Appendix Five: The ecology of Influenza A viruses in wild birds in southern Africa

(Appendix reference: Cumming, G. S., Caron, A., Abolnik, C., Catolli, G., L.W. Bruinzeel, C.E. Burger, K. Cecchettin, N. Chiweshe, B. Mochotlhoane, G.L. Mutumi, M. Ndlovu. 2011. The ecology of Influenza A viruses in wild birds in southern Africa. *EcoHealth* **8**(1): 4-13)

Introduction

Influenza A viruses have long been acknowledged as pathogens of global concern. In recent years, outbreaks of highly pathogenic avian influenza (HPAI) in populations of domestic and wild birds, and the related deaths of nearly 300 people (WHO 2010), have heightened fears of a new influenza pandemic in the human population (e.g. Enserink 2006, Pickles 2006). Assessment of the risks that are posed by avian influenza, and the development of appropriate response strategies in the event of an epidemic or pandemic, rely heavily on a fundamental scientific understanding of Avian Influenza Virus (AIV) dynamics in populations of domestic and wild birds (Dudley 2008).

Although low pathogenicity avian influenza (LPAI) viral prevalence in Western European and North American populations has been well documented (Olsen et al. 2006), it is unclear how the long-distance movements of migratory and nomadic bird species relate to larger-scale spatial and temporal variation in AIV recombination, maintenance, and epidemics (Kilpatrick et al. 2006, Krauss and Webster 2010). One of the largest single gaps in the geographical coverage of AIV sampling has been southern Africa (Kilpatrick et al. 2006, Olsen et al. 2006, Appendix Two - Gaidet et al. 2007), a region that is at risk following the detection of highly pathogenic strains in sub-Saharan Africa north of the Zambezi (Gaidet et al. 2008, Fasina et al. 2009). Although some intriguing data exist from South Africa (e.g. Sinclair et al. 2005, Abolnik et al. 2009, Abolnik et al. 2010), little relevant research has been carried out in most southern African countries.

By comparison to western Europe, southern Africa has a relatively mild winter; highly variable and often scarce rainfall; a higher diversity of bird species; no true geese or swans; and many nomadic waterbirds but no truly migratory afrotropical *Anas* ducks (Underhill et al. 1999, Cumming et al. 2008). We tested the predictions that (1) due to its more arid

environment and absence of migratory palearctic ducks, LPAI prevalence in wild waterbirds should be lower in southern Africa than in Europe; (2) due to the presence of many opportunistic, colonial, and nomadic waterbird species, and the lack of migratory corridors (Hockey 2000), LPAI prevalence in wild birds in southern Africa should show relatively little spatial variation along longitudinal and latitudinal gradients; and (3) the arrival of palearctic migrants in September, including charadriids known as potential LPAI reservoirs, should create a pulse in influenza occurrences in Afrotropical species.

While exploring these fundamental assumptions for the first time, we also provide a wealth of new and useful information on AIV and wild birds in southern Africa. Our results suggest that none of our starting assumptions can be strongly supported. Some re-thinking of prevailing assumptions about influenza A viruses in southern African bird populations thus appears necessary in planning health care and risk management strategies.

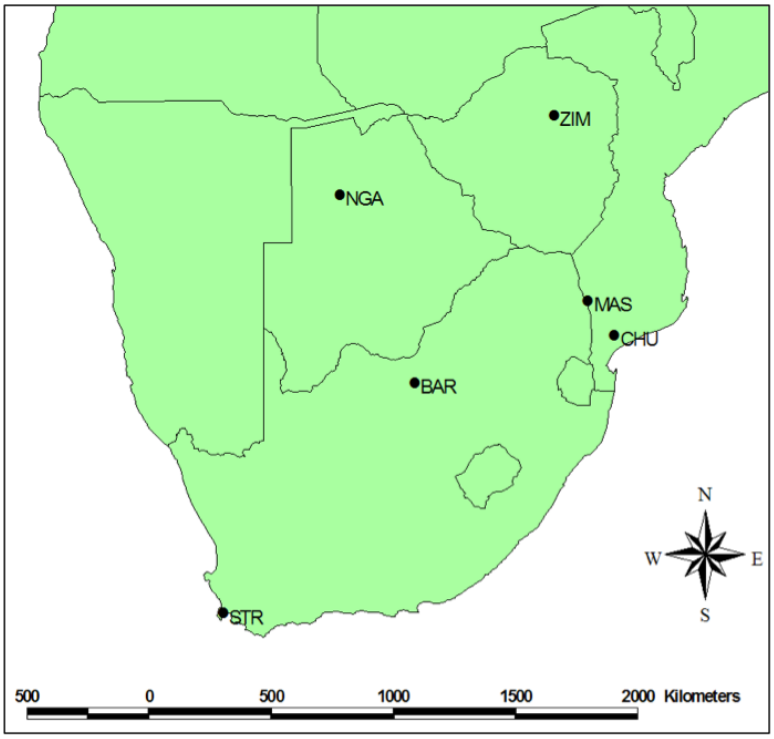
Methods

Project Design and Field Sites

Data were collected in Botswana, Mozambique, South Africa and Zimbabwe from March 2007 to May 2009. We worked at 5 different sites (Figure A5.1) and 12-15 sampling locations per site. We counted and sampled birds at daily, bimonthly, and annual time scales. Our three core sites (Barberspan and Strandfontein in South Africa, and the Manyame catchment in Zimbabwe) were sampled every two months and our Botswana site (Lake Ngami) and Mozambique site (Lake Chuali) every four months. Dates and coordinates of sampling are given in Appendices S1 and S2 (in Supporting Information) and additional information on study sites in Appendix A5.S3.

Figure A5.1: *Map of southern Africa showing sampling sites mentioned in this paper. Site codes: ZIM or ZW, Lakes Chivero and Manyame; NGA, Lake Ngami; MAS, Massingir Dam; CHU, Lake Chuali; BAR, Barberspan; STR, Strandfontein. Our three core sites were STR, BAR and ZIM, which fall in different biomes along a north-south latitudinal gradient.*

Figure 1



Counting protocols

Each site visit included 5-7 days of standardized bird counts followed by 7-10 days of bird captures in the same locations. Counts consisted of a 10-minute habituation period followed by a 30-minute counting period, during which the number and species of all birds within a 150m radius of the (stationary) observers were recorded. Each location was counted at 4 different times of day over a 5-day period prior to captures (additional details are in Appendix A5.S4). Over the two years of the study we completed 2,503 half-hour point counts. For each of our three core sites (Barberspan, Manyame/Chivero, and Strandfontein) the count data also provide estimates from 13 different points in time (i.e., every two months for two years), giving us a spatiotemporally balanced sampling design for exploring both spatial and temporal variation in the bird community.

