

ABSTRACT

Title of Dissertation: WILD BIRDS AND EMERGING DISEASES:
 MODELING AVIAN INFLUENZA TRANSMISSION
 RISK BETWEEN DOMESTIC AND WILD BIRDS IN
 CHINA

Diann J. Prosser, Doctor of Philosophy, 2012

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 Marine and Estuarine Environmental Sciences

Emerging infectious diseases in wildlife have become a growing concern to human health and biological systems with more than 75 percent of known emerging pathogens being transmissible from animals to humans. Highly pathogenic avian influenza (HPAI) H5N1 has caused major global concern over a potential pandemic and since its emergence in 1996 has become the longest persisting HPAI virus in history. HPAI viruses are generally restricted to domestic poultry populations, however, their origins are found in wild bird reservoirs (Anatidae waterfowl) in a low-pathogenic or non-lethal form. Understanding the spatial and temporal interface between wild and domestic populations is fundamental to taking action against the virus, yet this information is lacking. My dissertation takes two approaches to increase our understanding of wild bird and H5N1 transmission. The first includes a field component to track the migratory patterns of bar-headed geese (*Anser indicus*) and ruddy shelduck (*Tadorna ferruginea*) from the large H5N1 outbreak at Qinghai Lake, China. The satellite telemetry study revealed a new migratory connection between Qinghai Lake and outbreak regions in Mongolia, and

provided ecological data that supplements phylogenetic analyses of virus movement. The second component of my dissertation research took a modeling approach to identify areas of high transmission risk between domestic poultry and wild waterfowl in China, the epicenter of H5N1. This effort required the development of spatial models for both the poultry and wild waterfowl species of China. Using multivariate regression and AIC to determine statistical relationships between poultry census data and remotely-sensed environmental predictors, I generated spatially explicit distribution models for China's three main poultry species: chickens, ducks, and geese. I then developed spatially explicit breeding and wintering season models of presence-absence, abundance, and H5N1 prevalence for each of China's 42 Anatidae waterfowl species. The poultry and waterfowl datasets were used as the main inputs for the transmission risk models. Distinct patterns in both the spatial and temporal distributions of H5N1 risk was observed in the model predictions. All models included estimates of uncertainty, and sensitivity analyses were performed for the risk models.

WILD BIRDS AND EMERGING DISEASES: MODELING AVIAN INFLUENZA
TRANSMISSION RISK BETWEEN DOMESTIC AND WILD BIRDS IN CHINA

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Dedication

To Haley and Jessica.

I hope you always remember the words your Pau Pau told me throughout
life: “You can do anything you put your mind to!” I know you can.

Acknowledgements

Entrenched in the pages of this dissertation lies the support of an amazing array of mentors, colleagues, friends, and family who have helped me make its completion possible. First, I am grateful to my main advisor, Erle Ellis, for his razor sharp intellect, model for successful science, and can-do *modus operandi*. I am so grateful you accepted that first meeting many years ago. And to Mike Erwin, my long-time mentor at PWRC, for introducing me to the coastal world of waterbirds, and multiple examples of how to conduct science on difficult and mobile species. To boot, not only are you one of the best speakers I know, but one of the kindest people I have met. To both of you, I thank you for having confidence in my abilities (often when I could not see it), and for giving me the time and independence to make this project happen.

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I thank Judd Howell, former director of Patuxent Wildlife Research Center, who not only provided me the opportunity to enter the SCEP program at USGS, but also supported the telemetry work at Qinghai Lake well before we developed a funded program. Without his enthusiasm and support in those crucial beginning months, the initial connections and timely research that have blossomed into a large partnership across Asia, Africa, and the Middle East may not have occurred. And thanks are extended to Greg Smith, who before I had a research direction for the dissertation, gave insightful advice on the upcoming importance of avian influenza in the wildlife field (and well beyond). There are many others at PWRC to whom I owe thanks – Leslie Lammons and Marilyn Whitehead for exceptional administrative support (our Chinese colleagues love you, Marilyn!); Mary Ratnaswamy, Graham Smith, and John French who each in their own way have supported or fought for our avian influenza program; Elizabeth Cahoon who, among all her other duties, has navigated uncharted waters to set up international contracts; Lynda Garrett for invaluable library support; Kinard Boone, Greg Jones, Sabina Reseau and the

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Introduction

The emergence of highly pathogenic avian influenza (HPAI) H5N1 (hereafter H5N1) in south-east Asia has caused major global concern over a potential pandemic (Russell and Webster 2005, Lu 2006, 2006). Worldwide, 596 human cases and 350 deaths due to handling infected poultry have been confirmed since 2003 (World Health Organization 2012a). In addition to zoonotic concerns, the disease has caused serious economic loss within the poultry industry. Over 250 million poultry have been lost to infection and culling in south-east Asia alone (FAO 2011b). As new H5N1 outbreaks in poultry were reported across Russia and into Europe during fall 2005, wild birds were implicated as the main cause of disease spread (Normile 2006b). Many have questioned, however, the ability of wild migratory birds to transport virus under compromised condition due to infection (Liu et al. 2005, Butler 2006, Chen et al. 2006b, Fergus et al. 2006, Zhou et al. 2006). April 2005 marked the beginning of the largest known outbreak of avian influenza in wild birds in history – over 6,500 birds died at Qinghai Lake, an important colonial breeding area on the edge of the Qinghai-Tibetan plateau of China (Liu et al. 2005). More than half the mortalities occurred in bar-headed geese (*Anser indicus*) resulting in a 6% decline in their global population (Delany and Scott 2002, Liu et al. 2005, Chen et al. 2006b, Zhou et al. 2006). Other species affected included ruddy shelducks (*Tadorna ferruginea*), common cormorants (*Phalacrocorax carbo*), great-black headed and brown-headed gulls (*Larus ichthyaetus* and *L. brunnicephalus*).

Wild waterbirds are known natural reservoirs for low pathogenic forms of avian influenza, carrying and shedding the virus often without clinical signs of infection (Stallknecht and Shane 1988, Alexander 2000, Olsen et al. 2006). H5N1 is unique to

previous avian influenza viruses in three main regards: (1) spillback to wild birds had been a very rare event, yet H5N1 on multiple occasions has been reported in wild birds, presumably from spillback (Webster et al. 2007a); (2) previously it had been rare for an individual strain of avian influenza virus to infect more than one species; H5N1 has been detected in 178 species of 16 taxonomical Orders (USGS National Wildlife Health Center 2012); and (3) since its emergence in domestic geese in 1996 (Xu et al. 1999), the A/goose/Guangdong/1/96 (Gs/GD) virus lineage has become the longest recorded HPAI virus to remain endemic in poultry (Vijaykrishna et al. 2008), approximately 16 years at the time of this writing. In addition, the H5N1 virus has shown signs of mutation through decreased virulence and increased viral shedding in domestic ducks which are now considered silent reservoirs for the virus (Hulse-Post et al. 2005, Sturm-Ramirez et al. 2005). Given the potential for wild birds to contribute to disease spread and the ample opportunity for domestic poultry and wild birds to co-mingle in commercial and backyard farms within Asia, it is critical to determine the most likely places for H5N1 transmission between these 2 populations.

There are a number of factors related to H5N1 spread and persistence that make China a uniquely valuable region to focus this study. The closely interwoven relationship among humans, livestock, and the natural environment that occurs in the warmer regions of southern and eastern China provide ample conditions for virus development and evolution, as can be evidenced by the origin of H5N1 from domestic geese in Guangdong province (Xu et al. 1999). China is the world's top producer of poultry, accomplished by a mix of large and mid-scale farming, yet across China house-hold level back-yard farming puts billions of birds on the landscape in a free-range setting. This range in

complexity in farming systems is an important factor to consider in transmission risk models. Along with many other regions in Asia, H5N1 has become endemic in the poultry system causing an unrelenting threat. However, unlike the 63 other countries infected with H5N1 over the past 16 years, China has remote regions where domestic poultry is relatively non-existent and outbreaks occur among wild bird populations. Having an opportunity to examine transmission risk factors under these vastly different conditions allows us to answer the broadest questions regarding H5N1 transmission between wild and domestic bird populations, which is why we focus our study within China.

Proposed Research

In this dissertation, I have proposed study of the potential risk of HPAI transmission between wild and domestic waterfowl within China by combining field data, remote sensing, and modeling techniques. In order to do so, I developed geospatial data layers of estimated bird populations for wild waterfowl and domestic poultry at multiple spatial and temporal scales across China. The input layers served as the basis for the disease transmission risk model. An important component of this work has been to quantify the level of confidence in model output. The goals and products produced here have not changed from those outlined in my dissertation proposal: (1) to create high resolution spatial datasets for (a) wild waterfowl and (b) domestic poultry across China to serve as base inputs for (2) a disease transmission risk model focusing on potential spread of HPAI H5N1 between wild and domestic birds, and (3) to explore migratory behavior of wild waterfowl from the large outbreak area at Qinghai Lake in 2005.

Summary Table of Proposed Research

Chapter 1. Literature Review	Chapter 2. Satellite Telemetry	Chapter 3. Poultry Models	Chapter 4. Waterfowl Models	Chapter 5. Risk Model
Objective	Evaluate potential wild waterfowl migratory connections between outbreak areas in Mongolia and China	Characterize the distribution of major domestic poultry species in China in a spatial framework	Characterize the wintering and breeding distributions of China's Anatidae waterfowl (42 species) in a spatial framework	Determine areas of high risk of HPAI H5N1 transmission between domestic and wild birds in at multiple scales (1 km and 30 km)
Hypotheses	Ha ₁ : There is a direct migratory waterfowl connection between the HPAI outbreak areas of Qinghai Lake, China and Erkhel Lake, Mongolia.	Ha ₁ : Poultry densities differ among eight regional subdivisions of China (based on Verburg and Chen 2000). Ha ₂ : Ratios of domestic ducks, chickens, and geese differ among the 8 regions in China. Ha ₃ : Domestic duck densities will be greatest in SE China where the majority of rice paddy farming occurs.	Ha ₁ : A habitat relationship modeling approach can produce useful and accurate maps for Anatidae waterfowl Ha ₂ : Regions of high Anatidae species diversity differ for breeding and wintering seasons	Ha ₁ : Reported domestic bird outbreaks are correlated with areas of higher poultry density Ha ₂ : Reported wild bird outbreaks are not correlated with wild/domestic bird high-risk hotspots Ha ₃ : HPAI H5N1 transmission risk between domestic and wild birds differs across the China landscape. Ha ₄ : Patterns in transmission risk between wild and domestic poultry differ with resolution of analyses
Proposed Manuscript Title	<i>Satellite-marked waterfowl reveal migratory connection between H5N1 outbreak areas in China and Mongolia</i>	<i>Modelling the distribution of chickens, ducks, and geese in China</i>	<i>Species distribution models for China's wintering and breeding Anatidae waterfowl</i>	<i>A geospatially explicit model of HPAI H5N1 transmission risk between domestic and wild birds in China</i>
Proposed Journal	Ibis	Agriculture, Ecosystems, and the Environment	Diversity and Distributions or Ecosystem and Ecography	EcoHealth or Ecological Modelling
Draft Manuscript	Published 2009	Published 2011	In Prep	In Prep

CHAPTER 1. LITERATURE REVIEW

Avian Influenza

Type A Influenza

The avian influenza virus (AIV) is an RNA virus of the family Orthomyxoviridae. There are five genera in this family, including Thogotovirus, Isavirus and influenza types A, B, and C. The natural hosts of type A influenza are birds (hence the name, avian influenza or bird flu), although mammals such as humans, horses, pigs, cats and seals have also acquired infection from this virus. Type A influenza is the most commonly distributed of the group and can cause infections ranging from subclinical to lethal in its hosts.

The virus is spherical to filamentous in shape, having a protective outer protein layer (M1) with 3 glycoproteins protruding the surface: Hemagglutinin (HA), Neuraminidase (NA), and M2. The function of the HA protein is to aid the virus in attachment and penetration of the host cell. The NA protein assists with the release of newly replicated virus from the host cell. The M2 genes upon exposure to lower pH opens the virus shell to expose the virus contents within the cytoplasm of the host cell. Inside the shell are 8 RNA gene segments that encode for the production of 10 viral proteins that make up the virus. The replication process includes penetration of the surface of a host cell, invasion of the nucleus and replication of RNA, production of viral proteins in the host cell's cytoplasm, and bundling of the replicated material and exiting of the host cell (Palese and Shaw 2007).

