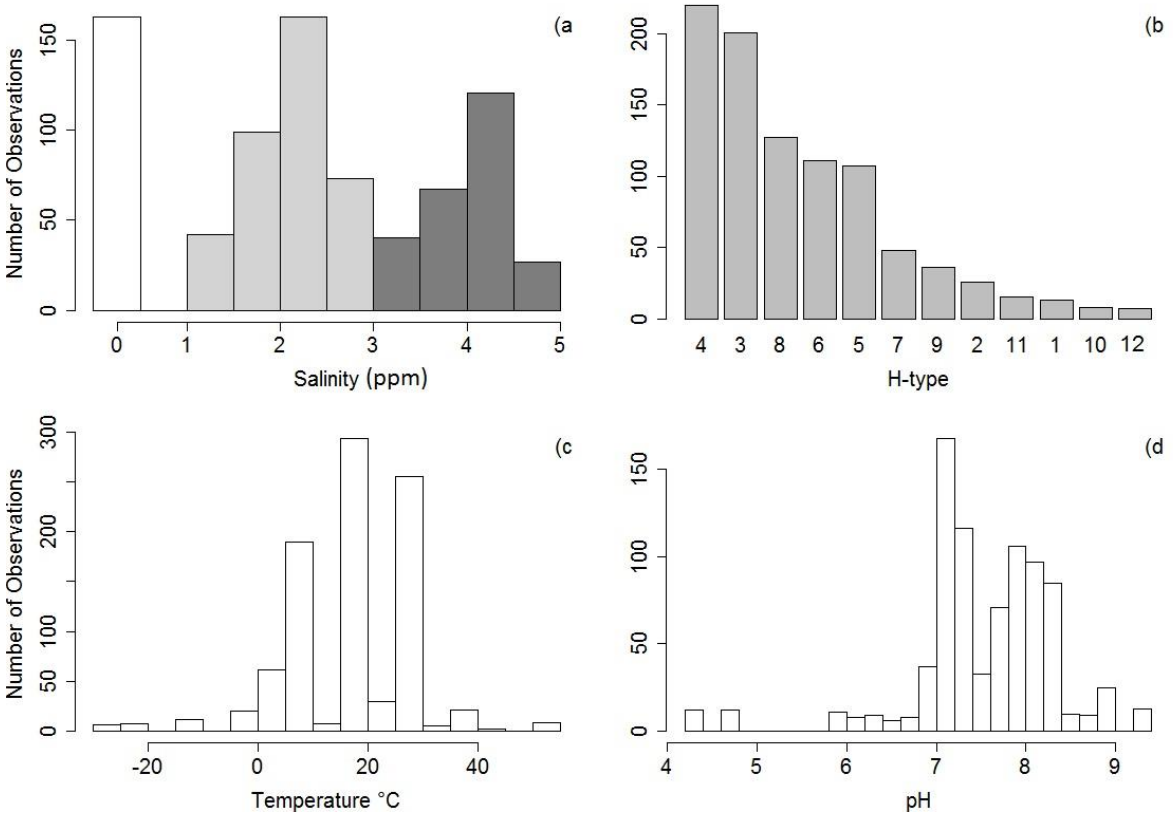

Figure 2.2. Distribution of observed values for the moderator variables across the full dataset: (a) $\log_{10}$ salinity (ppm); (white = 0ppm, grey = <1000ppm, dark grey = ≥ 1000ppm); (b) H-type; (c) temperature (°C); and (d) pH.

From each study I extracted the following summary statistics; or a subset when the full information was unavailable:

i) *Rt*; estimated duration of infectivity (in days) of the virus strain, being the time taken to achieve a 1-unit log-scale reduction in the $TCID_{50}$ (or EID_{50});

ii) *Regression slope and standard error*; the estimated slope coefficient from a reported linear regression model between 50% infective dose ($TCID_{50}$, or EID_{50}) and time (days);

iii) *Regression intercept*; the constant dependent value from the reported linear regression model (see ii);

iv) *n*; the number of experimental samples used to calculate Rt. Where *n* was not reported directly (2 out of 26 studies) I estimated it by multiplying the number of time points and the number of replicates, or counting the minimum number of time points for which I had clear evidence;

v) R^2; Coefficient of Determination from the reported linear regression model (see ii).

Table 1. Papers meeting all inclusion criteria for quantitative analysis, including all experimental outcomes, the number of reported results and model fit statistics. The primary author, year of publication, and country the study was conducted and published in are provided. 'H' is the number of different H-types reported in the experiment(s). Strains is the number of different strains (multiple version of one H-type might be used) in each study. Temperatures, pHs and salinities are the number of different levels recorded for each factor. Experimental combinations is the total number of temperature/pH/salinity/strain combinations that could be directly ascertained from the paper. Rt, R^2 and slope is the number of individual reports of each result in each paper. The origin and year of isolation for all strains used in each study are available in S1 Appendix 1.

Study	Year	Country	H	Strains	Temperatures	pHs	Salinities	Experimental combinations	Rt	R^2	Slope
Brown et al [42]	2007	N. America	2	8	2	1	3	48	48	48	48
Brown et al [24]	2009	N. America	12	12	3	5	5	900	60	0	0
Davidson et al [43]	2010	Israel	1	3	3			7	7	7	7
Graiver et al [44]	2009	N. America	1	1	3	2	4	8	8	0	8
Guan et al [45]	2009	Canada	6	1	4	2		8	8	0	0
Harris et al [46]	2010	N. America	2	2	2	2	2	12	10	0	0
Keeler et al [32]	2012	N. America	2	2	3	15	15	90	90	90	90
Keeler et al [33]	2013	N. America	2	11	1	1	1	27	27	0	0
Keeler et al [35]	2014	N. America	3	3	3	38	38	342	342	0	0
Lebarbenchon et al [47]	2011	N. America	2	2	5	3	2	18	18	18	0
Lebarbenchon et al [48]	2012	N. America	3	5	5	1	1	25	25	0	0
Mihai et al [49]	2011	Romania	1	1	3	3	3	27	9	9	9
Nazir et al [50]	2010	Germany	3	3	5	3	3	45	45	45	45
Nazir et al [51]	2010b	Germany	3	3	5	1	1	20	20	20	20
Nazir et al [52]	2011	Germany	3	3	4			32	32	0	0
Negovetich and Webster [53]	2010	N. America	1	7	2			3	0	0	21
Nielsen et al [54]	2013	Denmark	2	2	3	1	3	35	16	0	0
Shoham et al [55]	2012	Japan	2	2	2	3	3	12	12	12	12
Stallknecht et al [17]	2010	N. America	2	4	1	1	1	4	4	4	4
Stallknecht et al [56]	1990	N. America	3	3	2	8	7	95	22	22	22
Stallknecht et al [57]	1990b	N. America	5	5	3	1	1	11	11	11	11
Terregino et al [58]	2009	Italy	1	7	2			14		0	21
Webster et al [30]	1978	N. America	1	1	2	2	2	4	4	4	4
Zarkov & Urumova [59]	2013	Bulgaria	1	1	3	1	1	5	5	5	5
Zarkov [31]	2006	Bulgaria	2	2	5	5	5	20	20	0	0
Zhang et al [34]	2014	China	2	2	3	4	4	47	47	47	47
TOTALS								**1824**	**873**	**333**	**365**

Statistical analysis

I considered two measures of effect size: Rt (log-scale reduction in infective dose) and Zr (Fisher's z-transformed correlation coefficient). Rt was extracted, where possible, directly from the empirical results of the published studies. Zr was calculated from the correlation coefficients (see below) by converting the regression model R^2 values to correlation coefficients following equation (1) in Nakagawa[60]. All statistical analyses were conducted using the R software environment for statistical and graphical computing (v.3.1.0)[61].

Studies included in the meta-analysis did not always provide both an Rt and R^2 value (with associated slope, standard error and intercept), and some studies only provided a plotted figure (bivariate scatterplot) of the association between log $TCID_{50}$ (or EID_{50}) and time. Where a figure was provided and the diagnostic information was unavailable I data-mined the figures using a Plot Digitizer[62]. The figures were then reconstructed in the R software environment for statistical and graphical computing [61] and a simple linear regression model was fitted, allowing us to estimate the values for Rt, R^2, linear slope (and standard error), and model intercept, indirectly.

I used the meta-analysis package *metafor*[63] to transform and visualise the effect sizes, for model fitting, and for the calculation of within study variance (i.e., effect size heterogeneity). Correlation coefficients were transformed to their Fisher's *z*-transformed correlation coefficients (*Zr*), and their sampling variances calculated, using the *escalc* function in the package *metafor*. Where the sampling variances could not be calculated, due to small sample size ($n \leq 4$), they were excluded from further analysis.

I used an extension of Egger's regression test[64] to evaluate evidence for publication bias in our measure of the strength of the effect size of the persistence of LPAIV (Zr). The test was conducted by modifying the multi-level meta-regression models to include the square root of the sampling variance estimates associated with each effect size as an additional moderator variable. Where the intercept of the resulting model does not differ significantly from zero there is no evidence for publication bias. I did not apply the Egger regression test to our measure of the magnitude of the effect size (Rt) because the expected value of this effect size will always be greater than zero, given that persistence can only decrease with time, not increase, in the absence of transmission between hosts.

I followed a 'meta-regression' approach[65] to test the effects of multiple factors (including both continuous and categorical moderator variables) in a single model. I constructed a random-effects model to account for the random variation among studies (i.e., study ID was included as the among study random effect) and the non-independence of multiple data points from the same study (i.e., experiment within each study ID was included as the within study-level random effect). All moderator variables were included simultaneously as fixed effects in the model. All confidence intervals are 95% intervals. I refitted the meta-regression model using Bayesian estimation (using package R2JAGS) to extract the conditional between-study effect size estimates (and credible intervals) for plotting, as these were not available from the model output using *metafor*.

I evaluated the relative rankings of candidate models that included all possible subsets of the four predictor variable using an information-theoretic approach (AICc[66]) to determine the relative importance of each predictor. The sum of the Akaike weights

from all models in which a predictor variable was included was used as its measure of its relative importance.

I repeated the analysis on a subset of the data using only the specific H-types H3, H4 and H8 (n = 201, n = 220, n = 127, respectively), enabling me to test for differences in persistence between the three most commonly studied H types. I also compared the overall responses of the studies with those of the subset (of most commonly studied H-types), for confirmation of any observed patterns.

I conducted a contrast analysis for the meta-regression model to test for the influence of different levels of the moderator variables. When predicting the effects of a specific moderator variable the other moderators in the model were set to pre-determined reference values, based on a median temperature and pH of the available data, and baseline levels for water type and salinity. The reference (baseline) conditions, which I used to compare the effects of the individual moderators, were a temperature of 17°C, fresh, sterilised water and a pH of 7.6.

Further investigation of the effect of salinity, as a continuous variable, was conducted by removing the large number of laboratory-based 0ppm data points (n = 148).

The heterogeneity statistic, I^2, was used to quantify the relative proportions of among-study variation, within-study variation, and measurement variation[67]. This is particularly important in meta-analysis as it provides an estimate of model consistency[68]. Simple (rule-of-thumb) summary thresholds for the interpretation of I^2 are considered to fall into overlapping brackets; 0-40% low, 30-60% moderate, 50-90% substantial, and 75-100% considerable heterogeneity[69].

Results

Data Description

The quantitative meta-analysis included 26 studies that investigated the environmental variables affecting the persistence of LPAIV in water (Table 1). Just three temperatures (10, 17 and 28°C) accounted for 72% of all the individual temperatures studied (Figure 2.2b). Similarly, for pH 24% of the results were obtained from just two values (7.2 and 7.4; Figure 2.2d). Fresh water (Salinity of 0ppm) was associated with 17% of the extracted data. This non-uniform sampling distribution was also evident for H-type where H3 and H4 were the most frequently studied, accounting for 45.8% of all the observed data (Figure 2.2a).

There was a significant difference in persistence between the frozen (<4°C) and non-frozen (≥4°C) water temperatures ($t = 4.4$, $df = 44$, $p < 0.001$; Figure 2.3) with virus persisting for substantially longer (average= 691.6 days, s.d. = 158.21) in frozen compared to non-frozen samples (average = 22.9 days, s.d. = 40.5) (Figure 2.3). For all further analyses I only included samples with a temperature equal to or greater than 4°C; this temperature was the lowest temperature studied in liquid, rather than solid-state water.

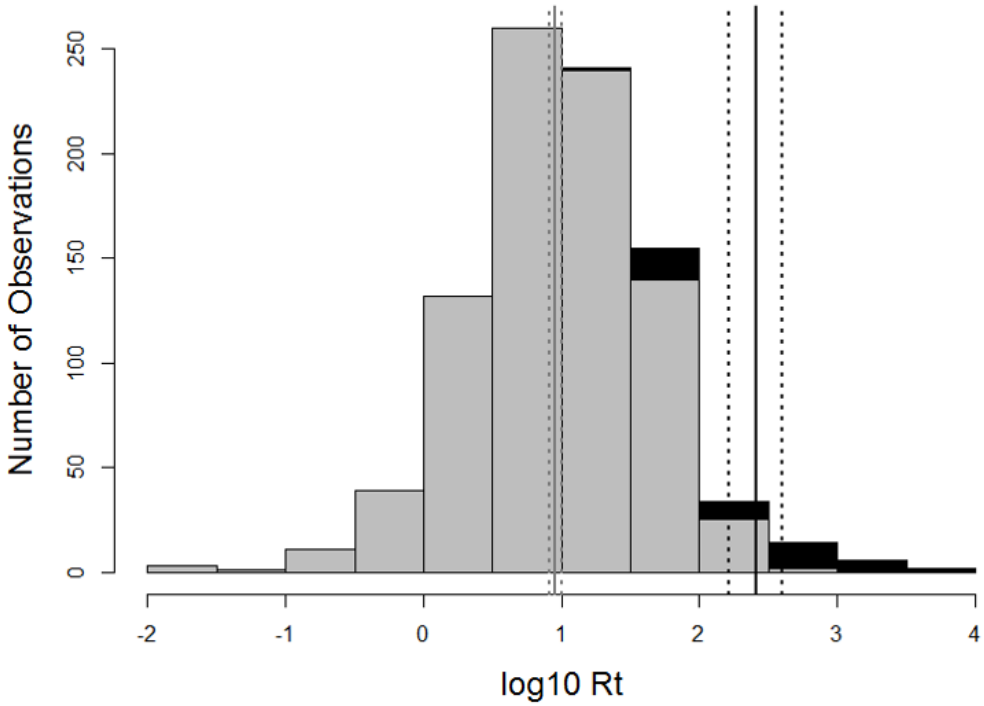

Figure 2.3. Distribution of $\log_{10}$ Rt for the two temperature classes; non-frozen (dark grey, >4°C), and frozen (black, <4°C). The mean persistence of each group is given by the solid line, 95%confidence intervals are denoted by the dotted lines ($\log_{10}$ dataset non-frozen average = 9 days; $\log_{10}$ dataset frozen average = 257 days)

The Egger's regression test for a zero intercept was marginally significant (P = 0.041), providing support for the possibility of publication bias in this effect size measure.

Meta-regression

Persistence (Rt)

The overall effect size of the persistence of LPAIV in water was 1.2 (95% CI = 0.9 - 1.5). The average effect size varied substantially across studies (Figure 2.4). The largest variance was attributable to within study heterogeneity ($I^2_{residual}$ = 43.6%; variance = 0.21) (Table 2.2). The addition of all of the moderator variables (in a full model) considerably reduced the amount of the variance attributable to within-study differences in effects, and the largest component of variance was then attributable to between study differences (I^2_{study} = 48.0%; variance = 0.110) (Table 2.2).

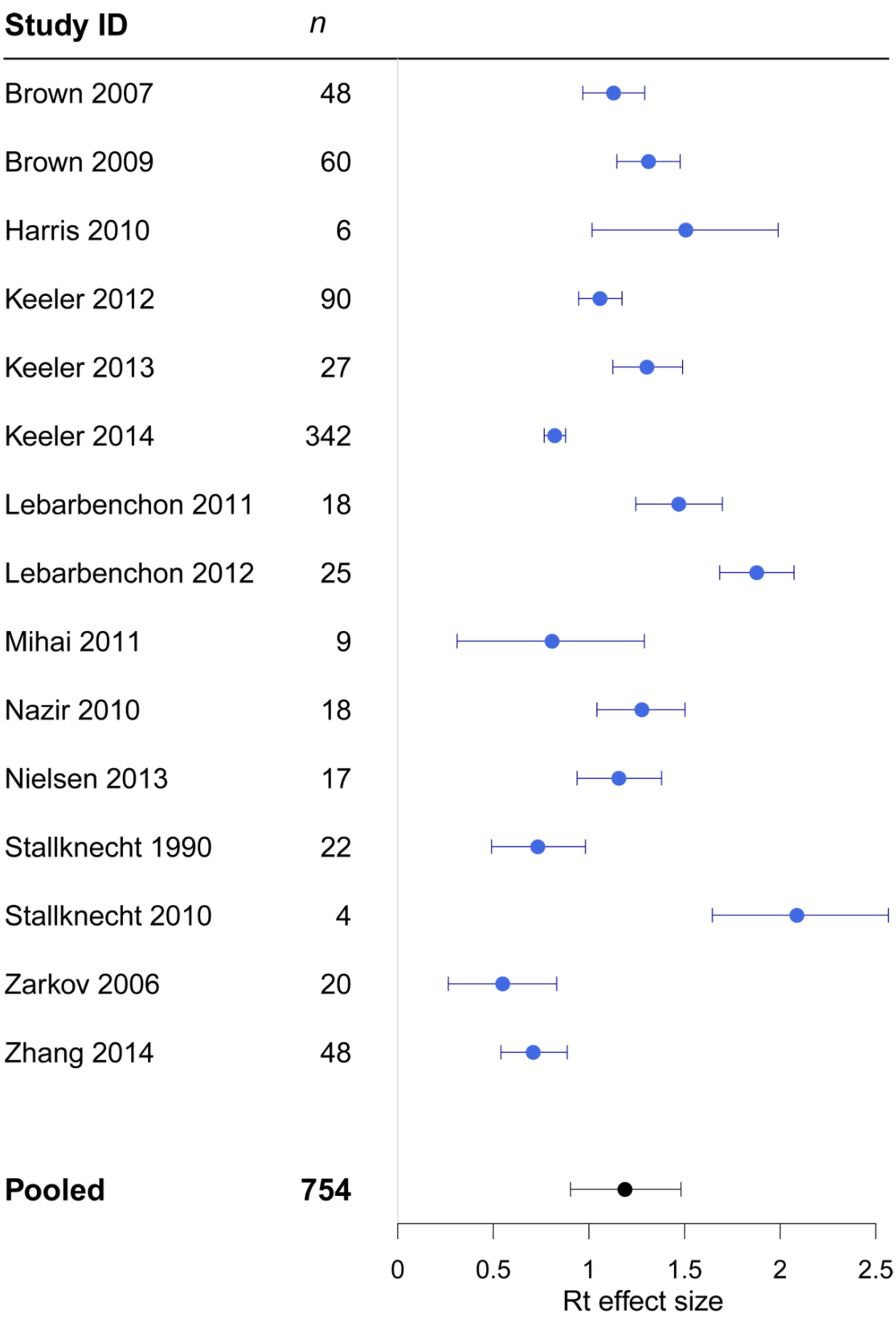

Figure 2.4. Forest plot showing heterogeneity between studies in the effect sizes for Rt; *n* = number of observations within each study; error bars show 95% credible intervals. The 'pooled' estimate provides the population-level average effect size.

Table 2.2. The heterogeneity measure I^2 (%) and variance for each model run with Rt and Zr accounted for by between study and within study variance, and measurement error. ID was included as the random effect, and the full model included moderator variables of temperature, pH, salinity and water type for the full dataset. The subset model included moderator variables of temperature, pH, salinity, water type and H-type for Rt; and temperature, pH, salinity, and H-type for Zr.

		Between study I^2 % (variance)	**Within study I^2 % (variance)**	**Measurement error I^2 % (variance)**
Rt	**Full dataset**			
	ID as random effect	41.9 (0.205)	43.6 (0.213)	14.4(0.071)
	Including moderator variables	48.0 (0.11)	21.4 (0.049)	30.6 (0.071)
	Subset			
	ID as random effect	34.3 (0.07)	33.3 (0.068)	32.4 (0.067)
	Including moderator variables	31.7 (0.061)	33.5 (0.064)	34.8 (0.067)
Zr	**Full dataset**			
	ID as random effect	27.8 (0.101)	42.8 (0.155)	29.5 (0.107)
	Including moderator variables	34.2 (0.12)	35.2 (0.123)	30.6 (0.107)
	Subset			
	ID as random effect	2.9 (0.01)	72.0 (0.247)	25.2 (0.086)
	Including moderator variables	0 (0)	70.2 (0.203)	29.8 (0.086)

Moderator variables that explained heterogeneity in the full model included a positive effect of pH on persistence of LPAIV (Figure 2.5a), a negative effect of temperature (Figure 2.5b), and lower persistence in filtered and unfiltered water (compared with sterilised and distilled water) (Figure 2.6). A continuous measure of salinity (after removing samples from the data set where salinity was equal to 0ppm) was positively related to persistence (Table 2.3). In this model, a significant effect of pH was not detected, and all other moderator variables maintained similar effects to those in the model fitted to the full data set (Table 2.3).

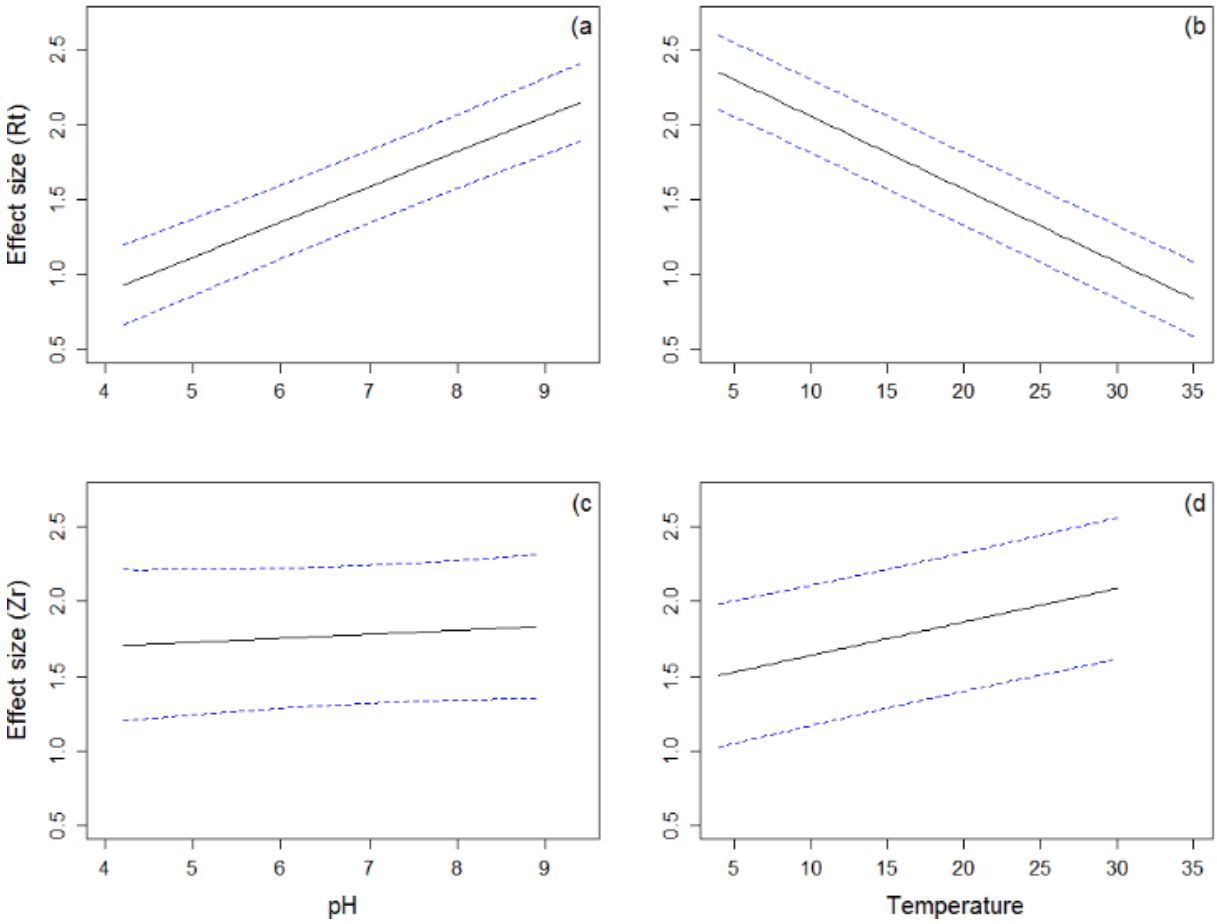

Figure 2.5. Predicted temperature and pH values from the contrasts model, with set priors of salinity and water type. Top panels represent Rt for (A) pH, and (B) temperature. Lower panels represent fisher's correlation coefficient, Zr for: (C) pH, and (D) temperature.

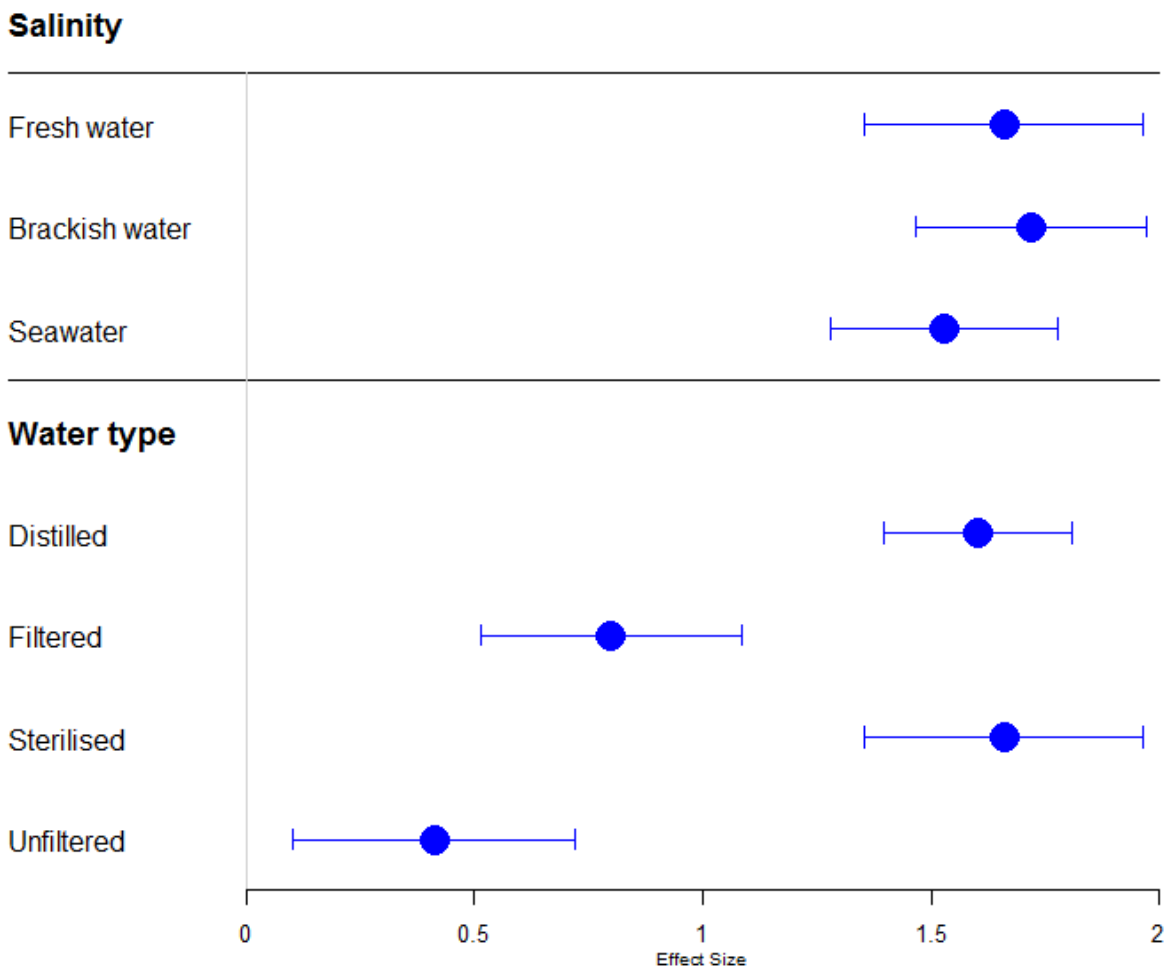

Figure 2.6. Meta-regression of the persistence of LPAIV using a mixed effects model. Average effect sizes for salinity group and water type are displayed for each individual level when continuous variables are set to temperature of 17°C, and a pH of 7.6. A smaller effect size indicates a more rapid degradation of the virus.

Table 2.3. Estimated effect size (Rt) from model predictions for the full dataset and subset dataset, using salinity as a categorical, and then continuous, variable. Influential moderator variables are highlighted in bold.

	Model variables	Effect size (Rt)	95% CI for Rt	z-value	p-value
Full dataset					
	pH	**0.235**	**0.194, 0.275**	**11.36**	**<0.0001**
	Temperature	**-0.049**	**-0.052, -0.045**	**-27.12**	**<0.0001**
	Salinity Group 1	0.0637	-0.113, 0.239	0.70	0.485
	Salinity Group 2	-0.127	-0.289, 0.036	-1.52	0.128
	Filtered water	**-0.804**	**-1.036, -0.572**	**-6.79**	**<0.0001**
	Unfiltered water	**-1.186**	**-1.446, -0.926**	**-8.93**	**<0.0001**
	Sterilised water	0.056	-0.193, 0.306	0.443	0.658
Salinity (>0 ppm) as a continuous variable					
	pH	0.046	-0.050, 0.142	0.94	0.348
	Temperature	**-0.054**	**-0.058, -0.049**	**-21.55**	**<0.0001**
	Salinity (log_{10})	**-0.113**	**-0.154, -0.073**	**-5.51**	**<0.0001**
	Filtered water	**-1.361**	**-1.787, -0.936**	**-6.27**	**<0.0001**
	Unfiltered water	**-1.358**	**-1.888, -0.828**	**-0.90**	**<0.0001**
	Sterilised water	-0.183	-0.583, 0.217	-5.02	0.369
H3,4,8 subset					
	pH	**0.261**	**0.216, 0.306**	**11.40**	**<0.0001**
	Temperature	**-0.050**	**-0.054, -0.046**	**-22.35**	**<0.0001**
	Salinity Group 1	0.284	-0.411, 0.978	0.80	0.424
	Salinity Group 2	0.122	-0.568, 0.812	0.35	0.729
	Filtered water	-0.681	-1.403, 0.043	-1.84	0.065
	Unfiltered water	**-1.618**	**-2.367, -0.869**	**-0.50**	**<0.0001**
	Sterilised water	-0.217	-1.063, 0.629	-4.23	0.615
	H4	-0.028	-0.101, 0.046	-0.74	0.458
	H8	**-0.186**	**-0.274, -0.099**	**-4.16**	**<0.0001**
Salinity (>0 ppm) as a continuous variable					
	pH	0.018	-0.052, 0.088	0.50	0.619
	Temperature	**-0.056**	**-0.063, -0.050**	**-17.19**	**<0.0001**
	Salinity (log_{10})	**-0.128**	**-0.169, -0.086**	**-6.07**	**<0.0001**
	Filtered water	**-0.750**	**-1.333, -0.168**	**-2.52**	**0.012**
	Sterilised water	-0.232	-0.878, 0.413	-0.71	0.481
	H4	-0.046	-0.162, 0.070	-0.77	0.441
	H8	**-0.123**	**-0.244, -0.002**	**-1.99**	**0.046**

The overall effect size for the subset of common H-types (H3, H4, H8) was 1.3 (95% CI 1.1 – 1.5). The variance was evenly distributed between the three components: (i) between study; (ii) within study; and (iii) measurement error (Table 2.2). Although temperature, pH, unfiltered water and H8 were associated with persistence of LPAIV (Table 2.3), they explained only small amounts of the heterogeneity observed between and within studies (Table 2.2).