Capture and sampling protocols

After the counts we spent 5-10 days catching and sampling birds, using standard procedures as detailed in Appendix A5.S4 (and Figure A5.2). We targeted ducks because they are considered the primary vectors of influenza in Europe and Asia. In addition to ancillary data (morphometry, photographs, blood, feathers) we collected two cloacal and two tracheal swabs per bird. Birds recaptured in the same week were not resampled. All swabs were placed in cryovials in viral transport medium (Hank's salt solution with antibitoics and fungicides) and frozen in liquid nitrogen within half an hour of collection.

The swabs were stored in a -70 freezer and transported in dry ice or liquid nitrogen to an FAO reference laboratory, either ARC-OVI (the Agricultural Research Council-Onderstepoort Veterinary Institute, Pretoria, South Africa) or IZSVe (Istituto Zooprofolattico Sperimentale delle Venezie, Padova, Italy) for analysis (see Appendix A5.S5 for details). Sets of swabs were randomised by laboratory; each received the first cloacal and second tracheal

Figure A5.2: *Example of a walk-in trap used to catch ducks. In this picture, XX (L) and YY (R) capture Egyptian geese at Strandfontein.*

Figure 2

swab from one bird and the second cloacal and first tracheal swab from the next bird. All samples from Botswana and Mozambique were analysed at IZSVe.

Sources of error included (1) failure to obtain a full complement of swabs, due to bird escapes or shortages of vials; (2) labelling errors; (3) loss or destruction of vials in transit; and (4) mistakes in allocation of vials to laboratories. Most of these errors were random and hence unbiased. We had fewer than 4 swabs per bird in just under 4% of cases. Samples were only sent to IZSVe on completion of the project, giving a delay between sampling and analysis of 2-24 months that may have affected the probability of AIV detection (Forster et al. 2008).

Data Analysis

Viral prevalence was too low to determine the influence of the number of swabs on viral detection probability. Since missing swabs were <4% and randomly distributed by species, we assumed that each sampled bird (rather than each swab) had an equal chance of viral detection. Prevalence was calculated as the ratio of the number of influenza viruses detected to the number of birds sampled. Since recaptures were not re-sampled during the same capture mission, and since each sampling effort was at least two months apart, we treated samples from recaptures (including birds that we had ringed and those ringed by others) as independent.

Having quantified viral prevalence for each species by site, we calculated overall prevalence for all bird species and all sites by dividing the total number of birds sampled by the number of samples that were positive for avian influenza viruses. Bird count summaries by site used the average number of birds counted across all point counts.

For the palearctic migrant analysis we included all birds found in our study sites that were both listed in class 6 (i.e., intercontinental and marine migrants) of the Roberts' database (Hockey et al. 2004) and associated with wetland and estuarine habitats. The total number of

foraging and non-foraging palearctic migrants for each sampling mission was converted to a mean abundance and we used Spearman's rank-order correlations to test for a significant relationship between these data, the abundance of anatids, and viral prevalence. Lastly, we multiplied data on prevalence by avian abundance to estimate the relative abundance of infected birds observed during an average half-hour point count.

Results

We sampled a total of 4,977 birds of 165 different species, including 158 recaptures. Captures were distributed unevenly across sites (Table A5.1) despite comparable sampling effort, with the Zimbabwean site yielding the most birds (n=1916), followed by Barberspan (n=1418) and Strandfontein (n=888). A full listing of the number of individuals of the 165 sampled species is provided in Table A5.S1.

Out of 4,977 sampled birds, 125 were positive for the presence of RNA from the influenza A virus group, giving a prevalence across all species and sites of 2.51%. The probability of an influenza-positive sample being from a cloacal or a tracheal swab was almost identical (n=125, p=0.48 *vs.* p=0.52 for cloacal and tracheal swabs respectively). Prevalence across different bird families was uneven (Table A5.2), with four families (Anatidae, Jacanidae, Charadriidae and Dendrocygnidae) together contributing 72.8% of positive samples; the same four families represented 67.5% of birds captured (Tables A5.2 and A5.3).

Reliable conclusions cannot be drawn from small sample sizes. We sampled over 20 individuals (i.e., the influence of an outlier was 5% or less) for 18 different bird families. From these families the highest mean prevalence values across all sites occurred in the

Table A5.1: *Numbers of birds sampled for avian influenza, by family and by site. BAR, Barberspan; CHU, Chuali; MAS, Massingir; NGA, Ngami; STR, Strandfontein; ZW, Zimbabwe (Chivero and Manyame). Sample sizes for Anatidae were similar across our three core sites (BAR, ZW, STR). Obvious differences include Jacanidae (jacanas; mostly ZW and CHU), Dendrocygnidae (whistling ducks; mostly ZW) and Rallidae (coots and rails; mostly BAR).*

Family	BAR	CHU	MAS	NGA	STR	ZW	Total
Accipitridae	0	0	0	0	2	1	3
Alaudidae	6	0	0	1	0	17	24
Alcedinidae	0	3	1	0	0	9	13
Anatidae	696	27	0	69	680	698	2170
Apodidae	1	0	0	0	0	0	1
Ardeidae	6	14	0	3	27	35	85
Burhinidae	1	1	0	0	0	1	3
Caprimulgidae	0	0	1	1	0	3	5
Cerylidae	1	10	0	4	0	25	40
Charadriidae	99	31	10	79	17	225	461
Ciconiidae	0	0	0	1	0	0	1
Cisticolidae	0	0	0	0	0	5	5

Columbidae	4	0	0	48	26	44	122
Coraciidae	0	0	0	0	0	4	4
Dacelonidae	0	0	0	0	0	8	8
Dendrocygnidae	5	13	1	9	0	206	234
Estrildidae	0	0	0	2	0	5	7
Fringillidae	0	0	0	0	0	1	1
Glareolidae	0	13	3	79	0	21	116
Haematopodidae	0	0	0	0	4	0	4
Hirundinidae	0	2	0	1	3	7	13
Indicatoridae	0	0	0	0	0	1	1
Jacanidae	1	116	0	39	0	337	493
Laniidae	1	0	0	0	0	2	3
Laridae	3	0	0	2	42	16	63
Lybiidae	1	0	0	0	0	2	3
Malaconotidae	0	0	0	0	0	2	2
Meropidae	0	0	0	1	0	0	1
Motacillidae	2	1	0	0	10	30	43
Muscicapidae	1	0	0	1	1	1	4
Numididae	10	0	0	0	8	5	23