Avian influenza viruses are classified into subtypes based on the type of HA and NA proteins found on the surface. Sixteen types of HA and 9 types of NA have been

described thus far, for a total of 144 potential combinations, all of which have been found in birds. Type A influenza viruses are identified by subtype combination, such as H5N1, H3N2, etc. Virus isolates are named using a standard convention: (1) antigenic type (A, B, or C), (2) type of animal host (if human, this part may be omitted), (3) geographic location of the isolate's origin (can range from city to country), (4) laboratory reference number, (5) year of isolation, (6) and the HA and NA subtypes often presented in parentheses. An example of virus isolated from bar-headed geese infected in during the spring 2005 avian influenza outbreak at Qinghai Lake, China is A/bar-headed goose/Qinghai/0510/05 (H5N1).

Classification by Pathogenicity: HPAI versus LPAI

Avian influenza viruses are classified into two groups based on their overall pathogenicity to domestic chickens: low pathogenic avian influenza (LPAI) causes mild disease in poultry such as mucosal infection and a decrease in egg production; highly pathogenic avian influenza (HPAI) causes severe mortality often with rapid spread resulting in 100% flock mortality within 48 hours of exposure. HPAI is measured as the level of pathogenicity caused in chickens (as opposed to other avian species) for 2 main reasons: (1) historically it was important to have an indicator of notifiable avian influenza viruses (NIA) to protect the economics of the poultry industry, and (2) avian influenza causes a variety of clinical signs depending on the species of host and its immune status, thereby necessitating a consistent measure of pathogenicity within a single host type (World Organization of Animal Health 2005). NIA cases are reported to the World Organization of Animal Health (formerly Office Internationale des Epizooties, OIE) for official control and stamping out of the virus. In brief, the OIE defines NIA's as: (1)

HPAI viruses causing at minimum 75% mortality in 4-8 week old chickens after intravenous infection; and (2) LPAI viruses having H5 or H7 subtypes (forms known to easily mutate from low pathogenic to highly pathogenic viruses). These designations, however, do not predict pathogenicity in other types of hosts such as wild birds, humans, or even other types of poultry such as domestic ducks.

Antigenic Drift and Shift

Influenza viruses are continuously changing and evolving. The majority of change occurs through a slow process of genetic mutation called antigenic drift. Small changes to specific gene segments, or point mutations, naturally occur during the virus replication process. Changes that occur to genes that produce the HA surface protein are of significance since change in the shape of the HA structure will affect the ability of the virus to attach to and penetrate the host cell. Antibodies that formerly attached to the HA protein no longer fit and the host remains unprotected. This is the reason that flu vaccines need to be updated each year. Antigenic drift results in new strains of a given subtype of AI.

Antigenic shift, on the other hand, is defined by an abrupt and major change in genetic material. This can occur if multiple virus types infect the same host and genetic material is swapped between viruses, producing a new virus. This process is called genetic reassortment and often results in a new subtype of influenza. Certain species, such as pigs, humans, and quail, are known to be viral mixing vessels, meaning that they can be infected by multiple virus types at one time, providing an opportunity for genetic reassortment (Webster et al. 1992, Makarova et al. 2003, Perez et al. 2003). The currently circulating Asian HPAI H5N1 virus, at this point in time, is not easily passed

from birds to humans, and human-to-human transmission is extremely rare (the World Health Organization (WHO) defines our current state of pandemic alert as Phase 3: “No or very limited human-to-human transmission” (World Health Organization 2012b)). Antigenic shift within this virus could result in efficient and sustained human-to-human transmission causing a worldwide pandemic.

LPAI viruses occur in all HA and NA subtypes, but HPAI viruses have been restricted to H5 and H7 subtypes, for reasons yet undetermined. Conversion from LPAI to HPAI is not common, but is believed to occur when LP forms circulate and rapidly replicate under dense poultry conditions (Perdue 2008), as was the case in the large outbreaks in poultry in Pennsylvania (H5N2) in 1983 (Bean et al. 1985, Brugh and Perdue 1991), Mexico (H5N2) in 1994 (Horimoto et al. 1995), and Canada (H7N2) in 2004 (Bowes et al. 2004, Hirst et al. 2004). One of the molecular causes for transition from LP to HPAI is the addition of amino acids at the HA cleavage site (location on the HA protein where, when split by specific proteases, activates the virus). The addition of such amino acids allows cleavage of the HA by multiple types of proteases (found within the host’s cells), allowing the virus to attack many different types of cells, causing systemic and often lethal infection within the host’s body.

Avian Influenza Virus Hosts for LPAI

Wild waterbirds, particularly those within the orders Anseriformes (waterfowl) and Charadriiformes (shorebirds and gulls) are the known natural hosts and reservoirs for LPAI (Stallknecht and Shane 1988, Alexander 2000, Clark and Hall 2006, Muzaffar et al. 2006, Olsen et al. 2006). These wild birds carry the low pathogenic form of avian

influenza often without showing clinical signs of infection. The LPAI virus is replicated in the intestinal tract, shed through feces, and transmitted via the fecal-oral route. Certain groups of species within the dabbling and diving duck subfamily (Anatinae) have higher prevalence of LPAI due to their feeding habits.

Table A.1 (Appendix A) provides a summary of species and prevalence rates from the three major review articles representing data from the Americas, Europe, and Asia/Africa. In the northern hemisphere, a number of surveillance efforts have been underway to determine prevalence of LPAI in wild birds over the past quarter century. LPAI has been isolated from more than 110 species of wild birds from 26 families (Olsen et al. 2006, Munster et al. 2007) although prevalence rates have been consistently highest within the orders Anseriformes and Charadriiformes. All HA and NA subtypes, with the exception of H13 and H16 (found in gulls), have been isolated from wild ducks, with the most common being H3, H4, H6, N2, N4, and N6. LPAI prevalence has been highest in ducks, particularly dabblers (ducks that feed in shallow waters by tipping their heads down and rumps up to reach the bottom surface) such as mallards (*Anas platyrhynchos*), northern pintails (*Anas acuta*), and blue-winged teal (*Anas discors*). Surveillance efforts show distinct geographic and temporal variations in prevalence between migrating waterfowl and shorebirds in the North American studies. Waterfowl had highest LPAI prevalence rates in fall and shorebirds had highest rates in spring (Stallknecht and Shane 1988, Slemons et al. 2003, Krauss et al. 2004). For ducks, LPAI prevalence was as high as 60% in late fall before the southward migration, with decreasing prevalence on wintering grounds (0.4 – 2%) and the northward return spring migration (0.3%). The high prevalence of LPAI in ducks in the fall is attributed to dense congregation of

juvenile waterfowl (immunologically naive individuals of the year) at fall staging areas. Prevalence in shorebirds was 14% in spring and 0.3% in the fall. The high prevalence of LPAI in shorebirds during spring migration has been suggested as a source of perpetuation of LPAI on the northern breeding grounds (Kawaoka et al. 1988, Krauss et al. 2004).

Extensive surveys were conducted in Europe over an eight year period from 1998 to 2006, including more than 36,000 birds (Munster et al. 2007). Anseriformes had the highest prevalence rates, followed by Charadriiformes. In contrast to the North American surveys, shorebirds did not exhibit high prevalence rates during spring migration. LPAI viruses were also distinct from those isolated in the Americas (Webster et al. 1992, Olsen et al. 2006, Munster et al. 2007).

Minimal surveillance has been conducted outside of North America and Europe prior to the recent HPAI H5N1 outbreaks. In 2006 the United Nations Food and Agriculture Organization (UN FAO) launched a surveillance program to sample wild birds across Eastern Europe, the Middle East, and Africa. The overall LPAI prevalence rate was 3.5% (>5000 samples) (Gaidet et al. 2007b).

Avian Influenza Virus Hosts for HPAI

While LPAI is recognized to be widely distributed in wild birds, the first case of HPAI (called fowl plague), occurred in domestic poultry in Italy in 1878 (Perroncito 1878). It wasn't until 1955 (Schafer 1955), however, that the cause of the outbreaks was determined to be highly pathogenic avian influenza. Since 1955, there have been 27 documented outbreaks of HPAI globally, all of subtypes H5 or H7 (Alexander 2000, United Nations Food and Agriculture Organization 2004, Swayne 2008). The majority of

reported outbreaks have occurred in Europe and North America (10 and 6, respectively) with the remainder in Australia (5), Asia (2), Pakistan (2), Africa (1), and South America (1) (Appendix A, Table A.2). Three quarters of the outbreaks have occurred since 1990.

Until H5N1, HPAI viruses were rarely observed in wild birds. The first case of HPAI identified in wild birds occurred in common terns (*Sterna hirundo*) in South Africa in 1961 (Rowan 1962). Over 1300 terns died on the wintering grounds of coastal South Africa in April after failing to migrate north in February. The virus was later identified as A/tern/South Africa/61 H5N3 (Becker 1966). Prior to the appearance of HPAI H5N1 in captive and free-living wild birds in Hong Kong in 2002 (Ellis et al. 2004), this has been the only outbreak reported in wild birds.

Our knowledge of pathogenicity and transmissibility of HPAI in wild birds is still quite limited, but appears to vary among species. A few challenge studies have been conducted on domestic species and farm-raised wild species. Perkins and Swayne (2002a, 2002b, 2003) challenged a suite of species including passerines, gulls, emus, and domestic ducks and geese with the 1997 Hong Kong virus (A/chicken/Hong Kong/220/97 H5N1). Zebra finches (*Taeniopygia guttata*) were most severely affected, exhibiting 100% mortality within 5 days of inoculation. House finches (*Carpodacus mexicanus*) and budgerigars (*Melopsittacus undulatus*) exhibited significant morbidity (disease) and mortality within 2 days of inoculation. House sparrows (*Passer domesticus*), emus (*Dromaius novaehollandiae*), and domestic geese (*Anser anser domesticus*) showed mild signs of infection but no mortality. European starlings (*Sternus vulgaris*), pigeons (*Columba livia*), laughing gulls (*Larus atricilla*) and domestic mallard ducks (*Anas platyrhynchos*) showed no signs of infection and no mortality.

Sturm-Ramirez et al. (2005) inoculated domestic mallards with 23 H5N1 viruses isolated in Asia between 2003 and 2004 and found that although pathogenicities varied, nearly all the viruses replicated and were transmitted to immunologically naive individuals.

Finally, Brown et al. (2008) tested 6 species of swans and geese with virus isolated from the outbreak in wild swans in Mongolia in 2005 (A/whooper swan/Mongolia/244/2005 H5N1): whooper swan (*Cygnus cygnus*), black swan (*Cygnus atratus*), trumpeter swan (*Cygnus buccinator*), mute swan (*Cygnus olor*), bar-headed goose (*Anser indicus*), and cackling goose (*Branta hutchinsii*). All of the swans showed 100% mortality, with a range of 2 to 8 days until death. Three-quarters of the cackling geese died, all within 4-8 days post infection (dpi). All of the bar-headed geese showed morbidity within 3-7 dpi, and 2 of 5 of the birds died (6-7 dpi). Cloacal shedding was detected in 4 of 5 of the bar-headed geese. Additional study of prevalence rates and pathogenicity for individual wild species would benefit efforts to understand avian influenza in wild bird populations.

Transmission Pathways for Avian Influenza

In wild birds, LPAI viruses replicate in the intestinal tract. Large amounts of virus are shed through the feces of infected birds, and transmission occurs via the fecal – oral route (Webster et al. 1978, Shortridge et al. 1998). HPAI H5N1 differs from many other strains in that the virus has evolved to replicate beyond the intestinal tract and into the respiratory system (Ellis et al. 2004, Sturm-Ramirez et al. 2004, Hulse-Post et al. 2005), allowing virus transmission to occur via both the fecal-oral route and bird-to-bird via aerosol particles. A significant change occurred with the second wave of spread in late 2003 to early 2004: pathogenicity of the virus decreased in domestic ducks while remaining highly virulent to chickens and humans (Hulse-Post et al. 2005). By allowing

its main host to survive, the virus increased its ability to spread. Not only could it replicate in ducks without causing major signs of disease (hence no mass culling) but ducks that survived shed virus for a longer period of time – an average of 17 days versus 2 to 5 days allowing increased perpetuation and spread of the disease (Li et al. 2004, Hulse-Post et al. 2005, Sturm-Ramirez et al. 2005).