Fisher's transformed correlation coefficient (Zr)

The overall estimate for the strength of the effect size was 1.77 (95% CI 1.45 - 2.14). The average effect size did not vary substantially across studies (Figure 2.7). The largest variance component was attributable to the within study variance ($I^2_{residual}$ = 42.8%). Only a small amount of the heterogeneity was accounted for by the moderator variables (Table 2.2).

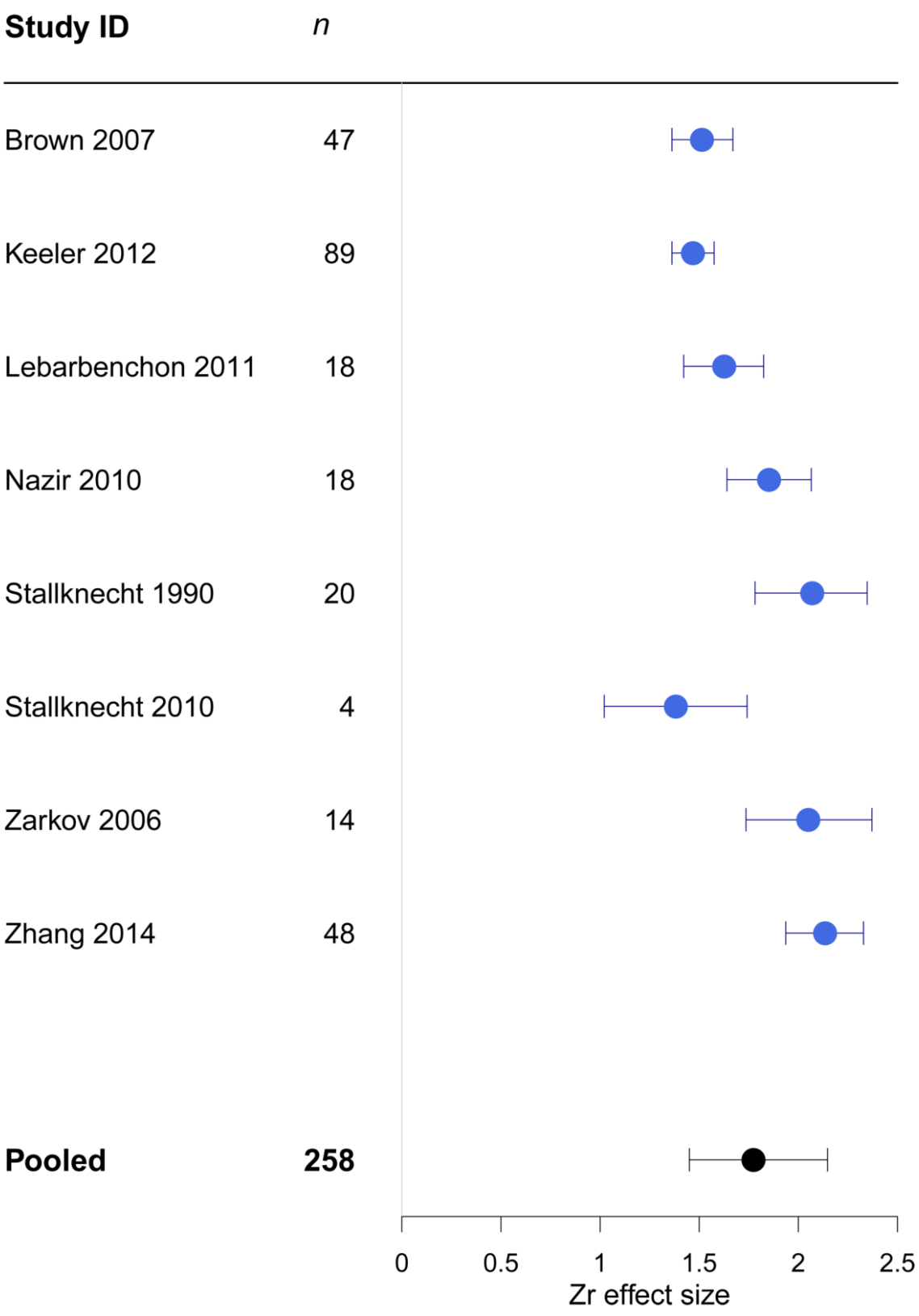

Figure 2.7. Forest plot showing heterogeneity between studies in the effect sizes for Zr. *n* = number of observations within each study; error bars show 95% credible intervals. The 'pooled' estimate shows the average population level effect size.

Moderator variables that positively influenced the strength of effect of persistence were warmer temperatures (Figure 2.5a), and higher salinities (>1000ppm) (Table 2.4). Both of these predictor variables were highly influential across all possible subsets of the full model (Supplementary material table 2). When the model was re-run with salinity as a continuous variable (i.e., without observations for 0ppm), the effect of salinity was no longer evident (Table 2.4).

Table 2.4. Estimated effect size (Zr) from model predictions for the full dataset and subset, using salinity as a categorical, and then continuous variable. Influential moderator variables are highlighted in bold.

	Model variables	Effect size (Zr)	95% CI for Zr	z-value	p-value
Full dataset					
	pH	0.027	-0.044, 0.098	0.74	0.461
	Temperature	**0.022**	**0.014, 0.031**	**5.17**	**<0.0001**
	Salinity Group 1	0.323	-0.043, 0.689	1.73	0.083
	Salinity Group 2	**0.372**	**0.085, 0.570**	**2.54**	**0.011**
	Filtered water	0.138	-0.472, 0.749	0.44	0.657
	Unfiltered water	0.069	-0.577, 0.715	-0.29	0.834
	Sterilised water	-0.060	-0.462, 0.342	0.21	0.771
Salinity (>0 ppm) as a continuous variable					
	pH	0.125	-0.032, 0.281	1.56	0.118
	Temperature	**0.026**	**0.010, 0.042**	**3.24**	**0.001**
	Salinity (log10)	0.133	-0.096, 0.362	1.14	0.256
	Filtered water	0.497	-0.103, 1.097	1.62	0.104
	Unfiltered water	0.270	-0.020, 1.055	0.75	0.452
	Sterilised water	0.517	-0.433, 0.974	1.89	0.059
H3,4,8 subset					
	pH	0.038	-0.510, 0.127	0.83	0.404
	Temperature	**0.028**	**0.015, 0.042**	**4.15**	**<0.0001**
	Salinity group 1	-0.137	-0.394, 0.120	-1.04	0.297
	Salinity group 2	0.098	-0.263, 0.458	0.53	0.596
	H4	-0.135	-0.338, 0.068	-1.30	0.193

Due to the smaller dataset for Zr (n = 302), I removed water type from the full model (see previous Results). The overall effect size for the subset of common H-types (H3, H4 and H8) was 1.5 (95% CI 1.3-1.7). The between study heterogeneity estimate

(I^2_{study}) was small (2.9%; variance = 0.010), due to the small number of studies (and observations) retained in the subset; for within study and measurement error variances see Table 2.2. Temperature was the only variable with a notable effect in the model (Table 2.4), and the explanation of heterogeneity in the model was not improved by including the moderator variables (Table 2.2).

Discussion

With any emerging disease it is fundamental that we develop a comprehensive understanding of the consequences of interplay between the host, the agent, and the environment[70]. This conceptual and quantitative understanding will help to ensure greater surveillance efficacy, as well as prevention of future outbreaks and more accurate prediction and prevention of pandemics[17, 71]. Influenza A virus is a disease of pandemic potential, with multiple host species and a rapidly mutating genome[72]. LPAIV naturally circulates in waterbird hosts and may often include an environmental component within its transmission dynamics[17, 19, 26, 28, 73]. The aquatic environment provides physical, chemical and biological challenges for LPAIV to overcome to ensure infectivity to a new recipient host[54].

Water type had a strong effect on the persistence of LPAIV, with unfiltered and filtered water significantly decreasing persistence relative to sterilised water (see also[32, 33, 36, 50, 52]). While the exact mechanism for the reduced persistence is not yet fully understood, Nazir et al (2010) suggested that virus particles may be both consumed by microbes, or adhere to particulate matter and no longer be infective, or become less infective, in more biologically active water. The biological content of water,

including filter feeders and other invertebrates, has been found to have an effect on the inactivation of echoviruses[74], polioviruses and coxsackieviruses[75].

The temperatures a virus can withstand are crucial to their persistence, whether inside a host or freely surviving in the environment[1], and a trade-off between persistence at low environmental temperatures and the ability to endure higher temperatures in avian hosts has been proposed[73]. Previous studies, as well as this one, have provided considerable insight into how LPAIV persistence and temperature are related. However, temperatures in natural environments are rarely constant. Locations may have widely varying temperatures throughout one single 24 hour period; e.g., rivers and shallow lakes that can observe a 10°C change between day and night in the surface temperature[76]. Although I found temperature to have a strong consistent negative influence on persistence of the virus, the majority of available data is centred on just three temperatures, which do not adequately represent conditions in large areas of the world. Researchers need to examine the local habitat differences that can affect the variability in water temperature, and subsequently persistence of the virus, as well as continue to expand the range of temperatures studied to allow full characterisation of the response.

I found salinity to be an influential continuous variable, but not when it was grouped as a categorical variable (i.e., including laboratory grade fresh water 0ppm). The inconsistent response to salinity, even between viruses of the same H and N types, has been observed previously[24]. The more rapid degradation of virus in salt water, relative to 'fresh' water, is most likely due to structural changes within the virus in the presence of higher salt concentrations that affect the conformation of the nucleocapsid segments[77].

Persistence of LPAIV was negatively associated with the acidity of the water sample and it has been suggested that LPAIV remains infective for the longest time between pH of 7.2 and 8.4[24, 56]. Viral fusion activity relies on pH to allow infection of a cell, with the haemagglutinin protein of the influenza virion experiencing a conformational change at low pH values that allows entry into the host cell[78]. Thus, the changes in the surface protein may go some way to explaining the more rapid loss of infectivity, and hence reduced persistence of LPAIV in low pH water.

Phenotypic diversity in response to temperature and pH have been suggested between individual viruses[24], and differences between strains at low temperatures have been proposed[17, 47, 56], but such differences have not yet been fully explored. Some differences between H-types have been noted under experimental conditions with different water types at low temperatures, however the difference is reduced when using unfiltered water [33]. I found strain-related differences for H8 compared to H3 when using a smaller dataset, however, I do not propose a mechanism for this difference as yet. Studies have suggested that viral genome composition has limited effects on virus persistence[48], and possibly no fitness cost to the wild bird populations[79]. Whilst there may be no fitness cost to the host, there may still be an evolutionary advantage to the virus if different strains are able to persist in different environments (e.g. temperatures and pH). This is a relationship that warrants further exploration.

Naming convention for LPAIV includes the species that the strain was first isolated from, but there is no known association with specific host species for individual strains. Alternatively, if there are differences between H-types with respect to their persistence under environmental conditions, they may be more likely to infect some hosts than

others due to the individual host ecology. The investigation of inter-strain differences across a wider range of H-types, under different naturalistic conditions, would be beneficial before we can rule out any differences between strains that may affect persistence in the aquatic environment.

Quantitative meta-analysis, using multiple studies, can provide important synthesis and agreement across replicate experiments, and provides the best evidence for cause-effect relationships[80]. By employing meta-analysis methods we can make predictions across a wide range of environmental conditions. These approaches also ensure the conclusions drawn are robust and markedly reducing Type II errors[81]. Meta-regression analysis has allowed me to investigate the effect of the environmental variables on infectivity of LPAIV in water, which may be accounting for the substantial heterogeneity in the dataset[82]. Unfortunately, despite reporting 1824 experimental outcomes, I was only able to estimate an effect size for half of these experiments (50.4%; n = 919 data points); because of the very poor reporting of individual results, and test statistics, across these studies.

Future reporting of studies should include the following minimum information: (i) Rt, and the method by which it was calculated (38.0% did not report Rt or an equivalent); (ii) the sample size (i.e., the number of time points used, and the number of replicate experiments performed), given explicitly in only two studies; (iii) reproducible descriptions, and definitions of all of the variables including water type, (provided by 50.0% studies) ; and (iv), where linear models are fitted to the data, the R^2 (given in all but one study where fitted) and standard errors of the slope estimate (reporting of the standard error of the slope was very limited). A comprehensive description of methods, including calculations, and transparency of results will allow comparison of

studies and assimilation of results to provide a wider basis for further analysis and translation of effects.

Environmental variables have been previously sampled across a very limited (or unrepresentative) range of values. Temperatures included in the studies have mostly reflected the average summer and winter temperatures of the North American breeding grounds of the natural waterbird hosts (28 and 17°C)[24, 42, 56, 57]. Other areas of the world are subject to very different temperature ranges, with some areas of the globe regularly reaching high 30-degrees for more than a week on end (e.g., my own part of South Australia, Adelaide), or having large variations between overnight and daytime temperatures.

A very small number of possible H-types (H3, H4, H8) accounted for more than five-hundred data points (59.6%) in our meta-analysis, and some strains were included in multiple studies (see Appendix 1 S1). It is unclear why these H-types have been the most utilised, but most likely it is representative of a geographic and taxonomic bias in field sampling, and by a relatively small number of researchers. It may also reflect a bias in the availability of stock virus for experiments, as the same stock virus was used for all four studies that examined the viral persistence of the H8 virus, and a similar situation is true of the H3 and H4 types. In any case, the same degree of coverage needs to be achieved for all H-types to ensure we can convincingly conclude whether (or not) there are any H-type related differences in persistence.

The majority of studies were conducted at neutral or near-neutral pH levels, 7.2 and 7.4, providing a good baseline, but providing little (or no) information on more acidic, (e.g. coastal lakes, pH ~5) or more alkaline waters (e.g. sea water, pH ~8.2)[83]. As

climates change around the world, and hosts alter their migration patterns, we are likely to detect hosts in new areas shedding virus into a variety of novel aquatic environments, as well as experiencing more acidic and higher salinity water in the traditional breeding locations[39]. I propose that studying a wider range of temperatures, pH and salinity, as well as H-types, would be informative.

While I found some support for publication bias in our measure of the strength of the effect size of the persistence of LPAIV (Zr), I interpret this with caution. My analysis included a large number of individual estimates across a reasonably small number of studies, with a high level of between- and within-study heterogeneity. However, I was not able to include half of these published results in this study, because of poor reporting statistics and effect size estimates. While it is clear that more studies need to be conducted to address the poor coverage of environmental variables, and resulting knowledge gaps, I am not convinced that this means there is a substantial publication bias (or 'file-drawer' problem).

All the experimental studies, included in my quantitative meta-analysis, were conducted under laboratory conditions. Without environmental realism, there are limitations to the applicability of the information gained from these experiments [17, 19]. This is particularly true for the large number of baseline studies of distilled water (0ppm, 17.7 %), conducted at a static pH and temperature. Such conditions are rarely, if ever, found in ex-situ systems, and as yet there have been very few published reports of experiments that have explicitly accounted for daily environmental variations, or fluctuations. Studies included here used static states for pH, temperature and salinity, with no inclusion of varying temperatures or salinities. There is one author[47, 48] who has begun the process of examining diurnal temperature variations; though the

findings were not consistent, demonstrating that diurnally varying conditions need much more investigation. The exception to this are those examining freeze-thaw degradation of the virus, though they were looking at large changes in temperature and a change in water state rather than diurnal maximums and minimums.

Diurnal variations in temperature, water flow rate and depth, ultraviolet light (UV) exposure, turbidity, and biological diversity are just some of the environmental variables that I suggest need to be considered in future work. Water flow rates, through areas where waterbirds are shedding virus via their faeces, are likely to have a dilution effect, reducing the number of infective particles available for ingestion by the next host, in a given area of water. In water treatment plants, and numerous other applications, UV light is used to disinfect physical surfaces and water. Viruses can be particularly resistant to UV[84], but the amount of exposure required to affect the persistence of LPAIV in water to date has not been investigated; although there has been some work on the human H7N9 where more than 30 minutes exposure to UV within 75cm of the light source caused the death of the virus[85]. Recently, researchers have included 'natural' water in infectivity experiments, i.e. water samples taken from natural water bodies[32, 33, 47]. However, these experiments were all maintained at single (static) temperatures, and whilst the physicochemical properties of the water are reported from *in situ* measurements, there was minimal reporting of the final 'laboratory' values (2 of 10 studies provided final values).

Whilst abiotic factors such as temperature and salinity have a role to play in the persistence of virus, they are only a fraction of the whole story. Biotic factors including filter feeders and invertebrates need to be considered when attempting to understand the role of the natural environment[86]. Investigation into the bioaccumulation and/or

inactivation of AIV by filter feeders and invertebrates has garnered interest in the last few years, with experiments using zebra mussels (*Dreissena polymorpha*)[87], freshwater Asiatic clams (*Corbicula fluminea*)[88] and water fleas (*Daphnia magna*)[89]. The results of the studies have been varied, with some providing evidence for bioaccumulation in the tissues of species which are a possible food source for waterbirds[87, 88], and others showing removal and inactivation of AIV by invertebrate communities[89].

Throughout this chapter I have highlighted the need for environmental realism, and in part this can be achieved through the use of meta-analysis to assimilate all available information allowing the extrapolation of expected results for a given set of circumstances. A further step forward would be the construction of mesocosms, with water quality parameters in line with the conditions found in the wild. Although mesocosms can only mimic the natural environment, and will have constraints which limit the overall realism that can be achieved, the ability to allow for biological content and broader (fluctuating) physicochemical conditions will be an advancement in the field.

The role that invertebrates, which share waterbodies with waterbirds, play in the maintenance and transmission of AIV in the environment needs greater investigation, and could be a substantial step towards understanding the interactions that occur between biotic and abiotic variables [86]. Subsequently, combining mesocosm studies with those involving invertebrates will take us much closer to an overall understanding of the persistence and transmission of AIV in the aquatic environment.

There are multiple factors influencing the persistence of viruses in the environment. Though I have focused on LPAIV, I have also described a methodology for health researchers and practitioners to apply meta-analytic techniques to wildlife diseases. I believe that these methods will continue to be particularly important when considering emerging diseases moving into new environments, or under anthropogenic environmental changes. Meta-analysis allows the consideration of the relationships among multiple variables, as well as determining the limitations of the sampling coverage to date. Some notable outbreaks and emergences in new areas, which may be ripe for meta-analysis include white nose syndrome in chiroptera [90, 91], Zika virus [92] and Ebola virus [93].

Conclusions

Environmental variables clearly impact the persistence of LPAIV in water, and although the current range of moderator variables studied is limited, some important conclusions can be drawn. Water type and temperature have significant effects on the persistence of the virus, with colder temperatures allowing for greater persistence in the environment and unfiltered water reducing infectivity. Salinity was shown to have a significant effect on the persistence of the virus. In addition, pH has an effect on infectivity, although the relationship is less clear when investigated in association with salinity. My study has highlighted that a small measured range, in a limited number of variables, accounts for the majority of research effort to date. I greatly hope that future experimental studies will continue to investigate outside these ranges. This is of particular importance for studies conducted outside the geographical range of past research (i.e., North America and Europe) where the range of conditions may be wider, the environment more variable, and the hosts following different life-histories from that

of the Northern hemisphere. I also hope that there will be a further shift towards environmental realism through the use of mesocosms and the integration of invertebrate accumulation and inactivation studies, and that eventually *in situ* experiments may be possible.

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Chapter 3.
Spatial LPAIV risk and surveillance implications for continental Australia

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Abstract

Surveillance for emerging and exotic diseases is an essential component of early detection and subsequent disease prevention. Influenza viruses are highly variable, and new strains occur readily, with low pathogenic avian influenza viruses (LPAIV) regularly isolated from wild bird species, particularly waterbirds and shorebirds. Australian waterbirds are largely nomadic, following resources and sharing habitat sites with migratory shorebirds. Ongoing surveillance for LPAIV in these taxa has been undertaken across Australia since 2005, with the selection of sites driven by a number of risk-based factors; however, environmental niche modelling has not been utilised. By combining environmental niche models for waterbirds and shorebirds with a model of LPAIV persistence, I evaluate how seasonal differences in host distribution and climate contribute to LPAIV risk across continental Australia. Austral summer has the lowest LPAIV risk compared to the other seasons, whilst it is greatest in the austral winter. I also show that current surveillance locations capture a cross-section of sites across the risk landscape. Based on the data available for analysis, the results suggest that a more targeted and cost-effective surveillance program could be developed, incorporating information on host distributions and LPAIV persistence and detectability. Surveillance targeting the southern Australian states during the austral autumn and winter could optimise the potential for better prediction of persistence and potential emergence of the virus.

Introduction

Emerging and re-emerging zoonotic diseases pose economic and public health risks globally[1]. Disease surveillance is often the responsibility of government agencies, with surveillance quality and capabilities being influenced by many factors[1]; including resourcing, accessibility and the availability of experienced personnel, all of which vary across different jurisdictions. Infrequently, surveillance includes wildlife and is largely triggered by the requirement to safeguard domestic livestock[2]. However, the monitoring of wildlife, and the investigation of disease outbreaks in non-domestic animals, is crucial to the early detection of emerging, newly introduced, or adapting pathogens[3]. Surveillance programs are costly and pose logistical challenges in large, climatically diverse countries such as Australia. Within Australia there have been active surveillance programs for diseases of economic importance including tuberculosis and brucellosis, and the wildlife surveillance undertaken for avian influenza across Australia, which commenced in 2005[4, 5].

Avian influenza viruses (AIVs) are found on every continent, in multiple wild bird species, and can cause significant morbidity and mortality in poultry[5, 6]. AIVs can also infect a multitude of non-avian species, including humans. Both low pathogenic (LPAIV) and high pathogenic avian influenza (HPAIV) forms have serious implications for public human health[5]. As pleomorphic, enveloped viruses, influenza viruses are prone to frequent genetic reassortment[7]. The transmission of LPAIV in birds involves a faecal-oral cycle, with an indirect environmental transmission component[8, 9]. The persistence of LPAI in water is dependent on multiple factors including temperature, water quality (including biological content), and the quantity of virus shed from the host

into the environment (see Chapter 2). The temperature of the water into which virus is shed has a significant impact on the rate of degradation of the virus, with higher temperatures leading to shorter persistence times (see Chapter 2) [6, 8]. The type or quality of the water also have an effect. In laboratory experiments, viral persistence is prolonged in distilled water, and shortened in water that has not been filtered or sterilised and water that more closely resembles the physicochemical qualities of natural sources (see Chapter 2).

Australian birdlife is both diverse and highly endemic, with considerable differences in species conservation status and distribution[10]. Waterfowl are members of the Anseriformes, including the ducks (Family: Anatidae) and geese (Family: Anseranatidae). Shorebirds, otherwise known as Charadriiformes, include, but are not limited to, gulls (Suborder: Lari), terns (Family: Sternidae) and waders (Suborder: Charadrii)[11]. Large numbers of migratory shorebirds visit Australia on an annual basis, travelling along the East Asian Australasian (EAA) flyway[12], passing through countries such as China and Hong Kong that have regular outbreaks of HPAI in poultry[13]. The ecology of many Australian waterbirds (waterfowl and shorebird) is a reflection of the country's climate and its seasonal unpredictability, which drives their breeding[14], feeding, moult and habitat use. Many Australian waterbirds exhibit considerable environmental plasticity, which is driven by the extreme and unpredictable climate patterns[15]. In contrast, northern hemisphere dynamics of AIV are largely driven by the regular movements and breeding patterns of waterfowl and shorebirds at known hotspots of infection[16]. Thus, the major differences between Northern and Southern Hemisphere circulation include the nomadic nature of the primary Anseriforme hosts such as the Pacific Black Duck (*Anas superciliosa*) and

Grey Teal (*Anas gracilis*), and the over-wintering, non-breeding nature of the migratory Charadriiformes, including the red-necked stint (*Calidris ruficollis*) and sharp-tailed sandpipers(*Calidris acuminata*), whilst in Australia[17]. In addition, the lack of regular site-specific annual breeding (providing an influx of naïve young to perpetuate infection dynamics) is a significant difference between the northern hemisphere and Australia[16].

Predictive environmental niche modelling (ENM) has been used to explore the distribution of animals[18-20], plants[21] and diseases[19, 22-25]. ENM using empirical reconstructions is based on associations of known occurrences and environmental characteristics of the environment at those locations[20]. When employed for disease modelling, ENM must also account for the interaction between disease agents and hosts. The environmental covariates considered for inclusion in such models need to be relevant biologically and hypothesised to affect either pathogen persistence or host distribution.

Waterbird distributions and movements across Australia are determined by multiple factors, many of which remain unexplained[15, 26]. The sites currently used for wild bird AIV surveillance in Australia are chosen based on a number of risk-based factors (e.g. host species, proximity to poultry farms and/or human populations) via a convenience sampling approach (e.g. for accessibility, and the known presence of bird populations)[5]. Our study aims to inform the current surveillance programs by providing information on spatial risk, and highlighting the areas of longest predicted persistence and highest predicted bird species richness. The principle objectives for this study are:

1. To generate models and mapped predictions of the species richness of LPAIV's natural reservoir hosts (waterfowl and shorebirds) across Australia.
2. To provide a visual, updatable resource for surveillance planning and targeted LPAIV monitoring in wild birds across Australia, based on the likelihood of virus survival in the aquatic environment, and the presence of wild birds.
3. To combine the output of aims 1 and 2 to produce quantitative maps of predicted LPAIV risk in water across the Australian landmass, by season.

Methods

Host species richness

All available bird survey data from BirdLife Australia (www.birdlife.org.au; Australian partner of BirdLife International) was downloaded from the Atlas of Living Australia (ALA, e-infrastructure funded by the Australian government via the National Collaborative Research Infrastructure Strategy). Bird distribution data provided by BirdLife Australia (www.birdlife.org.au/projects/atlas-and-birdata) are collected using standardised and validated bird-survey methods. The assumption was made that if a sighting of any bird species was recorded, then all sighted bird species would be recorded. Therefore when Anseriformes or Charadriiformes were not recorded they were presumed to be absent, for an estimation of species richness. The full data set was subsetted to include only validated surveys and sightings and where all relevant data was supplied. Surveys were considered to be unique if they had a unique combination of latitude, longitude and date.

Waterfowl and shorebird raster layers were generated from the BirdLife data by sub-setting based on order: 'Anseriformes' for water birds and 'Charadriiformes' for

shorebirds. Species richness data for all bird species from BirdLife surveys was collected, and rasterised to a 0.125° x 0.125° resolution and multiple surveys per cell were summed to give a single value per cell.

Covariates

Geographic covariates

Hydrogeological shape files were downloaded from *Geosciences Australia (www.ga.gov.au)* as geodatabases in point, polygon and line formats. They were transformed into raster layers using the R-software package *rgdal* [27] to create a layer of water bodies throughout Australia, which was subsequently used to calculate a distance from water layer using the R-software package '*raster*' [28]. To account for the elevation, or altitude, across the country, an Australia digital elevation model (DEM) was also used. I also constructed a layer for distance from the coast using the *raster* [28] and *oz* packages [29].

Climatic covariates

Gridded climatic data for Australia were obtained from the Bureau of Meteorology (BoM). Specifically, I obtained gridded monthly averages (spanning January 2008 to August 2016) at a resolution of 0.05° x 0.05°, and calculated austral seasonal (Table 3.1) and/or annual averages for:

i. *Maximum and minimum temperature* (°C); the average daily maximum and minimum air temperature, calculated on a seasonal basis. Air temperature was taken as a proxy for water temperature as the two are closely correlated

[30] and I confirmed this initially through comparison with water monitoring data from Queensland (see Appendix Figure 3.1);

ii. *Total precipitation* (mm); daily rainfall is nominally recorded at 9am local time and records the total precipitation for the preceding 24 hours. Seasonal rainfall was calculated by summing seasonal rainfall totals and dividing by the number of years in the period used[31];

iii. *Normalised difference vegetation index* (NDVI); an index of the "greenness" of vegetation derived by measuring the difference between the amount of visible light absorbed and solar energy reflected by living vegetation. It is calculated from red and near-infrared reflectance and always has a value between -1 and +1. NDVI decreased as leaves are stressed, diseased or die. Bare soil has values close to zero, and waterbodies have negative values[32]. Seasonal NDVI was calculated by summing the monthly datasets, then averaging into austral seasons;

iv. *Humidity (vapour pressure)*; the partial pressure of water vapour in the atmosphere, available for 9am and 3pm in monthly datasets. Seasonal humidity was calculated by summing the monthly datasets then averaging into austral seasons.

Data formatting

All data and layer formatting was conducted in the R-software (Version 3.3.0 R foundation 64-bit)[33] computing environment. Raster layers were created for each covariate listed above and disaggregated to a 0.0125 x 0.0125 resolution.

Table 3.1. Calendar months making up Austral seasons (Southern hemisphere).

Austral season	Months included
Spring	September, October, November
Summer	December, January, February
Autumn	March, April, May
Winter	June, July, August

Environmental Niche Modelling

Generalised additive models (GAMs) are an extension of generalised linear models with linear predictors relying on smooth functions of covariates [34, 35]. I used the *mgcv*[36] package in R to fit GAMs to predict waterbird and shorebird species richness across Australia. I considered a number of potential covariates; including: temperature, precipitation, normalised vegetation difference index (NDVI), austral season, distance from coast, distance from water, altitude, and humidity. Spatial dependence in the data was modelled using a 2D spatial spline, and we assumed a Poisson error distribution for these count data. A negative binomial error distribution was also tested, but made no difference to the model outcomes (Results not shown).

I evaluated a candidate set of models (Table 3.2) for the species richness of shorebirds and waterfowl, using corrected Akaike's Information Criterion (AICc) (lower numbers indicate better model performance). As a direct measure of the predictive capacity of different models, for each candidate model, I also conducted out-of-sample (OOS) validation, using 5 repeated 70:30 splits of the full dataset into training and hold-out data, and calculated the mean predictive deviance across the hold-out datasets (lower predictive deviance indicates better predictions). I then used a model-averaging

approach to predict the species richness of waterfowl and shorebirds across Australia, by weighting predictions from the candidate models by their AIC weights (wAIC) [37].

LPAIV persistence

Viral persistence is the time taken for there to be a 90% (1 log-unit) reduction in virus and is usually expressed as Rt in days. Persistence time of LPAIV was calculated based on the findings of a meta-analysis of viral persistence in water (see Chapter 2), and the seasonal maximum temperature. I used maximum temperature because higher temperatures reduce the persistence of LPAIV in water (see Chapter 2).

Predicted persistence of LPAIV in water across Australia was calculated from temperature across the country and the number of days required for a 1-log reduction (Rt) in viral load from Chapter 2. Predicted persistence time was then mapped across Australia, by season. Predicted persistence time and predicted bird species richness layers were multiplied to produce LPAIV risk layers by season, standardised to values between 0 and 1.

National Avian Influenza Wild Bird (NAIWB) surveillance program

The National Avian Influenza Wild Bird (NAIWB) surveillance program is coordinated by Wildlife Health Australia on behalf of the Commonwealth Department of Agriculture and Water Resources. The aim is to provide regular, Australia-wide, repeated sampling of birds directly or indirectly for LPAIV, providing information on the circulating strains, and prevalence through time (NAIWB Program Objectives: https://www.wildlifehealthaustralia.com.au/ProgramsProjects/WildBirdSurveillance.aspx). The program has been running since 2005, with 34 individual sampling sites used

during the subsequent years. Latitude and longitude for sampling sites were provided by NAIWB as a centroid within 50km of sampling sites encompassing multiple closely related sites within a state. The longitude and latitude coordinates for a subset of testing sites were used to extract the species richness and persistence risk information for each site.

I fitted a GAM to the complete NAIWB dataset as well as for State A and State B separately to model seasonal variation in the proportion of positive samples that could be expected at NAIWB sampling sites.