Passeridae	2	0	0	1	3	2	8
Phalacrocoracidae	0	1	0	0	5	2	8
Phasianidae	2	0	0	8	7	3	20
Phoenicopteridae	7	0	0	0	0	0	7
Ploceidae	25	17	0	32	10	81	165
Podicipedidae	0	1	0	0	0	1	2
Pycnonotidae	2	0	0	0	3	3	8
Rallidae	491	2	0	1	13	7	514
Recurvirosridae	4	0	0	4	8	0	16
Rostratulidae	1	6	0	25	0	0	32
Scolopacidae	36	11	0	48	0	86	181
Sturnidae	0	0	1	4	2	9	16
Sylviidae	3	2	0	1	0	7	13
Threskiornithidae	1	0	0	3	15	1	20
Tytonidae	4	0	0	0	1	2	7
Upupidae	2	0	0	0	0	1	3
Zosteropidae	0	0	0	0	1	0	1
TOTALS	1418	271	17	467	888	1916	4977

Table A5.2: *Numbers of birds that tested positive for avian influenza, by family and site. Information on viral strains is given in Table A5.4. BAR, Barberspan; CHU, Chuali; MAS, Massingir; NGA, Ngami; STR, Strandfontein; ZW, Zimbabwe.*

Family	BAR	CHU	MAS	NGA	STR	ZW	Total Positives	Total captures	Total prevalence %
Accipitridae	0	0	0	0	0	0	0	3	0
Alaudidae	0	0	0	0	0	3	3	24	12.5
Alcedinidae	0	0	0	0	0	1	1	13	7.7
Anatidae	8	0	0	1	8	35	52	2170	2.4
Apodidae	0	0	0	0	0	0	0	1	0
Ardeidae	0	0	0	0	0	0	0	85	0
Burhinidae	0	0	0	0	0	0	0	3	0
Caprimulgidae	0	0	0	0	0	0	0	5	0
Cerylidae	0	0	0	0	0	1	1	40	2.5
Charadriidae	0	0	0	0	0	12	12	461	2.6
Ciconiidae	0	0	0	0	0	0	0	1	0
Cisticolidae	0	0	0	0	0	0	0	5	0
Columbidae	0	0	0	0	0	0	0	122	0
Coraciidae	0	0	0	0	0	0	0	4	0

Dacelonidae	0	0	0	0	0	0	0	8	0
Dendrocygnidae	0	0	0	2	0	10	12	234	5.1
Estrildidae	0	0	0	0	0	0	0	7	0
Fringillidae	0	0	0	0	0	0	0	1	0
Glareolidae	0	0	0	0	0	0	0	116	0
Haematopodidae	0	0	0	0	0	0	0	4	0
Hirundinidae	0	0	0	0	0	1	1	13	7.7
Indicatoridae	0	0	0	0	0	0	0	1	0
Jacanidae	0	0	0	0	0	15	15	493	3.0
Laniidae	0	0	0	0	0	0	0	3	0
Laridae	0	0	0	0	0	0	0	63	0
Lybiidae	0	0	0	0	0	0	0	3	0
Malaconotidae	0	0	0	0	0	0	0	2	0
Meropidae	0	0	0	0	0	0	0	1	0
Motacillidae	0	0	0	0	0	2	2	43	4.7
Muscicapidae	0	0	0	0	0	0	0	4	0
Numididae	1	0	0	0	0	0	1	23	4.3
Passeridae	1	0	0	0	0	0	1	8	12.5
Phalacrocoracidae	0	0	0	0	0	0	0	8	0
Phasianidae	0	0	0	0	0	0	0	20	0
Phoenicopteridae	0	0	0	0	0	0	0	7	0

Ploceidae	0	0	0	0	0	5	5	165	3.0
Podicipedidae	0	0	0	0	0	0	0	2	0
Pycnonotidae	1	0	0	0	0	0	1	8	12.5
Rallidae	7	0	0	0	0	0	7	514	1.4
Recurvirosridae	0	0	0	0	0	0	0	16	0
Rostratulidae	0	0	0	0	0	0	0	32	0
Scolopacidae	0	0	0	0	0	7	7	181	3.9
Sturnidae	0	0	0	0	0	0	0	16	0
Sylviidae	0	0	0	0	0	2	2	13	15.4
Threskiornithidae	0	0	0	0	1	0	1	20	5
Tytonidae	0	0	0	0	0	0	0	7	0
Upupidae	1	0	0	0	0	0	1	3	33.3
Zosteropidae	0	0	0	0	0	0	0	1	0
TOTALS	19	0	0	3	9	94	125	4977	

Table A5.3: *Prevalence of avian influenza by avian family and by site over the period March 2007-April 2009. This table is derived from information given in Table A5.1 and Table A5.2. BAR, Barberspan; CHU, Chuali; MAS, Massingir; NGA, Ngami; STR, Strandfontein; ZW, Zimbabwe.*

Family	BAR	CHU	MAS	NGA	STR	ZW	Total Captures	Total Positives
Accipitridae	0.0	0.0	0.0	0.0	0.0	0.0	3	0
Alaudidae	0.0	0.0	0.0	0.0	0.0	17.6	24	3
Alcedinidae	0.0	0.0	0.0	0.0	0.0	11.1	13	1
Anatidae	1.1	0.0	0.0	1.4	1.2	5.0	2170	52
Apodidae	0.0	0.0	0.0	0.0	0.0	0.0	1	0
Ardeidae	0.0	0.0	0.0	0.0	0.0	0.0	85	0
Burhinidae	0.0	0.0	0.0	0.0	0.0	0.0	3	0
Caprimulgidae	0.0	0.0	0.0	0.0	0.0	0.0	5	0
Cerylidae	0.0	0.0	0.0	0.0	0.0	4.0	40	1
Charadriidae	0.0	0.0	0.0	0.0	0.0	5.3	461	12
Ciconiidae	0.0	0.0	0.0	0.0	0.0	0.0	1	0
Cisticolidae	0.0	0.0	0.0	0.0	0.0	0.0	5	0
Columbidae	0.0	0.0	0.0	0.0	0.0	0.0	122	0