Another important pathway for transmission of AIVs is through contaminated water or moist environments (Webster et al. 1978, Markwell and Shortridge 1982, Ito et al. 1995). The length of time an influenza virus can survive in wet conditions varies depending on the strain of virus and water conditions such as pH, temperature, and salinity. Some strains of avian influenza have been known to survive in water at 17° C for up to 207 days, and even longer at 4° C (Stallknecht et al. 1990). Stallknecht et al. (1990a) found that AIVs survived longer in water at lower temperatures (17 versus 28° C), and lower salinities (0 ppt versus 20 ppt). They also found an interaction effect between pH and salinity. Shortridge et al. (1998) found that AIV survived for 4 days in wet feces at 25° C. Brown et al. (2007b) were the first to study persistence of H5 and H7 AIVs in water (LPAI viruses from wild birds and HPAI H5N1 isolated from whooper swans in Mongolia and duck meat in Anyang). They determined that these viruses can persist for extended periods of time in water, that the persistence of these viruses is inversely proportional to temperature and salinity of water, and that a significant interaction exists between the effects of temperature and salinity on the persistence of these viruses (the effect of salinity is more prominent at lower temperatures). Some studies suggest that avian influenza viruses can survive in ice, allowing for persistence of the virus over winter (Zhang et al. 2006), although others discredit these findings due to

laboratory contamination (Worobey 2008). Lang (2008) successfully extracted a wide diversity of AIVs from sediments of ponds used heavily by waterfowl during spring, fall, and winter (under ice), although they did not test whether the viruses were viable and able to replicate. Despite the relatively small number of studies conducted on AIV persistence in water and environment, transmission of virus through this medium may be an important factor in the spread of AIV.

Traditionally, the main source of spread of HPAI has been through the poultry industry. Infected birds transmit the virus rapidly among densely concentrated populations, and depending on the quality of bio-security controls, movement can occur among farms and between farms and markets via contaminated vehicles and equipment. In certain parts of the globe (Asia in particular) virus is spread through the movement of feces sold as fertilizer for vegetable crops or as food for local aquaculture (Feare 2006). HPAI has also been transported through the importation of contaminated poultry meat (Tumpey et al. 2002) and illegal pet and wild bird trades. In most parts of Asia as well as Europe, the Middle East, and Africa, live bird markets are very common and are considered to be a contributor to AIV spread and source of human infection (Webby and Webster 2001). It is common to see domestic and wild birds sold under close conditions without bio-security controls, providing ample opportunity for virus spread and perpetuation.

Agricultural systems in Asia commonly integrate farming practices such as fish farming or poultry farming with rice paddy agriculture. In many areas, domestic ducks feed in rice paddies and adjacent wetlands for most of the year, creating opportunity for virus spread between wild and domestic birds either directly or through the water and

environment (Muzaffar et al. 2010). Such areas pose a high risk for AIV transfer within the poultry system as well as between wild and domestic birds. Gilbert et al. (2007) demonstrated a strong spatial relationship among free-grazing ducks, rice paddy agriculture, and HPAI H5N1 outbreaks in Thailand. China provides a unique situation to study AIV transmission risk between domestic poultry and wild waterfowl because it has both densely-farmed mixed agriculture systems as well as areas (such as Qinghai Lake in high-elevation western China) where large outbreaks have occurred in the absence of poultry.

Highly Pathogenic Avian Influenza H5N1

The first reported case of HPAI H5N1 of the Asian lineage was isolated from a sick goose in Guangdong province, southern China in 1996 (A/Goose/Guangdong/1/96)(Xu et al. 1999). Strict control measures were taken and it was believed that this form of H5N1 had been eradicated. In 1997, however, a related strain caused outbreaks in humans and poultry in Hong Kong (Claas et al. 1998, Shortridge et al. 1998), with 18 human cases, 6 of which were fatal. It is believed that the disease originated in live poultry markets. Over the next few years, the virus continued circulating in geese in southern China, and by 2000, domestic ducks had become infected with subsequent evolving genotypes (Guan et al. 2002). From 2000 through 2002, further outbreaks occurred in Hong Kong and other parts of China (Chen et al. 2004), and an increasing number of subclinical domestic ducks tested positive for H5N1 (Sims et al. 2003, Sims et al. 2005). The first cases of HPAI H5N1 in wild birds (in late 2002) were reported within a week of each other at 2 parks in Hong Kong (Ellis et al. 2004). Affected species included waterfowl, captive greater flamingo (*Phoenicopterus ruber*),

wild little egrets (*Egretta garzetta*), grey heron (*Ardea cinerea*), black-headed gull (*Larus ridibundus*), feral pigeon (*Columba livia*), and tree sparrow (*Passer montanus*). The outbreaks were contained using a combination of isolation, limited culling, and vaccination.

Late 2003 marked the beginning of multiple waves of HPAI H5N1 spread in poultry, humans, and wild birds, from China to other areas in Asia (2003-2004): South Korea, Vietnam, Japan, Thailand, Cambodia, Lao, Indonesia, Malaysia; then north to Mongolia and west to Europe (Aug – November 2005); and eventually south to Africa (early 2006). Li et al. (2004) linked the outbreaks to the 1997 virus isolated in Hong Kong based on a combination of genetic reassortments. The virus was reported to be endemic within China by 2004 and domestic ducks were revealed as key factors in the evolution and maintenance of the virus (Li et al. 2004). To date, hundreds of millions of poultry have died from infection and culling practices and 596 human cases (350 deaths) due to handling infected poultry have been confirmed since 2003 (World Health Organization 2012a).

The role of wild birds in the spread of HPAI H5N1 has been greatly debated and remains unclear (Bonn 2006, Butler 2006, Fergus et al. 2006, Normile 2006a, Fouchier et al. 2007). In April 2005, the largest known outbreak of HPAI in wild birds occurred in north-central China at Qinghai Lake - an area lacking domestic poultry. Over 6000 colonial nesting waterbirds died within a 2 month period, including 6 percent (3300 birds) of the world's population of bar-headed geese (*Anser indicus*), 1300 great cormorants (*Phalacrocorax carbo*), 930 great black-headed gulls (*Larus ichthyaetus*), 570 brown-headed gulls (*Larus brunnicephalus*), and 150 ruddy shelduck (*Tadorna*

ferruginea) (Chen et al. 2005, Liu et al. 2005, Chen et al. 2006b). A pattern in the timing of outbreaks occurred among the species, leading Chen et al. (2006b) to suggest that bar-headed geese brought the disease to Qinghai Lake. Bar-headed geese were the first to show clinical signs of infection (May 4) followed by brown-headed gulls and great black-headed gulls approximately 10 days later and Ruddy shelduck and great cormorant another 10 days after that. Chen et al. (2006b) argues that if the virus already existed at the lake, or was brought by a different bird species, that the bar-headed geese, gulls, cormorants and shelduck would have exhibited nearly simultaneous clinical infection because these species congregate in the same areas during breeding and would therefore be infected at the same time. Although this point is not without merit, two important factors are missing from this equation: (1) an understanding of the timing of bird migration and behavior, and (2) species specific virus factors such as latency time (incubation period), and amount and duration of viral shedding. Return of breeding species to Qinghai Lake is staggered, generally with bar-headed geese and ruddy shelduck returning first, followed by the gulls and cormorants (Hou, personal communication). In addition, birds returning to the breeding grounds of Qinghai Lake feed in areas outside the breeding colonies for a couple weeks before congregating at 3 nesting sites (Bird Island, San Kuai Shi, and Hai Xin Shan). The lake is the largest salt-water lake in China (6500 sq-km) with multiple large fresh-water streams and wetlands feeding into the system. In the weeks preceding nesting, bar-headed geese and ruddy shelduck feed together in the surrounding freshwater wetlands and wheat fields. Fish-eating cormorants and great black-headed gulls are usually found near the large river mouths draining into the lake. Brown-headed gulls, a generalist that feeds on grains to

invertebrates, returns to the lake a few weeks after the others, and can be found dispersed among the two groups of birds. Upon nesting, the birds separate into three main groups: bar-headed geese and gulls nest on the 3 breeding colonies mentioned above, cormorants on a separate island called Luci Dao, and ruddy shelduck in rocky crevices of mountains surrounding the lake (personal experience and Hou, personal communication). Based on the behavior of the birds and possible differences in reaction to the virus for each species, it is not unlikely that outbreaks among species would commence non-simultaneously even if infected after their return to the lake. Finally, the hypothesis that infected bar-headed geese migrated to Qinghai Lake with the virus can be questioned by the high pathogenicity exhibited among those infected in the outbreak: systemic infection with lesions in major internal organs such as heart, brain, pancreas, digestive tract, and ovaries (Chen et al. 2005, Liu et al. 2005, Chen et al. 2006b, Zhou et al. 2006).

The reported origin of H5N1 virus at Qinghai Lake differed among 4 research groups that isolated virus from the outbreak. A controversial paper published in Nature in July 2005 (Chen et al. 2005) reported close relation of virus isolates to those of domestic poultry in southern China in 2005 (A/chicken/Shantou/810/2005) despite a lack of reporting of HPAI H5N1 in domestic poultry during that time. Another study published in July 2005 by a separate group determined that 5 of 8 genomic segments were closely related to isolates from a dead peregrine falcon found in Hong Kong in 2004 (Liu et al. 2005). Zhou et al. (2006) analyzed a greater number of isolates and found their isolates to encompass those identified by both Chen et al. (2005) and Liu et al. (2005). Chen et al. (2006) (a different author than H. Chen of the 2005 Nature article) reported origin of the HA, NA, and nucleoprotein genes to be similar to those of

A/chicken/Jiangxi/25/2004. The 4 studies agreed that most viruses isolated from the Qinghai outbreak were similar in the following regards: (1) this virus was a new reassortant that included lysine at position 627 in the PB2 protein (a trait found in the human cases during the 1997 Hong Kong outbreak, and one that characterizes human virus), (2) that a series of amino acids were present at the HA cleavage site (RRRKKR) indicating HPAI, and (3) that a 20-amino-acid deletion on the NA stalk was present (also contributes to the virulence of the virus strain).

Unlike the other studies, however, (Chen et al. 2006) reported 4 distinct genotypes (genotypes represent groupings based on the combination of variation among all the internal genes) from the viruses isolated during the outbreak (15 viruses were sampled from 6 species). The PB2 genes were phylogenetically grouped into 3 clades (clades describe groupings based on a single internal gene of the virus): 2 bar-headed goose samples isolated early in the outbreak formed a clade with A/peregrine falcon/Hong Kong/2004 and A/chicken/Yamaguchi/7/2004; a single ruddy shelduck formed its own clade; and the remaining isolates formed a clade with those reported by Chen et al. (2005) and Liu et al. (2005). Based on the clades identified by the PB2 genes, and those based on a number of other internal genes, the 4 genotypes are as follows: A and B (isolated from bar-headed geese early in the outbreak), C (isolated from bar-headed geese, brown-headed gull, great black-headed gull, and common cormorant), and D (isolated from ruddy shelduck). Genotype C was also isolated from later outbreaks in Russia, Mongolia, Inner Mongolia, and Liaoning Province of China (Chen et al. 2006). The genotypes reported here outline differences in virus structure which helps to build an understanding of the history of H5N1 at this location, however, the addition of ecological

information from the host species, as well as species level physical and immunological response to exposure would help inform our understanding of the role the different species have in the epidemiology of the disease.

The autumn of 2005 marked the spread of HPAI H5N1 into geographic areas beyond southeast Asia, with first-case reports from Russia and Kazakhstan in July; Mongolia in August; Turkey, Romania, and Croatia, in October; Nigeria, Iraq, India, Greece, and Bulgaria in January (2006); Italy, Hungary, Germany, Slovenia, France, Austria, Albania, Bosnia Herzegovina, Egypt, Cameroon, Azerbaijan, Iran, Georgia, Niger, Pakistan, Sweden, Switzerland, in February; and Afghanistan, Poland, Denmark, Israel, Palestine, Jordan, and Scotland in March. During this time, media reports and many government officials implicated wild birds in the spread, without plausible evidence, and often contrary to known bird behavior and migration patterns. A global debate ensued, and in May 2006, the FAO and OIE organized a conference including 300 scientists from 100 nations to discuss the potential involvement of wild birds in the spread of HPAI H5N1. Conclusions from the meeting did not reveal a unidimensional answer, rather that the spread of the disease is complicated, including wild birds in some situations (Europe), poultry in others (Africa), and a combination in yet others. A vast gap in knowledge was recognized regarding susceptibility and transmissibility of wild birds to the virus, as well as general ecological information such as migration routes of individual species.

Following the FAO/OIE conference, a number of papers attempted to address the wild bird debate. Gauthier-Clerc et al.(2007) provided an examination of evidence supporting hypotheses of spread for both the poultry industry as well as through wild bird

migration. They concluded that although wild birds likely contribute to local spread of the virus, that the majority of the global spread has been due to the poultry industry, in particular along railway lines that connect Asia, Russia, Europe, and Africa. Kilpatrick et al. (2006) produced a comprehensive analysis exploring likely pathways of spread of HPAI H5N1. They investigated trade routes for poultry and wild birds, migratory patterns of wild birds, and phylogenetic relationships of virus isolates for 52 introduction events of HPAI H5N1 since 2003. They found that the majority of introductions into Asian countries were likely caused by poultry (9 of 21), whereas introductions to Europe were likely caused by wild birds (20 of 23), and introductions to Africa were caused by a combination (2 and 3 by poultry and migratory birds, respectively). Gilbert et al. (2006b) examined the timing of HPAI H5N1 spread from Russia and Kazakhstan to the Black Sea during autumn 2005. They concluded, based on timing of migration, and the absence of outbreaks in poultry in the vicinity of wild bird deaths, that waterfowl species could have spread the virus from Asia to Europe that year.