Results

Host species richness

The species richness of LPAIV's primary hosts, waterfowl and shorebirds, is greatest on the eastern and western coasts, and along the central south coast of Australia during summer. Species richness then increases to a more north eastern distribution in autumn. In spring there are up to 35 species predicted to be sighted in northern Western Australia and the Kimberley region (Figure 3.1A). There are also high numbers of species around Coongie Lakes, the Diamantina River and Cooper Creek in central Australia (Figure 3.1A). The seasonal changes in bird species will be enhanced by the arrival of migratory shorebirds from the EAA flyway during early spring and their departure for northern breeding grounds in late summer (Figure 3.1A and D).

Based on AICc rankings, the two top-performing models included the effects of temperature, precipitation, elevation, distance from the coast, NDVI and humidity (see

Table 3.2). Surprisingly, additionally including the presence or absence of water in the predictor set made little difference to model performance.

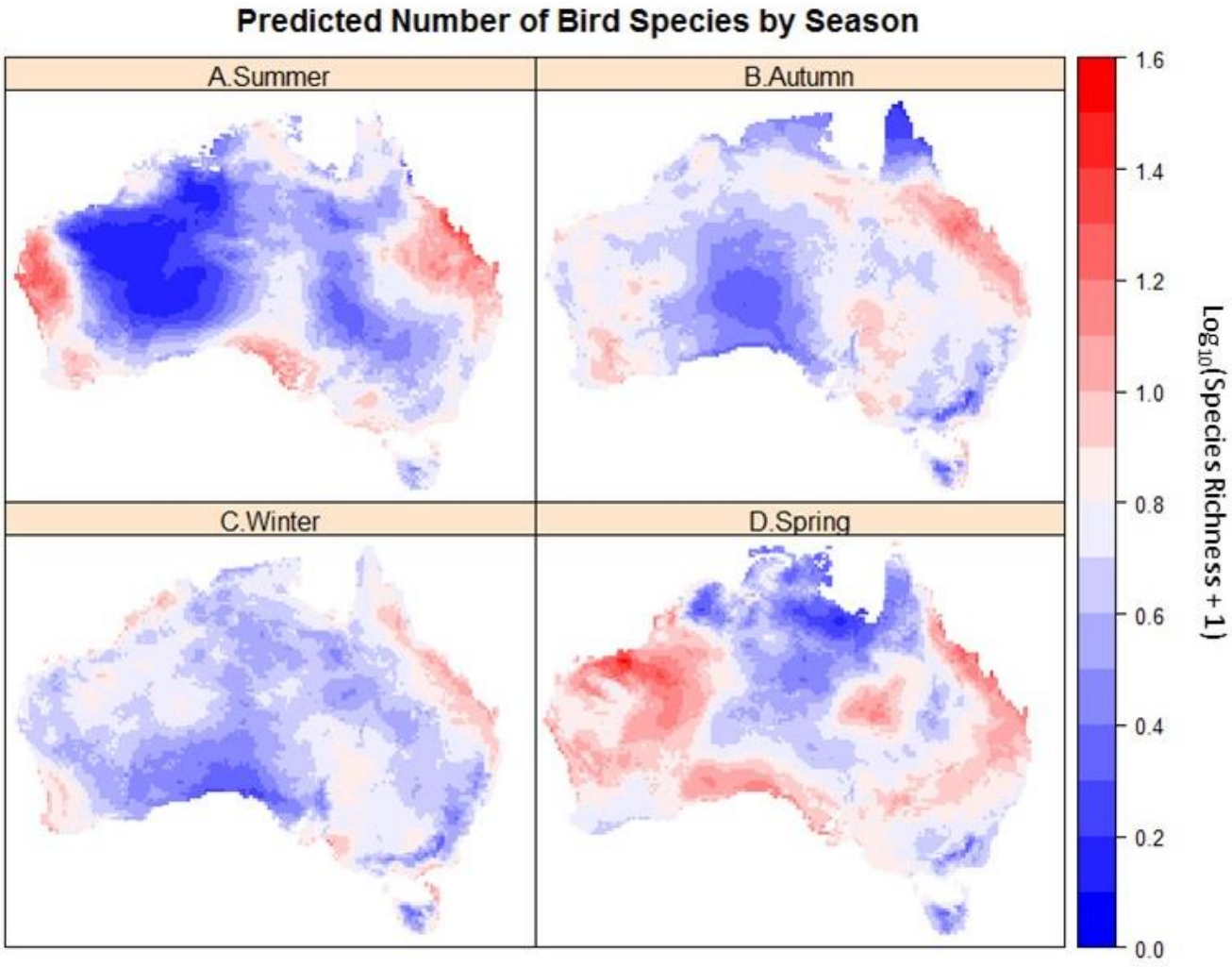

Figure 3.1. The predicted number of bird species, combined waterfowl and shorebirds, by season. These predictions were derived by model-averaging the top two models of seasonal species richness (see Table 3.2). Predictions are displayed at 0.25° x 0.25° resolution.

Table 3.2. Model selection table for generalised additive models for the combined richness of shorebirds and waterfowl across Australia. The following is shown for each candidate model: the model formula (x = longitude, y = latitude), the deviance explained; the mean predictive deviance from out-of-sample (OOS) validation (%); the corrected Akaike Information Criterion (AICc); the difference in AIC relative to the top-ranked model (ΔAIC); the AIC weight (wAIC). The s() terms in the models indicate the use of one- and two-dimensional spatial splines;

Model	Deviance Explained (%)	Mean (SE) OOS deviance explained	AICc	ΔAIC	wAIC
s(x,y, by = season) + s(Precipitation) + s(Temperature) + season + s(NDVI) + Coast_distance + s(Humidity9am) + Altitude	13.217	7.182	119685.6	0	0.726
s(x,y, by = season) + s(Precipitation) + s(Temperature) + season + s(NDVI) + water + Coast_distance + s(Humidity9am) + Altitude	13.217	7.129	119687.6	2.0	0.274
s(x,y, by = season) + s(Precipitation) + s(Temperature) + season + s(NDVI) + Altitude	12.728	7.179	120059.2	374	5.44E-82
s(x,y, by = season) + s(Precipitation) + s(Temperature) + season + s(NDVI) + water + Coast_distance	12.707	7.141	120078.5	393	3.49E-86
s(x,y, by = season) + s(Precipitation) + s(Temperature) + season	11.316	7.275	121177.6	1492	0
s(x,y, by = season) + s(Temperature) + season	10.800	7.298	121575	1889	0
s(x,y, by = season) + s(Precipitation) + season	10.787	7.252	121585.7	1900	0
s(x,y,by = season) + season	10.310	7.299	121951.7	2266	0

Spatial LPAIV risk

There is a clear pattern of lower LPAIV risk in summer (Figure 3.2A), with large areas of Australia having high temperatures during this period. Northern Australia has a lower LPAIV risk in the environment throughout the year, although in temperate regions the risk is consistently higher in spring, autumn and winter (Figure 3.2B, C, D).

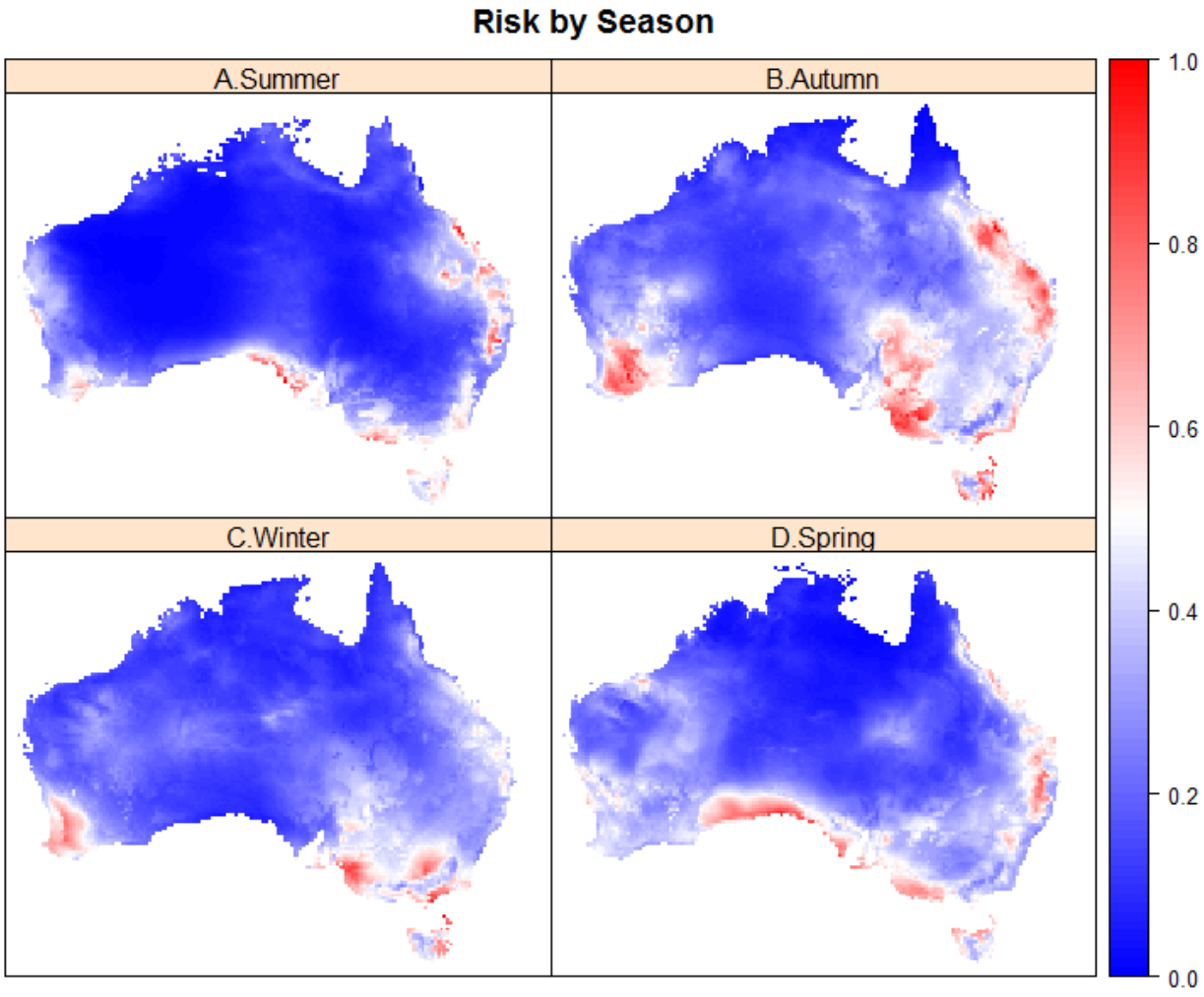

Figure 3.2. Risk profile across Australia calculated by multiplying predicted persistence time by combined bird species richness predictions. These predicted risk maps have been scaled from 0 to1 with 0 being the lowest likelihood of both bird population and environmental conditions being suitable for the shedding from host and maintenance of virus in the environment, and 1 being the highest likelihood of conditions and hosts being suitable.

NAIWB monitoring sites

The relationship between the NAIWB sampling locations provided and the number of species predicted, or the risk predicted in any season, was inconsistent, with some locations being low predicted risk areas, whilst others were sparsely sampled and in higher risk areas. Visualising the risk distribution across Australia showed that the sampling locations, based on those provided from the NAIWB program, were distributed across the risk spectrum, throughout the four seasons (see Figure 3.3).

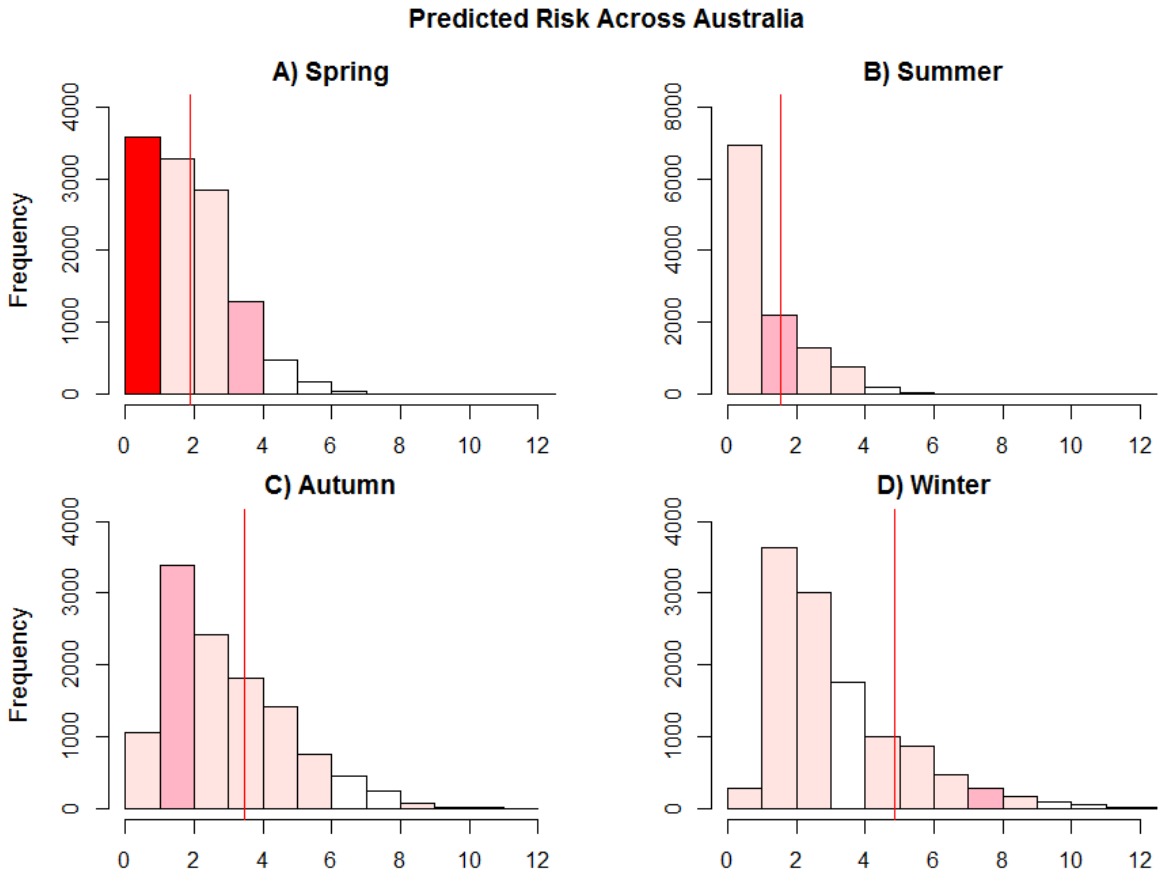

Figure 3.3. Histograms of LPAIV risk across Australia. The colour shading of each bar indicates the number of NAIWB monitoring sites that fall within the risk level bin, white indicates no NAIWB monitoring sites, light pink is 1-3 sites, dark pink is 4-5 sites, red is 6+ sites. The red line indicates the median predicted risk at the sites of NAIWB monitoring activities for the season.

Only one sampling site (within State A) was sampled with enough consistency (Figure 3.4), across the available timeframe, to allow a robust exploration of seasonality in the proportion of faecal samples that tested positive for LPAIV. The next most sampled site was in State B, although no sampling was performed in July or August at this site and the resulting prediction curve has large confidence intervals (Results not shown). The Australian dataset has 43933 samples, of which over 30% were from State A, meaning the resulting prediction curve is heavily influenced by the data from this state (see Figure 3.5).

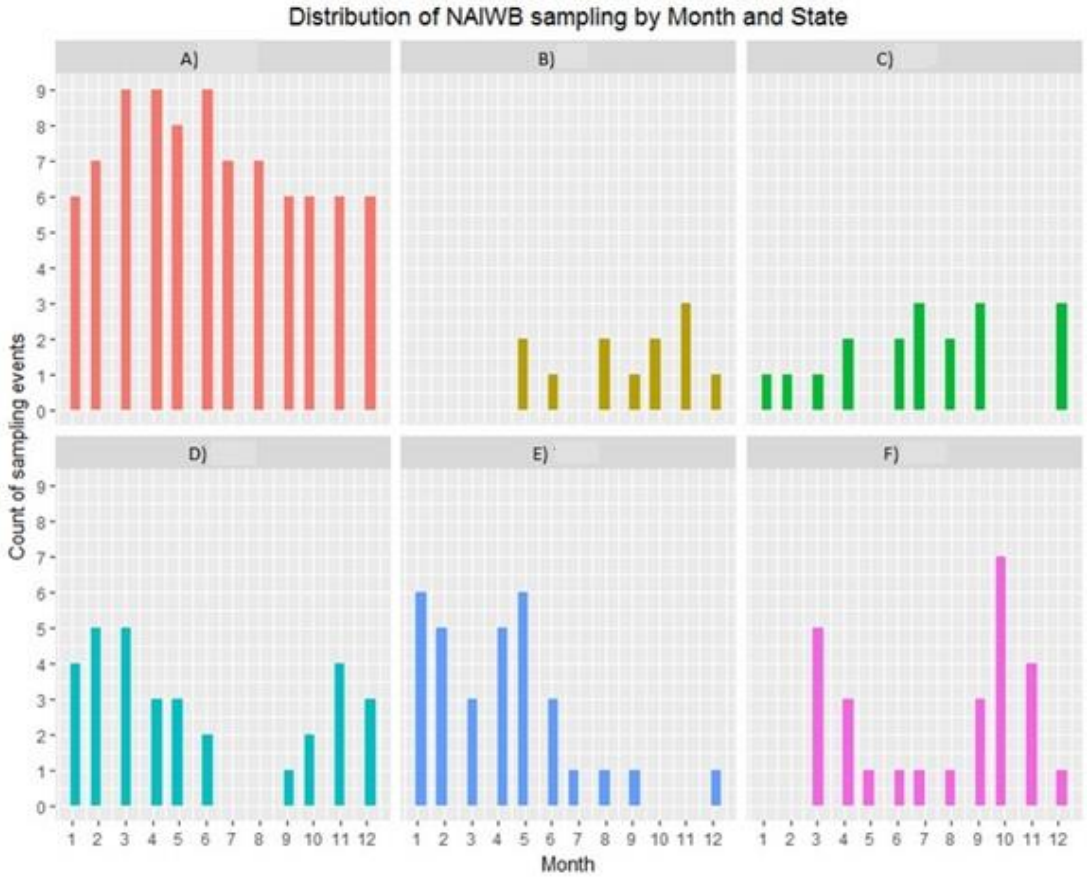

Figure 3.4. Sampling effort across all NAIWB sites by month showing the number of years each month has been sampled. States A-F: A) sampling events = 86, total samples = 15285; B) sampling events = 12, total samples = 3516; C) sampling events = 18, total samples = 4574; D) sampling events = 32, total samples = 9167; E) sampling events = 32, total samples = 2162; F) sampling events = 9199.

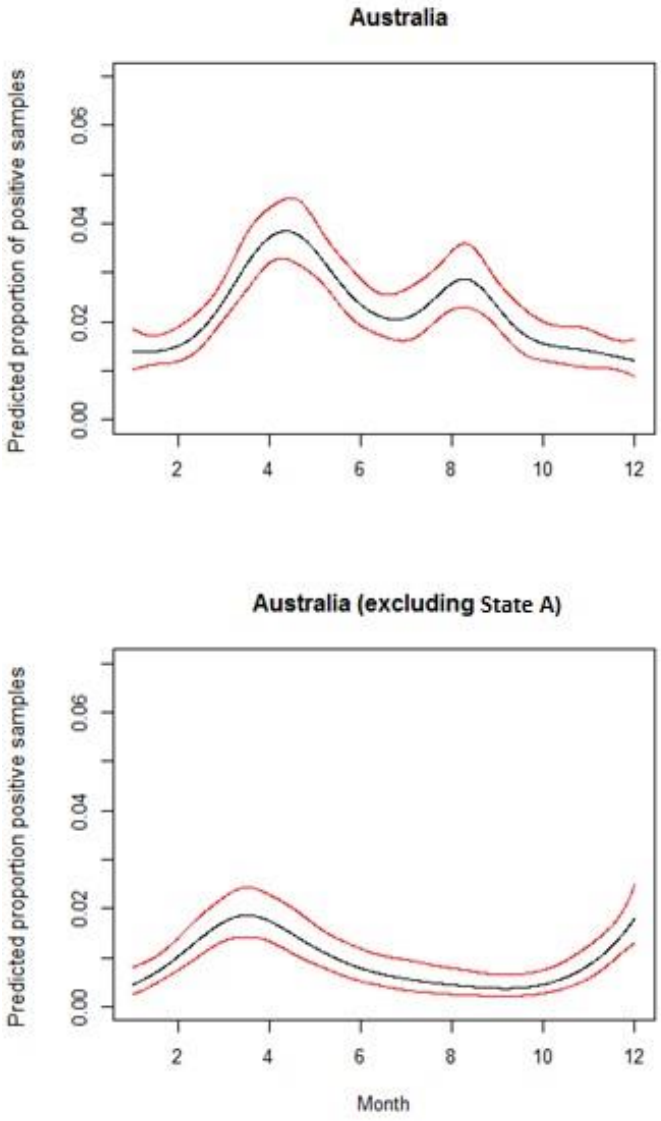

Figure 3.5 Prediction of positive proportion of samples by season for the Australian dataset, and Australia excluding State A. The red lines denote the 95% confidence intervals for the predictions.

Discussion

Surveillance for LPAIV in wild birds occurs across Australia, although not necessarily in the areas of highest risk, based on my analysis using ENM, and inconsistently in many states, based on the subset of available data that was provided. Here, I have considered, for the first time, both host distribution and persistence of virus in the environment, and quantitatively shown how the LPAIV risk varies in space and by season.

Seasonality has been recognised as a key driver for many infectious diseases [38, 39]. I have demonstrated seasonal differences in the predicted species richness of water and shorebirds, the natural hosts of LPAIV, around Australia. When coupled with the abiotic factors that play a role in the environmental persistence of LPAIV, I have predicted the areas that are most likely to have conditions conducive to the persistence and transmission of virus, through abundance of bird species present and most appropriate seasonal conditions. I found that combined waterfowl and shorebird species richness and distribution is related to; (i) seasonal movements of populations around the country; (ii) temperature; (iii) precipitation; (iv) vegetation (NDVI) and; (v) humidity (see appendix for effects plots). This is clearly seen with the high numbers of species seen on the west coast of Australia in summer and spring, and the high species richness along the south coast of Australia in spring, an area that has the lowest richness through autumn and winter.

I found that LPAIV persistence in the environment is influenced by season, with clear differences across Australia in the four seasons. Summer had the lowest persistence in the environment, with southern areas of Australia in winter likely to have the

conditions conducive to the highest persistence of LPAIV. The combined risk prediction layer (Figure 3.2) was constructed using both host species richness distributions and predictions of persistence of LPAIV in water. This produced a measure of the seasonal occurrence of potentially infected host species, and environmental conditions that would allow shed virus to persist for the longest time period. If the most likely hosts are not present, or the environmental conditions are going to lead to rapid degradation of the virus, the opportunity to pass virus from one host to another would be markedly reduced.

Precipitation was an important predictor of waterfowl and shorebird species richness across the country, with more species being expected where there has been greater precipitation (result not shown), which makes sense given the life history traits of water and shorebirds. Ferenczi *et al* [17] found that there was a delayed relationship between rainfall and bird abundance, and previous studies of Australian waterbirds noted different species responding to rainfall as a breeding cue at different rates[15, 17, 40-42]. We do not yet have information on how higher levels of precipitation, and subsequent run-off of water, affects the persistence of LPAIV in the environment. I suggest that it would reduce the concentration of virus present at any one location through simple dilution effects, which may help to explain why there is such a seasonal pattern for positive virus sample identification in the more temperate areas of Australia, with winter typically having a lower prevalence in NAIWB testing, though the virus would persist for longer in colder waters.

Surveillance sampling is not solely based upon the areas most conducive to persistence of a virus, but may also be affected by the accessibility of the location,

personnel expertise, available facilities, and environmental conditions, in addition to the number of birds available for sampling. The logistics of sampling are also contingent on the type of samples required. Sampling fresh faecal matter from the environment, which is the primary method used by the NAIWB surveillance program, is far easier than direct cloacal swabbing of birds and allows a larger number of samples to be collected on a more regular basis. The surveillance sampling in Australia for LPAIV is not conducted evenly across the country[5, 43], for multiple reasons, and the inferences that can be made are limited as a result. Surveillance data revealed a wide distribution across the risk profile of Australia, varying through seasons, and no direct relationship between sampling sites and predicted highest LPAIV risk as shown by the distribution of sampling sites (used in this analysis) across the predicted LPAIV risk based on the ENM (Figure 3.3). However, through modelling of the available surveillance data I have identified a seasonal pattern in LPAIV prevalence at surveillance sites with the most intensive sampling. These results suggest that future sampling to identify virus may benefit from a more seasonal approach, with autumn and spring yielding the most positive samples at the temperate sites in the dataset. I would call for more sampling in the highest risk seasons, but I would also call for more sampling across the board which would then allow further model refinement and the identification of the locations and seasons most likely to provide information.

I have presented my study in a seasonal format as the available data lends itself to an aggregated analysis. A variety of climatic variables and temporal scales were tested in preliminary modelling, however the data available was insufficient for robust models. A drawback of using a seasonal basis is the loss of finer detail in the movement pattern

of birds around Australia. Waterfowl and some shorebirds within Australia are known to be nomadic[26, 42, 44], and as such do not follow set routes. Some information is also lost by seasonally averaging precipitation and temperature across the years. Future work in this area would benefit from exploring the possibility of linking bird survey data, climate data and other factors, to more effectively describe and predict patterns of bird movement across Australia.

Spatial predictions of LPAIV persistence time were based on water temperature only, the most influential of abiotic variables invested (see Chapter 2) [45-47]. The degradation rate of LPAIV in water is also affected by pH and salinity, as well as the quality of the water. Currently the available water quality data across Australia is patchy at best, with individual states and territories being responsible for the maintenance of sites and collection of information. These data are held on individual state websites, and within states not all parameters are recorded for all sites. The overall time taken for the reduction or removal of infectious virus is also dependent on the number of birds present and shedding, as well as the volume of virus shed by each bird. Whilst I have been unable to fully elucidate the rate of degradation of the virus for all waterbodies and water types, my models and subsequent predictions can be updated in the future as further information is obtained for different virus types, and different abiotic conditions. An additional level of complexity could be achieved in future by modelling the differences across water profiles as conditions will likely vary dramatically depending on the depth of water, turbidity and size of waterbody.

Given the Birdlife bird survey data were available on a presence-absence basis, I predicted the species richness of hosts across the country. The inclusion of data on

absolute bird numbers, which is available for only small areas of the country and on an intermittent basis, would greatly refine my models and permit improved predictions of bird movements around the country. The number of birds, and the average amount of virus shed by different species[48], are important factors which improve the evaluation of spatial LPAIV risk.

Environmental persistence of LPAIV and the factors that affect it around the world continues to be investigated, but in Australia very little research about persistence in our climate is conducted. To that end, when bird samples are collected environmental variables should also be recorded, such as air and water temperature, number and species of birds, humidity, and the collection and testing of water samples, for both physicochemical parameters, and virus. Location-based information will allow finer scaled predictions to be made, and further refinement of surveillance programs.

The ability to predict the areas with environmental conditions appropriate for viral persistence, and with resources available required by the hosts gives an opportunity to focus surveillance resources on areas most likely to yield useful data. In a scientific world with limited financial resources available, and increasing demands on the time of appropriately skilled professionals, utilising risk-based ENM techniques and modelling of disease dynamics will become increasingly important [22, 49]. For emerging diseases, where the predictions need to be refined as more information becomes available, this is a particularly important application.

Conclusions

LPAIV circulates through the wild bird population of Australia, with waterbirds and shorebirds the most likely to carry the virus. Surveillance of these birds can be logistically difficult, time-consuming and expensive. Based on the locations provided, surveillance is patchily distributed across the country, and some areas are only sporadically tested. I have used the distribution of the host species, and the environmental conditions suitable for viral persistence, to identify areas of highest risk, and recommend that they may be used to inform future surveillance. There needs to be more sampling across all states, particularly in the austral autumn and winter, from which information can be fed back to improve this spatial risk framework.

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Overall percentage (%)	90
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Chapter 4.
A national survey of feral pigs (*Sus scrofa*) for influenza A virus exposure in Australia.

A subset of the data presented here has been previously published as:

Dalziel, Antonia E., Heidi A. Peck, Aeron C. Hurt, Julie Cooke and Phillip Cassey. "Proposed Surveillance for Influenza a in Feral Pigs." *EcoHealth* 13, no. 2 (2016): 410-4.

Abstract

Surveillance for exotic, emerging and re-emerging diseases in free-ranging wild and feral animals is an important component of disease biosecurity and for maintaining the credibility of disease-free status. Many species of mammals are susceptible to influenza A, and pigs in particular have the potential to be a mixing vessel for new viruses to emerge, i.e., if they are infected with more than one virus type and re-assortment occurs. We do not know how many different parasites and pathogens feral pigs come into contact with, and subsequently transport and transmit. Nasal swabs and blood samples were scavenged from feral pigs culled as part of private or government control programs between September 2014 and June 2015 across Australia. All swabs, tested negative for influenza virus using PCR assays. Blood samples were tested using serological assays for antibodies to influenza viruses. Positive serological samples were isolated in three consecutive years from one area of Australia (Innamincka, South Australia). I demonstrate the importance of considering feral pigs, as agents of transmission for influenza viruses, for inclusion in future disease surveillance programs.

Introduction

With the emerging threat of human pandemic influenza, and the potential costs that would be incurred, both in treatment and loss of earnings[1-3], it is becoming increasingly important to monitor broadly for reservoirs and transmission of virus. Whilst it remains critical to monitor the natural primary hosts of influenza A, i.e., waterbirds and shorebirds, biosecurity efforts must also consider potential novel reservoir species, which may facilitate the production of the perfect storm, a readily transmissible, pathogenic strain adapted to humans on a global scale[4, 5].

All influenza A strains originate from avian lineages, and largely occur in their primary reservoirs, wild birds; with Anseriformes (ducks and geese) and Charidriiformes (waders and gulls) the most commonly identified hosts[6-9]. Though all influenza A strains have an avian origin, there are multiple viral types that are transmitted between, and maintained within, mammalian species, such as equine, canine and swine influenza. In gaining specificity for mammalian species, these strains do not readily re-infect avian species[10-12]. To date, the more research undertaken in wild bird sympatric species, the more frequently reports of infection are obtained; including transmission of avian influenza viruses (AIV) in wild mammals; for example, Chinese pika[13-15], seals[16], ferrets[17], pine martens[18], skunks[19], and donkeys[20].

Throughout recent human history there have been numerous large scale-pandemics, with the loss of human life reaching millions, as in the 1918 ‘Spanish influenza’ H1N1 outbreak[21-23], and it has been suggested that intermediate mammalian hosts were responsible in this outbreak[24, 25].The recent outbreaks of low pathogenic avian influenza (LPAIV) H7N2 in a cattery in New York, USA[26], and the large-scale outbreak

of highly pathogenic avian influenza (HPAIV) H5N8 across continental Europe and the UK[27], highlights the considerable and urgent need for understanding the transmission and replication of LPAIV between hosts from different taxonomic groups. This includes humans, with one putative diagnosis as a result of transmission from feline to human[26].

Current surveillance for influenza A in Australia is predominantly focused on at wild bird monitoring, centred around areas known to include a dense poultry industry; thus safeguarding the poultry industry. To date, Australia has experienced just seven outbreaks of highly pathogenic influenza in poultry, all of the H7 type [28, 29]. There have been no reported outbreaks of HPAIV in wild birds; though some spillback to European starlings from an early outbreak might have occurred[30]. The small number of Australian outbreaks is most likely due to the strict biosecurity employed by commercial farmers[31], the quarantine requirements imposed by the government on the importation of poultry products, and the relative isolation of the Australian continent from other large landmasses with significant endemic and pandemic viral strains[32]. Although Australia and Asia are connected by shorebirds migrating along the East Asian Australasian Flyway, there is little evidence of the introduction of new strains by these birds, and they appear to be most likely infected once in Australia, rather than prior to arrival[32]. Influenza A is regularly isolated from wild water birds in Australia as part of the National Avian Influenza Wild Bird Surveillance program (see Chapter 3)[33, 34].