Coraciidae	0.0	0.0	0.0	0.0	0.0	0.0	4	0
Dacelonidae	0.0	0.0	0.0	0.0	0.0	0.0	8	0
Dendrocygnidae	0.0	0.0	0.0	22.2	0.0	4.9	234	12
Estrildidae	0.0	0.0	0.0	0.0	0.0	0.0	7	0
Fringillidae	0.0	0.0	0.0	0.0	0.0	0.0	1	0
Glareolidae	0.0	0.0	0.0	0.0	0.0	0.0	116	0
Haematopodidae	0.0	0.0	0.0	0.0	0.0	0.0	4	0
Hirundinidae	0.0	0.0	0.0	0.0	0.0	14.3	13	1
Indicatoridae	0.0	0.0	0.0	0.0	0.0	0.0	1	0
Jacanidae	0.0	0.0	0.0	0.0	0.0	4.5	493	15
Laniidae	0.0	0.0	0.0	0.0	0.0	0.0	3	0
Laridae	0.0	0.0	0.0	0.0	0.0	0.0	63	0
Lybiidae	0.0	0.0	0.0	0.0	0.0	0.0	3	0
Malaconotidae	0.0	0.0	0.0	0.0	0.0	0.0	2	0
Meropidae	0.0	0.0	0.0	0.0	0.0	0.0	1	0
Motacillidae	0.0	0.0	0.0	0.0	0.0	6.7	43	2
Muscicapidae	0.0	0.0	0.0	0.0	0.0	0.0	4	0
Numididae	10.0	0.0	0.0	0.0	0.0	0.0	23	1
Passeridae	50.0	0.0	0.0	0.0	0.0	0.0	8	1

Phalacrocoracidae	0.0	0.0	0.0	0.0	0.0	0.0	8	0
Phasianidae	0.0	0.0	0.0	0.0	0.0	0.0	20	0
Phoenicopteridae	0.0	0.0	0.0	0.0	0.0	0.0	7	0
Ploceidae	0.0	0.0	0.0	0.0	0.0	6.2	165	5
Podicipedidae	0.0	0.0	0.0	0.0	0.0	0.0	2	0
Pycnonotidae	50.0	0.0	0.0	0.0	0.0	0.0	8	1
Rallidae	1.4	0.0	0.0	0.0	0.0	0.0	514	7
Recurvirosridae	0.0	0.0	0.0	0.0	0.0	0.0	16	0
Rostratulidae	0.0	0.0	0.0	0.0	0.0	0.0	32	0
Scolopacidae	0.0	0.0	0.0	0.0	0.0	8.1	181	7
Sturnidae	0.0	0.0	0.0	0.0	0.0	0.0	16	0
Sylviidae	0.0	0.0	0.0	0.0	0.0	28.6	13	2
Threskiornithidae	0.0	0.0	0.0	0.0	6.7	0.0	20	1
Tytonidae	0.0	0.0	0.0	0.0	0.0	0.0	7	0
Upupidae	50.0	0.0	0.0	0.0	0.0	0.0	3	1
Zosteropidae	0.0	0.0	0.0	0.0	0.0	0.0	1	0

Alaudidae (larks; 24 birds, 3 positives, prevalence 12.5%) and the Dendrocygnidae (whistling ducks; 234 birds, 12 positives, prevalence 5.15%). Also of note were the Scolopacidae (sandpipers and snipes, 180 birds, 6 positives, prevalence 3.33%), Jacanidae (jacanas, 492 birds, 15 positives, prevalence=3.05%), Ploceidae (weavers, 165 birds, 5 positives, prevalence=3.03%), Charadriidae (plovers and lapwings; 458 birds, 12 positives, prevalence=2.62%), and Anatidae (ducks; 2168 birds, 52 positives, prevalence=2.4%). Conversely, despite reasonably large sample sizes, no AIV RNA was found in the Columbidae (pigeons and doves; n=122), Glareolidae (pratincoles and coursers; n=116) or Ardeidae (herons, egrets, and bitterns; n=88).

There was no spatial synchrony in influenza occurrences, with the prevalence of LPAI viruses in any two-month sampling period not being significantly correlated between any pair of sites (n=12 or 13, Spearman's rho < 0.43, *p* not significant to the 0.05 or 0.1 levels in all cases).

Two influenza viruses were isolated and several different strains identified (Table A5.4). An H1N8 influenza virus was isolated from an Egpytian goose *Alopochen aegyptiacus* caught at Barberspan and an H3N8 influenza virus from a Red-billed teal *Anas erythrorhyncha* caught at Strandfontein. Type-related information was obtained via rRT-PCR for an additional 22 viruses, which included 10 H5-positive and 10 H7-positive samples as well as two H6-positives. Amplicons from the reactions were insufficient for obtaining DNA sequences, and thus the amino acid sequence at the HA0 cleavage sites could not be determined; it is therefore unknown whether the H5 and H7 viruses were of high or low pathogenicity. H7 strains were only identified from Zimbabwe but were found in 5 different species.

Table 5A.4: *Information on viral strains and types. This table describes birds that tested positive, rather than positive samples; the 6 birds that tested positive for the same type on two different swabs provide 6 entries rather than 12. Blank cells are zeros rather than unknown values.*

Common name	Latin name	Family	Total +ves	H1 +ve	H3 +ve	H5 +ve	H6 +ve	H7 +ve	Typed
African hoopoe	*Upupa africana*	Upupidae	1						
African jacana	*Actophilornis africanus*	Jacanidae	15			2			
African pipit	*Anthus cinnamomeus*	Motacillidae	1						
African red-eyed bulbul	*Pycnonotus nigricans*	Pycnonotidae	1			1			
African snipe	*Gallinago nigripennis*	Scolopacidae	1						
African wattled lapwing	*Vanellus senegallus*	Charadriidae	3					1	
Barn swallow	*Hirundo rustica*	Hirundinidae	1						
Blacksmith lapwing	*Vanellus armatus*	Charadriidae	8			1		1	
Cape teal	*Anas capensis*	Anatidae	1						