Anatidae – Waterfowl

As one of the main reservoirs of low pathogenic avian influenza viruses (Clark and Hall 2006), and the fact that the highly pathogenic H5N1 has on multiple occasions spilled back to wild birds (Fergus et al. 2006, Webster et al. 2006), it is important to increase our understanding of waterfowl ecology and distribution. Below is a general introduction to the Anatidae waterfowl.

The family Anatidae (ducks, geese, and swans) belongs to the Order Anseriformes (waterfowl). There are 150 living species of waterfowl divided into 3 families: Anatidae, Anhimidae (screamers – 3 species found in South America only), and

Anseranatidae (magpie-goose – 1 species found in Australia/New Guinea). The Order Anseriformes has a global distribution and these birds are highly adapted for aquatic habitats. They have short legs, webbed feet, broad bills, short tails, and wings that are generally set well back on their body. Of the 3 families of Anseriformes, China has 42 species all within the Anatidae family (Mackinnon and Phillipps 2000).

Most of the Anatidae species are either long or short distance migrant species, meaning that they breed in the north and winter in the south often in order to follow critical food sources through the year. There are 2 major flyways for Anatidae species in Asia: the Central-South Asian flyway and the East Asian flyway (Figure 1.1). Detailed migratory patterns of waterfowl in these flyways are not well understood.

Wild Bird Distribution Maps

If we are to increase our understanding of how H5N1 may be transmitted within or between bird populations in a spatial context we must first understand how the populations are distributed. Global or even continental gridded population datasets do not exist for wild birds, perhaps because of the large amount of effort involved in producing such datasets. Winter and breeding distribution maps have been created for North America based on two ambitious expert volunteer programs, the Christmas Bird Count (CBC) and the Breeding Bird Atlas (BBA). Detailed surveys involving thousands of volunteers are conducted within 25 mile radius plots (CBC) or within quarter quadrangles (BBA) to produce 7.5 minute output maps for winter and breeding bird populations (Root 1988, Robbins and Blom 1996). Population surveys for North American waterfowl are estimated by federal, provincial, and state-run annual aerial counts covering more than 2.0 million square miles of breeding habitat (U.S. Fish and

Wildlife Service 2007). Bird surveys that involve the magnitude of effort required by the the aforementioned programs are not commonly implemented in areas outside of North America and parts of Europe.

Although the number of novice and expert bird watchers is growing in China, large scale programs such as the ones mentioned above have not yet taken hold. There does exist a large scale program across Asia called the Asian Waterfowl Census (AWC) administered by Wetlands International, a non-profit organization concerned with the conservation of wetlands and wildlife dependent on wetland systems (www.wetlands.org). The AWC is an annual international volunteer census of wintering waterbirds conducted in Asia each January since 1987 (Lopez and Mundkur 1997). Unfortunately, China is one of the least covered countries within the census; in 1994 to 1996, only 6, 14, and 4 sites were covered, respectively, by 3, 8, and 3 participants. Participation has increased, however, with a total of 300 sites visited over the 24 year period, 67 within the last report date of 2007.

The most common form of distribution information is reported as descriptive natural history such as the following example for the bar-headed goose (*Anser indicus*), a species negatively affected in the outbreak in Qinghai Lake in 2005. The bar-headed goose is a medium-sized goose (70cm) averaging 2200g with a global population of 60,000 individuals (Delany and Scott 2006). The bar-headed goose breeds in high elevation areas of central-western Mongolia and the Tibetan and Qinghai Plateaus of China and winters in northern India and Pakistan, southern India, Myanmar, and Thailand. Coarse range maps (defining the general areas where a species has been found in the past) can be found in field guides such as MacKinnon and Phillipps (2000). The

largest breeding colony (52,000 birds) was recorded at Qinghai Lake in 1999-2000 (Li 2001a). Numbers have decreased over the past 50 years and although the bird is sacred to the Tibetan Buddhists, it is reported to be hunted by other groups. Habitat loss is a large problem for this species, particularly due to the loss of riverine wetlands by dams built to support hydroelectric plants and channelization of rivers.

Modeling approaches such as habitat suitability assessments and species distribution modeling (Scott et al. 1993, Csuti 2000) utilize alternative methods for drawing inferences about species populations by taking advantage of large-scale datasets such as remotely sensed land cover and elevation. As an example, the Gap Analysis Program (Scott et al. 1993) incorporates existing information on species range limits, known location data, habitat modeling, and expert opinion to predict the presence of a species in a particular geographic area. For each species, the following steps are taken: (1) obtain location records and attribute their source in a geodatabase, (2) delineate the range extent for each species using the best available information and subdivide the extent into known occurrence and extrapolated occurrence areas, (3) conduct expert review of the range maps, (4) develop a database of habitat use for each species, which can be used to develop a (5) Wildlife Habitat Relationship Model (WHRM) based on available GIS data (watersheds, mountain ranges, land cover classes, elevation, slope, soil types, min max temperatures, etc.), (6) conduct an expert review of the WHRM, and (7) integrate range limits and habitat association into a predicted species distribution map using GIS habitat data. Spatial output is in the GAP hexagon format. Predicted distributions are meant to be treated as testable hypotheses which are aimed to have an accuracy of 80% or higher. Accuracy of the predicted models is tested using multiple

levels of validation data including species checklists, species occurrence records, and field surveys. Scott et al. (1993) compared predicted species lists for 3 managed areas in the state of Idaho and found GAP modeling efforts to have an overall omission error of 11% (45 species) and commission error of 21% (88 species) (omission error here represents the number of species predicted to be absent that were actually present and commission error represents the number of species predicted to be present that actually were absent). Edwards et al. (1996) compared species predictions against known checklists for 8 national parks in Utah and reported accuracy rates ranging between 81 and 95% for 353 bird species.

Seonane et al. (2004) examined the question whether existing vegetation maps derived from land cover data are adequate to predict bird distributions. Predictive models for 54 bird species were built using generalized additive models (GAMs), using landscape and vegetation structure variables as predictors. They compared for each bird species the predictive accuracy of the best model derived from each map. They used vegetation structure measured at bird sample points as ground-truth data for comparing the accuracy of vegetation maps. Although maps differed in their resolution and accuracy, results showed that all maps produced similarly accurate bird distribution models, and that a mixed map produced using both thematic and satellite information performed the best. Their results suggest that land-use/land-cover maps can be accurate enough to derive bird distribution models and that there is a certain limit to improve vegetation maps above which no effect is observed in power to predict bird distribution.

Gottschalk et al. (2005) provides a comprehensive review of published studies that used satellite imagery for avian applications over the past 30 years. Over 120 published

papers were found on the topic, most of which aimed to describe relationships between bird species records and habitat characteristics. Modeling approaches to predict species distribution or abundance were applied in nearly half of the studies. Most of the studies were conducted in North America and Europe. Less than one quarter of the papers reported any type of accuracy assessment of the classification process, which varied between 60 and 99%. Types of bird species data included presence/absence (20% of studies), presence only (15%), relative abundance (65%) and bird densities (only 1 study). Satellites used in the studies included (in decreasing order) Landsat Thematic Mapper (TM), NOAA Advance Very High Resolution Radiometer (AVHRR), Landsat Multi-Spectral Scanner (MSS), Systeme Probatoire pour l'Observation de la Terre (SPOT), Indian Remote Sensing (IRS), European Remote Sensing Satellite radar image (ERS), and Meteosat High Resolution Radiometer (HRR). The newer high resolution (<4m) satellites such as IKONOS (launched in 1999), Quickbird (in 2001), and Orbview (in 2003) were not used in the reviewed studies but were noted as promising options by the authors. Statistical analyses used to create wildlife-environment relationships included Bayesian models, expert opinion techniques, traditional statistics such as Chi-square tests, T-tests, Mann-Whitney tests, and logistic and multiple regression.

Gridded Population Models

Gridded population models are a concise way to visualize and analyze large census datasets in a spatial context. Within the past 15 years, a handful of projects have emerged that model populations in high-resolution global gridded format, the most prominent being human population projects such as the Gridded Population of the World

(GPW) series (Tobler et al. 1997, Deichmann et al. 2001, Balk and Yetman 2004), and LandScan (Dobson et al. 2000) as well as the recently released Gridded Livestock of the World (GLW) which models global livestock populations (Robinson et al. 2007, United Nations Food and Agriculture Organization 2007). The GPW series (versions 1 through 3) offer a “lightly modeled” approach whereby census data are converted into spatial gridded format using a direct proportional allocation and the population number within a political boundary is apportioned evenly across the entire area. LandScan and GLW, in contrast, employ more complex population reallocations based on other input factors such as roads, urban areas, and environmental factors.

The first version of GPW was created in the mid 1990’s by the National Center for Geographic Information and Analysis (NCGIA) at the University of California, Santa Barbara. The model transformed population data from native spatial units (defined by administrative boundaries) to a global grid of quadrilateral latitude-longitude cells at a resolution of 2.5 arc minutes (approximately 5 km at the earth’s equator). They employed proportional allocation, or areal weighting, which works on the assumption that the variable being modeled is distributed evenly over the administrative unit. Grid cells were assigned a portion of the total population for the administrative unit dependent on the proportion of the area of administrative unit that the grid cell covered. For example an administrative unit with a population of 10,000 covering 100 grid cells would contain 100 persons per cell. They implemented this gridding routine for each country and merged the national grids to produce continental and global raster data sets of population counts (i.e., persons residing in each grid cell). Because the grids were based on a latitude-longitude reference system, grids varied in size as a function of latitude – the

farther from the equator, the smaller the size of the grid. Grids are approximately 21, 15, and 5 square kilometers (sq km) in area at the equator, 45° and 75 °, respectively. They created a separate area grid containing the total land area within each cell based on the latitude. In addition, cells that contained large uninhabitable areas (such as water or ice) were masked using a filter and the area of habitable land was calculated for each cell. The population grid was multiplied by the area grid to produce a population density grid. In the years 2000 and 2004, Columbia University's Center for International Earth Science Information Network (CIESIN) updated the GPW to versions 2 and 3, respectively, using new census data and slight modifications in model processing. In 2005, CIESIN and 2 other institutes built upon GPWv3 to create the Global Rural-Urban Mapping Project (GRUMP) which modeled rural and urban areas at a 30 arc second resolution (approximately 1 km at the equator). Deichmann et al. (2001) describes the different sources of error in their modeling process. These included error in: (1) population census data (such as accuracy of interpolation method which assumes a constant rate of growth between intervals, timeliness of census estimates, number of estimates, and accuracy of the estimates), (2) boundary accuracy (timeliness of the boundary in relation to the population census), and (3) positional accuracy (areal weighting assumes homogeneity in population distribution within a political boundary which might not be true particularly within boundaries covering large areas). They note that error analyses are rarely performed for existing spatial population databases (including their own). The areal weighting technique they used does not allow for cross-validation methods such as kriging (where interpolated point data are evaluated by comparing the point to neighboring data points) because cells within an administrative polygon are identical.

They suggest one approach that entails modeling population distribution at a higher aggregation level than the input values then comparing the resulting totals at the lower level with recorded information. They admit that such error measures are not as reliable as cross-validating residuals for point data (eg. kriging) but the sensitivity analysis could help identify problem regions.

The LandScan Global Population Project was developed by Oak Ridge National Laboratory (ORNL) in 2000 (Dobson et al. 2000). The group produced a 30 arc second (approx 1 km at the equator) global spatial population database for 1998. The unit of measure was *ambient* population, or the estimated population over a 24 hour period, as opposed to traditional population estimates that focus on residential or nighttime use only. The authors chose this measure because they felt that an ambient measure of population would be more useful for predicting risk analyses for emergency response. For example, they argue that it's more important to classify a cell that contains a multi-lane highway passing through an uninhabited area (such as a desert) as some level of 'populated' even though no-one lives there because of the risk to lives should an emergency occur. They used a distribution allocation based on probability coefficients including road proximity, slope, land cover, and night-time lights. The coefficients were weighted values independent of the population census data. Examples include (1) road proximity (weighted distance from cells to roads), (2) slope (weighted by favorability of slope categories), (3) land cover (weighted by type with exclusions for certain land cover types), and (4) Nighttime Lights of the World (weighted by frequency). Probability weights were customized for different regions based on economic, physical, and cultural factors. Recognizing that verification of global spatial population databases are difficult

to perform based on a lack of suitable reference datasets, they attempted to use indirect measures to verify their data and validate their models. They compared high-resolution population estimates with indicators of population such as buildings, settlements, and land cover classes (high intensity developed, low-intensity developed, cultivated, etc.). They also reported verifying, validating, and conducting sensitivity analyses for input data (land cover, elevation, roads, and nighttime lights).