Surveillance for exotic, emerging and re-emerging diseases in free-ranging wild and feral animals is an important component of biosecurity for parasites and pathogens,

and in the maintenance of disease-free status. In Australia, there are many diseases that are of significance to the agricultural industry, and which can be contracted and transmitted by feral pigs. Feral pigs *(Sus scrofa)* are found across much of mainland Australia, with the population estimated up to 20 million individuals[35, 36]. They range from the northern tropical regions of Queensland and the Northern Territory, to the cooler temperate states of New South Wales and South Australia[37]. Currently, there are no known feral pig populations in Tasmania, although there is a population on Flinders Island in Bass Strait[37]. Feral pigs occupy many different habitats, and have a close association with water, sharing the environment with many other species including waterfowl and shorebirds.

Pigs are susceptible to influenza A, including species-specific influenza A[38]. They have the potential to be a mixing vessel[39], within which new viruses could emerge following infection by more than one virus type. This provides the conditions for reassortment, and the exchange of gene segments giving rise to new strains of IAV. What is not currently known about the millions of feral pigs in Australia is how much contact they have with domesticated animals, and how many different parasites and pathogens they are in contact with. Nor do we know how often these are subsequently transported and transmitted to livestock and humans.

Australia has 65 wetlands of international importance under the Ramsar convention (1971)[40]. The absolute requirement pigs have for fresh water means that wetlands provide vital areas for pigs, ensuring that pigs and waterbirds are often in close proximity. Feral pigs are a shy species and so have limited interaction with humans. I predict we are most likely to observe environmental interactions leading to exposure

to influenza viruses at wetlands where avian hosts and feral pigs are able to interact frequently; both directly and indirectly.

In 2014 a pilot program was undertaken to determine evidence of influenza A exposure in feral pigs in wetland areas of South Australia. The study did not yield virus, however, three serological positive samples were identified at two locations [41]. The number of feral pigs in South Australia is a small percentage of the national population, and very little of South Australia is known to provide habitat for feral pigs[37], though the state-level population is subject to fluctuations due to highly seasonal water and food resource availability.

The objectives of this study were to: i) expand upon previous work and assess the national seroprevalence of influenza A in pigs,using a network of registered sports shooters and government control agents to obtain samples from as wide a range of habitats as possible; ii) identify any demographic differences in seropositive feral pigs; and iii) identify and sequence shed virus.

Methods

Samples were scavenged from feral pigs culled as part of private or government control programs between September 2014 and February 2017.

Sampling network - collectors

To enable as much coverage of Australia as possible, samples were collected by three groups: i) the principal researcher (AE Dalziel) on shoots in South Australia (Figure 4.1); ii) local and federal government employees in New South Wales, Queensland

and the Northern Territory, whose participation was achieved by direct approach; and iii) recreational hunters with locations in New South Wales, Queensland and the Northern Territory. The recruitment of hunters was achieved through the development of a working relationship with the Sporting Shooters Association of Australia, and advertisement to their members via their regular web publication and hard-copy print in their quarterly magazine.

Inclusion as a sample collector required hunters to satisfy the following requirements: i) to have regular hunting areas through established relationships with landholders; ii) to be able to obtain, appropriately store and transport samples during the planned project; and iii) to be able to provide samples from areas not covered by other departments or hunters. Sample kits, comprising personal protective equipment, sample collection consumables, and collection and return instructions were dispatched to all parties. Each kit also contained record forms for the collection of data on the sex (male/female), approximate age (piglet/juvenile/adult) and location (GPS waypoint coordinates) of each sample.

Over 40 individual private shooters contacted us about the project; 25 were identified as likely to be able to collect samples from desired areas. Sampling kits, 10 to 20 packs per kit, were dispatched to the collectors (n = 200 kits) across Australia over the course of the study. Some collectors were unable to obtain samples from their regular hunting grounds due to altered environmental conditions, and many did not return any kits (68% of collectors did not return kits).

Sampling locations - Australia

Designation of planned locations for sample collection were based on the availability of control shoots and regular hunting grounds of private hunters, as well as the known general distribution of feral pigs from the Invasive Animals Cooperative Research Centre website FeralScan[37]. All sample locations were recorded with decimal degree latitude and longitude coordinates, as provided by the sample collectors. In total 231 feral pigs were sampled, with 217 serum samples and 107 nasal swabs collected from across the sampling network.

Sampling – South Australia

Annual control program shoots in and around the Innamincka region of South Australia conducted by the NRM Arid Lands pest management team were utilised as a source of feral pigs. Three control shoots were completed during the study period allowing for three sampling rounds in the same location. Each program of pest control was conducted at a different time of year due to climatic factors and the dispersal of animals after flooding of the region. Innamincka is in the remote north-east of South Australia (Figure 4.1), approximately 1000km from the capital city, Adelaide. The large distances involved, along with difficult terrain and inaccessibility, necessitated the use of helicopters for the cull and collection of samples (Figure 4.1). Through the cooperation of all marksmen and pilots it was possible to obtain samples from across the control area. All samples obtained in the three Innamincka sampling sessions were processed at base camp and chilled to 4°C post collection and post initial processing.

Sample collection

Pigs were sampled within thirty minutes of being culled. Whole blood samples were collected from the jugular or directly from the heart using a 10ml syringe (Terumo®) and 19 gauge 1.5” needle (Terumo®), and transferred to a serum clot activator tube (Vacuette® Z Serum Separator Clot Activator) before being chilled for storage and transport.

A sterile plastic-shaft cotton-tip non-media viral swab (COPAN Transystem™) was inserted as far as possible into the nostril of the pig, rotated 3-4 times, ensuring contact with the tissues within the nasal cavity, and then removed and replaced into the protective sheath. RNAlater® was added to the swab sheath to preserve any viral RNA present on the swab. Samples taken in the second year from Innamincka (South Australia) were preserved with viral transport media (VTM) rather than RNAlater® to allow for viral culture. Samples taken in the third year from Innamincka (South Australia) were duplicated, with one swab preserved with VTM and one swab preserved with RNALater® for each pig.

Figure 4.1: The principal researcher (A. Dalziel) collecting samples from feral pigs culled as part of pest management programs operated by the Natural Resource Management Arid Lands Board of South Australia. The central map shows the location of Innamincka in relation to the capital city (Adelaide) of South Australia.


Australia

Sample processing and analysis

Samples were maintained at a minimum of 4°C post-collection and they were subsequently sent to a central collection point at the University of Adelaide for storage at -80°C. Samples were transported to the CSIRO Australian Animal Health Laboratories (AAHL) in Geelong, Australia, for further analysis. The second set of samples collected by the Northern Australian Quarantine Strategy (NAQS) were sent directly to AAHL.

Blood

Blood samples in clot activator tubes were centrifuged to separate the serum and cellular component. The serum was then decanted into Eppendorf tubes and stored in a -80°C freezer prior to sample transport to AAHL. Samples from the NAQS surveillance program were returned as serum samples already centrifuged and decanted.

Serum samples were tested with blocking ELISA to identify samples with antibodies to influenza A. Positive samples were then tested with haemagglutination inhibition tests. Haemagglutination inhibition tests use a separate antigen to test for antibodies to each virus subtype. The virus subtypes (Table 4.1) were chosen based on likelihood of presence, expert opinion and availability of the virus for testing in Australia.

Table 4.1. The 16 virus types included in the panel for HI testing, their individual designation and species of origin for each strain.

Virus	Designation	Origin
H1N1	A/California/7/2009	Human (pandemic)
H1N1	A/New Jersey/8/76	Human
H1N2	A/Swine/WA2577896X/2012	Swine
H3N2	A/Swine/WA/2577766G/2012	Swine
H3N8	A/Avian/WA/699/78	Avian
H4N4	A/Grey Teal/1840/WA/79	Avian
H4N6	A/Duck/Victoria/1/2010	Avian
H5N1	A/Chicken/Konawe Selatain/BBVM204/2005	Avian
H5N3	A/Duck/Victoria/1462/2008	Avian
H6N1	A/Whistling Duck/WA/2009	Avian
H6N5	A/Shearwater/Australia/1/1972	Avian
H7N4	A/Emu/NSW/75/1997	Avian
H8N4	A/Mallard/South Korea/2A/2006	Avian
H9N2	A/Turkey/NSW/10/2012	Avian
H9N2	A/Turkey/Wisconsin/66	Avian
H10N7	A/Chicken/NSW/Australia/CV10-1004-12/2010	Avian

Swabs

Nasal swab samples in RNALater® or VTM were stored in a -80°C freezer prior to transport to AAHL. Swabs in RNALater® were processed for RNA extraction and the extracted RNA was analysed using a TaqMan™ PCR assay; a one-step reverse transcriptase PCR assay that incorporates primers and probes specific for influenza type A and subtypes H5 and H7. The PCR assay was run with at least one positive control on all occasions. Swabs in VTM were collected for processing in egg culture if the PCR on the RNALater® sample indicated the presence of influenza virus, no samples were processed this way.

Results

Coverage of Australia

Samples were returned by 8 collectors (for distribution of samples see Figure 4.2). Samples were also collected as part of routine culling and sampling operations by the local land services (LLS) officers in central NSW, and as part of the biosecurity monitoring operations conducted by the Northern Australia Quarantine Service, a federal government initiative. A total of 238 blood samples were collected across four states (Figure 4.3) and 130 nasal swab samples were collected (Figure 4.4)

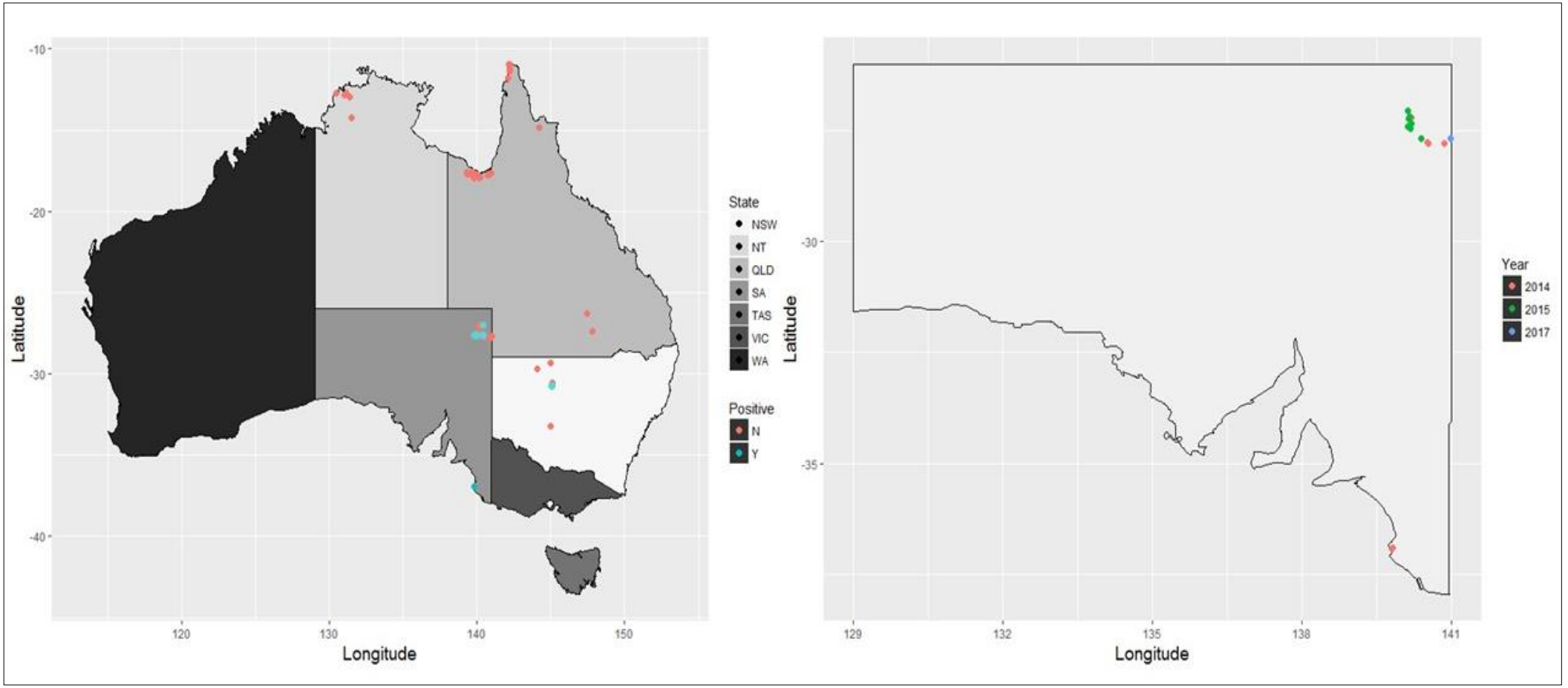

Figure 4.2. Distribution of sampling across Australia (panel A). The orange dots represent the location of negative samples, and the blue dots represent the positive samples collected across the four states Panel B shows the samples taken in South Australia only over the three fieldwork sessions, the orange dots represent samples from 2014, green samples from 2015 and blue samples from 2017.

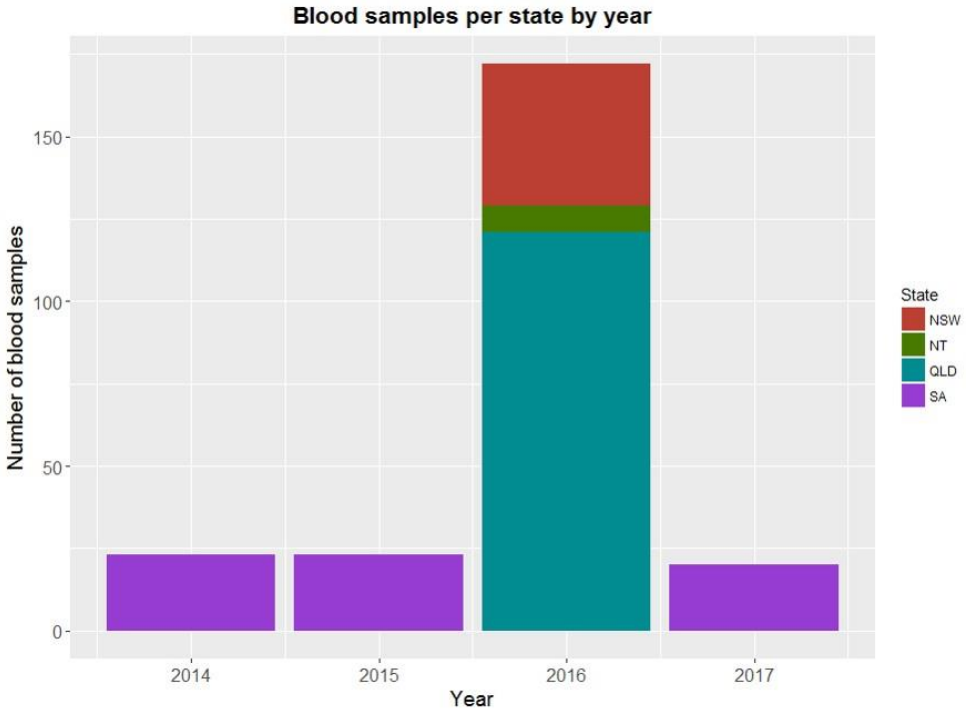

Figure 4.3 The total number of blood samples collected per year by state between 2014 and 2017, (n = 238)

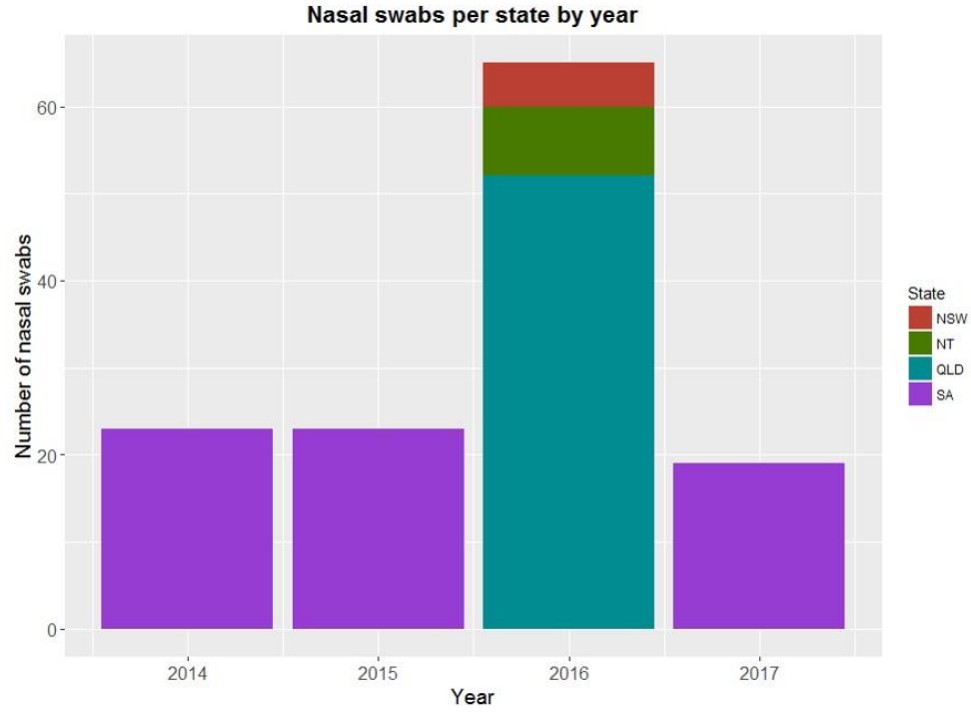

Figure 4.4 Nasal swab samples collected by year per state between 2014 and 2017, (n = 130)

Serology

Of the 233 samples tested, 7 were positive on bELISA for antibodies to influenza A. All but one sample was collected in South Australia, with the six positives collected across three years of sampling (2014 = 3 across the two sites (Innamincka and Kingston SE), 2015 = 2, 2017 = 1). The seventh positive sample was collected in New South Wales. Haemagglutination inhibition tests were inconclusive for the identification of an H-type. No swabs were positive for viral RNA on PCR, therefore no further testing was conducted on the swabs.

Discussion

Identification of seropositive animals at Innamincka (north-eastern South Australia), in close proximity over three years of sampling, is of considerable interest. The landscape is largely arid land, though there are some areas with permanent water, such as Coongie Lakes (a Ramsar wetland[42]) fed by the Cooper Creek. The lake and river system supports a large diversity and abundance of water and shorebirds, and a variable population of feral pigs (*Sus scrofa*), camels (*Camelus* spp.), donkeys (*Equus asinus*) and horses (*Equus caballus*), as well as cats (*Felis catus*), rabbits (*Oryctolagus cuniculus*) and dogs (*Canis familiaris*). There is limited opportunity for contact with humans as it is a remote location, and though the lakes are a popular tourist destination, the permanent population of humans is very small (approximately 20 people), and all agriculture is on an extensive grazing basis[43]. The seropositive detection from New South Wales was in a broadly similar habitat type, with limited human population, and expansive arid or semi-arid habitat.

Identification of H-type would greatly aid in understanding the influenza strains the culled pigs had been exposed to in life. Currently I cannot be specific about the origin of the virus that has caused the serological signal, however, it is important to remember the potential for pigs to be mixing vessels for new strains, and the consequences this may have[44]. H-type identification in a feral species, from serological samples, is a difficult prospect. Without the correct virus and anti-sera, a closely associated, but antigenically different virus will not be identified and the available stock viruses in Australia are relatively limited in both number of strains of virus and amount of anti-sera available (D. Eagles, AAHL, private communication). The panel of 16 viruses used for haemagglutination inhibition testing of the seropositive samples was derived through expert consultation, and in light of the available stocks of virus and anti-sera, with reference to the strains that were deemed most likely to be present at the sampling locations.

There are many possible explanations for the results obtained in this study, including an unidentified influenza A strain circulating in eastern SA and western NSW, and for the differences between arid, semi-arid areas and tropical environments. As previously shown, the quality of the water influenza virus is shed into is key to the persistence of the virus for long enough to find a new host (see Chapter 2). With changing seasons water bodies expand and contract changing the physicochemical qualities of the water will including salinity, temperature and pH, potentially affecting the duration of viability of the virus. Water availability, and related resources in a relatively inhospitable environment for an animal with an absolute requirement for fresh water must play a role.

The seropositive pigs were in areas of limited water flow. In times of low flow, standing waterholes will contract in size, in turn concentrating all animals with water requirements into smaller areas. Both feral pig and waterbird population dynamics are driven by resource availability including fresh water, with birds often displaying nomadic tendencies and following resources (see chapter 3). By pushing pigs and waterbirds closer together, water scarcity could increase the probability of pigs encountering infective shed virus. When paired with a likely increase in physiological stress due to resource limitation, the potential for infection would be greater.

As an omnivorous species, pigs will make use of diverse food resources, preferentially grazing green vegetation, but also consuming fruits, nuts and other animals[45]. In arid areas the availability of green vegetation can be limited, and pigs will dig in the land-water interface for tubers and, at Innamincka, for bivalves (AE Dalziel personal observation). The possibility for infection via ingestion or inhalation of virus by disturbing the ground at the land-water interface is viable and should be considered. In more tropical areas, the stressors at play may differ as water and resources are more abundant, so the concentration around water for food resources will not be as great.

Logistical difficulties of coordinating disparate collectors, in non-ideal conditions, with limited cooling capabilities and large distances between both the sampling and the dispatch points, create serious constraints in a pilot study of this kind. The size of Australia, the distribution of feral pigs, the relationship required with land holders to shoot on private and public properties, and the resources required to conduct a study on a national scale would have made this project impossible without the cooperation

of multiple agencies and individuals. The difficulties of using hunter networks have previously been discussed [46], but with this study it was the most efficient way to collect numerous samples from a wide-ranging landscape. Whilst samples were collected and stored as efficiently and appropriately as possible, there were periods of up to seven days between collection and centrifugation, and decanting of the serum. Therefore, the quality of the samples could have degraded over time.

The economic costs and environmental damage that feral pigs can cause has been a subject of discussion and management for many years[46-48]. Diseases of economic importance, which occur in feral pigs, remain subject to surveillance; including brucellosis and leptospirosis[49]. The culling of feral pigs was an important part of the tuberculosis eradication program[50], and is a crucial part of spatial epidemiological model simulations for foot and mouth disease control[51, 52], and classical swine fever[53] incursions, which would be potentially devastating to the agricultural industry in Australia.

In Australia, influenza in poultry or swine has fortunately been of limited impact through rapid intervention and the enforcement of biosecurity measures. However, it is not unreasonable to expect that further outbreaks will occur, and that feral pigs may become involved in sustaining and transmitting new virus strains. Whilst I acknowledge that in the event of a large-scale outbreak IAV control will not be primarily in pigs, I do believe it is important to consider the potential for them to become part of control methods/programs. As pigs are known to carry numerous diseases, surveillance for influenza virus in feral pigs would be a valuable addition to the current monitoring which is undertaken.

Conclusions

Antibodies to influenza A virus have been isolated from blood samples taken from feral pigs in successive years, and across three locations (Kingston SE and Innamincka in South Australia, and Mid-north New South Wales). The close association of feral pigs and natural avian hosts of influenza A in arid Australia suggests a role of environment in the exposure of pigs to IAV. Although I cannot conclude that feral pigs are instrumental in the maintenance of influenza as a reservoir host, their potential to be the host of new strains, with greater virulence for people, should be considered, and any future surveillance of feral pigs should also include serology for influenza A antibodies.

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Chapter 5.

A semi-quantitative risk analysis for the transmission of low pathogenic avian influenza from free-ranging wild birds to public display captive populations

Prepared for publication in Australian Veterinary Journal

Animal Ethics: All sample collection procedures were performed in accordance with the animal ethics approvals (S-2016-104 and S-2014-197) from the University of Adelaide Animal Ethics Committee.

Abstract

Disease risk assessments are an important decision-tool in wildlife translocations and species survival programs. Their use in zoological institutions is often informal, with the consideration of disease transmission and biosecurity risk being a component of the quarantine process each institution implements with all acquisitions. Low pathogenic avian influenza (LPAIV) is carried by many wild birds and the shedding of virus from its natural hosts is most often through contaminated faeces. I used a semi-quantitative disease risk analysis to estimate the prevalence of, and likely hazards for, LPAIV infection in avian species housed in a major public Australian capital city zoological institution. I identified hazards for exposure to LPAIV from different avian enclosure types, including: (i) the opportunity to have direct and/or indirect contact with wild birds; and (ii) the difference in species composition within the zoo aviaries. Enclosures were categorised based on the identification of five major hazards and provided with a score for the likelihood of the release of the virus. I also undertook screening of enclosures by collecting faecal swabs from all enclosures, and exposure screening of all birds that presented to the animal health department within the study period (October 2016 to January 2017, n = 76). No swabs were positive for LPAIV on PCR. Serology testing identified four birds from the zoo and one (out of 7) sampled free-ranging Pacific Black Ducks (*Anas superciliosa*) were seropositive for LPAIV. All four zoo birds were in high risk enclosures, the probability of having all four positive samples from a single risk category by chance was 1.8%. I recommend that the risk of LPAIV exposure can be extensively mitigated through the regular cleaning of water and food sites, and circulation of water within ponds, as well as the continued training of keepers who maintain daily records on birds in each enclosure. Future research to

confirm my work, by comparing multiple institutions and husbandry techniques, should be considered.

Introduction

The application of risk assessment, and the complete risk analysis framework, has greatly increased in wildlife veterinary practice[1, 2]. In particular, the veterinary profession understands, and has embraced, the need for risk assessments in preventing disease emergence; for example, when developing conservation management breeding programs and conducting wildlife translocations[2-5]. Risk assessments are structured processes that can aid decision makers to evaluate questions of both consequence and likelihood[6]. Risk assessments can be quantitative, assigning probabilities to all levels and events; semi-quantitative, assigning probabilities to some areas of the events; or qualitative, with expert opinion utilised extensively, and subjective levels used to assign risk[7, 8].

Quantitative risk assessments are data-intensive and require a large amount of quantifiable information; a situation rarely possible when working with natural systems and wildlife[2, 4, 9]. The majority of veterinary and wildlife risk assessments are subsequently qualitative[9], because the available data do not provide enough detail to enable the assignment of robust quantitative risk probabilities[7]. Semi-quantitative risk assessments are similar to categorical qualitative risk assessments, however, the risk categories are assigned a probability based on expert opinion or a technical understanding of the agent that is being considered. Although semi-quantitative risk assessments overcome one of the major weaknesses of qualitative risk assessments, by attaching a specific quantitative meaning to the probability terms[7], they are still not

commonly used for managing wildlife health. Assigning estimated values to hazards, and then using semi-quantitative methods, can allow for more robust, repeatable and updateable assessments to be used[7].

Avian influenza is a member of the Orthomyxoviridae commonly identified by two surface proteins, haemagglutinin (H, or HA) and neuraminidase (N, or NA), and the degree of pathogenicity the virus exhibits in poultry, i.e., highly pathogenic (HPAIV) or low pathogenic (LPAIV)[10]. There have been 16 H-types and 9 N-types identified[11, 12], and two further influenza-like H-types identified from bats[13, 14]. There are 144 possible combinations of the avian H and N types, and each combination event gives rise to a new, genetically distinct strain, meaning the number of individual influenza viruses is in the thousands. Strains of LPAIV have been identified on all continents, including Antarctica. In the northern hemisphere, regular annual phenological events facilitate the perpetuation and transmission of the virus[15, 16]; including predictable migratory pathways and the congregation of multiple species in massive numbers on waterbodies[16], and the breeding of waterfowl and shorebird hosts (tied to the northern hemisphere seasons.

In Australia, there are many unanswered questions regarding the perpetuation of LPAIV in wild bird populations (see Chapter 1), as well as the sporadic emergence of HPAIV in poultry flocks[17]. In contrast to the northern hemisphere, HPAIV outbreaks are typically restricted to single locations and to date have been successfully controlled at early stages in Australia. The means of introduction into poultry is unknown; although contamination of poultry flocks by wild birds is the prime suspect[18]. Surveillance is key to understanding the historic, current and future potential threats

to biosecurity. Consequently, Australian States and Territories are involved in the National Avian Influenza Surveillance Program, administered by Wildlife Health Australia on behalf of the Department of Agriculture and Water Resources. Sampling as part of this program is conducted across Australia, throughout the year, and centralised to provide information and advice with regards to the present status of avian influenza[15].

Biosecurity preparedness is a focus for the veterinary and human public health fields, and disease risk assessments are used to inform the decisions made by animal managers and government agencies. Zoological institutions are acutely aware of biosecurity and quarantine concerns, as they are involved in many animal relocations, which occur both domestically and internationally every year between zoos. Zoos do not exist in isolation. As educational and entertainment venues, they are easily accessible by the public, and are often located in central metropolitan areas. Zoos are also readily accessible by, and likely highly attractive to natural and exotic free-ranging wild animals (e.g., birds) that inhabit the local environment. When attempting to ensure adequate precautions are in place, to prevent exposure and/or infection, consideration needs to be given to both the direct physical interactions (i.e., bird to bird contact), and indirect interactions such as shared water or food resources, or the contamination between enclosures. LPAIV can remain infectious in water for many days[19-23] as discussed in Chapter 2, so the potential for environmental contamination is always present.

There are many factors to consider with regards to the impact of an infection with LPAIV in bird species. There are marked differences in susceptibilities between

species, with the most susceptible to infection and transmission widely accepted to be the Anseriformes, ducks and geese[24], and the next most susceptible being the Charadriiformes, or shorebirds. Neither Anseriformes nor Charadriiformes commonly display signs of infection and are usually considered to be asymptomatic carriers of disease. Galliformes, which includes chickens, quail and pheasants, are susceptible to disease and are more likely to exhibit symptoms. Clinical signs can vary in nature and extent, including mild upper respiratory signs, a drop in egg production, and even sudden death, dependent on which strain of the virus is contracted[25].

Australia has guidelines in place for emergency animal disease (EAD) responses, AUSVETPLAN; aimed at the control of economically important diseases within the agricultural sector[26]. In addition to the avian influenza EAD manual[27], a Zoo Enterprise manual has been produced[28]. Many of the animals within a zoo are either of conservation importance, part of reintroduction schemes, or insurance populations, making the culling of at risk individuals a complex issue. Detection of AIV within a commercial poultry flock would lead to the immediate culling of infected individuals and at-risk populations. However, in a zoo there is a caveat which allows for case-by-case determination of the best course of action, and further involvement of the state Chief Veterinary Officer.

Each pathogen has different host and environment requirements and will pose variable risks dependent on the individual situation. The traditional application of disease risk analysis has examined multiple pathogens for a single host, and assigned risk of exposure, release and consequence across a range of hazards. There are some pathogens, which lend themselves to an alternative viewpoint because of their

significant public health concern, particularly a zoonotic pathogen of pandemic potential such as influenza A. In this chapter, I present a pathogen-specific, institution-based risk assessment that can be applied to multiple situations with minimal alteration. I implement a modified semi-quantitative risk assessment method that considers the enclosure to be the primary hazard, with population, enclosure type and design, and husbandry methods all influencing the likelihood of event, release and consequence.

Methods and Materials

Adelaide Zoo

Zoos South Australia (ZoosSA) has two public collection sites; Monarto zoo, and Adelaide zoo, a traditional metropolitan zoo and the location of this study/. Adelaide zoo covers 20 acres, is located to the east of the central district of Adelaide and is bounded by the river Torrens and the Botanical Gardens of South Australia (Figure 5.1). The zoo is home to approximately 2500 animals with birds accounting for at least 50% of this number at any one time and has an average of 290 animal movements each year (ZoosSA unpublished data).