Chestnut-backed sparrowlark	*Eremopterix leucotis*	Alaudidae	3			1			
Common ringed plover	*Charadrius hiaticula*	Charadriidae	1						
Common sandpiper	*Actitis hypoleucos*	Scolopacidae	2						
Egyptian goose	*Alopochen aegyptiaca*	Anatidae	7	1					H1N8
Fulvous duck	*Dendrocygna bicolor*	Dendrocygnidae	2				1		
Glossy ibis	*Plegadis falcinellus*	Threskiornithidae	1						
Helmeted guineafowl	*Numida meleagris*	Numididae	1						
Hottentot teal	*Anas hottentota*	Anatidae	3			1			
Little rush-warbler	*Bradypterus baboecala*	Sylviidae	1						
Little stint	*Calidris minuta*	Scolopacidae	3					1	
Malachite kingfisher	*Alcedo cristata*	Alcedinidae	1			1			
Pied kingfisher	*Ceryle rudis*	Cerylidae	1						

Red-billed quelea	*Quelea quelea*	Ploceidae	1						
Red-billed teal	*Anas erythrorhyncha*	Anatidae	35		1			2	H3N8
Red-knobbed coot	*Fulica cristata*	Rallidae	7						
South African shelduck	*Tadorna cana*	Anatidae	2			1	1		
Southern grey-headed Sparrow	*Passer diffusus*	Passeridae	1						
Southern masked-weaver	*Ploceus velatus*	Ploceidae	1						
Spur-winged goose	*Plectropterus gambensis*	Anatidae	2						
Village weaver	*Ploceus cucullatus*	Ploceidae	2						
White-faced duck	*Dendrocygna viduata*	Dendrocygnidae	10			1		5	
Willow warbler	*Phylloscopus trochilus*	Sylviidae	1						

Wood sandpiper	*Tringa glareola*	Scolopacidae	1			1			
Yellow bishop	*Euplectes capensis*	Ploceidae	1						
Yellow-billed duck	*Anas undulata*	Anatidae	2						
Yellow-throated longclaw	*Macronyx croceus*	Motacillidae	1						
Totals			125	1	1	10	2	10	(2)

Table A5.5: *Palearctic migrants observed during point counts. The 'total' column sums foraging and non-foraging birds (e.g. whimbrel were only observed in flight). Abundance by location were divided by four to average over different samples, but since total numbers of birds are more relevant to the role of palearctic migrants in influenza transmission, we have not divided by the number of locations per site.*

Common name	Latin name	Family	Total
Amur falcon	*Falco amurensis*	Falconidae	27
Barn swallow	*Hirundo rustica*	Hirundinidae	1847
Black-tailed godwit	*Limosa limosa*	Scolopacidae	0
Common greenshank	*Tringa nebularia*	Scolopacidae	89
Common redshank	*Tringa totanus*	Scolopacidae	0
Common ringed plover	*Charadrius hiaticula*	Charadriidae	57
Common sandpiper	*Actitis hypoleucos*	Scolopacidae	138
Common swift	*Apus apus*	Apodidae	18
Common tern	*Sterna hirundo*	Laridae	49
Common whimbrel	*Numenius phaeopus*	Scolopacidae	0
Curlew sandpiper	*Calidris ferruginea*	Scolopacidae	372
Eurasian curlew	*Numenius arquata*	Scolopacidae	1
European roller	*Coracias garrulus*	Coraciidae	4

Green sandpiper	*Tringa ochropus*	Scolopacidae	11
Grey plover	*Pluvialis squatarola*	Charadriidae	2
Lesser black-backed gull	*Larus fuscus*	Laridae	2
Lesser grey shrike	*Lanius minor*	Laniidae	1
Lesser kestrel	*Falco naumanni*	Falconidae	2
Little stint	*Calidris minuta*	Scolopacidae	1741
Marsh sandpiper	*Tringa stagnatilis*	Scolopacidae	44
Marsh warbler	*Acrocephalus palustris*	Sylviidae	0
Montagu's harrier	*Circus pygargus*	Accipitridae	3
Red-backed Shrike	*Lanius collurio*	Laniidae	4
Ruddy turnstone	*Arenaria interpres*	Scolopacidae	1
Ruff	*Philomachus pugnax*	Scolopacidae	1728
Sand martin	*Riparia riparia*	Hirundinidae	81
Sanderling	*Calidris alba*	Scolopacidae	15
Sandwich tern	*Sterna sandvicensis*	Laridae	672
Sedge warbler	*Acrocephalus schoenobaenus*	Sylviidae	3
Steppe buzzard	*Buteo vulpinus*	Accipitridae	1
Terek sandpiper	*Xenus cinereus*	Scolopacidae	2
White-winged tern	*Chlidonias leucopterus*	Laridae	658

Willow warbler	*Phylloscopus trochilus*	Sylviidae	5
Wood sandpiper	*Tringa glareola*	Scolopacidae	458
Yellow wagtail	*Motacilla flava*	Motacillidae	7

Influenza viruses are in circulation across the subregion throughout the year (Figure A5.3), with no obvious pattern in relation to temperature or rainfall. Patterns between years also appear to be inconsistent, with peaks in viral prevalence in December 2007 and January 2008 in Zimbabwe and Barberspan not present in 2008-2009.

These data should be interpreted within the context of the sampled bird communities. We had relatively high numbers of influenza-positive birds from each of four avian families: Anatidae, Charadriidae, Dendrocygnidae, and Jacanidae. The birds in each of these families show differing seasonal trends in abundance as well as considerable spatial variation between our three core sites (Figure A5.4).

For the Anatidae, we were able to obtain an estimate of changes in the relative abundance of influenza viruses across our three sites at different times of year (Figure A5.5). This figure presents a different portrayal of viral risk from a simple prevalence estimate, as well as giving a rough guide to the number of infected birds that occur in an average point count at each site. Using additional bird count data, such as the African Waterfowl Census, these data could be extrapolated to estimate the total number of birds infected with LPAI in our study sites at different times of year.

During counts we recorded 32,153 individuals belonging to 32 different palearctic migrant bird species from 12 avian families (Table A5.5). Numbers of palearctic migrants showed a strong peak in the southern African summer (Figure A5.6) with varying levels of between-site consistency between years. Comparison of the abundance of palearctic migrants and the prevalence of viruses from the same site and time, treating each site as an independent sample at each time step, found no dependency of viral prevalence on numbers of migrants (Spearman's $r=0.039$, $p<0.8$, $n=42$). Numbers of anatid ducks were also independent of viral prevalence (Spearman's $r=-0.1$, $p<0.5$, $n=42$). At time lags of 2 and 4 months, and excluding

the Lake Ngami data, the relationship remained insignificant (2 months, Spearman's r=0.2, p<0.22, n=35; and at 4 months, r=0.1, p<0.57, n=33).