Tian et al. (2005) posed the hypothesis that a reliable human population dataset could be modeled for China using land cover predictor variables. They used county-level census data, land cover data derived from Landsat TM imagery ((Liu et al. 2002), Appendix C), digital elevation models (GTOPO30), temperature (National Weather Bureau), and ancillary data such as railways, highways, rivers, and cities. All of their input data were converted to 1 km raster GRID cells in Albers Equal Area map projection. They used separate approaches to modeling human densities within rural and urban areas. For cells in rural areas, they used multivariate regression models to create population probability coefficients. For cells in urban areas they used a power exponential decay model based on city size and distance from urban center to calculate population probability coefficients. Analyses were conducted for 12 agro-ecological zones of China. They determined that human population densities for China could be predicted with reasonable accuracy using land cover predictor variables. This is one of the few studies that completed an accuracy assessment. The mean relative error was 3.1% for rural areas and 5.3% urban areas. They also found their model accuracy to be higher than other existing models (LandScan and GPW) at the cell, county, and province scales.

The United Nations Food and Agriculture Organization's Animal Production and Health Division (UN FAO APHD) in conjunction with the Environmental Research Group of Oxford created the first global, sub-national resolution spatial dataset of major agricultural livestock including cattle, buffalo, sheep, goats, pigs, and poultry: Gridded Livestock of the World (GLW) (UN FAO 2007). Output is in ESRI GRID format at 3 arc minutes (approx 1 km at the equator) as animal densities (number of animals per sq km) for each type of livestock. For each country, sub-national (usually provincial level) livestock census data and administrative boundary data were digitized and entered in an Oracle database. Missing values were approximated using data from a higher administrative level (usually national) and subtracting the available sub-national values to produce an estimate for the remaining area or by filling gaps using predictor data as described below. Once the dataset was complete, they identified areas where livestock production was known to be zero based on political or environmental factors (for example, pigs are banned in Islamic countries, and livestock are absent from ice areas). They removed these areas and calculated densities by distributing the number of animals over the remaining area of land within the administrative unit (a technique termed suitability masking). Livestock were grouped into 2 categories including (1) ruminant agriculture and ruminant livestock (cattle, buffalo, sheep, goats) and (2) monogastric livestock (pigs and poultry). Example environmental datasets used to mask suitable lands for the poultry group included protected areas, population density (>1500 people km^{-2}), LandScan lights (>90), elevation ($>4750\text{m}$), and LandScan land cover classes (water, developed, partly developed, wetlands, wooded wetlands, tundra, snow, and ice). Output at this stage included 'observed' densities of livestock type per administrative unit

converted into 1 km raster GRID format. Distributions of these densities were then disaggregated based on modeling statistical relationships between livestock numbers and environmental predictor variables. They created training datasets by extracting a series of regularly spaced sample points for each ecological zone within a country. Values for observed livestock densities and predictor variable were extracted for each point. A series of stepwise multiple regression analyses was performed to establish statistical relationships between the observed densities and predictor variables. The statistically significant predictor variables were applied across all pixels within the ecological zone to create the predicted distributions. This process was completed for each ecological zone within each country and the outputs combined into a total global predicted livestock density map.

The descriptions above of large-scale gridded population modeling offer some detail on how such models are produced. For the poultry modeling chapter (Chapter III.), I worked with the GLW team to produce new poultry maps on a species basis for China. The map sets are included in the updated resources offered through the FAO website.

Disease Risk Models

Epidemiology, the study of how disease is distributed in populations and the factors that cause these patterns in distribution (Gordis 2004), has come a long way since the mid-1800's when John Snow solved the mystery of London's cholera outbreaks by mapping incidence locations against source of water supply. Advances in mapping, spatial analysis, and remote sensing technology, provide sophisticated tools for using the relationship between space, time, and disease factors to understand disease spread.

Below is a description of four recently published papers that address spatial risk analyses of avian influenza spread, followed by a discussion of how they relate to other published H5N1 risk models and the goal of modeling transmission risk between wild and domestic birds in China.

Boender et al. (2007) created geographic risk maps for the spread of HPAI H7N7 between poultry farms in the Netherlands in 2003. They based their analyses on the spatial location of farms and epidemiologic factors of infected farms such as status of infection during the outbreak (uninfected (S), infected but not infectious (E), infected and infectious (I), and removed(R)); number of barns; number, type and age of animals; number of sick and dead animals per day; timing of mortality increase; and timing of culling. An infection matrix (C) was created ($n \times t$, where $n = 5360$ farms, and $t = 78$ days). A distance matrix (D) was created containing pair-wise distances between farms. The individual unit for the risk model was the farm. The key function of their model is called the transmission kernel and is defined as the infection hazard posed by farm i to farm j as a function of inter-farm distance. The kernel is estimated from D and C using maximum likelihood. They selected the best transmission function by comparing alternatives using Akaike's Information Criterion (AIC), a model selection tool that measures goodness of fit of an estimated statistical model (Burnham and Anderson 2002b). They used Mathematica 5.2 for all data processing, modeling, and statistical analyses. Their model predicted two high risk areas of endemic spread within the country, which was in close agreement with the outbreak data. The main factor driving the model was distance to outbreak. Sensitivity analyses were conducted to test the effect of uncertainty of the *moment of infection of infected farms* (m) variable. In the analysis,

they increased m by two days and although the transmission kernel increased, the overall spatial map of predicted infected farms was remarkably similar to the original output. They also examined the potential effectiveness of two control strategies – culling and vaccination. They found that culling within 1 km of infection was not effective; culling within the 3km ring was somewhat effective (100 farms were still classified as high risk in the output); and culling within a 5km ring was fully effective (although admittedly nearly impossible to enforce in a real-world situation). They determined that vaccination would not be effective once an infection reached a farm within a densely populated poultry region because of the amount of time it would take to vaccinate the large numbers of poultry (1 week to vaccinate, and 7-14d for the vaccine become effective). They did, however, determine that use of vaccine as a preventative measure in the high-density poultry areas before an outbreak occurred would be effective in controlling the spread of the disease.

Gilbert et al. (2008) took another approach to risk mapping. They outlined three main objectives for the risk analysis of H5N1 in 2004-2005 in Vietnam and Thailand: (1) to compare five potential risk factors associated with H5N1 outbreaks in the two countries, (2) to evaluate the value of adding a rice cropping variable to the model, and (3) to evaluate the predictive power of an HPAI H5N1 risk map by developing it on data from Thailand, testing it on Vietnam, and applying it to the Mekong region. They examined epidemiologic data (incidence) for each country from January 2004 – December 2005 and grouped these data into three outbreak waves based on clustering of timing of outbreak events: wave I, January 2004 to May 2004; wave II, June 2004 to May 2005; and wave III, June 2005 to December 2005. Instead of examining outbreaks based

on distance measures, they chose five potential risk factors to correlate with outbreak data: elevation, human population, chicken and duck abundance, and rice cropping intensity (number of rice crops produced per year with values ranging from 0 to 3). For elevation, they used the publicly available SRTM 90-m elevation data (Farr et al. 2007) and obtained human population data at the sub-district level from each country. Poultry data for Vietnam were obtained at the commune level for (a) chickens and (b) ducks and geese. Thailand poultry data were extracted from a detailed survey of farms in October to mid-November 2004 during the outbreak which included variables such as numbers of free-grazing ducks, farm ducks, native chickens, and industrial production chickens (layers and broilers). They used Xiao et al.'s (2006) algorithm to identify rice paddy areas and estimates of the number of rice crops per year from MODIS data (<http://modis.gsfc.nasa.gov/>). For the response variable, they converted outbreak data into a spatial data layer of presence/absence of disease for each of the three outbreak waves. They then performed a logistic regression analysis between the five predictor variables and the outbreak response variable. In order to account for spatial autocorrelation in the response variable, they added an autoregressive term as a covariate in the regression model. This was accomplished by averaging the presence/absence values among a group of neighbors (defined by the limit of autocorrelation) and weighting the value by the inverse of the Euclidean distance (Augustin et al. 1996).

Gilbert et al. (2008) found a significant association between human population, elevation, rice cropping intensity, and to a lesser extent duck abundance. Chicken abundance was not significantly associated with risk except in wave III in Vietnam (an area where chickens are highly abundant). A positive association existed between both

duck numbers and rice cropping intensity and H5N1 presence. A negative association existed between elevation and H5N1 presence. In Thailand, data precision allowed for an analysis of farm ducks versus free-grazing ducks against H5N1 presence; the farm ducks were not significantly associated with virus presence, but the free-grazing ducks were. They also found that the model based on presence of H5N1 in Thailand during wave II can be applied to other areas in the Mekong region.

Goutard et al. (2007) examined the risk of introduction of HPAI H5N1 into the poultry population surrounding two lakes in Ethiopia during the wild bird migratory season (December to March). They divided the process into three steps by assessing: (1) risk release via migratory birds and the legal or illegal poultry-product marketing chains, (2) risk exposure between imported and exposed poultry and among wild and domestic birds, and (3) risk consequences considering the introduction of disease into the poultry industry and the probability of it escaping detection. Steps 1 and 2 were evaluated using multiple data sources including expert opinion (epidemiologists, ornithologists, and wildlife specialists), published literature, and preliminary field visits. They used @Risk software (©2006 Palisade Europe) to calculate the risk of exposure of domestic poultry at the two lakes based on the (a) density wild birds potentially exposed to disease divided by the poultry densities in the two areas, under the assumption that wild birds can act as a source of HPAI H5N1. Density of potentially exposed birds (a) was calculated by multiplying the proportion of migratory birds potentially infected with the disease multiplied by the probability of resident birds being exposed to the virus through infected migratory birds. Their model did not measure absolute risk, rather it measured the relative risk of HPAI H5N1 transmission into the poultry system between the two lake

areas. Their results indicate a relatively low risk of transmission to either lake, although one lake had three times the level of risk than the other. This lake (Awassa) is an important area used by migrating wild birds, and also houses a large poultry system locally.

Ferguson et al. (2006) developed an individual based model (IBM) to predict HPAI H5N1 spread patterns and timing in humans in the U.S. and Great Britain, in the event that a pandemic should occur. They incorporated high-resolution population densities and travel patterns to make predictions about potential disease spread. Other measures included transportation movements, population densities and geographic patterns, transmission rates for households, schools, and communities, influenza reproduction number (R_0 , estimated from 1918 and 1957 pandemic influenza mortality data), and classification of clinical cases (assumed 50% of those infected are ill enough to be classified clinical). They modeled the geographic spread, and number of cases expected should the pandemic reach either the U.S. or Great Britain. They also examined effectiveness of travel restrictions and school closures, and determined the timeliness in which vaccines need to be distributed in order to have a significant impact. The model provides a way to determine which factors are important in the disease spread, and to determine different outcome scenarios in advance of an actual pandemic.

Each of the 4 studies described above take very different approaches to modeling avian influenza transmission risk including SIR disease modeling, identifying risk factors by drawing statistical relationships between outbreaks and agro-environmental predictors, risk assessment of introduction to poultry using uncertainty analysis, and individual based modeling to predict potential spread patterns in the human population. Components of

the SIR disease models and the uncertainty models are useful approaches that could be employed in the wild bird transmission models for China. For example, we used viral uptake and shedding rates taken directly from two SIR models for use in the H5N1 transmission risk equations. A major feature of our risk models was to incorporate uncertainty measures directly in the modeling process using Monte-Carlo analysis. Although we would have preferred to use a data-driven approach to modeling transmission risk (such as in the Gilbert paper), a lack of data precluded us from doing so. The reason for this is that training data such as locations of virus outbreaks may be recorded by general type (human, poultry, wild bird, etc.) but we cannot determine the transmission pathway that caused the event; for example, whether an outbreak in poultry was caused by exposure from infected poultry, wild birds, or other. Even if a perfect surveillance system was in place, it would be difficult to design it to determine the transmitting host. For this reason, we took an approach that allows us to understand where overlap between wild (waterfowl) and domestic (poultry) populations is most likely to occur.

In a recent review, Gilbert and Pfeiffer (2012. *In Press*) note a lack of published studies that explicitly incorporate wild birds in the modeling process. Data on wild birds - particularly high resolution spatial and temporal data – is difficult to find, which explains why so few studies have formally integrated them. The transmission risk models produced in this study will be among the first to specifically focus on risk the interface between wild birds and domestic poultry.