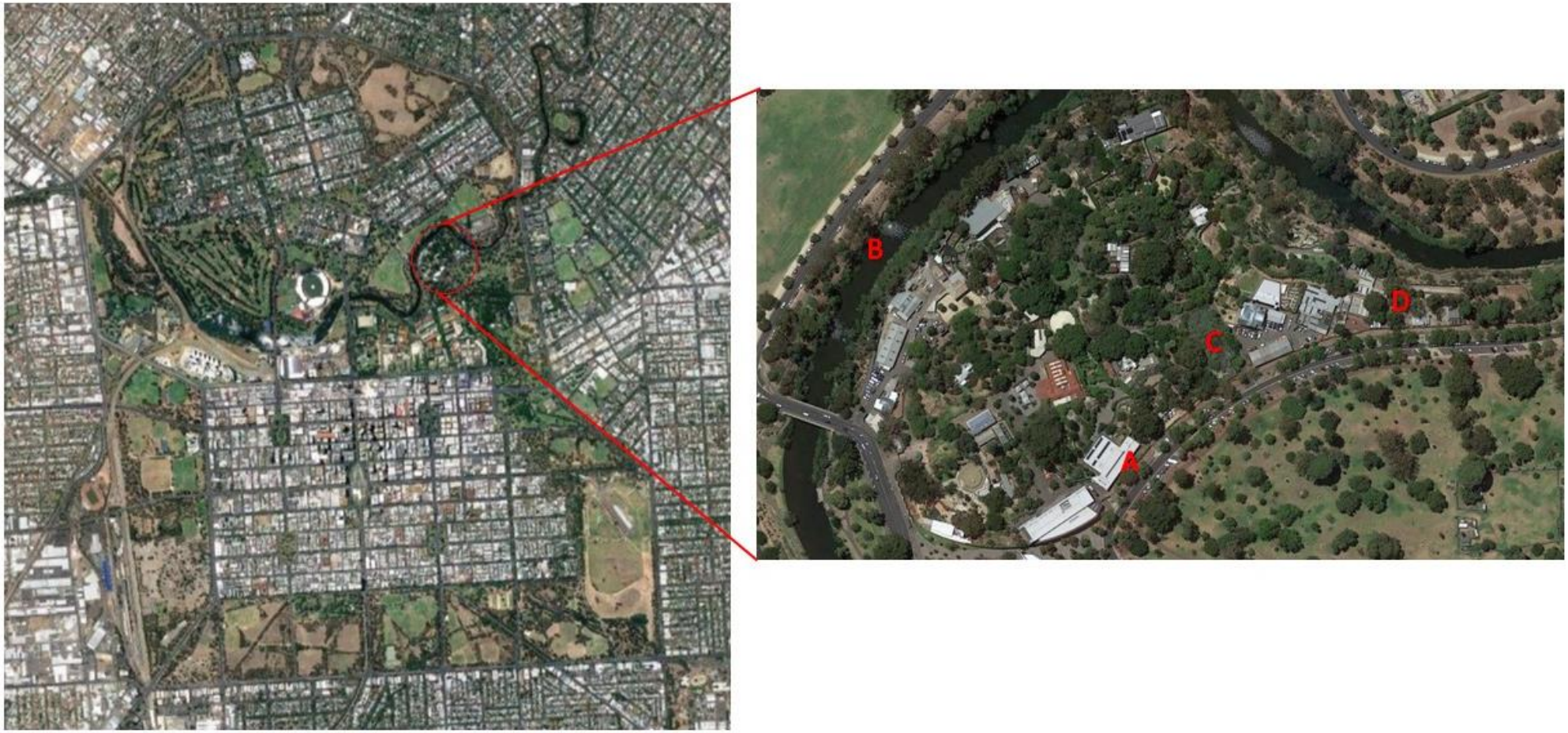

Figure 5.1. The location of Adelaide Zoo in relation to city centre of Adelaide (left panel). The right panel shows an aerial view of Adelaide Zoo, the main entrance (A), the River Torrens (B), the Australian walk through aviary (C) and the Children's zoo (D).

Risk analysis process

Risk analysis is a complex multi-step process; see Figure 5.2 for a simplified overview. Full explanation of the process of disease risk analysis has been discussed previously; for example see Sainsbury *et al*[9] and Jakob-Hoff *et al*[23] for full descriptions of each step in the process:

i) Problem description

Birds in zoological institutions comprise a novel captive population of birds with extremely varied eco-evolutionary histories and biogeographical origins living in close quarters. Depending on the enclosure structure and husbandry decisions there can also be opportunities for captive birds to come into direct and indirect contact with free-ranging wild birds. Contact of this nature is of particular concern with waterfowl (Anseriformes), shorebirds (Charadriiformes) and poultry (Galliformes), which are susceptible to LPAIV and can be asymptomatic shedders and may then become sources of infection for other birds, and mammals, in the collection. Zoos aim to maintain their animals in good health, reducing the occurrence of disease by husbandry management and preventative health programs.

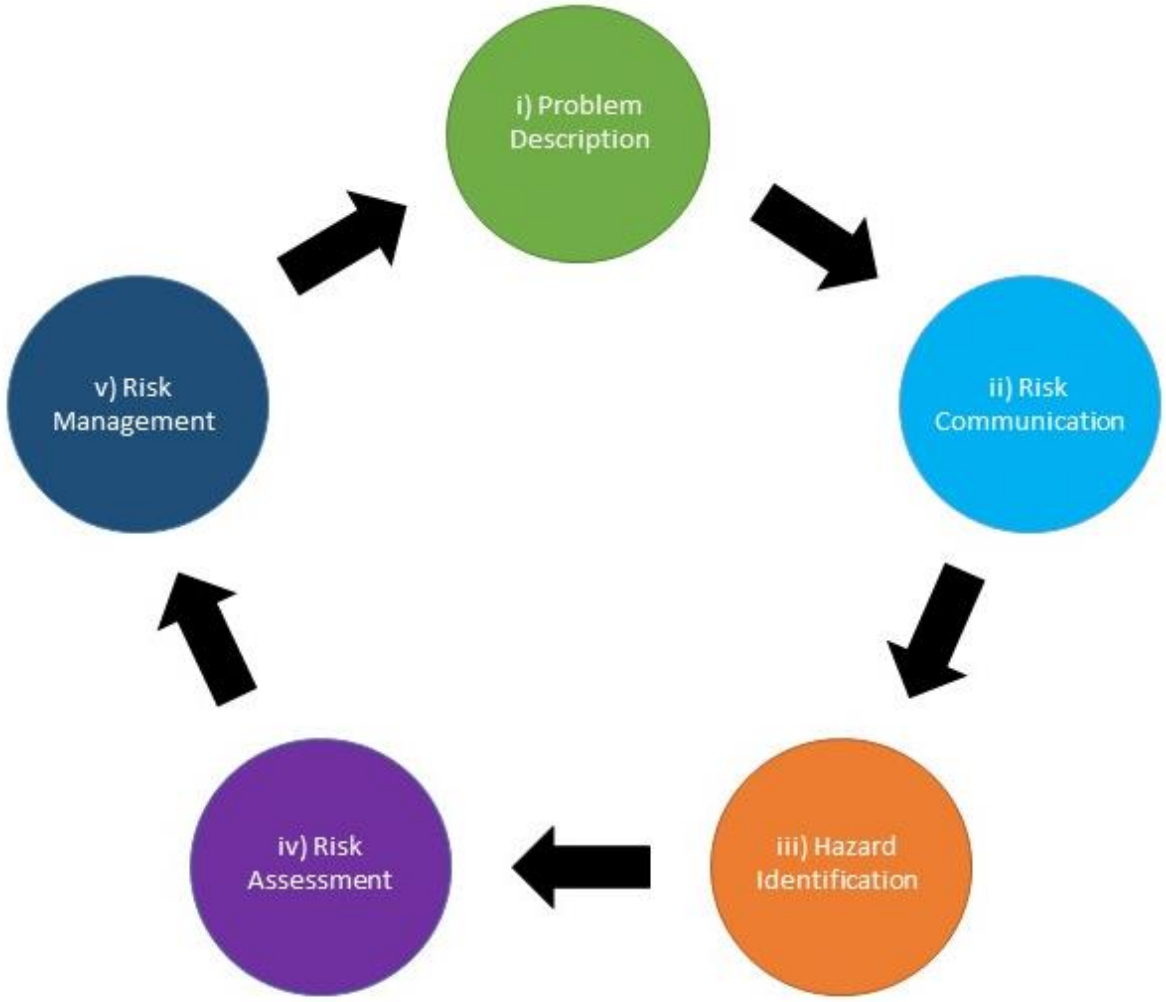

Figure 5.2. The pathway for risk analysis, adapted from Jakob-Hoff[23]. The steps include: i) *problem description*; defining the context, goal and scope of the risk analysis including the assumptions, limitations and the acceptable level of risk; ii) *risk communication*; identification of experts and communication plan for findings and recommendations to stakeholders; iii) *hazard identification*; listing the identified hazards, describing their key characteristics and categorising the hazards; iv) *risk assessment* (RA); assessment of the probability of release of a hazard, the probability of exposure to the hazard, and an assessment of the consequence of release and exposure, including hazard prioritisation; v) *risk management*; identification of options and the prediction and evaluation of outcomes, making of decisions and recommendations.

ii) Risk Communication

This chapter, and future publication, serves as the risk communication.

iii) Hazard identification

I considered multiple factors within the zoo, on a daily basis, to identify the hazards to be included in the study. These included: (i) the environment the birds are housed in; (ii) the husbandry requirements in light of the life history traits of the birds housed; (iii) the level of public interaction with enclosures, i.e. how much access members of the public have, allowing more opportunity for the introduction of wild bird faeces into the enclosures; and (iv) species composition within an enclosure, e.g. single species such as the little penguin (*Eudyptula minor*) enclosure, a single family enclosure housing parrots (psittacines) or a truly multi-species mix with multiple families in a single space.

I identified five features/circumstances of the zoo enclosures that may affect the risk of release of virus:

i. *Roof construction* (two categorical levels): open with no roof structure, but mesh allowing for wild bird faeces to fall into the enclosure, and wild birds to perch on top of the enclosure; or solid roof preventing any faecal contamination or interaction of birds (see Figure 5.1 panels B/C/F/G).
ii. *Species in enclosure* (two categorical levels): single species or multiple species.
iii. *Open water* (two categorical levels): enclosure includes a pond, pool or stream or the enclosure has no waterbodies, LPAIV is known to persist in water (see Figure 5.1 panels D and G).

iv. *Food provision* (two categorical levels): uncovered area for bowl/tray allowing faeces from wild birds to potentially contaminate food, or covered area for bowl/tray (see Figure 5.1 panels A/E/F).
v. *Public access* (two categorical levels): walk through aviary for public interaction and experience, or restricted enclosure with access only available to staff.

iv) Risk assessment

My risk assessment procedure is adapted from FAO/OIE guidelines and consists of the following components: (a) *assessing the probability of release*, will the virus become a circulating infection in the population?; (b) *assessing probability of exposure*, are the animals in the population going to come into contact with the virus?; (c) *assessment of the consequence of exposure*, if the population is coming into contact with the virus what will the outcome be?; and (d) *hazard prioritisation*, hazards likely to have the greatest impact on release or consequence.

In particular, I have employed the following definitions for our risk assessment of the likelihood of captive zoo population birds contracting LPAIV from wild free-ranging birds.

(1) An **event** is defined as the direct or indirect interaction of a captive-population individual bird and a free-ranging wild bird. Direct contact is limited by enclosure roof construction type; however, indirect contact is possible in numerous enclosures (see enclosure classification below).

(2) An **exposure** is defined as an individual bird, from the captive population, encountering contaminated faeces, water or food.

(3) An **impact** is defined as the effect an influenza infection has on the individual bird. The impact is variable dependent on the species of bird [29], and upon the variant of LPAIV [30]. For example, whilst psittacines are not considered to be particularly susceptible to AIV, pathological infection in the critically endangered Orange-Bellied Parrot (*Neophema chrysogaster*) could result in a very high impact. Impact also considers the conservation importance and the impact on a population of loss of a genetically valuable individual.

Table 5.1. Definition of the levels of probability for an event per year per enclosure; the number of exposures per enclosure per year; and the impact in the event of an exposure. Severity score is calculated as the sum of each component risk score, the exposure event and the impact scores. Adapted from FAO/OIE guidelines[31]

Category (Score)	Probability of event per year	Exposures per year	Impact description	Severity Score
Negligible (0)	Indistinguishable from 0	Indistinguishable from 0	No effect	0
Very Low (1)	$< 10^{-4}$, except 0	1-2	Transient ill thrift	1 to 3
Low (2)	10^{-3} to 10^{-4}	3-10	Mild respiratory signs	4 to 6
Medium (3)	10^{-2} to 10^{-3}	10-20	Respiratory/GI signs requiring supportive care; important species for institution	7 to 9
High (4)	10^{-1} to 10^{-2}	20-50	Hospitalisation and chronic sequelae; Endangered species	10 to 12
Very High (5)	$>10^{-1}$, not 1	>50	Death; Critically endangered species, breeding colony	13 to 15
Certain (6)	1			18

Figure 5.3. Examples of the avian enclosure types at Adelaide zoo: A) An uncovered food bowl in an enclosure housing White browed-wood swallow (*Artamus superciliosus*), Mallee fowl (*Leipoa ocellata*), Purple-crowned lorikeets (*Glossopsitta porphyrocephala*) and Regent parrot (*Polytelis anthopeplus*); B) mesh roof with trees evident above and behind; C) feral rock pigeons (*Columba livia*) helping themselves to food placed in the pelican (*Pelicanus conspicillatus*)and Cape Barren goose (*Cereopsis novaehollandiae*) enclosure; D) open pool in the pelican enclosure, no roof to the area; E) a covered food tray in a mesh-roofed enclosure; F) mesh roof structure and covered water and food bowls; G) large mesh roof structure with open water in a walk-through enclosure with waterbirds. All photos by A. Dalziel.

Data Collection and Analysis

Enclosure classification

Enclosures included in the risk assessment were restricted to those containing birds. Classification of avian enclosure types were based on the hazards previously identified in the hazard identification step.

Sixty individual enclosures were identified within the institution. The breeding and holding blocks were not included in our assessment as they have impervious roof structures, and have no access for wild birds. The remaining spaces were then aggregated, by varying husbandry requirements for the species housed, into larger enclosures and maintained with open doors between sections to give a total of 20 enclosures for assessment. The birds housed separately for the free-flight exhibitions were excluded from the assessment, leaving 19 enclosures for assessment.

The enclosures were assessed based on the identified hazards using a decision tree (see Appendix 3, Figure S3.3), with a binary score (0, 1) at each categorical level and the overall score for the enclosure achieved by summing across each level. Using this method I assessed one enclosure as having negligible exposure risk score, it is not possible to eliminate all risks and so a zero exposure risk score cannot be assigned, two a very low exposure risk score, six with a low exposure risk, five with medium exposure risk and five with a high exposure risk. I did not identify any enclosures with a very high exposure risk.

Family risk classification

The bird population of the zoo was classified as either high or low risk based on their taxonomic family membership. For example, Anseriformes and Galliformes were designated as high risk due to being natural hosts (Anseriformes)[24] or their susceptibility to the disease (Galliformes)[10], whilst Passeriformes and Psittaciformes were classed as low risk as they have only rarely been identified with the virus[10, 32, 33], usually as the result of spillover during large scale poultry infection events[29, 34]. The full table is provided in the supplementary material (Appendix 2).

All analyses were conducted in the R-software (Version 3.3.0 R Foundation 64-bit)[35]. I performed Welch two sample t-tests to determine if there were statistically significant differences in: (i) the average enclosure scores of high and low risk bird families, as defined in this study; and (ii) the number of individuals (log_{10}) of each family risk category. I then used a generalised linear model to examine the influence of family risk, a two level categorical variable, and enclosure score, an ordinal numerical variable (0-5), on the log_{10} number of individuals kept in the zoo.

Wild bird disease prevalence – South Australia

The prevalence of LPAIV in South Australia information was obtained from published records [36, 37], and current unpublished faecal testing data from within South Australia as part of the national surveillance project (C. Dickason, Primary Industries and Research South Australia).

Wild bird disease prevalence – Central Metropolitan Adelaide

I directly sampled free-living waterfowl and waders in hand (n = 27) from the immediate public area surrounding the zoo (within 500m). Birds were trapped using a walk-in funnel trap baited with commercial poultry grain, and cloacal swabs and blood samples were taken by a wildlife veterinarian (AD), with restraint by an avian biologist (Dr. R. L. Boulton). Blood samples were taken from the cutaneous ulnar vein, (Terumo 1ml insulin syringe with 27G needle) and stored as described for the zoo bird samples. Each bird sampled was individually identified by the application of a leg band following the guidelines of the Australian Bird and Bat Banding Scheme (ABBBS).

Institution prevalence investigation

During the study period (October 2016 to February 2017) the bird population at Adelaide Zoo was c.1000 individual birds across 120 species. The bird population was split into various groups based on husbandry, management and exhibit requirements, and housed between 19 major enclosure sets with some containing a single individual, and others housing over 150 individuals.

Virological and serological study

All birds admitted to the Animal Health Department for routine health screening, quarantine health checks or on medical grounds between October 2016 and January 2017 were potential candidates for inclusion in the screening study. Sampling for this study was at no time the primary reason for capture or anaesthesia. In all cases, samples were obtained as secondary considerations, and only when appropriate, given the health status, body size, some birds were too small to obtain extra blood

samples from, and history of the bird, if it had been in the hospital more than once within the study period samples were taken only once.

Blood samples were collected into serum microtubes (MiniCollect®), centrifuged and the serum decanted. Alternatively, where blood was not able to be separately collected, plasma was decanted from centrifuged lithium heparin tubes, subsequent to institutional requirements. All serum or plasma samples were individually labelled and stored at -80°C within an hour of collection (n = 34). Supplemental historical plasma samples were obtained from the institution archive (n = 26). A total of 60 serum and plasma samples were tested for antibodies to LPAIV.

Cloacal swab samples were obtained from all individual birds included in the study (n = 76) whether manually or chemically restrained. Individuals that were anaesthetised for examination had combined choanal-cloacal swabs taken. A sterile swab was placed into the cloaca, rotated and removed for cloacal swabs. Choanal-cloacal swabs followed the same procedure except the swab was inserted into the choana and rotated prior to insertion into the cloaca. Swabs were then placed in PBS-based viral transport medium, labelled and stored at -80°C within 30 minutes.

Fresh faecal environmental swab samples were taken from all bird enclosures across the institution (n = 99, 3 swabs pooled into each sample to give 33 samples). Fresh faeces was identified by visual inspection and a swab was rotated in the sample, a minimum of three swabs per enclosure, then treated in the same manner as choanal-cloacal swabs, see above.

Sample testing

All swab and serum/plasma samples and cloacal/choanal-cloacal samples were submitted to the laboratory (Gribbles VETLAB South Australia) for AIV serology and viral PCR under standard testing conditions in accordance with OIE test guidelines. RNA was extracted from all swabs using a QIAGEN MagAttract 96 cador pathogen kit on an Applied Biosystems MagMax Express 96 platform. PCR was performed using a QuantiTect® multiple RT-PCR NR kit in a RotorGene-Q (QIAGEN). Serum/plasma samples (n = 86) were tested using an Influenza A blocking ELISA kit provided by the CSIRO Australian Animal Health Laboratory (AAHL).

Results

Risk assessment and Severity Score

Five enclosures obtained a very high severity score (Table 5.2), both with an open enclosure or/and multiple species. Seven enclosures were assessed as having a high severity score, four having open roof structures, and three having mesh roof structures. Four enclosures had a medium severity score, with all four having mesh roof structures. Eight enclosures had a low severity score, three with an open roof structure and five with a mesh structure.

Table 5.2. Enclosure scoring and probability calculation risk table. Severity score is the sum of the 3 component scores (enclosure/exposure, event and impact)

	Enclosure	Enclosure Class/Exposure Score	Event Score	Impact Score	Severity Score
Open Roof	• Emu • Cassowary (female) • Cassowary (male)	2 (Low)	1 (Very low)	3 (Moderate)	6 (Low)
	• Flamingo • Little Blue Penguins	3 (Medium)	4 (High) 3 (Medium)	4 (High)	11 (High) 10 (High)
	• Pelican, Pygmy goose, • Barn poultry (chickens and quail)	4 (High)	4 (High)	4 (High)	12 (High)
Mesh	• Orange Bellied Parrots (OBP)	0 (Negligible)	0 (Negligible)	5 (Very High)	5 (Low)
	• Kingfisher/Kookaburra • Conure/Cockatoos	1 (Very Low)	2 (Low)	2 (Low)	5 (Low)
	• Lorikeet/Regent Parrot, Sacred Kingfisher • Macaw/Conures • OBP/Dotterel/Finches	2 (Low)	2 (Low)	2 (Low) 2 (Low) 5 (Very High)	6 (Low) 6 (Low) 9 (Medium)
	• Duck/Lory/Parrot/Lady Amherst Pheasant (LAP) • Swift parrot/Stilt/Quail/wWren • Nicobar pigeons/LAP/Pygmy goose	3 (Medium)	2 (Low)	4 (High)	9 (Medium)
	• South East Asia walk through • Australian woodland walk through • Australian wetland walk through	4 (High)	3 (Medium)	5 (Very High)	12 (High)

Wild bird infection prevalence

National monitoring indicates that South Australian-state infection level of LPAIV in wild birds between 2007 and 2012 ± standard error, varied between 0.4 ±0.3% and 3.9 ±1.1% with an overall average of 1.2 ± 0.2% [15]. In 2015-2016 prevalence in South Australia varied between 1.0 and 3.7% with an average of 2.02% from a single site, over three sampling events with 300 samples per event (C. Dickason, Primary Industries and Research South Australia, pers. comm.)

I calculated seroprevalence from our own sampling, from 30 wild birds trapped within 500m of the metropolitan Adelaide zoo, to be 3.7% overall, with the seroprevalence in Pacific Black ducks (n = 7) 16.7%, a species known to have a particularly high prevalence of LPAIV[38].

Institution prevalence

Four positive samples for influenza A from birds in the Adelaide Zoo collection were identified on serological testing, three from a group of columbids housed in a walk-through exhibit, and one galliform from an open walk-through barn exhibit. All faecal swabs of enclosures, and swabs taken directly from birds were negative on PCR and were not tested further. I conducted a bespoke randomisation test (k = 10,000) to assess the probability of obtaining four seropositive samples from class 4 enclosures (see Table 5.2) by chance. The result was a 1.8% probability of obtaining all positive samples from a class 4 enclosure.

Birds in low risk families were largely held in lower risk enclosures (t = -4.99, df = 92.12, *P* = 2.80e-06) (Figure 5.4). We also investigated the number of individuals that

were held for each risk type, high and low. Birds of high risk families were typically held in lower numbers (t = 2.31, df = 109.36, *P* = 0.023) (Figure 5.4), compared to the birds of lower risk families across enclosures. The generalised linear model indicated that, overall, there were more individuals housed in higher risk enclosures (*b* = 0.104, *t* = -3.55, *P* <0.01), but they tended to be of low risk families (intercept = -0.261, *t* = 4.30, *P* <0.01) (Figure 5.5).

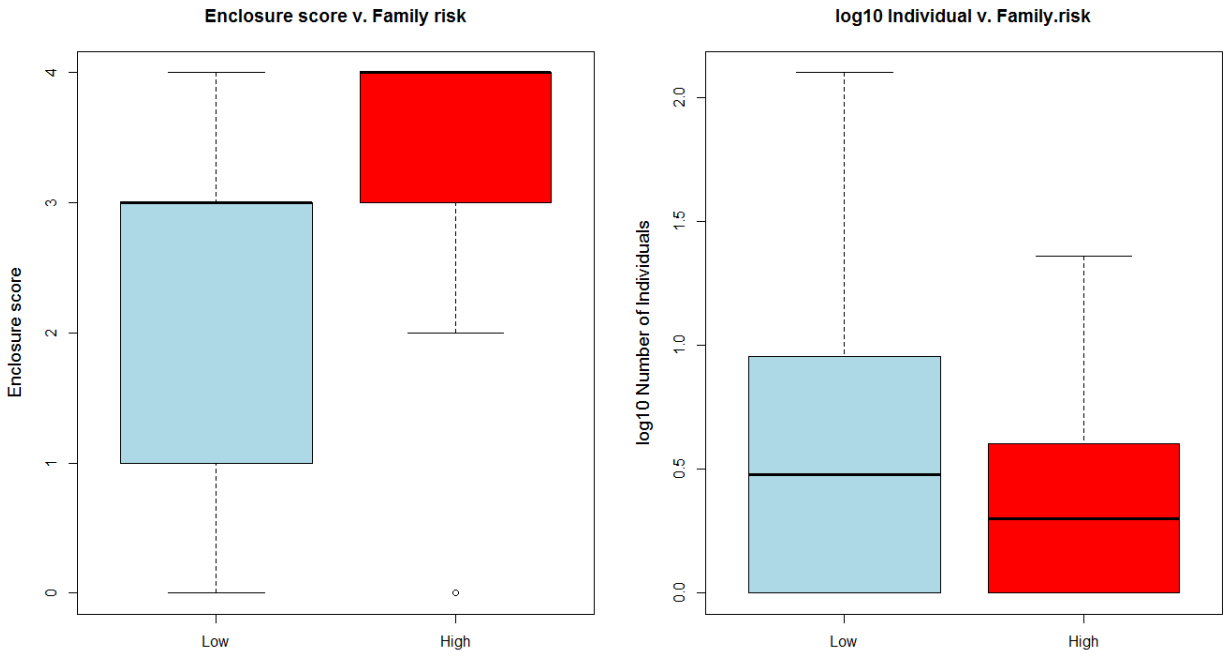

Figure 5.4. Boxplots of the enclosure score against family risk (left) and $\log_{10}$ number of individuals against Family risk of LPAIV (right).

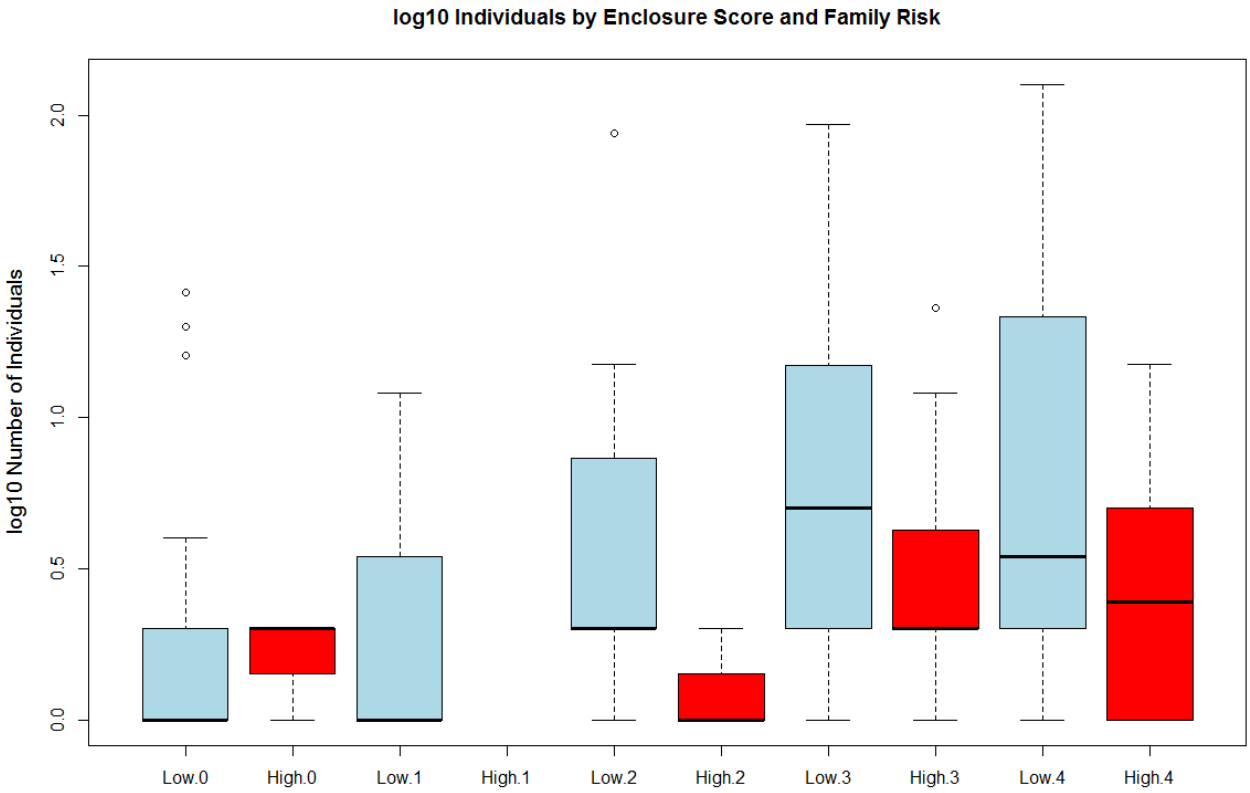

Figure 5.5. A boxplot of the $\log_{10}$ number of individuals in each enclosure class displayed by family risk.

Discussion

Implementing a pathogen-specific institution-based approach I proposed five major hazards that could likely influence the risk of transmission of LPAIV from between wild free-range birds and native and exotic captive birds within a zoo. Each enclosure housing birds within the institution was assessed based on the hazards identified and given a score from 0 to 5. The overall risk assessment identified six enclosures with an overall high risk, three with no barriers to wild bird access, and three with access for the public as walk-through aviaries.

Birds from high risk families are being housed in smaller numbers, but in higher risk enclosures. The majority of these birds are larger bodied, such as pelicans (Pelicanidae), ducks (Anatidae), emus and cassowaries (Casuariidae), and so require more space to house them than many of the lower risk (smaller-bodied) bird species, e.g. passerines and columbids, particularly those which spend part of their time on or in water. The smaller bodied passerine species are housed in secure free-flight exhibits, and often in larger numbers.

Surveillance sampling in South Australia regularly identifies virus from faecal swabs of wild birds. The testing did not detect any shed virus within any of the enclosures, and individuals with antibodies to influenza A were housed in only two enclosures. The opportunistic nature of the serological study did not allow for testing every bird within all enclosures, however, the lack of detectable virus within the enclosures and the general husbandry and maintenance conducted by staff will reduce the risk of exposure and mitigate the risk levels identified in the risk assessment.

The seropositive galliform was in an open enclosure that also has free public access and no barriers to wild birds for access to the area, food or water sources. This fits with the current knowledge of transmission pathways and susceptibility of Galliformes, and with the very high severity score identified in the risk assessment. The three columbids identified as seropositive provide an interesting result, they were housed in a multi-species walk-through free-flight aviary, which was identified as high risk in the assessment, and no reports of clinical signs were made to the veterinary staff. Columbids are considered to be ‘dead end’ hosts for avian influenza viruses and have not previously demonstrated clinical signs in infection studies[30, 39].

Enclosure design considers many competing interests from multiple avenues, with priorities moving to more naturalistic exhibits benefitting the animals that live in them, which may subsequently compromise the view for paying visitors[40]. More naturalistic design can also present difficulties for hygiene and biosecurity, for example, the competing requirements for a natural flooring (such as leaf litter and soil), which does not allow for daily replacement compared to concrete floor which can be washed on a daily basis.

A risk assessment by the veterinary department of an institution, following the same format could be performed for any transmissible pathogen, where there is contact between wild and captive animals. The findings of my risk assessment highlighted multiple enclosures, which could require future mitigation. Many of the potential mitigation steps that can be implemented are part of the daily maintenance and husbandry practiced by keepers for each enclosure. For example, mitigation could include: (i) replacement of substrate on a regular basis removing faecal contamination from carrier hosts; (ii) medical assessment for any birds displaying clinical signs of illness, removing potentially infected, and infectious, birds; (iii) cleaning of food trays and water bowls daily, particularly important for those in open locations; and (iv) cleaning of paths used by members of the public within enclosures and requiring visitors to walk through foot baths prior to entering a walk-through enclosure.