Discussion

The overall prevalence of LPAI influenza viruses that we found in anatid ducks across southern Africa is 2.4%. The range in PCR prevalence in anatids reported from Northern Europe is between 2.1% and 3.8% (Munster et al. 2006, Munster and Fouchier 2009); and an extended survey in EU member states documented an overall LPAI prevalence in Europe of 1.87% (Breed et al. 2007). Some studies have found higher prevalences, ranging from 4% in Switzerland (Baumer et al. 2010) through 6.1% for European dabbling ducks (Munster et al. 2007) to as high as 12-15% (Olsen et al. 2006, Terregino et al. 2007, Wallensten et al. 2007). Estimates depend on the time of year when sampling occurred and the species that were tested (Olsen et al. 2006); our results are within the range of northern hemisphere estimates rather than notably lower.

One of our most interesting results is the lack of a predictable annual spike in prevalence. In Canada, for example, AIV prevalence in anatids may be as high as 60% on breeding grounds in early fall (Olsen et al. 2006). Our highest prevalence across all birds for any one sampling event was 21.43%, in summer in Zimbabwe; but in the same month in the following year, albeit with a relatively small sample size, prevalence was zero (Figure A5.3). We attribute this unpredictability to the relatively stochastic nature of southern African seasonality and the flexible movement strategies of nomadic southern African ducks.

Figure A5.3: *Prevalence by site and month across all captured birds. Sites are BAR, Barberspan; STR, Strandfontein; NGA, Ngami; and ZW, Zimbabwe (Manyame and Chivero). Note that (1) another 294 birds were sampled in Mozambique over the same period, with no AIV positives found; and (2) BAR, STR and ZW were sampled every 2 months and NGA every 4 months, so birds were not sampled in some months. The coloured squares at the top of the chart indicate when a given site was sampled, using the same colour codes as the bars.*

Figure 3

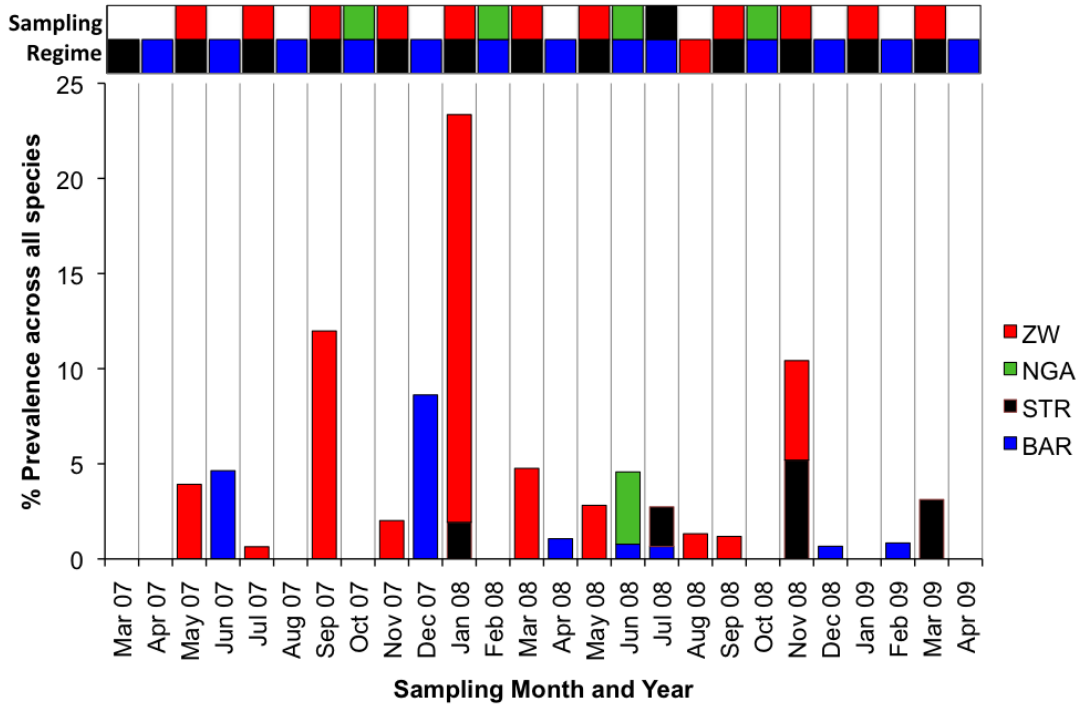
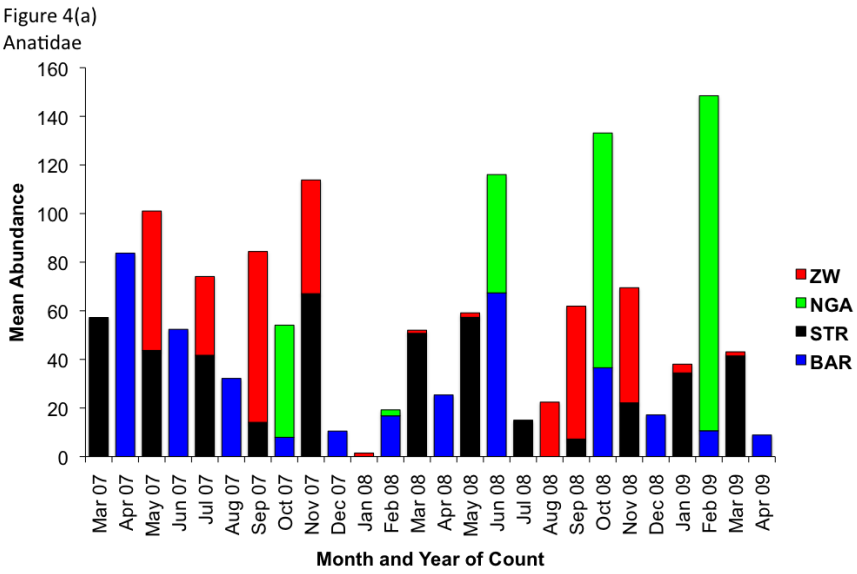


Figure A5.4: *Relative abundance of (a) Anatidae, (b) Charadriidae, (c) Jacanidae and (d) Dendrocygnidae at each of the four sites from which we obtained AIV positive samples (Strandfontein, Barberspan, Lakes Manyame and Chivero, and Lake Ngami) as estimated by monthly point counts at each site during the sampling period. We undertook bird counts but not captures at Lake Ngami in February 2009, so that count is not reflected in Figure A5.3.*