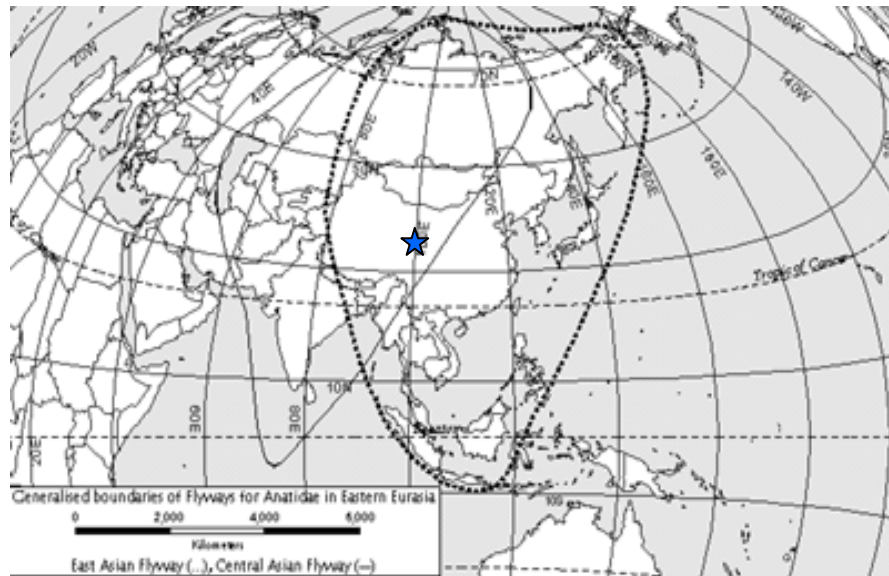
Application of this work beyond HPAI H5N1

Our global population continues to grow and countries such as China and India, which house over a third of the world's people, are undergoing rapid economic change. With these developments comes an increase in standard of living and demand for more dietary protein such as meat products (Delgado 2003). The issue of disease in agricultural populations will only continue to intensify with this increasing demand and the highly globalized nature of our society in recent times (van der Zijpp 1999). We should expect to see more threats of potentially devastating diseases evolving as we continue to commercialize and produce larger, denser meat-producing farms. Climate change and habitat loss are two other global issues that affect the health of ecosystems and emergence of new diseases (Colwell et al. 1998, Zell 2004). Building a model that helps us understand the important factors involved in H5N1 transmission between domestic and wild birds is a good exercise in outlining the general steps and thought processes involved that can be applied to other disease situations. Each of these issues demands an interdisciplinary approach, calling upon experts from various fields such as virology, ecology, epidemiology, climate, remote sensing, agriculture, human health, spatial analysis, modeling, etc. to work together to solve these issues (Patz et al. 1996, Macdonald and Laurenson 2006, Melville and Shortridge 2006). This work is a small step in that direction which can be learned from, improved upon, adapted, and applied to future global studies.

The building blocks of this work (geospatial poultry and waterfowl population estimates and satellite telemetry studies of waterfowl migratory patterns) each have value independent of avian influenza risk modeling. Spatial maps of poultry distributions could

be useful to those in the poultry industry as well as epidemiologists studying other types of poultry-related disease. Little is known about Anatidae distributions in China and this work will be useful to those interested in species conservation, habitat management, and human-wildlife interactions. Virtually no information exists on detailed movements, timing, and habitat use of waterbird species such as the bar-headed geese, ruddy shelduck, and great black-headed gulls and the results of this work will contribute to the base of knowledge for these species.

Figure 1.1. Central-South Asian flyway (solid line) and East Asian flyway (dotted line) for waterfowl in Asia. Blue star indicates location of Qinghai Lake, China.



CHAPTER 2. SATELLITE TELEMETRY

Manuscript Title: Satellite-marked Waterfowl Reveal Migratory Connection Between H5N1 Outbreak Areas in China and Mongolia

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Abstract

The role of wild birds in the spread of highly pathogenic avian influenza H5N1 has been greatly debated and remains an unresolved question. However, analyses to determine involvement of wild birds have been hindered by the lack of basic information on their movements in central Asia. Thus, we initiated a programme to document migrations of waterfowl in Asian flyways to inform hypotheses of H5N1 transmission. As part of this work, we studied migration of waterfowl from Qinghai Lake, China, site of the 2005 H5N1 outbreak in wild birds. We examined the null hypothesis that no direct migratory connection existed between Qinghai Lake and H5N1 outbreak areas in central Mongolia, as suggested by some H5N1 phylogeny studies. We captured individuals in 2007 from two of the species that died in the Qinghai Lake outbreaks and marked them with GPS satellite transmitters: Bar-headed Geese *Anser indicus* (n = 14) and Ruddy Shelduck *Tadorna ferruginea* (n = 11). Three of 25 marked birds (one Goose and two Shelducks) migrated to breeding grounds near H5N1 outbreak areas in Mongolia. Our results describe a previously unknown migratory link between the two regions and offer new critical information on migratory movements in the region.

Keywords: highly pathogenic avian influenza H5N1, satellite telemetry, waterfowl, wild birds.

Introduction

Highly pathogenic avian influenza (HPAI) H5N1 virus (hereafter H5N1) continues to threaten societies worldwide with the potential for a human pandemic. The precursor variant to the currently circulating H5N1 was first discovered in an outbreak in domestic geese in southeastern China in 1996 (Guo et al. 1998, Xu et al. 1999, Zhao et al. 2008). In 1997, the reassorted virus emerged in Hong Kong causing the first documentation of human fatalities from a purely avian influenza virus (Claas et al. 1998). H5N1 has since continued to circulate in poultry in Asia under multiple re-emergence events (Guan et al. 2002, Li et al. 2004, Sims et al. 2005, Chen et al. 2006a, Sims and Brown 2008, Wang et al. 2008). However, in April 2005 more than 6000 wild birds, including 3,300 Bar-headed Geese (*Anser indicus*) and 145 Ruddy Shelducks (*Tadorna ferruginea*), died of H5N1 infection on nesting grounds at Qinghai Lake, north-central China (Chen et al. 2005, Liu et al. 2005, Chen et al. 2006b). This outbreak marked a turning point in the evolution and spread of H5N1. For the first time since its emergence, wild birds were infected with H5N1 in a major epizootic, raising concerns over whether they have the ability to spread the disease along migratory corridors (Fergus et al. 2006, Normile 2006b, Weber and Stilianakis 2008).

Radiation of H5N1 out of Asia and into Russia, Europe, and Africa occurred subsequent to this event (Chen et al. 2006a, Gilbert et al. 2006b, Sims and Brown 2008). In August 2005, H5N1 was reported in north-central Mongolia with deaths of an additional 89 waterfowl including Bar-headed Geese and Whooper Swans (*Cygnus cygnus*) at Erkel and Khunt Lakes (OIE 2005, Wildlife Conservation Society 2005) 1200 km north of Qinghai Lake. The general lack of poultry in the Qinghai Lake and

Mongolia outbreak regions (FAO 2007a;b) raised questions about how the disease spread to these areas. Several reviews have pointed to the domestic poultry system as the primary mechanism of H5N1 spread (Muzaffar et al. 2006, Normile 2006a, Feare 2007, Gauthier-Clerc et al. 2007), although wild bird involvement could not be ruled out and in certain events likely played a significant role (Gilbert et al. 2006b). Phylogenetic analyses have been used to suggest routes of H5N1 movement based on genetic relationships of virus isolates (Chen et al. 2006a, Kilpatrick et al. 2006, Janies et al. 2007, Wallace et al. 2007), however, these analyses do not specify mechanisms underlying disease transmission. Large gaps in the knowledge of wild bird migratory patterns in Asia and the ecology of H5N1 in their populations have limited our understanding of how this disease spreads (Muzaffar et al. 2006, Olsen et al. 2006, Yasue et al. 2006, Alexander 2007).

The Asian flyways are the least studied in the Palearctic (Mundkur 2006) with little known about specific waterfowl migration routes (Miyabayashi and Mundkur 1999a, Kear 2005, Popovkina 2006). The goal of our work is to document wild bird migration patterns in Asian flyways to inform hypotheses regarding H5N1 transmission. In 2007, we initiated satellite telemetry studies at Qinghai Lake on migration pathways of two waterfowl species extensively affected in the outbreaks: the Bar-headed Goose and Ruddy Shelduck. Here we tested a null hypothesis that no migratory connection exists between Qinghai Lake and Mongolia in these species.

Methods

Study Area

We conducted research at the Qinghai Lake National Nature Reserve, Qinghai Province, in north-central China (36.82°N, 99.81°E). The 495,000 ha reserve was established in 1975 as a provincial reserve and listed as a Wetland of International Importance in 1992 (Ramsar Convention Secretariat 2007). Qinghai Lake, located on the eastern edge the Qinghai-Tibet Plateau, is China's largest salt water lake. Elevation of the lake is 3200 m and annual precipitation is 35 cm (majority of rainfall occurring May through September) (Xu et al. 2007). The Qinghai Lake climate is characterized by long, cold, dry winters (October through April), strong winds, high solar radiation, and average annual temperatures of -0.7° C (Xu et al. 2008). Qinghai Lake has long been recognized for its important position in the intersection of the East Asian and Central Asian flyways and has been designated a key breeding site for colonial nesting waterbirds such as the Bar-headed Goose (greater than 15% of the global population use this area, (Miyabayashi and Mundkur 1999a)), Ruddy Shelducks, Brown-headed Gulls (*Larus brunnicephalus*), Pallas's Gulls (*L. icthyaetus*), and Great Cormorants (*Phalacrocorax carbo*) (Li 2001a).

Capture and Marking

We captured Bar-headed Geese and Ruddy Shelducks in March 2007 and September 2007 on the western and southern edges of Qinghai Lake, China. Birds were captured outside of the breeding season to reduce disturbance to nesting birds and to increase opportunity of marking potential migrants using Qinghai Lake as a migratory stopover. We captured individuals with monofilament leg nooses (made by Indian trappers) and a remotely activated net launcher (Coda Enterprises, Mesa, Arizona, USA).

Upon capture, birds were immediately removed, placed in individual cloth bags, and processed. We recorded mass, flat wing, diagonal tarsus, sex, and age for each bird. Adult birds and equal numbers of males to females were targeted for marking. Virology sampling was conducted for birds marked in September only (due to logistical constraints) and included cloacal and tracheal swabs and blood samples for each bird following standard sampling and transport procedures (FAO 2007c). Analyses included: (a) type A influenza with an ELISA test (OD_{630} above 0.23 as positive), (b) H5 subtype with RT-PCR (see Fouchier et al. 2000), and (c) H5, H7, H9, and H10 antibodies with HI inhibition following OIE standards (OIE 2004a). Laboratory analyses were conducted by the Chinese Academy of Sciences, Wuhan Institute of Virology. Geese and shelducks were marked with 45 or 30g (respectively) GPS solar-powered Platform Terminal Transmitters (PTTs: solar-GPS PTT-100, Microwave Telemetry, Inc., Columbia, MD, USA) affixed with Teflon harnesses (Bally Ribbon Mills, Bally, PA, USA). Transmitter packages averaged 2.1 and 2.4 percent of the bird's body weight (geese and ducks, respectively). Birds were released as close to capture locations as possible within 1 hour of capture. Procedures for capture, handling, and marking were reviewed and approved by the USGS Patuxent Wildlife Research Center Animal Care and Use Committee and University of Maryland Baltimore County Institutional ACUC (Protocol EE070200710).

Satellite Telemetry Locations

PTTs were programmed to record GPS locations every 2 hours and data were uploaded to the Argos satellite tracking system every 2 days (CLS America Inc., Largo, MD, USA). For this analysis, we used a subset of locations that examined connectivity between Qinghai and Mongolia from March through June 2007 for geese and September

2007 through July 2008 for shelducks. We used ArcGIS 9.2 (Environmental Systems Research Institute, Inc., Redlands, California, USA) and Google Earth 4.3 (Google, Mountain View, California, USA) to plot and analyze the telemetry locations. Migratory stopover sites were defined as areas where birds moved less than 20 km within a 24 h or greater period of time.

Results

We marked 25 birds with PTTs at Qinghai Lake in 2007: 14 geese (12 adults: 4 males, 8 females; 2 first-year: one male, one female) and 11 shelducks (6 adults: 3 males, 3 females; 5 hatch-year: 2 males, 3 females). Three of 25 (12%) migrated to breeding grounds in central Mongolia, including one goose and two shelducks.