Animals in zoos are a mix of species with varied phylogenetic and geographical life histories housed in enclosures that are grouped dependent on the intention of the institution and the managers. The presentation of animals in multi-class, multi-species exhibits provides a more naturalistic and engaging experience for members of the

public, and the design of the exhibits aims to provide all the residents with suitable habitat for them to express normal behaviours. New enclosures can be designed with biosecurity, husbandry and the public in mind. By including all these considerations we can reduce risk to the lowest possible level and aim to prevent communicable disease outbreaks in captive populations.

Conclusions

Infection of collection birds with LPAIV contracted from free-ranging wild birds could lead to reduced success in breeding programs, morbidity or possibly mortality of captive species. Zoos do a very good job of maintaining the health of their animals, often in less than ideal circumstances, where natural migration cycles cannot be accommodated, species mixes are approximations at best, and nesting is in artificial environments. Risk of infection can never be completely eliminated, however, there are important steps that can be taken to mitigate them. Risk assessments should be updated in light of changes in management practices, or changes in the virulence or nature of the pathogen. If a particularly virulent strain of avian influenza were to be identified in Australia this risk assessment could be revised and further testing of the bird population undertaken to allow consideration of any further mitigation required.

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Chapter 6.

General Discussion

Viral diseases are abundant across the world, in plants, invertebrates and animals[1, 2]. The potential for mass mortalities from pandemic influenza real, and the prevention of pandemics is dependent on our ability to understand and predict where and when they are going to occur. Currently, the World Health Organisation uses expert opinion and genetic mapping of influenza virus evolution to inform their manufacture of influenza vaccine in advance of isolation of the viruses responsible for the next seasons influenza outbreaks, with varying success [3, 4]. Knowledge of the fundamental factors that contribute to viral persistence and transmission is essential to refine our ability to predict influenza outbreaks.

The trend towards, and the expansion of, the 'One Health' approach over the last few years, where it is understood that environmental, human and animal health are intrinsically linked, allows us to consider problems in a more holistic way[5]. This has largely been driven by the realisation that a large proportion of emerging diseases are zoonotic in nature (60%), and of those, a high proportion are from a wildlife source (75%)[6]. Large-scale habitat loss, land-use change and land clearance have facilitated our increasing interaction with, and displacement of, wildlife[7]. In turn these interactions have permitted exposure to, and contraction of, various highly infectious and pathogenic diseases[8]. Many of these 'emerging' diseases have previously only been observed in sporadic cases and certainly not in the epidemic proportions seen recently for Ebola[9], Zika[10] and West Nile[11] viruses. One Health is also concerned with food and water security[12, 13]. With the potential for carnivores to become infected with IAV via food sources[14], and wild animals to be exposed via water sources, elucidating the risks must become a further priority.

In this thesis, I have highlighted the areas of environmental research that need to be further investigated for LPAIV in Australia, providing suggestions for ways in which this can move forward. By sampling feral pigs I have postulated they are a potential secondary host for influenza A in the Australian environment. The presence of antibodies to IAV in feral pigs was previously unknown and I hope that future surveillance of feral pigs for diseases of economic importance will include, at a minimum, serology for influenza viruses. The area identified to have feral pigs with influenza antibodies is arid, with ephemeral water sources, lakes that fill following high rainfall events elsewhere, and high concentrations of mammalian pests occurring at water sources in the hottest times of the year. Australia has phylogenetically distinct circulating influenza viruses, and it may be that we have an influenza A virus endemic to the feral pigs in central arid Australia. In addition, I have examined the risk pattern for persistence and subsequent isolation of LPAIV across Australia. This research has concentrated on the Australian context, however, the results of my studies may be equally applicable to other regions in the world, and are expandable as more information is made available.

The highly changeable nature of influenza viruses, and a wide variety of potential hosts, means understanding how they persist in an environment is possibly the best hope we have for improving the control and prevention of disease in both humans and non-human animals. Influenza virus can be transmitted through indirect environmental means, and persistence is intrinsically linked to the conditions encountered by the virus once external to the original host.

There has been speculation, with justification, that the next human influenza pandemic will likely originate from an animal host[15], whether directly from interactions with bird species, as occurred with HPAI H5N1 in Hong Kong in the 1990s, or via a secondary host, such as pigs[16]. Predicting where the most likely emergence will occur requires knowledge of the circulating viruses (through surveillance), evolutionary and genetic studies, and knowledge of their interaction with the environment; particularly through realistic environmental mesocosm experiments. The more information we have, the better the predictions we can make, and the greater the possibility of preventing mass mortalities through having preventatives in place.

Although numerous physicochemical conditions of water have been included in laboratory-based research, there are still many that have yet to be investigated. I examined the known factors affecting persistence, and provided a framework to further enhance prediction and surveillance capabilities. Research conducted so far has understandably concentrated on the northern hemisphere. Thus, the isolation of southern hemisphere-specific viruses[17], and recognition of the differences in the host species natural history, and their interactions with the environment, in the Southern Hemisphere, has been relatively recent[18, 19]. Until further information about Southern Hemisphere viruses, and results from more environmentally realistic studies are available, progress in predictive studies will be limited. Continued publication of new findings, related to individual strains of virus, and/or isolation of antibodies from secondary hosts, such as feral pigs in the USA[16] illustrates the large body of work still to be undertaken. It also demonstrates the difficulty we face when attempting to predict and implement control measures for a disease entity we do not fully understand, and that is so capable of changing rapidly.

Active surveillance for LPAIV, and by extension HPAIV, is limited by the ability to: (i) access areas; (ii) find experienced samplers; (ii) isolate virus (an issue in Australia); and (iii) to provide repeatability at sampling sites. Whilst there is a push to monitor close to poultry operations[20-22], the focused view this takes may not facilitate the effective functioning of a full monitoring network. I argue that it would be more appropriate to expand the network, where possible, to surveil at areas with the appropriate host species, and environmental conditions conducive to viral persistence in water, and to supplement this surveillance regime by sampling close to operations. With a One Health view, we should also be developing abilities to test (environmental) waterbodies for influenza viruses[23, 24], particularly those with larger gatherings of known hosts such as the Ramsar wetlands{Ramsar} across the country. Although Australian birds do not show strong site fidelity for breeding, aggregations of birds do occur in some locations with regularity, and further sampling and detection efforts within these areas would enhance our knowledge base.

Key findings and recommendations

1. Avian influenza, with either low or high pathogenicity, is found across the world. Australia is fortunate to have experienced very few outbreaks of HPAIV, and this is in part due to the relative isolation of the island continent. The differences in our wild bird ecology, with nomadic lifestyles being common amongst waterbirds, can allow us to tease out some of the ecology and drivers of LPAIV, in contrast to the northern hemisphere where the seasonal breeding and mass congregations of naïve or immunocompromised birds adds to the burden of disease.

2. The knowledge base regarding LPAIV in natural environmental waters, and the factors that affect persistence, is limited. Expanding the range of abiotic factors tested, including the examination of the impact of UV exposure on viral persistence, and moving towards a more realistic experimental system will aid in understanding the many complex interactions that are likely to be occurring.
3. More information on environmental transmission, immunology of the natural hosts, and continued targeted surveillance with the collection of ancillary environmental data when surveillance occurs, will improve our ability to quantify risk in space and time, and allow more effective targeting of limited resources.
4. In the case of influenza, feral pigs have been proposed as a potential host for years, but until recently little to no investigation had been done to quantify their infection status across an extensive natural environment. Feral species should not be overlooked as either spillover or reservoir hosts for emerging (or re-emerging) infectious diseases.
5. Assessments focusing on the disease agent, rather than considering risk of exposure to a particular pathogen as part of an array of possible disease agents, affords the opportunity to identify and potentially mitigate specific hazards that could make it more likely to occur. The use of disease risk assessments, and adaptation of current qualitative assessment processes, as well as wider dissemination of completed risk assessments, will increase our ability as practitioners to be confident in the decisions made.

I recommend maintaining the current surveillance network through the NAIWB working group, and expanding this to include the collection of environmental variables and water samples for viral isolation at each wild bird sampling point. The collation of

ground-truthing information would help in parameterising the models I have presented in Chapter 3, and as a consequence refine the predictions to provide a greater degree of confidence of appropriate allocation of resources. I also recommend the continuation of surveillance for influenza virus exposure in feral pigs. Though there may currently be limitations due to the number of samples available, the publication of North American data from long-term surveillance of feral swine has identified infection of pigs with avian influenza viruses. The changeable climate and variable resources that affect pig numbers in Australia will also have effects on the persistence of influenza virus in the environment, and on the movements of the natural avian hosts of the virus. As extremes of weather and resource availability continue there may be more opportunity for spillover of influenza viruses into feral swine, and further risk for reassortment and evolution of the virus.

Conclusions

With a subject as changeable and evasive as influenza virus it is highly unlikely we will ever be able to completely explain and predict all changes in the pathogenicity, host range or genetics of the virus, nor will we be able to prevent all further outbreaks. However, progress can be made towards a broader knowledge base, along with a more in depth understanding of the conditions the virus needs to persist in the environment and transmit between hosts.

Temperature and water quality measures can be used to make some predictions about the duration of influenza persistence in environmental waters, and bird surveys can be used to make predictions about the presence or absence of primary host species. As more species are identified as secondary hosts, these too could be incorporated to

improve viral risk across the landscape, and particularly the identification of higher risk locations. If we hope to be able to predict outbreaks, and prevent countless deaths as a result of pandemic influenza, understanding the fundamental basics of virus survival in the environment, as well as maintenance and transmission within and between natural hosts, has to be a priority.

The central goal of this thesis was to advance the field of understanding IAV pandemics and pandemic preparedness. During the course of this work, using statistical modelling, field surveys and laboratory assays, I identified environmental variables that affect the persistence of virus, applied this across Australia and showed that feral pigs in Australia may be exposed to influenza virus. I have argued during this thesis that understanding the ecology of the virus, and the range of hosts that can be infected, as well as investigating with environmental realism are pivotal to prediction and preparedness. The implication of my work is whilst there is much research being conducted into vaccines against influenza, the ability to understand, predict and prevent outbreaks must be striven for if we hope to get ahead of the next pandemic.

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Appendix 1

Chapter 2

Additional material

S1. Study, H-type, N-type, year of isolation, species of isolation, Country and State of isolation and designation of each viral subtype use in all included studies.

Study	Year	Country	H	strains	Temps	pHs	Salinities	Experimental combinations	Rt	R2	Slope
Brown et al	2007	N. America (Georgia)	2	8	2	1	3	48	48	48	48
Brown et al	2009	N. America (Georgia)	12	12	3	5	5	900	60	0	0
Davidson et al	2010	Israel	1	3	3			7	7	7	7
Graiver et al	2009	N. America (Nebraska)	1	1	3	2	4	8	8	0	8
Guan et al	2009	Canada	6	1	4	2		8	8	0	0
Harris et al	2010	N. America (Georgia)	2	2	2	2	2	12	10	0	0
Keeler et al	2012	N. America (Georgia)	2	2	3	15	15	90	90	90	90
Keeler et al	2013	N. America (Georgia)	2	11	1	1	1	27	27	0	0
Keeler et al	2014	N. America (Georgia)	3	3	3	38	38	342	342	0	0
Lebarbenchon et al	2011	N. America (Georgia)	2	2	5	3	2	18	18	18	0
Lebarbenchon et al	2012	N. America (Georgia)	3	5	5	1	1	25	25	0	0
Mihai et al	2011	Romania	1	1	3	3	3	27	9	9	9
Nazir et al	2010	Germany	3	3	5	3	3	45	45	45	45
Nazir et al	2010b	Germany	3	3	5	1	1	20	20	20	20
Nazir et al	2011	Germany	3	3	4			32	32	0	0
Negovetich and Webster	2010	N. America	1	7	2			3	0	0	21
Nielsen et al	2013	Denmark	2	2	3	1	3	35	16	0	0
Shoham et al	2012	Japan	2	2	2	3	3	12	12	12	12
Stallknecht	2010	N. America (Georgia)	2	4	1	1	1	4	4	4	4
Stallknecht et al	1990	N. America (Louisiana)	3	3	2	8	7	95	22	22	22
Stallknecht et al	1990b	N. America (Louisiana)	5	5	3	1	1	11	11	11	11
Terregino et al	2009	Italy	1	7	2			14		0	21
Webster et al	1978	N. America (Tennessee)	1	1	2	2	2	4	4	4	4
Zarkov & Urumova	2013	Bulgaria	1	1	3	1	1	5	5	5	5
Zarkov	2006	Bulgaria	2	2	5	5	5	20	20	0	0
Zhang et al	2014	China (Wuhan)	2	2	3	4	4	47	47	47	47
TOTALS								**1824**	**873**	**333**	**365**

S2. Model selection table for Zr using showing model structure, AICc and weights for each model, accounting for combinations of the four moderator variables included in the full model.

Model number	Model	AICc	Weights
1	yi ~ 1 + Salinity group + Temperature	409.90	6.07e-01
2	yi ~ 1 + Salinity group + pH + Temperature	411.36	2.92e-01
3	yi ~ 1 + Salinity group + Water type + Temperature	415.84	3.10e-02
4	yi ~ 1 + Temperature	416.08	2.76e-02
5	yi ~ 1 + Salinity group + WT + pH + Temperature	417.37	1.45e-02
6	yi ~ 1 + pH + Temperature	417.79	1.17e-02
7	yi ~ 1 + Water type + Temperature	417.88	1.12e-02
8	yi ~ 1 + Water type + pH + Temperature	419.39	5.26e-03
9	yi ~ 1 + Salinity group	432.53	7.36e-06
10	yi ~ 1 + Salinity group + pH	434.03	3.48e-06
11	yi ~ 1 + Salinity group + Water type	438.20	4.33e-07
12	yi ~ 1	438.81	3.20e-07
13	yi ~ 1 + pH	439.93	1.82e-07
14	yi ~ 1 + Salinity group + Water type+ pH	440.36	1.47e-07
15	yi ~ 1 + Water type	440.48	1.39e-07
16	yi ~ 1 + Water type + pH	442.03	6.39e-08

S3. The full dataset used for the meta-analysis.

Study	Year	ID	Rt	H-type	pH	Temperature	Salinity	Water	WT	Slope	Intercept	R2	n
Brown	2009	BR09	77.7	1	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	176.2	2	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	94.6	3	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	71	4	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	31.6	5	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	57.5	6	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	104.5	7	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	92.4	8	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	54.7	9	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	26.8	10	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	18	11	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	69.8	12	7.2	4	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	6.3	1	7.2	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	8.4	2	7.2	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	6.3	3	7.2	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	9.6	4	7.2	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	6.7	5	7.2	28	5000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	5.2	6	7.2	28	10000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	7.4	7	7.2	28	15000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	10	8	7.2	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	5.6	9	7.2	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	7.7	10	7.2	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	4.8	11	7.2	28	20000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	7.5	12	7.2	28	5000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	51.8	1	7.2	17	0	1	distilled	NA	NA	NA	4

Brown	2009	BR09	54.3	2	7.2	17	10000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	32.4	3	7.2	17	15000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	47	4	7.2	17	20000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	35.2	5	7.2	17	15000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	13.3	6	7.2	17	5000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	78.8	7	7.2	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	34.5	8	7.2	17	5000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	30.4	9	7.2	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	30.8	10	7.2	17	20000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	9.4	11	7.2	17	15000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	22.2	12	7.2	17	20000	1	distilled	NA	NA	NA	4
Brown	2009	BR09	11.9	1	7.4	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	6.6	2	7.4	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	11.7	3	7.4	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	7.9	4	7.4	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	11.2	5	7.8	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	10.9	6	8.6	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	9.6	7	7.8	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	7.7	8	7.8	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	3.3	9	7.4	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	10.1	10	7.4	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	4.6	11	7.4	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	8.7	12	7.4	28	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	55.3	1	7.4	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	32.5	2	7.4	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	34.2	3	7.4	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	47.6	4	7.8	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	41.5	5	8.2	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	32.7	6	8.2	17	0	1	distilled	NA	NA	NA	4

Brown	2009	BR09	74.7	7	7.4	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	36.4	8	7.4	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	30.8	9	7	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	19.9	10	7.4	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	12.3	11	8.2	17	0	1	distilled	NA	NA	NA	4
Brown	2009	BR09	30.7	12	7.4	17	0	1	distilled	NA	NA	NA	4
Brown	2007	BR07	71	5	7.4	17	0	1	distilled	-0.014	5.375	0.28	11
Brown	2007	BR07	53	5	7.4	17	0	1	distilled	-0.019	5.318	0.41	9
Brown	2007	BR07	38	5	7.4	17	0	1	distilled	-0.026	6.265	0.57	9
Brown	2007	BR07	48	5	7.4	17	0	1	distilled	-0.021	4.029	0.12	9
Brown	2007	BR07	36	7	7.4	17	0	1	distilled	-0.028	6.075	0.7	11
Brown	2007	BR07	29	7	7.4	17	0	1	distilled	-0.034	6.517	0.65	9
Brown	2007	BR07	111	7	7.4	17	0	1	distilled	-0.009	5.469	0.15	11
Brown	2007	BR07	32	7	7.4	17	0	1	distilled	-0.031	5.109	0.71	9
Brown	2007	BR07	21	5	7.4	17	15000	1	distilled	-0.047	4.695	0.71	9
Brown	2007	BR07	56	5	7.4	17	15000	1	distilled	-0.018	5.549	0.53	9
Brown	2007	BR07	63	5	7.4	17	15000	1	distilled	-0.016	5.049	0.61	9
Brown	2007	BR07	21	5	7.4	17	15000	1	distilled	-0.048	3.815	0.62	9
Brown	2007	BR07	22	7	7.4	17	15000	1	distilled	-0.045	6.474	0.64	9
Brown	2007	BR07	22	7	7.4	17	15000	1	distilled	-0.045	5.973	0.8	9
Brown	2007	BR07	24	7	7.4	17	15000	1	distilled	-0.042	5.619	0.75	9
Brown	2007	BR07	23	7	7.4	17	15000	1	distilled	-0.043	4.68	0.81	9
Brown	2007	BR07	11	5	7.4	17	30000	1	distilled	-0.095	5.057	0.86	9
Brown	2007	BR07	19	5	7.4	17	30000	1	distilled	-0.063	6	0.85	9
Brown	2007	BR07	14	5	7.4	17	30000	1	distilled	-0.071	5.495	0.84	9
Brown	2007	BR07	10	5	7.4	17	30000	1	distilled	-0.103	4.135	0.89	9
Brown	2007	BR07	15	7	7.4	17	30000	1	distilled	-0.065	6.427	0.9	9
Brown	2007	BR07	18	7	7.4	17	30000	1	distilled	-0.056	6.282	0.97	9
Brown	2007	BR07	18	7	7.4	17	30000	1	distilled	-0.057	5.926	0.91	9

Brown	2007	BR07	29	7	7.4	17	30000	1	distilled	-0.035	5.37	0.75	9
Brown	2007	BR07	20	5	7.4	28	0	1	distilled	-0.051	5.278	0.56	9
Brown	2007	BR07	14	5	7.4	28	0	1	distilled	-0.071	4.767	0.6	9
Brown	2007	BR07	9	5	7.4	28	0	1	distilled	-0.114	6.619	0.9	9
Brown	2007	BR07	6	5	7.4	28	0	1	distilled	-0.167	4.379	0.75	9
Brown	2007	BR07	12	7	7.4	28	0	1	distilled	-0.086	5.49	0.81	9
Brown	2007	BR07	10	7	7.4	28	0	1	distilled	-0.1	6.054	0.82	9
Brown	2007	BR07	11	7	7.4	28	0	1	distilled	-0.09	5.668	0.89	9
Brown	2007	BR07	4	7	7.4	28	0	1	distilled	-0.252	5.611	0.99	9
Brown	2007	BR07	10	5	7.4	28	15000	1	distilled	-0.098	4.691	0.78	9
Brown	2007	BR07	9	5	7.4	28	15000	1	distilled	-0.108	5.65	0.9	9
Brown	2007	BR07	8	5	7.4	28	15000	1	distilled	-0.128	5.199	0.99	9
Brown	2007	BR07	7	5	7.4	28	15000	1	distilled	-0.143	4.352	0.99	9
Brown	2007	BR07	5	7	7.4	28	15000	1	distilled	-0.205	6.409	0.92	9
Brown	2007	BR07	3	7	7.4	28	15000	1	distilled	-0.32	6.006	0.83	9
Brown	2007	BR07	4	7	7.4	28	15000	1	distilled	-0.233	5.744	0.97	9
Brown	2007	BR07	4	7	7.4	28	15000	1	distilled	-0.242	5.153	0.89	9
Brown	2007	BR07	5	5	7.4	28	30000	1	distilled	-0.214	5.31	0.96	9
Brown	2007	BR07	4	5	7.4	28	30000	1	distilled	-0.279	6.184	0.93	9
Brown	2007	BR07	4	5	7.4	28	30000	1	distilled	-0.23	5.364	0.84	9
Brown	2007	BR07	7	5	7.4	28	30000	1	distilled	-0.143	4.071	0.75	9
Brown	2007	BR07	5	7	7.4	28	30000	1	distilled	-0.188	6.306	0.84	9
Brown	2007	BR07	5	7	7.4	28	30000	1	distilled	-0.209	5.934	0.89	9
Brown	2007	BR07	4	7	7.4	28	30000	1	distilled	-0.269	6.258	0.97	9
Brown	2007	BR07	4	7	7.4	28	30000	1	distilled	-0.231	5.08	0.93	9
Davidson	2010	DA10	0.57	9	NA	37	NA	1	NA	-1.7432	8.1459	0.9783	21
Davidson	2010	DA10	0.35	9	NA	37	NA	1	NA	-2.8833	8.2558	0.969	15
Davidson	2010	DA10	0.42	9	NA	37	NA	1	NA	-2.3601	8.5594	0.9227	15
Davidson	2010	DA10	13.14	9	NA	20	NA	1	NA	-0.0761	8.3916	0.6216	27

Davidson	2010	DA10	37.99	9	NA	4	NA	1	NA	-0.7896	8.5425	0.9582	27
Davidson	2010	DA10	13.41	9	7	20	NA	1	NA	-0.5221	7.4446	0.6384	12
Davidson	2010	DA10	28.91	9	7	4	NA	1	NA	-0.2421	7.5388	0.2537	12
Graiver	2009	GR09	55.81	6	8	4	4540	0	NA	-0.01792	6.017503	0.8242	54
Graiver	2009	GR09	55.84	6	8	4	0	1	NA	-0.01791	6.101463	0.7203	54
Graiver	2009	GR09	7.16	6	8	21	4540	0	NA	-0.13966	5.73091	0.9285	54
Graiver	2009	GR09	20.75	6	8	21	0	1	NA	-0.04819	5.83145	0.8944	54
Graiver	2009	GR09	9.25	6	8	37	4540	0	NA	-0.10812	5.05915	0.8478	54
Graiver	2009	GR09	10.03	6	8	37	0	1	NA	-0.09974	5.465102	0.9684	54
Graiver	2009	GR09	24.86	6	6	21	4540	0	NA	-0.04022	4.82495	0.9896	54
Graiver	2009	GR09	48.92	6	6	21	0	1	NA	-0.02044	4.797816	0.8215	54
Graiver	2009	GR09	9.43	6	8	21	4540	0	NA	-0.1061	5.555701	0.9429	54
Graiver	2009	GR09	21.04	6	8	21	0	1	NA	-0.04754	5.827224	0.8889	54
Graiver	2009	GR09	14.5	6	NA	21	2114	0	NA	-0.06896	4.995718	0.9153	54
Graiver	2009	GR09	18.71	6	NA	21	2114	1	NA	-0.05346	5.1411	0.9545	54
Graiver	2009	GR09	9.39	6	NA	21	5025	0	NA	-0.10645	5.55474	0.9441	54
Graiver	2009	GR09	20.62	6	NA	21	5025	1	NA	-0.04851	5.10731	0.8746	54
Graiver	2009	GR09	15.23	6	NA	21	18572	0	NA	-0.06564	4.36691	0.7985	54
Graiver	2009	GR09	17.16	6	NA	21	18572	1	NA	-0.05827	5.163887	0.8401	54
Keeler	2013	KE13	65.9	3	7.2	17	0	1	distilled	NA	NA	NA	48
Keeler	2013	KE13	73.7	3	7.2	17	0	1	distilled	NA	NA	NA	48
Keeler	2013	KE13	78.8	3	7.2	17	0	1	distilled	NA	NA	NA	48
Keeler	2013	KE13	59.1	3	7.2	17	0	1	distilled	NA	NA	NA	48
Keeler	2013	KE13	68.6	8	7.2	17	0	1	distilled	NA	NA	NA	48
Keeler	2013	KE13	75.6	4	7.2	17	0	1	distilled	NA	NA	NA	48
Keeler	2013	KE13	69.2	4	7.2	17	0	1	distilled	NA	NA	NA	48
Keeler	2013	KE13	66.6	4	7.2	17	0	1	distilled	NA	NA	NA	48
Keeler	2013	KE13	46.2	4	7.2	17	0	1	distilled	NA	NA	NA	48
Keeler	2013	KE13	29.2	3	7.2	17	12	1	filtered	NA	NA	NA	39

Keeler	2013	KE13	27.1	3	7.2	17	12	1	filtered	NA	NA	NA	39
Keeler	2013	KE13	36.9	3	7.2	17	12	1	filtered	NA	NA	NA	39
Keeler	2013	KE13	41.4	3	7.2	17	12	1	filtered	NA	NA	NA	39
Keeler	2013	KE13	26.2	8	7.2	17	12	1	filtered	NA	NA	NA	39
Keeler	2013	KE13	24.4	4	7.2	17	12	1	filtered	NA	NA	NA	39
Keeler	2013	KE13	29.3	4	7.2	17	12	1	filtered	NA	NA	NA	39
Keeler	2013	KE13	29.9	4	7.2	17	12	1	filtered	NA	NA	NA	39
Keeler	2013	KE13	25.6	4	7.2	17	12	1	filtered	NA	NA	NA	39
Keeler	2013	KE13	3.7	3	7.2	17	12	1	unfiltered	NA	NA	NA	18
Keeler	2013	KE13	3	3	7.2	17	12	1	unfiltered	NA	NA	NA	18
Keeler	2013	KE13	3.2	3	7.2	17	12	1	unfiltered	NA	NA	NA	15
Keeler	2013	KE13	3.2	3	7.2	17	12	1	unfiltered	NA	NA	NA	18
Keeler	2013	KE13	2.3	8	7.2	17	12	1	unfiltered	NA	NA	NA	9
Keeler	2013	KE13	3.5	4	7.2	17	12	1	unfiltered	NA	NA	NA	15
Keeler	2013	KE13	2.8	4	7.2	17	12	1	unfiltered	NA	NA	NA	12
Keeler	2013	KE13	3.2	4	7.2	17	12	1	unfiltered	NA	NA	NA	12
Keeler	2013	KE13	3.1	4	7.2	17	12	1	unfiltered	NA	NA	NA	15
Nazir	2011	NA11	11	4	7.9	30	NA	0	NA	NA	NA	NA	45
Nazir	2011	NA11	7	5	7.9	30	NA	0	NA	NA	NA	NA	45
Nazir	2011	NA11	5	6	7.9	30	NA	0	NA	NA	NA	NA	45
Nazir	2011	NA11	4	1	7.9	30	NA	0	NA	NA	NA	NA	45
Nazir	2011	NA11	43	4	7.9	10	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	47	5	7.9	10	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	54	6	7.9	10	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	19	1	7.9	10	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	18	4	7.9	20	NA	0	NA	NA	NA	NA	45
Nazir	2011	NA11	13	5	7.9	20	NA	0	NA	NA	NA	NA	45
Nazir	2011	NA11	17	6	7.9	20	NA	0	NA	NA	NA	NA	45
Nazir	2011	NA11	16	1	7.9	20	NA	0	NA	NA	NA	NA	45

Nazir	2011	NA11	66	4	7.9	0	NA	0	NA	NA	NA	NA	39
Nazir	2011	NA11	118	5	7.9	0	NA	0	NA	NA	NA	NA	39
Nazir	2011	NA11	394	6	7.9	0	NA	0	NA	NA	NA	NA	39
Nazir	2011	NA11	46	1	7.9	0	NA	0	NA	NA	NA	NA	39
Nazir	2011	NA11	2	4	NA	30	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	2	5	NA	30	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	2	6	NA	30	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	1	1	NA	30	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	21	4	NA	10	NA	0	NA	NA	NA	NA	36
Nazir	2011	NA11	16	5	NA	10	NA	0	NA	NA	NA	NA	36
Nazir	2011	NA11	14	6	NA	10	NA	0	NA	NA	NA	NA	36
Nazir	2011	NA11	18	1	NA	10	NA	0	NA	NA	NA	NA	36
Nazir	2011	NA11	4	4	NA	20	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	4	5	NA	20	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	7	6	NA	20	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	4	1	NA	20	NA	0	NA	NA	NA	NA	42
Nazir	2011	NA11	60	4	NA	0	NA	0	NA	NA	NA	NA	39
Nazir	2011	NA11	75	5	NA	0	NA	0	NA	NA	NA	NA	39
Nazir	2011	NA11	52	6	NA	0	NA	0	NA	NA	NA	NA	39
Nazir	2011	NA11	47	1	NA	0	NA	0	NA	NA	NA	NA	39
Nazir	2010	NA10A	8	4	7.8	30	0	1	distilled	-0.129	5.224	0.917	20
Nazir	2010	NA10A	5	5	7.8	30	0	1	distilled	-0.198	5.05	0.916	20
Nazir	2010	NA10A	13	6	7.8	30	0	1	distilled	-0.078	5.587	0.828	20
Nazir	2010	NA10A	15	4	7.8	20	0	1	distilled	-0.069	5.764	0.869	18
Nazir	2010	NA10A	14	5	7.8	20	0	1	distilled	-0.071	5.026	0.864	18
Nazir	2010	NA10A	37	6	7.8	20	0	1	distilled	-0.027	5.485	0.904	18
Nazir	2010	NA10A	121	4	7.8	10	0	1	distilled	-0.008	4.709	0.83	18
Nazir	2010	NA10A	85	5	7.8	10	0	1	distilled	-0.012	5.019	0.909	18
Nazir	2010	NA10A	197	6	7.8	10	0	1	distilled	-0.005	5.512	0.851	18