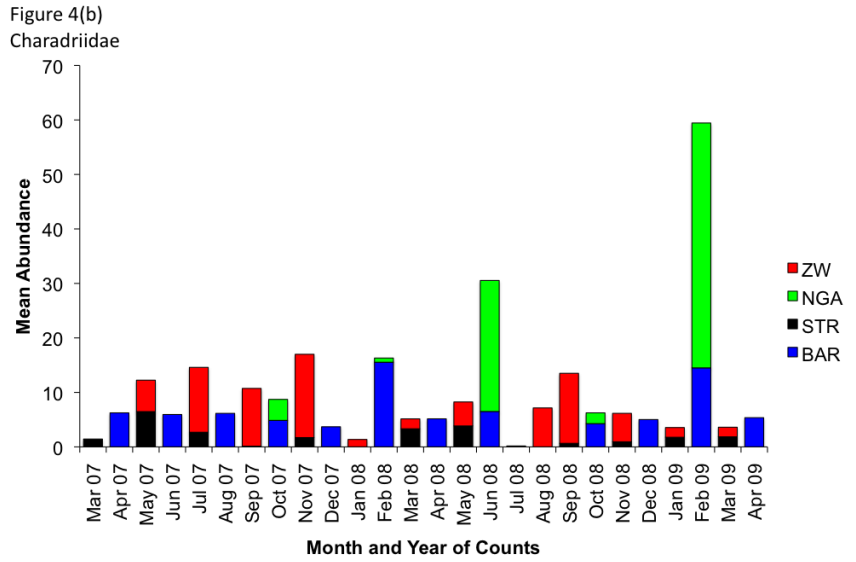


Figure 4(c)
Jacanidae

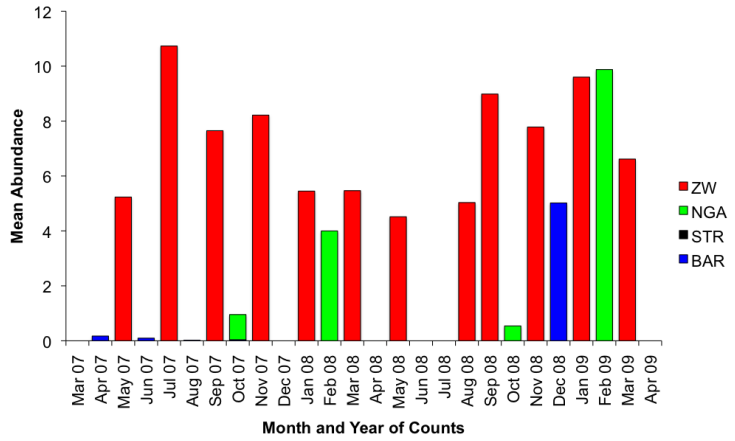


Figure 4(d)
Dendrocygnidae

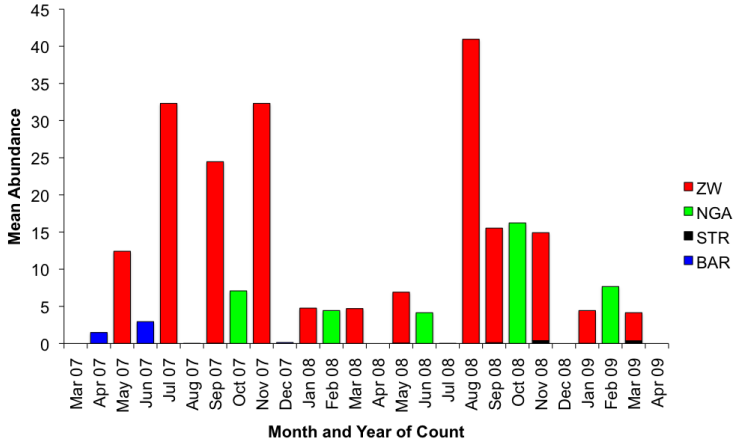


***Figure A5.5**: Relative abundance of infected anatids per half-hour point count by site and month. Note how the impression given by this figure differs from that in A5.s 2 and A5.3(a).*