Of the geese, female (#67693) migrated from Qinghai Lake to central Mongolia while the other 13 marked geese remained at Qinghai Lake through the 2007 breeding season (April-June). Goose #67693 was captured and marked at a creek on the western edge of the lake on March 26. It remained at Qinghai Lake for 25 d using freshwater wetlands and wheat fields before migrating north on April 20. Between April 20 and May 7, the bird flew 1200 km to a complex of small lakes 200 km south of Erhel Lake (47.99°N, 99.88°E), Arhangay Province, Mongolia (Figure 2.1). The 17 d migration included three stopovers ranging from 1 to 7 d each (Table 2.1) and distances flown between stopovers ranged from 108 to 755 km. Goose #67693 remained on the Mongolia breeding grounds within an area of 200 km² until the PTT ceased transmitting on June 5, 2007.

Of the 11 shelducks, two migrated to breeding grounds in central Mongolia (Figure 2.1). Female #74808 and male #74810 were caught and marked on September 13 and remained within 5 km of the capture site until commencing separate fall migrations. On November 11, the female migrated 59 km southwest for a 12 d stopover, then flew 1300 km to wintering grounds (25.15°N, 97.22°E) in Kachin State, Myanmar arriving on November 23 (Table 2.2). On November 18, the male shelduck flew 763 km southwest to a riverine floodplain where it remained for 16 d before migrating an additional 576 km south to wintering grounds in the same vicinity as the female. From December to March, both birds used riverine wetlands and agricultural fields within a 370 km² area on the northern tip of Myanmar (22.19°N, 97.06°E). On March 12, 2008 they began the northward spring migration together, flying 1378 km to an area of wetlands 100 km west of Qinghai Lake. They remained here for 47 d before flying 1423 km north to breeding grounds in Mongolia, 70 km southwest of Erhel Lake (49.30°N, 99.57°E).

Virology results for the 11 shelducks sampled revealed no positives for type A influenza and two positives for H5 antibodies (inhibition at serum dilution of 1:32 and 1:64, respectively). All birds handled appeared healthy, showing no symptoms of influenza infection and virology tests of the two shelducks that migrated to Mongolia (#74808, and #74810) were negative for avian influenza (results were unavailable for goose #67693).

Discussion

Disease models have offered competing hypotheses to explain H5N1 movements from China to Mongolia, Russia, and Kazakhstan in late 2005 (Chen et al. 2006a,

Kilpatrick et al. 2006, Webster and Govorkova 2006, Janies et al. 2007, Wallace et al. 2007). Wallace et al. (2007) were among the first to use phylogenetic analyses to map patterns of hypothesized H5N1 dispersal within and beyond Asia from available genetic sequences of viral isolates. They reported on two significant H5N1 movement patterns from Asia including Qinghai Lake to Novosibirsk, Russia, and Qinghai Lake to Astrakhan, Russia. However, none of the 25 geese and shelducks tracked in our study migrated along these pathways to indicate that wild birds traveled those routes. Instead, three of our 25 birds (12%) documented a migration route from Qinghai Lake to Mongolia, a pathway that Wallace et al. (2007) reported as a possible route for movement of H5N1, but one which they found statistically insignificant.

The timing of migration from our study also provides supporting information for hypotheses put forth by Kilpatrick et al. (2006). Their integrated analyses combined molecular phylogenies, poultry routes, and wild bird patterns to suggest that H5N1 infection in Mongolia occurred through wild bird movements from China a few months before the outbreaks. The geese and shelducks in our study moved from Qinghai Lake to central Mongolia in early May, approximately 3 months before outbreaks in wild geese and swans were reported.

Establishment of the migratory connection between these two regions, however, does not prove that an infected bird could survive and shed virus along the 1200 km migration (Weber and Stilianakis 2007). Laboratory trials have shown varying rates of mortality and viral shedding among species, including geese shedding virus for several days before dying and some migrant ducks and previously-exposed swans shedding virus without any clinical signs of infection (Sturm-Ramirez et al. 2005, Brown et al. 2008,

Keawcharoen et al. 2008). Despite extensive global surveillance efforts over the past 5 years (Alexander 2003, Ellis et al. 2004, Chen et al. 2006a, Globig et al. 2006, Buranathai et al. 2007, Gaidet et al. 2007a, Gaidet et al. 2007b, Munster et al. 2007, Nagy et al. 2007, Wallensten et al. 2007, Wildlife Conservation Society 2007), detection of H5N1 in healthy wild birds is rare (Chen et al. 2006a, L'Vov D et al. 2006, Saad et al. 2007). However, successful migration of an HPAI (H5N2) infected White-faced Whistling duck (*Dendrocygna viduata*) recently documented in Nigeria demonstrates the possibility for wild bird movement of HPAI (Gaidet et al. 2008).

Two of the 11 shelduck exhibited H5 antibodies, suggesting that these individuals survived prior infection of a type A influenza with H5 hemagglutinin (although this does not indicate that the subtype was H5N1 nor that the birds migrated while infected). Challenge studies by Brown et al. (2008) suggest relatively low susceptibility of Bar-headed Geese to A/whooper swan/Mongolia/244/2005 (H5N1) with exposed birds shedding virus for 5-8 d and three out of five inoculated geese making full recoveries. If these results from captive-reared geese relate to wild birds, geese that survived the 2005 outbreaks could have moved the virus to other places. The marked birds in our study migrated in multiple segments among stopover areas within a few days; conditions that could permit viral transmission among individuals sharing these stopover habitats.

Our work documents existence of a migratory connection between Qinghai Lake and Mongolia and provides new insights into the ecology of these species as well as informing hypotheses of H5N1 spread. Only when we have a greater understanding of the ecology of migratory populations in these Asian flyways will the possible role of wild birds as vectors for H5N1 be revealed.

Acknowledgements

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Tables

Table 2.1. Location, duration of stopover, duration of flight, and habitat used by Bar-headed Goose (#67693) during 2007 spring migration between HPAI H5N1 outbreak areas of Qinghai Lake (QL), China and central Mongolia.

Date	Location	No. Days at Location	No. Flight Days	Province, Country	Km Flown	Coordinates (Decimal Degrees)	Habitat
3/26	Capture site, sw edge of QL	<1		Qinghai China	0	36.76°N 99.77°E	Small stream, oxbow lake
3/26 - 4/20	Western edge of QL	25		Qinghai China	0	37.22°N 100.02°E	Freshwater wetlands and wheat fields
4/20	Stopover A	1	1	Qinghai China	108	38.13°N 99.53°E	100m lakes
4/22	Stopover B	1	1	Qinghai China	196	39.71°N 99.32°E	Riverine wetlands
4/27 - 5/4	Stopover C	7	4	Bayanhongor Mongolia	755	46.45°N 100.21°E	150m lakes
5/7 - 6/5*	Breeding grounds	28	3	Arhangay Mongolia	172	47.99°N 99.88°E	150-700m lakes

*Last signal received from PTT on 5 June 2007.

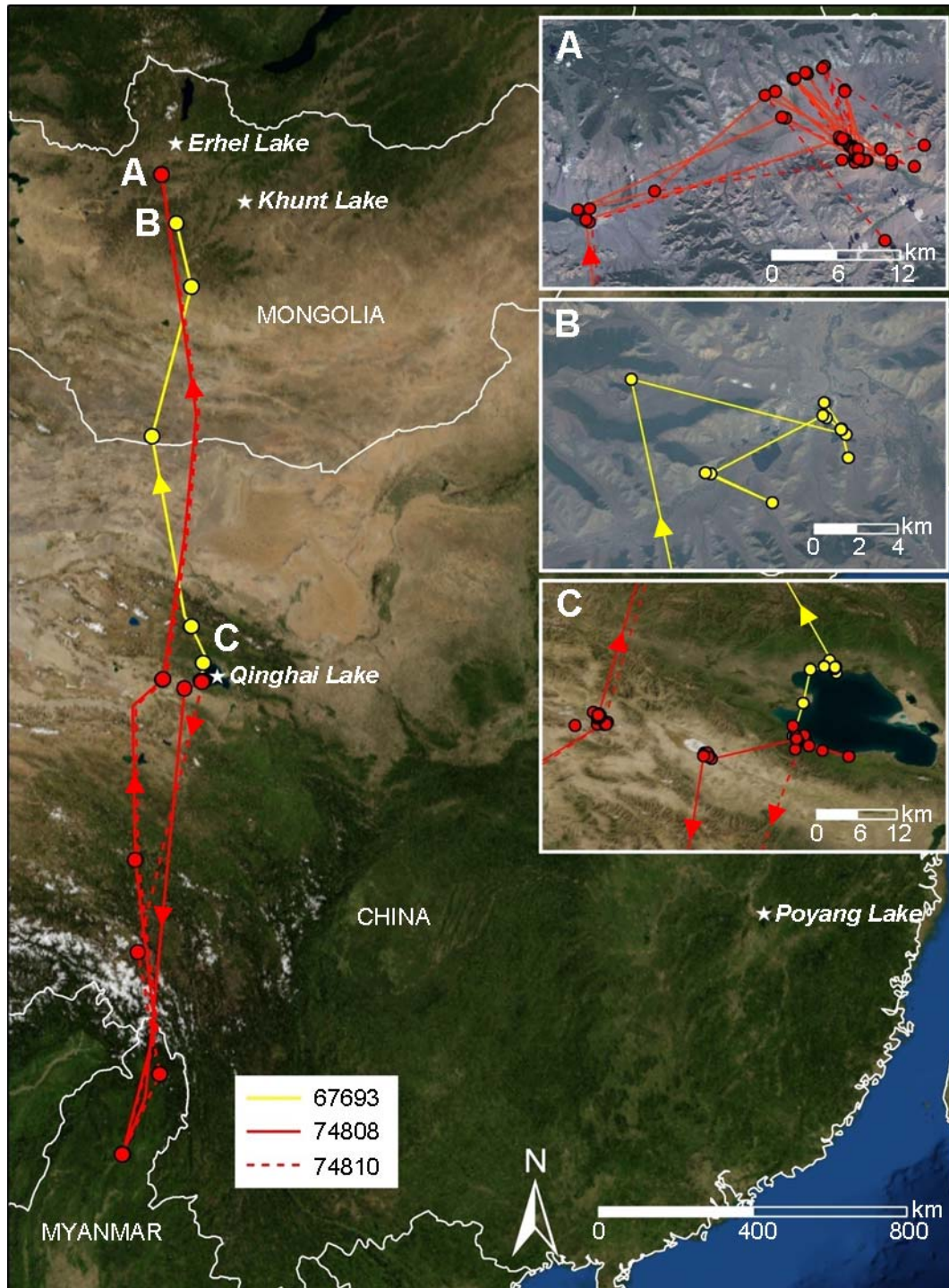
Table 2.2. Location, duration of stopover, duration of flight, and habitat used by Ruddy Shelduck (#74808 and #74810) during fall 2007 and spring 2008 migrations between HPAI H5N1 outbreak areas of Qinghai Lake (QL), China and central Mongolia. Southward fall migrations were flown separately. Birds were paired on wintering grounds, during the northward spring migration, and on the breeding grounds.

Date 2007-2008	Location	No. Days at Location	No. Flight Days	Province, Country	Km Flown	Coordinates (Decimal Degrees)	Habitat
<i><u>Ruddy Shelduck #74808, female – 2007 fall migration</u></i>							
9/13- 11/11/07	Capture site, Hei Ma He	28		Qinghai China	0	36.74°N 99.80°E	Lake edge
11/11- 11/23/07	Stopover A	12	<1	Qinghai China	59	36.64°N 99.18°E	Freshwater wetlands
11/24/07- 3/12/08	Wintering Site	109	1	Kachin Myanmar	1300	25.15°N 97.22°E	Riverine wetlands, agricultural fields
<i><u>Ruddy Shelduck #74810, male – 2007 fall migration</u></i>							
9/13- 11/18/07	Capture site, Hei Ma He	35		Qinghai China	0	36.74°N 99.80°E	Lake edge
11/18- 12/5/07	Stopover A	16	<1	Tibet China	763	30.19°N 97.44°E	Riverine wetlands
12/5/07 3/12/08	Wintering Site	98	<1	Kachin Myanmar	576	25.15°N 97.22°E	Riverine wetlands, agricultural fields
<i><u>Ruddy Shelduck #74808 and #74810 – 2008 paired spring migration</u></i>							
3/14- 4/30/08	Stopover A	47	2	Qinghai China	1378	36.86°N 98.50°E	Wetlands and small lake
5/2- 7/15/08	Breeding Grounds	74	2	Hovsgol Mongolia	1423	49.30°N 99.57°E	Riverine wetlands, mountain cliffs

Figures

Figure 2.1. Migration routes of 1 Bar-headed Goose (yellow) and 2 Ruddy Shelducks (red) from Qinghai Lake to central Mongolia. Qinghai, Erhel, and Khunt Lakes are locations of large wild bird HPAI H5N1 outbreaks in 2005, marking the spread of the disease beyond Asia. Insets provide enlarged view of local habitats for the following areas: (a) Ruddy Shelducks breeding grounds, (b) Bar-headed Goose breeding grounds, (c) Qinghai Lake local bird movements. Circles and lines demarcate breeding, wintering, and migration locations. Poyang Lake, location of suspected progenitors to Qinghai HPAI H5N1 isolates is indicated in the lower right of the map.