Nazir	2010	NA10A	443	4	7.8	0	0	1	distilled	-0.002	5.059	0.79	22
Nazir	2010	NA10A	383	5	7.8	0	0	1	distilled	-0.003	5.967	0.526	22
Nazir	2010	NA10A	558	6	7.8	0	0	1	distilled	-0.002	5.429	0.721	22
Nazir	2010	NA10A	642	4	7.8	-10	0	1	distilled	-0.002	5.008	0.654	38
Nazir	2010	NA10A	576	5	7.8	-10	0	1	distilled	-0.002	4.996	0.667	38
Nazir	2010	NA10A	594	6	7.8	-10	0	1	distilled	-0.002	5.425	0.799	38
Nazir	2010	NA10A	6	4	7.2	30	9000	1	sterilised	-0.172	4.426	0.915	20
Nazir	2010	NA10A	6	5	7.2	30	9000	1	sterilised	-0.179	4.63	0.943	20
Nazir	2010	NA10A	2	6	7.2	30	9000	1	sterilised	-0.419	5.869	0.956	20
Nazir	2010	NA10A	12	4	7.2	20	9000	1	sterilised	-0.084	5.316	0.954	18
Nazir	2010	NA10A	13	5	7.2	20	9000	1	sterilised	-0.079	5.13	0.948	18
Nazir	2010	NA10A	7	6	7.2	20	9000	1	sterilised	-0.146	5.312	0.958	18
Nazir	2010	NA10A	59	4	7.2	10	9000	1	sterilised	-0.017	5.014	0.929	18
Nazir	2010	NA10A	68	5	7.2	10	9000	1	sterilised	-0.015	4.725	0.87	18
Nazir	2010	NA10A	70	6	7.2	10	9000	1	sterilised	-0.014	4.911	0.91	18
Nazir	2010	NA10A	190	4	7.2	0	9000	1	sterilised	-0.005	5.155	0.957	22
Nazir	2010	NA10A	185	5	7.2	0	9000	1	sterilised	-0.005	5.244	0.954	22
Nazir	2010	NA10A	169	6	7.2	0	9000	1	sterilised	-0.006	5.44	0.88	22
Nazir	2010	NA10A	321	4	7.2	-10	9000	1	sterilised	-0.003	5.12	0.703	38
Nazir	2010	NA10A	236	5	7.2	-10	9000	1	sterilised	-0.004	5.244	0.913	38
Nazir	2010	NA10A	233	6	7.2	-10	9000	1	sterilised	-0.004	5.488	0.796	38
Nazir	2010	NA10A	2	4	NA	30	NA	1	unfiltered	-0.434	3.479	0.828	14
Nazir	2010	NA10A	2	5	NA	30	NA	1	unfiltered	-0.469	3.57	0.735	12
Nazir	2010	NA10A	2	6	NA	30	NA	1	unfiltered	-0.423	4.037	0.809	20
Nazir	2010	NA10A	4	4	NA	20	NA	1	unfiltered	-0.266	3.522	0.9	14
Nazir	2010	NA10A	3	5	NA	20	NA	1	unfiltered	-0.32	3.522	0.824	12
Nazir	2010	NA10A	3	6	NA	20	NA	1	unfiltered	-0.305	4.704	0.928	18
Nazir	2010	NA10A	14	4	NA	10	NA	1	unfiltered	-0.07	3.536	0.914	16
Nazir	2010	NA10A	10	5	NA	10	NA	1	unfiltered	-0.099	3.485	0.834	12

Nazir	2010	NA10A		14	6	NA		10	NA		1	unfiltered	-0.074	4.35	0.84	18
Nazir	2010	NA10A		31	4	NA		0	NA		1	unfiltered	-0.032	3.909	0.776	14
Nazir	2010	NA10A		35	5	NA		0	NA		1	unfiltered	-0.029	3.328	0.693	22
Nazir	2010	NA10A		34	6	NA		0	NA		1	unfiltered	-0.029	4.374	0.835	22
Nazir	2010	NA10A		55	4	NA		-10	NA		1	unfiltered	-0.018	4.449	0.895	32
Nazir	2010	NA10A		58	5	NA		-10	NA		1	unfiltered	-0.017	4.376	0.893	32
Nazir	2010	NA10A		66	6	NA		-10	NA		1	unfiltered	-0.015	5.313	0.788	38
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.031	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.038	NA	NA	21
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.028	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.026	NA	NA	24
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.039	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.039	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.026	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.031	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.041	NA	NA	21
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.029	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.025	NA	NA	24
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.031	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.028	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		37	NA		0	NA	-0.036	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		55	NA		0	NA	-0.031	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		55	NA		0	NA	-0.053	NA	NA	21
Negovetich	2010	NE10	NA		2	NA		55	NA		0	NA	-0.047	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		55	NA		0	NA	-0.042	NA	NA	24
Negovetich	2010	NE10	NA		2	NA		55	NA		0	NA	-0.036	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		55	NA		0	NA	-0.047	NA	NA	18
Negovetich	2010	NE10	NA		2	NA		55	NA		0	NA	-0.032	NA	NA	18
Nielsen	2013	NI13		5	5		7.1	4		0	1	filtered	NA	NA	NA	30

Nielsen	2013	NI13	4	5	7.1	4	8000	1	filtered	NA	NA	NA	36
Nielsen	2013	NI13	5	5	7.1	4	20000	1	filtered	NA	NA	NA	45
Nielsen	2013	NI13	11	7	7.1	4	0	1	filtered	NA	NA	NA	27
Nielsen	2013	NI13	7	7	7.1	4	8000	1	filtered	NA	NA	NA	36
Nielsen	2013	NI13	4	7	7.1	4	20000	1	filtered	NA	NA	NA	33
Nielsen	2013	NI13	90	5	7.1	4	8000	1	sterilised	NA	NA	NA	45
Nielsen	2013	NI13	14	5	7.1	17	8000	1	sterilised	NA	NA	NA	45
Nielsen	2013	NI13	4	5	7.1	25	8000	1	sterilised	NA	NA	NA	45
Nielsen	2013	NI13	55	5	7.1	4	20000	1	sterilised	NA	NA	NA	54
Nielsen	2013	NI13	34	5	7.1	17	20000	1	sterilised	NA	NA	NA	54
Nielsen	2013	NI13	53	7	7.1	4	8000	1	sterilised	NA	NA	NA	45
Nielsen	2013	NI13	26	7	7.1	17	8000	1	sterilised	NA	NA	NA	45
Nielsen	2013	NI13	11	7	7.1	25	8000	1	sterilised	NA	NA	NA	45
Nielsen	2013	NI13	104	7	7.1	4	20000	1	sterilised	NA	NA	NA	54
Nielsen	2013	NI13	14	7	7.1	17	20000	1	sterilised	NA	NA	NA	54
Nielsen	2013	NI13	5	7	7.1	25	20000	1	sterilised	NA	NA	NA	54
Stallknecht	1990	ST90A	4.35	6	6.2	28	20000	1	distilled	-0.23	4.79	0.9	6
Stallknecht	1990	ST90A	3.45	6	7.2	28	20000	1	distilled	-0.29	5.06	0.93	5
Stallknecht	1990	ST90A	1.49	6	8.2	28	20000	1	distilled	-0.67	5.31	0.99	3
Stallknecht	1990	ST90A	2	6	6.2	28	0	1	distilled	-0.5	3.62	0.86	3
Stallknecht	1990	ST90A	7.69	6	7.2	28	0	1	distilled	-0.13	4.75	0.98	6
Stallknecht	1990	ST90A	8.33	6	8.2	28	0	1	distilled	-0.12	5.09	0.99	6
Stallknecht	1990	ST90A	12.5	6	6.2	17	20000	1	distilled	-0.08	5.37	0.9	6
Stallknecht	1990	ST90A	9.09	6	7.2	17	20000	1	distilled	-0.11	5.48	0.96	6
Stallknecht	1990	ST90A	5.26	6	8.2	17	20000	1	distilled	-0.19	5.48	0.98	6
Stallknecht	1990	ST90A	3.85	6	6.2	17	0	1	distilled	-0.26	441	0.97	5
Stallknecht	1990	ST90A	9.09	6	7.2	17	0	1	distilled	-0.11	5.48	0.96	6
Stallknecht	1990	ST90A	16.67	6	8.2	17	0	1	distilled	-0.06	5.03	0.9	6
Stallknecht	1990	ST90A	9.09	6	8.2	17	500	1	sterilised	-0.11	5.07	0.85	7

Stallknecht	1990	ST90A	9.09	6	7.7	17	2200	1	sterilised	-0.11	5.09	0.88	7
Stallknecht	1990	ST90A	2.5	6	7.8	17	19900	1	sterilised	-0.4	5.35	0.97	7
Stallknecht	1990	ST90A	5.88	6	7.4	17	2400	1	sterilised	-0.17	5.19	0.97	7
Stallknecht	1990	ST90A	8.33	6	7.1	17	0	1	distilled	-0.12	5.19	0.91	7
Stallknecht	1990	ST90A	3.13	6	8.2	28	500	1	sterilised	-0.32	5.23	0.93	7
Stallknecht	1990	ST90A	2.94	6	7.7	28	2200	1	sterilised	-0.34	4.92	0.94	7
Stallknecht	1990	ST90A	1.59	6	7.8	28	19900	1	sterilised	-0.63	5.06	0.99	7
Stallknecht	1990	ST90A	2.63	6	7.4	28	2400	1	sterilised	-0.38	5.09	0.98	7
Stallknecht	1990	ST90A	3.57	6	7.1	28	0	1	distilled	-0.28	4.96	0.96	7
Stallknecht	1990	ST90B	32.26	3	NA	17	NA	1	distilled	-0.031	5.11	0.83	16
Stallknecht	1990	ST90B	10.87	3	NA	28	NA	1	distilled	-0.092	5.38	0.85	16
Stallknecht	1990	ST90B	35.71	4	NA	17	NA	1	distilled	-0.028	4.55	0.7	16
Stallknecht	1990	ST90B	13.33	4	NA	28	NA	1	distilled	-0.075	4.72	0.97	16
Stallknecht	1990	ST90B	35.71	6	NA	17	NA	1	distilled	-0.028	4.55	0.78	16
Stallknecht	1990	ST90B	15.38	6	NA	28	NA	1	distilled	-0.065	4.78	0.89	16
Stallknecht	1990	ST90B	20.83	12	NA	17	NA	1	distilled	-0.048	4.54	0.66	16
Stallknecht	1990	ST90B	5.08	12	NA	28	NA	1	distilled	-0.197	5.8	0.83	16
Stallknecht	1990	ST90B	2.44	10	NA	17	NA	1	distilled	-0.41	5.6	0.89	16
Stallknecht	1990	ST90B	1.69	10	NA	28	NA	1	distilled	-0.59	5.32	0.83	16
Stallknecht	1990	ST90B	250	10	NA	4	NA	1	distilled	-0.004	4.84	0.15	8
Zhang	2014	ZH14	14.29	5	6.95	4	989.44	1	unfiltered	-0.07	2.5	0.94	6
Zhang	2014	ZH14	5.56	5	6.95	16	989.44	1	unfiltered	-0.18	2.4	0.96	6
Zhang	2014	ZH14	1.22	5	6.95	28	989.44	1	unfiltered	-0.82	2.6	0.97	6
Zhang	2014	ZH14	20	5	6.95	4	989.44	1	filtered	-0.05	2.5	0.96	6
Zhang	2014	ZH14	7.69	5	6.95	16	989.44	1	filtered	-0.13	2.5	0.95	6
Zhang	2014	ZH14	1.47	5	6.95	28	989.44	1	filtered	-0.68	2.2	0.95	6
Zhang	2014	ZH14	16.67	9	6.95	4	989.44	1	unfiltered	-0.06	2.1	0.96	6
Zhang	2014	ZH14	5.88	9	6.95	16	989.44	1	unfiltered	-0.17	2.2	0.89	6
Zhang	2014	ZH14	1.43	9	6.95	28	989.44	1	unfiltered	-0.7	2.3	0.94	6

Zhang	2014	ZH14	25	9	6.95	4	989.44	1	filtered	-0.04	2	0.98	6
Zhang	2014	ZH14	7.69	9	6.95	16	989.44	1	filtered	-0.13	1.7	0.85	6
Zhang	2014	ZH14	1.67	9	6.95	28	989.44	1	filtered	-0.6	2.2	0.95	6
Zhang	2014	ZH14	14.29	5	7.06	4	416.64	1	unfiltered	-0.07	2.4	0.97	6
Zhang	2014	ZH14	5	5	7.06	16	416.64	1	unfiltered	-0.2	2.5	0.98	6
Zhang	2014	ZH14	1.19	5	7.06	28	416.64	1	unfiltered	-0.84	2.6	0.96	6
Zhang	2014	ZH14	20	5	7.06	4	416.64	1	filtered	-0.05	2.3	0.98	6
Zhang	2014	ZH14	8.33	5	7.06	16	416.64	1	filtered	-0.12	2.3	0.96	6
Zhang	2014	ZH14	1.67	5	7.06	28	416.64	1	filtered	-0.66	2.2	0.97	6
Zhang	2014	ZH14	14.29	9	7.06	4	416.64	1	unfiltered	-0.07	2.1	0.97	6
Zhang	2014	ZH14	5.56	9	7.06	16	416.64	1	unfiltered	-0.18	2	0.97	6
Zhang	2014	ZH14	1.39	9	7.06	28	416.64	1	unfiltered	-0.72	2.4	0.95	6
Zhang	2014	ZH14	25	9	7.06	4	416.64	1	filtered	-0.04	2	0.97	6
Zhang	2014	ZH14	7.69	9	7.06	16	416.64	1	filtered	-0.13	2	0.92	6
Zhang	2014	ZH14	1.79	9	7.06	28	416.64	1	filtered	-0.56	2.2	0.97	6
Zhang	2014	ZH14	12.5	5	6.93	4	227.2	1	unfiltered	-0.08	2.3	0.94	6
Zhang	2014	ZH14	5.88	5	6.93	16	227.2	1	unfiltered	-0.17	2.3	0.98	6
Zhang	2014	ZH14	1.16	5	6.93	28	227.2	1	unfiltered	-0.86	2.8	0.97	6
Zhang	2014	ZH14	16.67	5	6.93	4	227.2	1	filtered	-0.06	2.4	0.98	6
Zhang	2014	ZH14	7.69	5	6.93	16	227.2	1	filtered	-0.13	2.1	0.97	6
Zhang	2014	ZH14	1.47	5	6.93	28	227.2	1	filtered	-0.68	2.3	0.97	6
Zhang	2014	ZH14	16.67	9	6.93	4	227.2	1	unfiltered	-0.06	2.1	0.97	6
Zhang	2014	ZH14	5.26	9	6.93	16	227.2	1	unfiltered	-0.19	2.1	0.93	6
Zhang	2014	ZH14	1.39	9	6.93	28	227.2	1	unfiltered	-0.72	2.5	0.92	6
Zhang	2014	ZH14	20	9	6.93	4	227.2	1	filtered	-0.05	2	0.94	6
Zhang	2014	ZH14	6.67	9	6.93	16	227.2	1	filtered	-0.15	1.9	0.95	6
Zhang	2014	ZH14	1.69	9	6.93	28	227.2	1	filtered	-0.59	2.3	0.98	6
Zhang	2014	ZH14	7.69	5	8.89	4	18060.8	1	unfiltered	-0.13	2.7	0.93	6
Zhang	2014	ZH14	2.94	5	8.89	16	18060.8	1	unfiltered	-0.34	2.1	0.95	6

Zhang	2014	ZH14	1.16	5	8.89	28	18060.8	1	unfiltered	-0.86	2.8	0.9	6
Zhang	2014	ZH14	8.33	5	8.89	4	18060.8	1	filtered	-0.12	2.7	0.93	6
Zhang	2014	ZH14	2.94	5	8.89	16	18060.8	1	filtered	-0.34	2.5	0.99	6
Zhang	2014	ZH14	1.19	5	8.89	28	18060.8	1	filtered	-0.84	2.3	0.96	6
Zhang	2014	ZH14	11.11	9	8.89	4	18060.8	1	unfiltered	-0.09	2.1	0.89	6
Zhang	2014	ZH14	4.76	9	8.89	16	18060.8	1	unfiltered	-0.21	1.8	0.96	6
Zhang	2014	ZH14	1.43	9	8.89	28	18060.8	1	unfiltered	-0.7	2.3	0.97	6
Zhang	2014	ZH14	11.11	9	8.89	4	18060.8	1	filtered	-0.09	2.1	0.93	6
Zhang	2014	ZH14	4.55	9	8.89	16	18060.8	1	filtered	-0.22	2.1	0.98	6
Zhang	2014	ZH14	1.47	9	8.89	28	18060.8	1	filtered	-0.68	2.3	0.96	6
Keeler	2012	KE12	21.2	3	7.2	10	40	1	filtered	-0.0472	5.3	0.613	12
Keeler	2012	KE12	22.8	3	7.2	17	40	1	filtered	-0.0439	4.71	0.471	12
Keeler	2012	KE12	2.1	3	7.2	28	40	1	filtered	-0.476	4.61	0.977	12
Keeler	2012	KE12	16.8	4	7.2	10	40	1	filtered	-0.0594	4.55	0.77	12
Keeler	2012	KE12	16.5	4	7.2	17	40	1	filtered	-0.0607	4.51	0.951	12
Keeler	2012	KE12	1.6	4	7.2	28	40	1	filtered	-0.612	4.8	0.33	12
Keeler	2012	KE12	24.9	3	7.3	10	40	1	filtered	-0.0401	6.56	0.972	12
Keeler	2012	KE12	20.6	3	7.3	17	40	1	filtered	-0.0486	6.32	0.993	12
Keeler	2012	KE12	6.7	3	7.3	28	40	1	filtered	-0.149	6.08	0.918	12
Keeler	2012	KE12	32.4	4	7.3	10	40	1	filtered	-0.0309	5.01	0.823	12
Keeler	2012	KE12	25.7	4	7.3	17	40	1	filtered	-0.0389	4.37	0.727	12
Keeler	2012	KE12	6.9	4	7.3	28	40	1	filtered	-0.144	5.25	0.753	12
Keeler	2012	KE12	15.8	3	7.4	10	110	1	filtered	-0.0633	6.65	0.961	12
Keeler	2012	KE12	11.9	3	7.4	17	110	1	filtered	-0.084	6.1	0.914	12
Keeler	2012	KE12	7.9	3	7.4	28	110	1	filtered	-0.127	5.67	0.767	12
Keeler	2012	KE12	23.2	4	7.4	10	110	1	filtered	-0.0431	4.67	0.57	12
Keeler	2012	KE12	9.6	4	7.4	17	110	1	filtered	-0.104	4.85	0.86	12
Keeler	2012	KE12	8.1	4	7.4	28	110	1	filtered	-0.123	3.1	0.551	12
Keeler	2012	KE12	58.1	3	6.8	10	60	1	filtered	-0.0172	5.71	0.517	12

Keeler	2012	KE12	44.8	3	6.8	17	60	1	filtered	-0.0223	5.44	0.753	12
Keeler	2012	KE12	6.5	3	6.8	28	60	1	filtered	-0.153	6.09	0.997	12
Keeler	2012	KE12	61.4	4	6.8	10	60	1	filtered	-0.0163	4.72	0.351	12
Keeler	2012	KE12	37.2	4	6.8	17	60	1	filtered	-0.0269	4.99	0.679	12
Keeler	2012	KE12	9.7	4	6.8	28	60	1	filtered	-0.103	4.96	0.887	12
Keeler	2012	KE12	57.5	3	7.3	10	70	1	filtered	-0.0174	5.63	0.593	12
Keeler	2012	KE12	43.9	3	7.3	17	70	1	filtered	-0.0228	5.46	0.709	12
Keeler	2012	KE12	7.8	3	7.3	28	70	1	filtered	-0.129	5.84	0.97	12
Keeler	2012	KE12	61.7	4	7.3	10	70	1	filtered	-0.0162	4.74	0.552	12
Keeler	2012	KE12	44.8	4	7.3	17	70	1	filtered	-0.0223	4.59	0.779	12
Keeler	2012	KE12	10	4	7.3	28	70	1	filtered	-0.0998	4.85	0.823	12
Keeler	2012	KE12	88.5	3	7.4	10	60	1	filtered	-0.0113	5.77	0.667	12
Keeler	2012	KE12	47.4	3	7.4	17	60	1	filtered	-0.0211	5.89	0.863	12
Keeler	2012	KE12	9.5	3	7.4	28	60	1	filtered	-0.105	5.69	0.793	12
Keeler	2012	KE12	68	4	7.4	10	60	1	filtered	-0.0147	4.52	0.629	12
Keeler	2012	KE12	73	4	7.4	17	60	1	filtered	-0.0137	4.69	0.606	12
Keeler	2012	KE12	12.7	4	7.4	28	60	1	filtered	-0.0789	4.51	0.987	12
Keeler	2012	KE12	5.7	3	4.7	10	30	1	filtered	-0.177	2.94	0.646	12
Keeler	2012	KE12	3.9	3	4.7	17	30	1	filtered	-0.257	2.88	0.522	12
Keeler	2012	KE12	1.9	3	4.7	28	30	1	filtered	-0.525	4.55	0.829	12
Keeler	2012	KE12	2.6	4	4.7	10	30	1	filtered	-0.383	3.65	0.816	12
Keeler	2012	KE12	4.1	4	4.7	17	30	1	filtered	-0.242	3.54	0.493	12
Keeler	2012	KE12	1.2	4	4.7	28	30	1	filtered	-0.805	3.52	0.835	12
Keeler	2012	KE12	51	3	6.9	10	60	1	filtered	-0.0196	5.55	0.584	12
Keeler	2012	KE12	49.8	3	6.9	17	60	1	filtered	-0.0201	5.52	0.535	12
Keeler	2012	KE12	7	3	6.9	28	60	1	filtered	-0.142	5.78	0.894	12
Keeler	2012	KE12	79.4	4	6.9	10	60	1	filtered	-0.0126	4.64	0.748	12
Keeler	2012	KE12	66.7	4	6.9	17	60	1	filtered	-0.015	4.58	0.658	12
Keeler	2012	KE12	11.6	4	6.9	28	60	1	filtered	-0.0865	4.86	1	12

Keeler	2012	KE12	30.6	3	7.6	10	2800	1	filtered	-0.0327	4.9	0.693	12
Keeler	2012	KE12	18.3	3	7.6	17	2800	1	filtered	-0.0548	5.27	0.837	12
Keeler	2012	KE12	5.1	3	7.6	28	2800	1	filtered	-0.197	5.63	0.998	12
Keeler	2012	KE12	24.1	4	7.6	10	2800	1	filtered	-0.0415	3.51	0.58	12
Keeler	2012	KE12	14.7	4	7.6	17	2800	1	filtered	-0.0679	4.23	0.854	12
Keeler	2012	KE12	6.9	4	7.6	28	2800	1	filtered	-0.144	3.93	0.752	12
Keeler	2012	KE12	12.9	3	4.8	10	2740	1	filtered	-0.0773	4.91	0.789	12
Keeler	2012	KE12	18	3	4.8	17	2740	1	filtered	-0.0557	4.83	0.853	12
Keeler	2012	KE12	2.1	3	4.8	28	2740	1	filtered	-0.478	4.68	0.893	12
Keeler	2012	KE12	11.8	4	4.8	10	2740	1	filtered	-0.0846	4	0.924	12
Keeler	2012	KE12	10.6	4	4.8	17	2740	1	filtered	0.0943	4.04	0.155	12
Keeler	2012	KE12	2.2	4	4.8	28	2740	1	filtered	-0.458	3.98	0.917	12
Keeler	2012	KE12	0.7	3	4.3	10	50	1	filtered	-1.39	4.03	0.864	12
Keeler	2012	KE12	0.8	3	4.3	17	50	1	filtered	-1.25	3.82	0.814	12
Keeler	2012	KE12	0.3	3	4.3	28	50	1	filtered	-3.16	3.9	0.938	12
Keeler	2012	KE12	3.8	4	4.3	10	50	1	filtered	-0.263	2.15	0.407	12
Keeler	2012	KE12	0.7	4	4.3	17	50	1	filtered	-1.53	3.21	0.939	12
Keeler	2012	KE12	0.2	4	4.3	28	50	1	filtered	-6.32	3.5	0.683	12
Keeler	2012	KE12	0.9	3	4.2	10	50	1	filtered	-1.14	4.09	0.509	12
Keeler	2012	KE12	1	3	4.2	17	50	1	filtered	-1.04	3.8	0.903	12
Keeler	2012	KE12	0.7	3	4.2	28	50	1	filtered	-1.48	3.56	0.648	12
Keeler	2012	KE12	3.6	4	4.2	10	50	1	filtered	-0.276	2.66	0.583	12
Keeler	2012	KE12	1.8	4	4.2	17	50	1	filtered	-0.555	3.24	0.964	12
Keeler	2012	KE12	0.2	4	4.2	28	50	1	filtered	-6.44	3.97	0.96	12
Keeler	2012	KE12	73	3	7.2	10	120	1	filtered	-0.0137	5.91	0.515	12
Keeler	2012	KE12	41.3	3	7.2	17	120	1	filtered	-0.0242	5.94	0.93	12
Keeler	2012	KE12	9.2	3	7.2	28	120	1	filtered	-0.109	5.72	0.942	12
Keeler	2012	KE12	62.1	4	7.2	10	120	1	filtered	-0.0161	5.07	0.771	12
Keeler	2012	KE12	48.8	4	7.2	17	120	1	filtered	-0.0205	4.55	0.577	12

Keeler	2012	KE12	10	4	7.2	28	120	1	filtered	-0.0998	5.18	0.886	12
Keeler	2012	KE12	70.9	3	7.4	10	80	1	filtered	-0.0141	5.87	0.774	12
Keeler	2012	KE12	51.3	3	7.4	17	80	1	filtered	-0.0195	5.72	0.787	12
Keeler	2012	KE12	18.3	3	7.4	28	80	1	filtered	-0.0546	4.9	0.608	12
Keeler	2012	KE12	63.3	4	7.4	10	80	1	filtered	-0.0158	4.87	0.426	12
Keeler	2012	KE12	48.1	4	7.4	17	80	1	filtered	-0.0208	4.94	0.89	12
Keeler	2012	KE12	11.1	4	7.4	28	80	1	filtered	-0.0901	4.99	0.995	12
Keeler	2012	KE12	26	3	7.6	10	130	1	filtered	-0.0385	5.97	0.816	12
Keeler	2012	KE12	28.1	3	7.6	17	130	1	filtered	-0.0356	5.49	0.646	12
Keeler	2012	KE12	7	3	7.6	28	130	1	filtered	-0.143	5.68	0.784	12
Keeler	2012	KE12	50.3	4	7.6	10	130	1	filtered	-0.0199	4.83	0.758	12
Keeler	2012	KE12	52.9	4	7.6	17	130	1	filtered	-0.0189	4.66	0.508	12
Keeler	2012	KE12	8.2	4	7.6	28	130	1	filtered	-0.122	5.09	0.844	12
Lebarbenchon	2012	LE12	245	3	7.2	4	0	1	distilled	NA	NA	NA	48
Lebarbenchon	2012	LE12	323	4	7.2	4	0	1	distilled	NA	NA	NA	48
Lebarbenchon	2012	LE12	346	6	7.2	4	0	1	distilled	NA	NA	NA	48
Lebarbenchon	2012	LE12	274	6	7.2	4	0	1	distilled	NA	NA	NA	48
Lebarbenchon	2012	LE12	309	6	7.2	4	0	1	distilled	NA	NA	NA	48
Lebarbenchon	2012	LE12	202	3	7.2	10	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	242	4	7.2	10	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	241	6	7.2	10	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	159	6	7.2	10	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	272	6	7.2	10	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	83	3	7.2	17	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	109	4	7.2	17	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	80	6	7.2	17	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	61	6	7.2	17	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	72	6	7.2	17	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	44	3	7.2	23	0	1	distilled	NA	NA	NA	24

Lebarbenchon	2012	LE12	47	4	7.2	23	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	45	6	7.2	23	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	43	6	7.2	23	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	44	6	7.2	23	0	1	distilled	NA	NA	NA	24
Lebarbenchon	2012	LE12	14	3	7.2	28	0	1	distilled	NA	NA	NA	20
Lebarbenchon	2012	LE12	13	4	7.2	28	0	1	distilled	NA	NA	NA	20
Lebarbenchon	2012	LE12	14	6	7.2	28	0	1	distilled	NA	NA	NA	20
Lebarbenchon	2012	LE12	13	6	7.2	28	0	1	distilled	NA	NA	NA	20
Lebarbenchon	2012	LE12	10	6	7.2	28	0	1	distilled	NA	NA	NA	20
Lebarbenchon	2011	LE11	13	3	6.2	17	0	1	distilled	NA	NA	0.86	24
Lebarbenchon	2011	LE11	6	3	6.2	28	0	1	distilled	NA	NA	0.6	20
Lebarbenchon	2011	LE11	211	3	7.2	4	0	1	distilled	NA	NA	0.86	56
Lebarbenchon	2011	LE11	175	3	7.2	10	0	1	distilled	NA	NA	0.86	24
Lebarbenchon	2011	LE11	79	3	7.2	17	0	1	distilled	NA	NA	0.78	24
Lebarbenchon	2011	LE11	51	3	7.2	23	0	1	distilled	NA	NA	0.85	24
Lebarbenchon	2011	LE11	11	3	7.2	28	0	1	distilled	NA	NA	0.95	20
Lebarbenchon	2011	LE11	22	3	8.2	17	0	1	distilled	NA	NA	0.93	24
Lebarbenchon	2011	LE11	8	3	8.2	28	0	1	distilled	NA	NA	0.84	20
Lebarbenchon	2011	LE11	9	4	6.2	17	0	1	distilled	NA	NA	0.83	24
Lebarbenchon	2011	LE11	3	4	6.2	28	0	1	distilled	NA	NA	0.97	20
Lebarbenchon	2011	LE11	270	4	7.2	4	0	1	distilled	NA	NA	0.78	56
Lebarbenchon	2011	LE11	193	4	7.2	10	0	1	distilled	NA	NA	0.84	24
Lebarbenchon	2011	LE11	102	4	7.2	17	0	1	distilled	NA	NA	0.61	24
Lebarbenchon	2011	LE11	51	4	7.2	23	0	1	distilled	NA	NA	0.86	24
Lebarbenchon	2011	LE11	13	4	7.2	28	0	1	distilled	NA	NA	0.92	20
Lebarbenchon	2011	LE11	18	4	8.2	17	0	1	distilled	NA	NA	0.77	24
Lebarbenchon	2011	LE11	7	4	8.2	28	0	1	distilled	NA	NA	0.91	20
Zarkov	2006	ZA06	2.98	6	7.76	5.5	11072	1	unfiltered	-0.33593	1.62085	0.9891	4
Zarkov	2006	ZA06	2.98	6	7.34	5.5	1178	1	unfiltered	-0.33593	1.62085	0.9891	4

Zarkov	2006	ZA06	4.23	6	7.78	5.5	417	1	unfiltered	-0.23622	1.6139	0.9872	5
Zarkov	2006	ZA06	8.06	6	7.61	5.5	156	1	unfiltered	-0.12403	1.640695	0.9704	8
Zarkov	2006	ZA06	0.61	6	9.34	11	513	1	unfiltered	-1.63	1.63	NA	2
Zarkov	2006	ZA06	4.12	6	7.76	5.5	11072	1	filtered	-0.24299	1.53756	0.9608	5
Zarkov	2006	ZA06	4.08	6	7.34	5.5	1178	1	filtered	-0.245	1.67	0.943	5
Zarkov	2006	ZA06	5.05	6	7.78	5.5	417	1	filtered	-0.19814	1.7089	0.9661	6
Zarkov	2006	ZA06	10.05	6	7.61	5.5	156	1	filtered	-0.0995	1.5885	0.9894	9
Zarkov	2006	ZA06	0.61	6	9.34	11	513	1	filtered	-1.63	1.63	NA	2
Zarkov	2006	ZA06	1.87	11	7.76	5.5	11072	1	unfiltered	-0.5361	3.1495	0.8481	5
Zarkov	2006	ZA06	3.18	11	7.34	5.5	1178	1	unfiltered	-0.31414	3.2319	0.889	7
Zarkov	2006	ZA06	3.01	11	7.78	5.5	417	1	unfiltered	-0.33203	3.35072	0.9099	7
Zarkov	2006	ZA06	3.75	11	7.61	5.5	156	1	unfiltered	-0.2669	3.9139	0.9481	9
Zarkov	2006	ZA06	0.26	11	9.34	11	513	1	unfiltered	-3.83	3.83	NA	2
Zarkov	2006	ZA06	2.25	11	7.76	5.5	11072	1	filtered	-0.4443	3.73959	0.956	6
Zarkov	2006	ZA06	3.76	11	7.34	5.5	1178	1	filtered	-0.2658	3.2569	0.9205	8
Zarkov	2006	ZA06	4.26	11	7.78	5.5	417	1	filtered	-0.23458	3.33969	0.9416	9
Zarkov	2006	ZA06	4.14	11	7.61	5.5	156	1	filtered	-0.24139	4.25284	0.9507	10
Zarkov	2006	ZA06	0.26	11	9.34	11	513	1	filtered	-3.83	3.83	NA	2
Harris	2010	HA10	94.64	3	7.2	4	0	1	distilled	NA	NA	NA	5
Harris	2010	HA10	31.82	3	7.2	17	0	1	distilled	NA	NA	NA	3
Harris	2010	HA10	13.5	3	6.6	17	0	1	distilled	NA	NA	NA	3
Harris	2010	HA10	23.42	3	6.6	4	NA	0	NA	NA	NA	NA	5
Harris	2010	HA10	4	3	6.6	17	NA	0	NA	NA	NA	NA	3
Harris	2010	HA10	92.42	8	7.2	4	0	1	distilled	NA	NA	NA	5
Harris	2010	HA10	31.82	8	7.2	17	0	1	distilled	NA	NA	NA	3
Harris	2010	HA10	26.74	8	6.6	17	0	1	distilled	NA	NA	NA	3
Harris	2010	HA10	18.35	8	6.6	4	NA	0	NA	NA	NA	NA	5
Harris	2010	HA10	5.51	8	6.6	17	NA	0	NA	NA	NA	NA	3
Webster	1978	WE78	7.33	3	NA	4	NA	0	NA	-0.13649	8.79642	0.6756	9