Figure 5

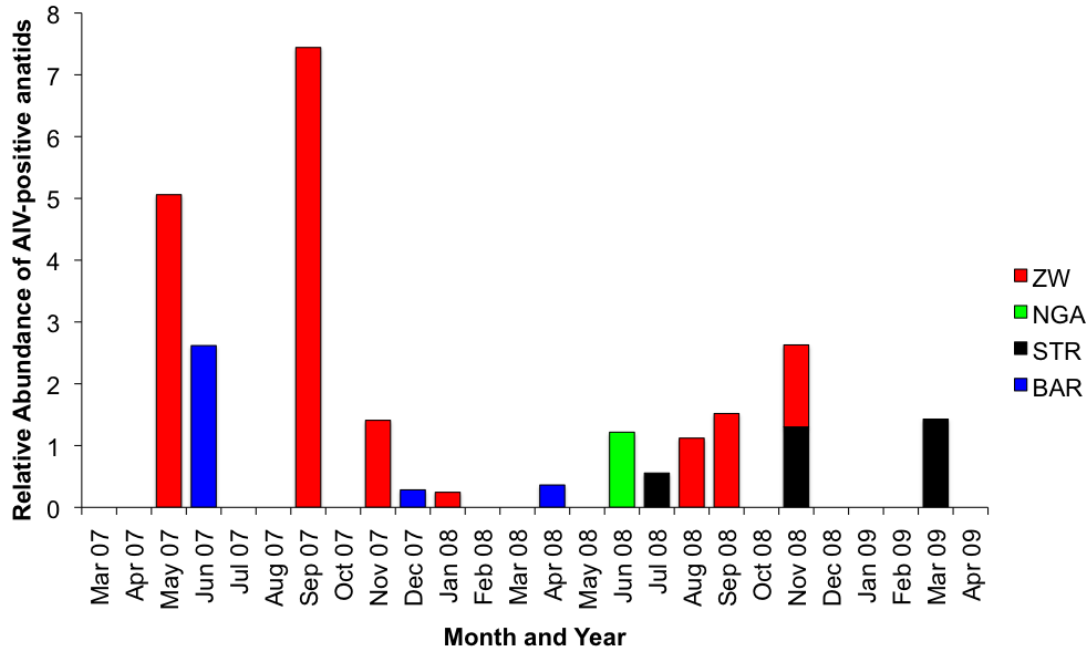
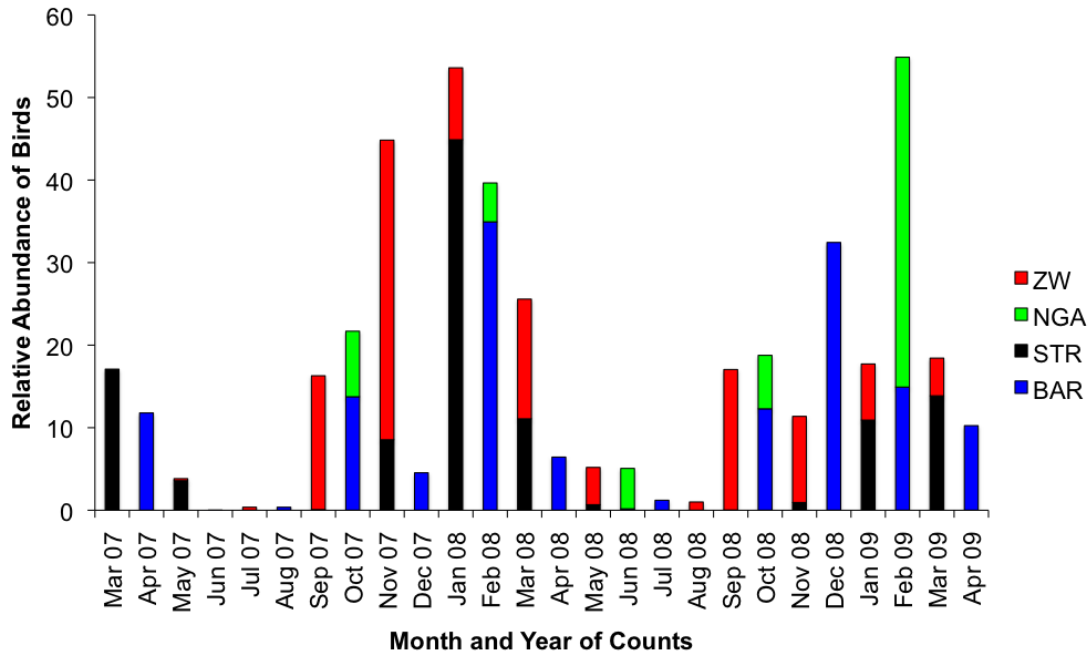


Figure A5.6: *Relative abundance of palearctic migrants per half-hour point count by site. A breakdown by species is given in Table A5.5. Birds arrive earlier at more northern sites (ZW and NGA); seasonal peaks coincide with the austral summer and the boreal winter.*

Figure 6



The prevalence of influenza A viruses in southern Africa appears to be twice as high in dendrocygnid (whistling) ducks (5.2%) as in anatid ducks (2.4%), although this result may be partly an artefact of whistling ducks having been sampled in largest numbers at the site with the highest overall viral prevalence. Most of our dendrocygnid samples were from white-faced duck *Dendrocygna viduata*, but fulvous duck *Dendrocygna bicolor* are common (if almost uncatchable) at Lake Chuali and Lake Ngami. Whistling ducks are less abundant in South Africa but we observed both fulvous and white-backed duck *Thalassornis leuconotus* as far south as Strandfontein, and Barberspan periodically hosts flocks of >20 white-faced duck. White-faced and fulvous duck have an extensive pan-African range and individuals from populations north of the equator may migrate annually to western Europe. Gaidet et al. (2007)(Appendix Two) reported an AI prevalence of 3% in west African dendrocygnids, and Gaidet et al. (2008) found HPAI H5 genomes in white-faced duck in west Africa. Given their high abundance and mobility (Cumming et al. 2008), whistling ducks may play an important regional role in the dynamics of AIV.

Although no HPAI viruses were positively identified, potentially virulent H5 and H7 strains are in circulation in southern Africa in resident wild bird populations. There is some hint of a latitudinal gradient in prevalence, with Manyame > Barberspan > Strandfontein; but data from Mozambique and Botswana do not fit this pattern, although the sample sizes (n=271 and 467 birds respectively) are too small to draw strong inferences.

Most studies of avian influenza have focused on Anseriformes and Charadriiformes (ducks and waders), but other waterbirds may play a role in maintaining AIV in southern Africa. Rallids and jacanids (e.g. Red-knobbed Coot *Actophilornis africanus* and African Jacana *Fulica cristata*) occur year-round in high abundances in many wetlands and frequently forage near to dabbling and diving ducks. Cormorants and darters (Phalacrocoridae) are common in our study sites, mobile, and frequently roost with ducks. Risks of transmission to

humans are increased by their capture in fishing nets. A variety of other species, such as Sacred ibises (*Threskiornis aethiopicus*; Threskiornithidae) also share foraging habitats with grazing and dabbling ducks; Sacred ibises in particular may feed on carcasses, making them potentially vulnerable to AIV epidemics.

For the Passeriformes, a prevalence of 4.5% (14 positives out of 308 birds) suggests a potential role in influenza epidemiology. Most of the AIV positive species that we found in this order are residents (yellow throated longclaw, chestnut-backed sparrowlark, red-billed quelea, and village weaver; Table A5.4) but barn swallows and willow warblers are paleartic migrants. Our data and those from other studies (Chapter Three - Caron et al. 2010) suggest that some passeriform families (e.g. Alaudidae and Ploceidae) may contribute to the persistence and spread of AIV in southern African ecosystems.

In practical terms, our results preclude the assumptions of an annual cycle of viral circulation and strong seasonal variation in wild bird-related risks that hold in many northern hemisphere regions. From a health care perspective, AIV epidemics in wild birds appear to be possible at any time of the year. The opportunistic behavioural responses of waterbird populations to environmental drivers, and the lag between rainfall and bird and pathogen responses may nonetheless make it possible to obtain short-term predictions of AIV risks using information on rainfall.

Supporting Information (not displayed in the thesis)

Additional Supporting Information may be found in the online version of this article:

Appendix A5.S1.: Dates of each sampling mission.

Appendix A5.S2.: Coordinates of all counting and capture sites.

Appendix A5.S3.: Background information on study sites.

Appendix A5.S4.: Additional information on the bird counting and capture protocols.

Appendix A5.S5.: Detailed information on laboratory methods.

Table A5.S1.: The number of individuals of each of the 165 species that we sampled during this study (includes scientific, common, and family names).

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