Webster	1978	WE78	7.47	3	6.8	4	NA	1	unfiltered	-0.13386	8.23925	0.7965	5
Webster	1978	WE78	1.76	3	NA	22	NA	0	NA	-0.5686	7.6691	0.6369	9
Webster	1978	WE78	0.89	3	6.8	22	NA	1	unfiltered	-1.125	8.1	1	5
Stallknecht	2010	ST10	192.31	5	7.4	4	0	1	distilled	-0.0052	5.163	0.844	96
Stallknecht	2010	ST10	256.41	5	7.4	4	0	1	distilled	-0.0039	5.733	0.477	96
Stallknecht	2010	ST10	222.22	7	7.4	4	0	1	distilled	-0.0045	6.237	0.794	96
Stallknecht	2010	ST10	178.57	7	7.4	4	0	1	distilled	-0.0056	5.644	0.669	96
Stallknecht	2010	ST10	45.98	5	7.4	-20	0	1	distilled	NA	NA	NA	2
Nazir	2010	NA10B	4	4	7.84	30	NA	1	unfiltered	-0.253	4.42	0.83	8
Nazir	2010	NA10B	4	5	7.84	30	NA	1	unfiltered	-0.239	5.39	0.98	8
Nazir	2010	NA10B	5	6	7.84	30	NA	1	unfiltered	-0.217	5.76	0.92	8
Nazir	2010	NA10B	7	4	7.84	20	NA	1	unfiltered	-0.141	5.04	0.87	8
Nazir	2010	NA10B	7	5	7.84	20	NA	1	unfiltered	-0.138	5.52	0.88	8
Nazir	2010	NA10B	12	6	7.84	20	NA	1	unfiltered	-0.085	5.62	0.94	8
Nazir	2010	NA10B	14	4	7.84	10	NA	1	unfiltered	-0.073	4.24	0.96	8
Nazir	2010	NA10B	18	5	7.84	10	NA	1	unfiltered	-0.056	4.9	0.81	8
Nazir	2010	NA10B	18	6	7.84	10	NA	1	unfiltered	-0.057	5.35	0.75	8
Nazir	2010	NA10B	74	4	7.84	0	NA	1	unfiltered	-0.014	4.27	0.89	8
Nazir	2010	NA10B	53	5	7.84	0	NA	1	unfiltered	-0.019	5.46	0.82	8
Nazir	2010	NA10B	66	6	7.84	0	NA	1	unfiltered	-0.015	5.66	0.79	8
Nazir	2010	NA10B	160	4	7.84	-10	NA	1	unfiltered	-0.006	2.63	0.89	8
Nazir	2010	NA10B	206	5	7.84	-10	NA	1	unfiltered	-0.005	3.29	0.41	8
Nazir	2010	NA10B	253	6	7.84	-10	NA	1	unfiltered	-0.004	2.73	0.26	8
Keeler	2014	KE14	20.1	3	8.35	10	125	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	12.9	3	7.23	10	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	62.9	3	8.13	10	93	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	10.1	3	8.4	10	42477	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	33.3	3	7.98	10	144	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	7.4	3	7.95	10	37171	1	filtered	NA	NA	NA	14

Keeler	2014	KE14	26.8	3	8.3	10	186	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	29.2	3	8.37	10	299	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	37	3	8.22	10	1889	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	20.7	3	8.01	10	32282	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	19.9	3	8.18	10	13215	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	44	3	8.12	10	3679	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.6	3	7.67	10	561	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	13.2	3	7.99	10	22072	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	81.6	3	8	10	350	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	25.1	3	7.54	10	31	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	41.9	3	7.75	10	176	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	38	3	7.52	10	216	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.6	3	8.12	10	194	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	25.9	3	8.27	10	22844	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.6	3	8.12	10	16333	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5	3	8.17	10	251	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.5	3	7.82	10	192	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	23.2	3	8.21	10	155	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	8.2	3	9.4	10	910	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	16.1	3	8.68	10	1076	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	40.1	3	8.22	10	309	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	14.9	3	8.31	10	29020	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	23.6	3	8.03	10	7576	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	11.2	3	7.74	10	197	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	82	3	8.17	10	217	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	51.5	3	7.76	10	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	13.7	3	6.25	10	17	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	18.4	3	8.95	10	3254	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	92.6	3	8.52	10	346	1	filtered	NA	NA	NA	14

Keeler	2014	KE14	3.2	3	5.84	10	66	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	11.2	3	7.85	10	101	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	51.9	3	8	10	85	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	15.3	3	8.35	17	125	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	14	3	7.23	17	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	25.6	3	8.13	17	93	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.4	3	8.4	17	42477	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	19	3	7.98	17	144	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.4	3	7.95	17	37171	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	23.5	3	8.3	17	186	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	15.9	3	8.37	17	299	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	17.4	3	8.22	17	1889	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	13.6	3	8.01	17	32282	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	12.4	3	8.18	17	13215	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	22.5	3	8.12	17	3679	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.5	3	7.67	17	561	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	10.2	3	7.99	17	22072	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	30.1	3	8	17	350	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	16.3	3	7.54	17	31	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	21.8	3	7.75	17	176	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	26.6	3	7.52	17	216	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.1	3	8.12	17	194	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	15.6	3	8.27	17	22844	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.4	3	8.12	17	16333	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4	3	8.17	17	251	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.5	3	7.82	17	192	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	24.2	3	8.21	17	155	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.7	3	9.4	17	910	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	11.8	3	8.68	17	1076	1	filtered	NA	NA	NA	14

Keeler	2014	KE14	19.2	3	8.22	17	309	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.8	3	8.31	17	29020	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	17.9	3	8.03	17	7576	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.3	3	7.74	17	197	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	44.5	3	8.17	17	217	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	29	3	7.76	17	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	8.9	3	6.25	17	17	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	11.2	3	8.95	17	3254	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	43.1	3	8.52	17	346	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.7	3	5.84	17	66	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.9	3	7.85	17	101	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	38	3	8	17	85	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.3	3	8.35	28	125	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.5	3	7.23	28	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.8	3	8.13	28	93	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.4	3	8.4	28	42477	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.2	3	7.98	28	144	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.8	3	7.95	28	37171	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.1	3	8.3	28	186	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.9	3	8.37	28	299	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6	3	8.22	28	1889	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.4	3	8.01	28	32282	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.7	3	8.18	28	13215	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.8	3	8.12	28	3679	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.2	3	7.67	28	561	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.4	3	7.99	28	22072	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.1	3	8	28	350	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.8	3	7.54	28	31	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.8	3	7.75	28	176	1	filtered	NA	NA	NA	14

Keeler	2014	KE14	3.9	3	7.52	28	216	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.9	3	8.12	28	194	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.9	3	8.27	28	22844	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1	3	8.12	28	16333	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.1	3	8.17	28	251	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.8	3	7.82	28	192	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.7	3	8.21	28	155	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.3	3	9.4	28	910	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.7	3	8.68	28	1076	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.3	3	8.22	28	309	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1	3	8.31	28	29020	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.3	3	8.03	28	7576	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.5	3	7.74	28	197	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.9	3	8.17	28	217	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.5	3	7.76	28	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.7	3	6.25	28	17	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.6	3	8.95	28	3254	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3	3	8.52	28	346	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.3	3	5.84	28	66	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.6	3	7.85	28	101	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.4	3	8	28	85	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	19.6	4	8.35	10	125	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	21	4	7.23	10	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	54.8	4	8.13	10	93	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	8.6	4	8.4	10	42477	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	11.2	4	7.98	10	144	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.5	4	7.95	10	37171	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	25.2	4	8.3	10	186	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	25.3	4	8.37	10	299	1	filtered	NA	NA	NA	14

Keeler	2014	KE14	44	4	8.22	10	1889	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	18.7	4	8.01	10	32282	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	19.3	4	8.18	10	13215	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	45	4	8.12	10	3679	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	7.4	4	7.67	10	561	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	15.3	4	7.99	10	22072	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	69.1	4	8	10	350	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	27.6	4	7.54	10	31	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	28.8	4	7.75	10	176	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	38.8	4	7.52	10	216	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.9	4	8.12	10	194	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	22.5	4	8.27	10	22844	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.6	4	8.12	10	16333	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.2	4	8.17	10	251	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.1	4	7.82	10	192	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	22	4	8.21	10	155	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.4	4	9.4	10	910	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	22.3	4	8.68	10	1076	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	29.7	4	8.22	10	309	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	13.8	4	8.31	10	29020	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	15.4	4	8.03	10	7576	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.4	4	7.74	10	197	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	86.2	4	8.17	10	217	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	46.3	4	7.76	10	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	16.9	4	6.25	10	17	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	22.9	4	8.95	10	3254	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	86.2	4	8.52	10	346	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1	4	5.84	10	66	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	9.7	4	7.85	10	101	1	filtered	NA	NA	NA	14

Keeler	2014	KE14	50	4	8	10	85	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	21.1	4	8.35	17	125	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	15.4	4	7.23	17	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	31	4	8.13	17	93	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.2	4	8.4	17	42477	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	15	4	7.98	17	144	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.9	4	7.95	17	37171	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	26.7	4	8.3	17	186	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	17.5	4	8.37	17	299	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	17.1	4	8.22	17	1889	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	9.7	4	8.01	17	32282	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	8	4	8.18	17	13215	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	29.1	4	8.12	17	3679	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.4	4	7.67	17	561	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	8.3	4	7.99	17	22072	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	32.7	4	8	17	350	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	25.6	4	7.54	17	31	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	25.2	4	7.75	17	176	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	27.6	4	7.52	17	216	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.7	4	8.12	17	194	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	18.1	4	8.27	17	22844	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.1	4	8.12	17	16333	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.4	4	8.17	17	251	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.1	4	7.82	17	192	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	18.8	4	8.21	17	155	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.2	4	9.4	17	910	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	14.4	4	8.68	17	1076	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	17.8	4	8.22	17	309	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.8	4	8.31	17	29020	1	filtered	NA	NA	NA	14

Keeler	2014	KE14	15.2	4	8.03	17	7576	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.1	4	7.74	17	197	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	49.2	4	8.17	17	217	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	37.8	4	7.76	17	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	7.2	4	6.25	17	17	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	11.4	4	8.95	17	3254	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	49.3	4	8.52	17	346	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.9	4	5.84	17	66	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.7	4	7.85	17	101	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	46.4	4	8	17	85	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.8	4	8.35	28	125	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.1	4	7.23	28	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.4	4	8.13	28	93	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.8	4	8.4	28	42477	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.8	4	7.98	28	144	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.5	4	7.95	28	37171	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.6	4	8.3	28	186	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.2	4	8.37	28	299	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.3	4	8.22	28	1889	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.8	4	8.01	28	32282	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.9	4	8.18	28	13215	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	7.2	4	8.12	28	3679	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.2	4	7.67	28	561	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.3	4	7.99	28	22072	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.1	4	8	28	350	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.1	4	7.54	28	31	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.7	4	7.75	28	176	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4	4	7.52	28	216	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.4	4	8.12	28	194	1	filtered	NA	NA	NA	14

Keeler	2014	KE14	2.8	4	8.27	28	22844	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.4	4	8.12	28	16333	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.1	4	8.17	28	251	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.7	4	7.82	28	192	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.5	4	8.21	28	155	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.3	4	9.4	28	910	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.5	4	8.68	28	1076	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3	4	8.22	28	309	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.9	4	8.31	28	29020	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.6	4	8.03	28	7576	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.8	4	7.74	28	197	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.6	4	8.17	28	217	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.5	4	7.76	28	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.5	4	6.25	28	17	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.7	4	8.95	28	3254	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.6	4	8.52	28	346	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.3	4	5.84	28	66	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.2	4	7.85	28	101	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.3	4	8	28	85	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.3	8	8.35	10	125	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	18.2	8	7.23	10	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	71	8	8.13	10	93	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	8.7	8	8.4	10	42477	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	11.2	8	7.98	10	144	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.8	8	7.95	10	37171	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	20.9	8	8.3	10	186	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	7.2	8	8.37	10	299	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	37.3	8	8.22	10	1889	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	17.1	8	8.01	10	32282	1	filtered	NA	NA	NA	14

Keeler	2014	KE14	15.5	8	8.18	10	13215	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	26.5	8	8.12	10	3679	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.1	8	7.67	10	561	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5	8	7.99	10	22072	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	48.4	8	8	10	350	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	8.5	8	7.54	10	31	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	9.2	8	7.75	10	176	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	35.7	8	7.52	10	216	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.2	8	8.12	10	194	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	36.4	8	8.27	10	22844	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.8	8	8.12	10	16333	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.4	8	8.17	10	251	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	7.5	8	7.82	10	192	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	26.4	8	8.21	10	155	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.2	8	9.4	10	910	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	17.9	8	8.68	10	1076	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	24.7	8	8.22	10	309	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	14.3	8	8.31	10	29020	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	12	8	8.03	10	7576	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.9	8	7.74	10	197	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	46.6	8	8.17	10	217	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.9	8	7.76	10	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	8.9	8	6.25	10	17	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	22.1	8	8.95	10	3254	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	20.3	8	8.52	10	346	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.5	8	5.84	10	66	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	9.7	8	7.85	10	101	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	14.3	8	8	10	85	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.7	8	8.35	17	125	1	filtered	NA	NA	NA	14

Keeler	2014	KE14	15.2	8	7.23	17	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	38.8	8	8.13	17	93	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.8	8	8.4	17	42477	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.6	8	7.98	17	144	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.6	8	7.95	17	37171	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	24.9	8	8.3	17	186	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.4	8	8.37	17	299	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	15.2	8	8.22	17	1889	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.3	8	8.01	17	32282	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.3	8	8.18	17	13215	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	16.1	8	8.12	17	3679	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2	8	7.67	17	561	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.4	8	7.99	17	22072	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	20.1	8	8	17	350	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	12.5	8	7.54	17	31	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	9	8	7.75	17	176	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	27	8	7.52	17	216	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.4	8	8.12	17	194	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	25.9	8	8.27	17	22844	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.6	8	8.12	17	16333	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4	8	8.17	17	251	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.5	8	7.82	17	192	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	22.5	8	8.21	17	155	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.1	8	9.4	17	910	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	9.7	8	8.68	17	1076	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	18.9	8	8.22	17	309	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	6.1	8	8.31	17	29020	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	14.7	8	8.03	17	7576	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.3	8	7.74	17	197	1	filtered	NA	NA	NA	14

Keeler	2014	KE14	12.4	8	8.17	17	217	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.1	8	7.76	17	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.1	8	6.25	17	17	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	8.8	8	8.95	17	3254	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	11.8	8	8.52	17	346	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.4	8	5.84	17	66	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	7.4	8	7.85	17	101	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4	8	8	17	85	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.5	8	8.35	28	125	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.8	8	7.23	28	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.9	8	8.13	28	93	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1	8	8.4	28	42477	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.8	8	7.98	28	144	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.7	8	7.95	28	37171	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	33	8	8.3	28	186	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1	8	8.37	28	299	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.7	8	8.22	28	1889	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.9	8	8.01	28	32282	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.3	8	8.18	28	13215	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.2	8	8.12	28	3679	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.2	8	7.67	28	561	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.5	8	7.99	28	22072	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.2	8	8	28	350	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.2	8	7.54	28	31	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.5	8	7.75	28	176	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.7	8	7.52	28	216	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.6	8	8.12	28	194	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.1	8	8.27	28	22844	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.4	8	8.12	28	16333	1	filtered	NA	NA	NA	14

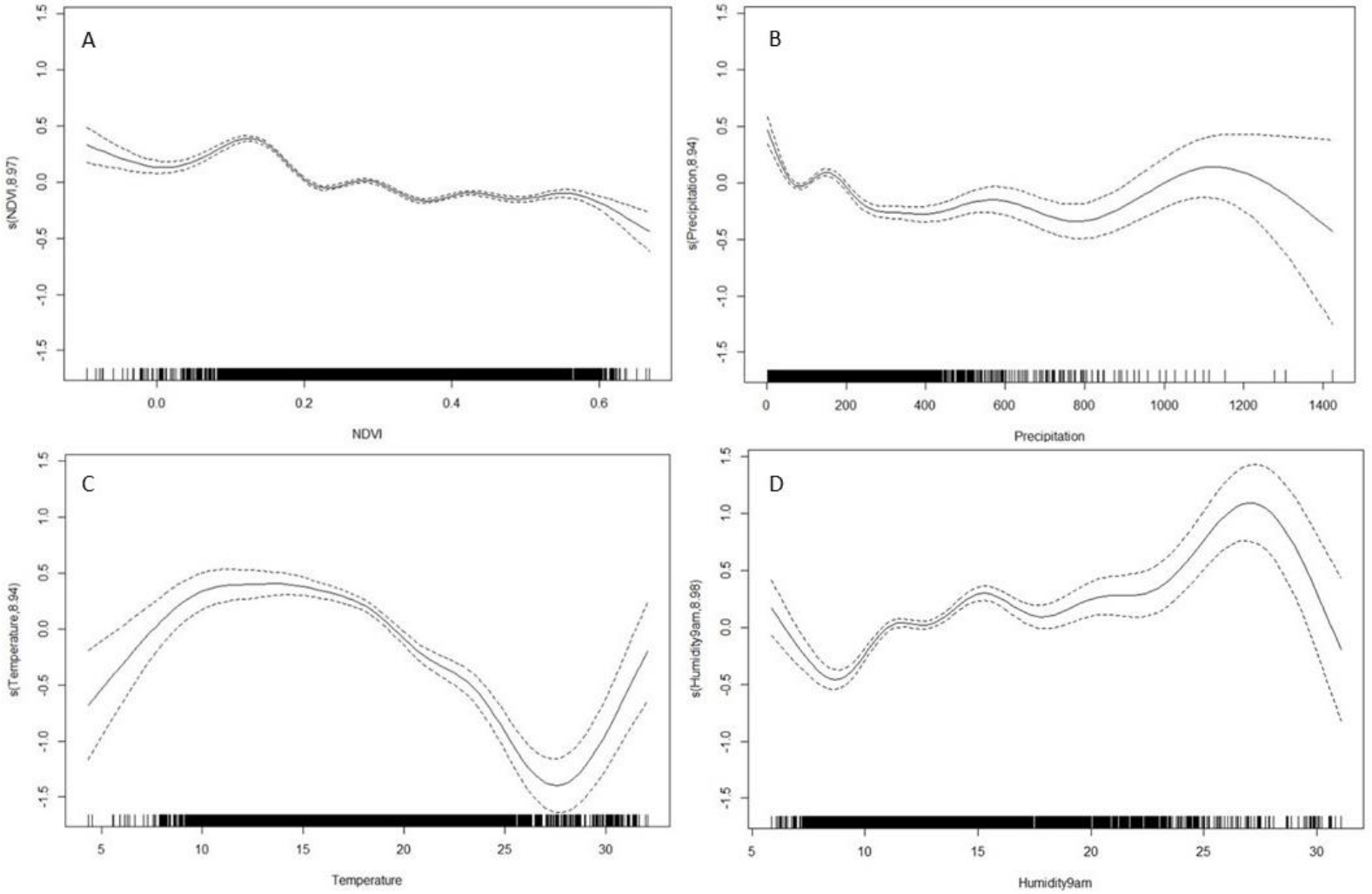
Keeler	2014	KE14	1.8	8	8.17	28	251	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.4	8	7.82	28	192	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.9	8	8.21	28	155	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.4	8	9.4	28	910	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.4	8	8.68	28	1076	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.6	8	8.22	28	309	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.5	8	8.31	28	29020	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.1	8	8.03	28	7576	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.4	8	7.74	28	197	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.5	8	8.17	28	217	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	1.7	8	7.76	28	60	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	4.3	8	6.25	28	17	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	3.2	8	8.95	28	3254	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.9	8	8.52	28	346	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	0.2	8	5.84	28	66	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	2.1	8	7.85	28	101	1	filtered	NA	NA	NA	14
Keeler	2014	KE14	5.6	8	8	28	85	1	filtered	NA	NA	NA	14
Terregino	2009	TE09	1.847473	7	NA	37	NA	0	NA	-0.54128	6.10168	0.9907	39
Terregino	2009	TE09	2.83519	7	NA	37	NA	0	NA	-0.35271	7.09478	0.9877	54
Shoham	2012	SH12	1829.08	5	7.1	-20	0	1	unfiltered	-0.00311	6.68477	0.1244	13
Shoham	2012	SH12	4610.98	7	7.1	-20	0	1	unfiltered	-0.00132	7.06344	0.03208	13
Shoham	2012	SH12	2678.86	5	7.1	-30	0	1	unfiltered	-0.00193	6.159492	0.08807	13
Shoham	2012	SH12	2517.1	7	7.1	-30	0	1	unfiltered	-0.00246	7.179487	0.1314	13
Shoham	2012	SH12	1553.99	5	7.93	-20	9000	1	unfiltered	-0.00354	6.505804	0.1612	13
Shoham	2012	SH12	3481.73	7	7.93	-20	9000	1	unfiltered	-0.00168	6.845819	0.04936	13
Shoham	2012	SH12	839.87	5	7.93	-30	9000	1	unfiltered	-0.00502	5.215324	0.212	13
Shoham	2012	SH12	965.87	7	7.93	-30	9000	1	unfiltered	-0.00483	5.661291	0.1906	13
Shoham	2012	SH12	1467.79	5	8.4	-20	0	1	unfiltered	-0.0038	6.576139	0.1931	13
Shoham	2012	SH12	2794.16	7	8.4	-20	0	1	unfiltered	-0.00218	7.085688	0.086	13

Shoham	2012	SH12	897.49	5	8.4	-30	0	1	unfiltered	-0.00572	6.130977	0.3154	13
Shoham	2012	SH12	959.61	7	8.4	-30	0	1	unfiltered	-0.00599	6.746161	0.3942	13
Zarkov	2013	ZA13	1.29	6	NA	15	NA	0	NA	-1.9978	3.5836	0.8847	3
Zarkov	2013	ZA13	0.75	6	NA	15	NA	0	NA	-4	4	NA	3
Zarkov	2013	ZA13	11.71	6	NA	4	NA	0	NA	-0.23986	3.80914	0.9368	9
Zarkov	2013	ZA13	6.54	6	NA	15	NA	0	NA	-0.4904	4.2067	0.9763	9
Zarkov	2013	ZA13	2.63	6	NA	22	NA	0	NA	-0.9849	3.5894	0.9223	9
Mihai	2011	MI11	3.8	5	7.4	6	231	1	unfiltered	-0.26325	3.94872	0.726	2
Mihai	2011	MI11	2.7	5	8.02	6	621	1	unfiltered	-0.37009	4.75385	0.9596	2
Mihai	2011	MI11	3.87	5	7.6	6	16208	1	unfiltered	-0.25812	3.67179	0.8387	2
Mihai	2011	MI11	5.65	5	7.4	22	231	1	unfiltered	-0.17707	3.54937	0.928	2
Mihai	2011	MI11	4.71	5	8.02	22	621	1	unfiltered	-0.2122	4.4084	0.9792	2
Mihai	2011	MI11	6.8	5	7.6	22	16208	1	unfiltered	-0.14709	3.45372	0.7842	2
Mihai	2011	MI11	5.84	5	7.4	35	231	1	unfiltered	-0.17109	3.31631	0.8682	2
Mihai	2011	MI11	4.93	5	8.02	35	621	1	unfiltered	-0.2028	4.454	0.9227	2
Mihai	2011	MI11	5.78	5	7.6	35	16208	1	unfiltered	-0.1731	3.1418	0.9397	2
Guan	2009	GU09	0.0125	6	9	55	NA	1	unfiltered	NA	NA	NA	2
Guan	2009	GU09	0.0375	6	9	45	NA	1	unfiltered	NA	NA	NA	2
Guan	2009	GU09	0.2625	6	9	35	NA	1	unfiltered	NA	NA	NA	2
Guan	2009	GU09	0.904167	6	9	25	NA	1	unfiltered	NA	NA	NA	2
Guan	2009	GU09	0.0125	6	7	55	NA	1	unfiltered	NA	NA	NA	2
Guan	2009	GU09	0.025	6	7	45	NA	1	unfiltered	NA	NA	NA	2
Guan	2009	GU09	0.979167	6	7	35	NA	1	unfiltered	NA	NA	NA	2
Guan	2009	GU09	3.204167	6	7	25	NA	1	unfiltered	NA	NA	NA	2

Appendix 2

Chapter 3

Additional Material



S2.1 Effects plots for the fitted GAM for combined shorebird and waterbird species richness across Australia. a)NDVI, b) precipitation (mm), c) temperature (°C), d) humidity at 9am (%).

Appendix 3

Chapter 5

Additional Material

S3.1 Incident risk classification tables. Table A scores the level of impact against the event likelihood to produce an Impact Event score. Table B uses the Impact Event score against the consequence score to produce an overall risk score classification

IMPACT						
VHI		7	8	8	10	11
HI		5	6	7	8	9
MED		4	5	6	7	8
LO		3	4	5	6	7
VLO		2	3	4	5	6
NIL						
	NIL	VLO	LO	MED	HI	VHI

EVENTS

HIGH
MEDIUM
LOW

A

CONSEQUENCE										
VHI - 5	7	8	9	10	11	12	13	14	15	16
HI - 4	6	7	8	9	10	11	12	13	14	15
MED - 3	5	6	7	8	9	10	11	12	13	14
LO - 2	4	5	6	7	8	9	10	11	12	13
VLO - 1	3	4	5	6	7	8	9	10	11	12
NIL	2	3	4	5	6	7	8	9	10	11
	2	3	4	5	6	7	8	9	10	11

IMPACTEVENT

VERY HIGH
HIGH
MEDIUM
LOW

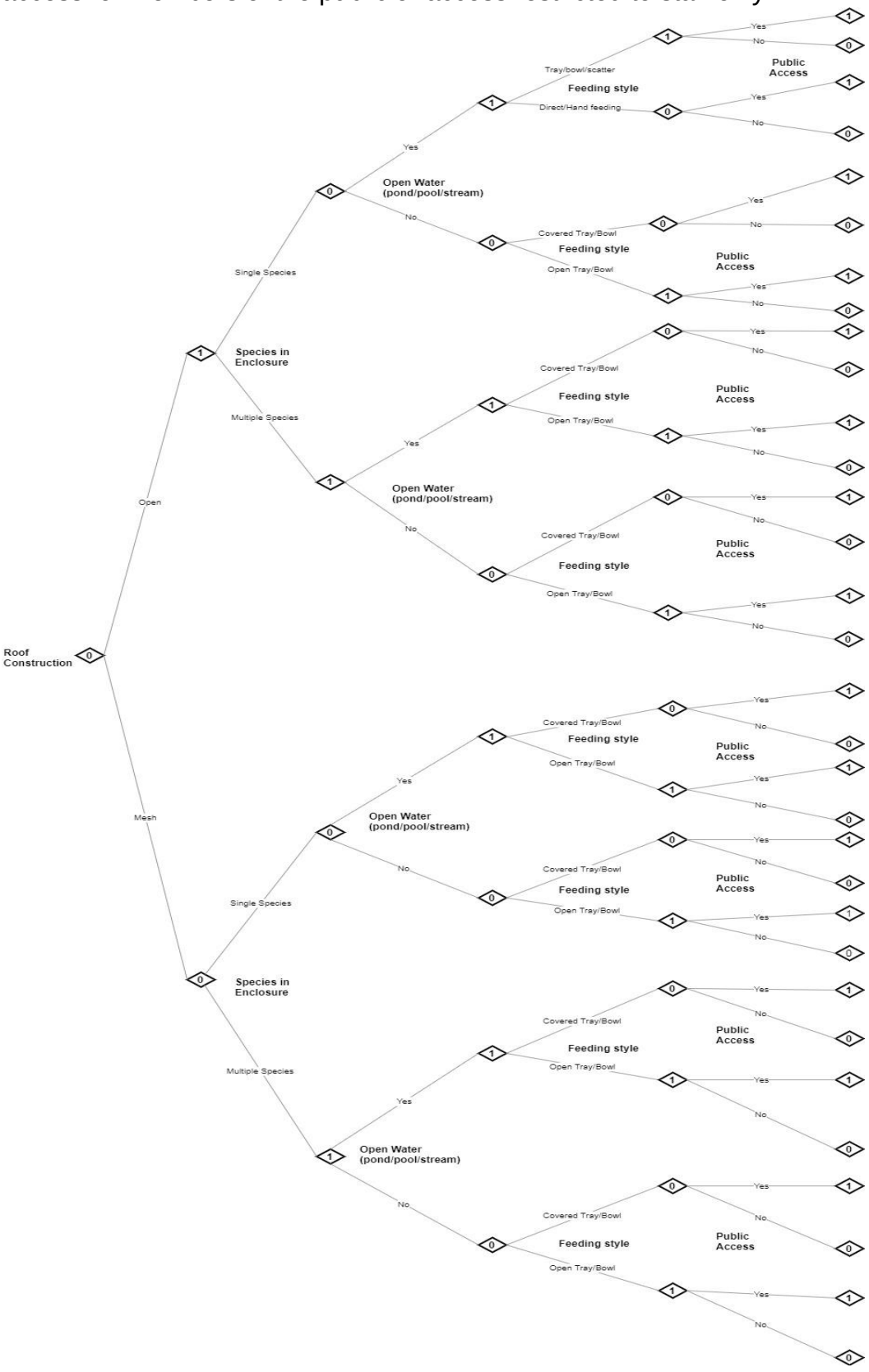
B

.

S3.2 Summary table of the number of species, number of individuals, swab and blood samples taken for Adelaide Zoo bird population by enclosure score

Enclosure.score	No.of.Cages	No.of.individuals	Swab.enclosure	Swab.bird	Blood.sample	Seropos
0	3	88	6	5	4	0
1	1	43	6	12	7	0
2	5	172	12	3	3	0
3	5	564	24	36	4	0
4	6	427	57	12	12	4
NA	0	4	0	0	0	0

S3.3 The flow chart for enclosure classification. Each decision is a binary option, (i) open or mesh roof structure, (ii) single or multiple species, (iii) open water or drinking water source only, (iv) tray/bowl/scatter feeding or direct hand feeding, and (v) access for members of the public or access restricted to staff only.



S3.4 Family risk classifications for the bird species housed in the Adelaide Zoo collection.

Family	Family.risk
Accipitridae	Low
Alcedinidae	Low
Anatidae	High
Ardeidae	High
Artamidae	Low
Burhinidae	High
Cacatuidae	Low
Casuariidae	High
Charadriidae	High
Columbidae	Low
Coraciidae	Low
Estrildidae	Low
Fringillidae	Low
Maluridae	Low
Megapodiidae	Low
Meliphagidae	Low
Numididae	High
Oreoicidae	Low
Oriolidae	Low
Pelecanidae	High
Phalacrocoracidae	High
Phasianidae	High
Phoenicopteridae	High
Pittidae	Low
Ploceidae	Low
Podargidae	Low
Psittacidae	Low
Psittaculidae	Low
Psophodidae	High
Ptilonorhynchidae	Low
Rallidae	High
Recurvirostridae	High
Spheniscidae	High
Sturnidae	Low
Threskiornithidae	High
Turnicidae	High
Tytonidae	Low