Figure 2.1.



CHAPTER 3. POULTRY MODELS

Manuscript Title: Modelling the distribution of chickens, ducks, and geese in China

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Abstract

Global concerns over the emergence of zoonotic pandemics emphasize the need for high-resolution population distribution mapping and spatial modelling. Ongoing efforts to model disease risk in China have been hindered by a lack of available species level distribution maps for poultry. The goal of this study was to develop 1 km resolution population density models for China's chickens, ducks, and geese. We used an information theoretic approach to predict poultry densities based on statistical relationships between poultry census data and high-resolution agro-ecological predictor variables. Model predictions were validated by comparing goodness of fit measures (root mean square error and correlation coefficient) for observed and predicted values for ¼ of the sample data which was not used for model training. Final output included mean and coefficient of variation maps for each species. We tested the quality of models produced using three predictor datasets and 4 regional stratification methods. For predictor variables, a combination of traditional predictors for livestock mapping and land use predictors produced the best goodness of fit scores. Comparison of regional stratifications indicated that for chickens and ducks, a stratification based on livestock production systems produced the best results; for geese, an agro-ecological stratification produced best results. However, for all species, each method of regional stratification produced

significantly better goodness of fit scores than the global model. Here we provide descriptive methods, analytical comparisons, and model output for China's first high resolution, species level poultry distribution maps. Output will be made available to the scientific and public community for use in a wide range of applications from epidemiological studies to livestock policy and management initiatives.

Keywords: poultry; China; distribution modelling; population estimates; GIS; epidemiology

Introduction

Globalization and a growing demand for meat products in developing regions in recent years have led to rapid expansion of the livestock sector, particularly pork and poultry meat in Asia. With these changes come an increased threat of emerging zoonotic diseases and a need for improved food safety and the implementation of appropriate biosecurity measures. Epidemiological efforts, livestock sector planning, and policy development all require knowledge of livestock distributions and abundance, information that is often difficult to obtain in a consistent spatial format. For example, epidemiological modelling of highly pathogenic avian influenza (HPAI) type H5N1 (hereafter HPAI H5N1) in hot zones of re-emergence such as China is hampered by a lack of available data on spatial distributions of its main host, domestic poultry. HPAI H5N1 first emerged in 1996 in domestic geese of southeastern China (Xu et al. 1999). From 1997 to 2003, the virus continued to evolve and in early 2004, an extensive wave of outbreaks erupted across China and seven additional Asian countries (OIE 2004b). The

virus showed varying degrees of pathogenicity and transmissibility among chickens, ducks, and geese, with ducks potentially serving as silent propagators of the virus (Li et al. 2004, Sturm-Ramirez et al. 2005). Fourteen years later, HPAI H5N1 has spread from Asia to parts of Europe and Africa, and remains active in many regions, including China.

Since HPAI H5N1's first emergence in 1996, China has reported nearly 200 outbreaks in poultry and wild birds (primarily the former), and 39 cases in humans (OIE 2010, World Health Organization 2010). Strong government control efforts, including mass vaccination programs, a national active surveillance program, and culling of more than 35 million poultry, have led to a decrease in the number of outbreaks reported over the past year. The disease persists, however, with some human outbreaks occurring in regions without concurrent outbreak reports in poultry, raising questions as to whether underreporting of outbreaks or asymptomatic viral replication is occurring within the poultry population. High resolution distribution maps of individual poultry species would provide important input factors for disease risk modelling and vaccination strategies. To date, however, no such data have been available.

In 2007, the Food and Agriculture Organization of the United Nations (FAO) released the *Gridded Livestock of the World* (GLW): the first standardized, global, sub-national resolution population maps of livestock species, including poultry (FAO 2007a). An unprecedented accomplishment, these raster maps provide 3 arc-minute resolution livestock density estimates (approximately 5 km at the equator) based on disaggregation of agricultural census data (Robinson et al. 2007, Neumann et al. 2009). Until now, these were the only poultry distribution maps available that encompassed the whole of China. However, the temporal, spatial, and species resolutions available through GLW are not

ideal for epidemiological modelling of HPAI H5N1 in China. The current version of the GLW uses poultry data from China in 1990s. Given that poultry production increased substantially from the 1990s to 2000s in China (<http://kids.fao.org/glipha/>), and HPAI H5N1 modelling efforts target this same timeframe, it is important to have distribution models based on updated poultry figures. In addition, the GLW dataset groups all poultry into one category. As chicken, duck and geese respond differently to HPAI H5N1 virus infection (Sturm-Ramirez et al. 2005), and their production systems have different spatial distributions, mapping poultry distributions at the species level is important for epidemiological modelling efforts.

In this study, we aimed to produce 1 km resolution population distribution maps for chickens, ducks, and geese across the extent of China. We hypothesized that strong statistical relationships exist between poultry populations and agro-ecological variables, which in turn could be used to spatially disaggregate census data. Building from previous work (FAO 2007a), we investigated quality of model output using remotely sensed predictors of meteorological data (Hay et al. 2006, Scharlemann et al. 2008) compared to ones that might offer more intuitive interpretation such as land cover variables. We also explored the effects of building predictive models within varying regional stratifications, and validated our data using a subset of the observed poultry data. Finally, in concert with related distribution modelling efforts for ducks across much of Monsoon Asia (Van Boeckel et al. 2011), we compared the efficacy of using data solely from within China versus that from China and surrounding countries to determine whether the inclusion of outside data would improve model results.

The poultry distribution maps produced in this study are valuable for a variety of uses including epidemiological modelling, guiding policy decisions, livestock management, biosecurity and food safety, conflict resolution, and environmental impacts. We have made these data freely available through the USGS Patuxent Wildlife Research Center and FAO Geonetwork websites.

Materials and Methods

Poultry Data

We aimed to obtain nationwide county level (administrative level 3) statistics for the 3 major types of poultry produced in China: chickens, ducks, and geese. Poultry statistics for China are published annually by the National Statistics Bureau (NSB) and the Ministry of Agriculture's Animal Husbandry Bureau (AHB). Both agencies report standard poultry statistics including: number of individuals sold per year (SOLD), number of individuals existing at the end of the calendar year (residual poultry; RESID), and meat and egg production by weight. Annual counts of each poultry type are collected from farms and households at the township level and are reported up through county, prefecture, and provincial administrative units with final submission to the national level. These data are publicly released as aggregated total poultry figures in provincial rural and statistical yearbooks (China National Bureau of Statistics 2007). Differences between NSB and AHB statistics are attributed to the level of administrative unit for reporting and the type of poultry reported: NSB publishes aggregated estimates of total poultry (all species combined) at the county or prefecture level (levels 3 or 2, respectively) in

provincial yearbooks; AHB publishes both aggregated (total poultry) and species level statistics (chickens, ducks, geese) at the coarser, provincial scale (level 1).

We extracted poultry census data from 96 rural and statistical yearbooks (printed in Chinese) for years 2003 through 2005 (reference list provided in Supplementary Table S3.1). Data were gathered for each of China's 22 provinces, 5 autonomous regions, and 4 municipalities (hereafter referred to as 31 provinces). We accessed yearbooks from the National Library of China in Beijing, the National Agricultural Information System of the Chinese Academy of Agricultural Sciences Agricultural Institute, the China National Knowledge Infrastructure (<http://www.global.cnki.net/grid20/index.htm>), and the United States Library of Congress in Washington, D.C.

Of the standard metrics reported, we used RESID poultry for the modelling process for 2 reasons: (a) RESID counts are conducted at the end of the calendar year at peak production prior to national Spring Festival holidays, and (b) RESID was the most comprehensive metric reported. In contrast to SOLD poultry, which comprised mainly poultry raised for meat consumption (broilers), RESID poultry provides a more complete representation of the poultry populations by including egg layers, meat poultry, and backyard poultry (poultry raised by households for personal consumption). As defined by the National Statistics Bureau, residual poultry is the number of poultry held in rural and urban areas at the end of the calendar year and includes “all size and breeds of poultry... from rural cooperative economic organizations, State-operated farms, rural individuals, organizations, groups, schools, industrial/mining companies, government departments and units and raised by urban citizens” (China National Bureau of Statistics 2007).

We employed a standardized protocol for filling gaps in available poultry statistics (See Figure 3.1a and Results). In order of priority, 6 methods were used to create a complete set of poultry data for China: (1) county level RESID poultry; (2) prefecture level RESID poultry; (3) conversion of county level SOLD poultry to RESID poultry estimates; (4) conversion of prefecture level SOLD poultry to RESID poultry estimates; (5) provincial level RESID poultry; and (6) conversion of provincial level AHB RESID poultry to NSB RESID estimates (see Supplementary Fig. S3.1 for correlations between NSB and AHB provincial RESID poultry census data). We then divided total poultry figures into species estimates (chickens, ducks, and geese) using provincial species ratios from the 2006 Agricultural Census (China National Bureau of Statistics 2008) which have not yet been released to the public. Poultry census estimates were converted to geospatial format using ArcGIS 9.3 (Environmental Systems Research Institute, Inc., Redlands, CA, USA).

The Modelling Process

We modeled distributions of domestic chickens, ducks, and geese in China using the following steps modified from the GLW processing chain (FAO 2007a) (Figure 3.1b): (1) obtain poultry census data; (2) fill data gaps, develop species level estimates, and convert to geospatial format (3) mask unsuitable areas and calculate adjusted observed densities for each poultry species; (4) extract dependent (poultry) and independent (predictor) training and validation data using a stratified random sampling scheme; (5) establish statistical relationships between dependent poultry estimates and predictor covariates; (6) create predicted poultry distribution maps using equations from

statistical relationships; and (7) assess model goodness of fit using sample points omitted from the training set.

After preparing the poultry census data for input into the modelling process, we calculated observed poultry densities for each administrative unit by correcting for the area of land unsuitable for poultry production. Suitability masks for chickens, ducks, and geese were modified from original GLW monogastric livestock (pigs and poultry) masks (FAO 2007a). Our suitability masks were restricted to exclude only the most environmentally unsuitable areas for production (e.g., extreme high elevations, tundra, ice, etc; Table S3.2) but did not exclude heavily populated locations as certain phases of poultry production may occur in urban areas, such as chick hatcheries located within city limits.

We created a stratified random sampling frame that included one point per polygon (reporting administrative unit) and an average of 20 points per decimal degree across the extent of China. Sample points were bootstrapped to create 25 data sets to be used in assessing model variation. At each sample point, poultry estimates and predictor covariates were extracted. Seventy five percent of the points were used for training models and 25 percent were reserved for model validation.

We used an information theoretic approach to choose best models at iterative steps in a multivariate regression procedure (Burnham and Anderson 2002a, Whittingham et al. 2006). Dependent variables were log transformed for normality, and each independent variable was paired with its quadratic term to accommodate curvilinear relationships (Rawlings et al. 1998). The stepwise procedure began with a null model followed by inclusion of the predictor pair defined by the best Akaike Information

Criterion (AIC). The process was successively repeated for each remaining pair of predictors until one of 2 conditions was met: i) improvement in AIC score for 2 successive models was less than 1%, or ii) a threshold minimum number of unique data values was not available for each predictor pair entered in the model (i.e., 15 data points per variable pair). Coefficients from the top regression models were then applied to the predictor imagery to create predicted maps of distributions for each species. Means and coefficients of variation (standard deviation divided by mean) were estimated from the 25 bootstrapped predictions. Two goodness of fit indicators were used to assess quality of model output: root mean square error (RMSE) and correlations (COR) between predicted and observed values. Lower RMSE and higher COR indicated better fits. Correction by country totals were applied to the final maps.

Environmental and demographic conditions relevant to poultry production vary widely across the extent of China. We therefore performed regression models within stratification zones chosen to reflect regional differences in association with poultry production. Model predictions for four stratification schemes were compared: i) global livestock production systems (LPS), ii) data driven ecozones (EZ) using unsupervised classification of Moderate Resolution Imaging Spectroradiometer (MODIS) remote sensing variables and Shuttle Radar Topography Mission (SRTM) digital elevation models, iii) China Agro-ecological Regions (CAR), and iv) a combination of the first three (All.BestRSE). The LPS regions, updated from those initially developed by Sere and Steinfeld (1996) and mapped by Thornton et al. (Thornton et al. 2002), represent 14 classes of livestock production based on grassland, mixed farming, and landless systems. The EZ regions consist of 4 hierarchical levels of clustering for Asia: EZ5, EZ12, EZ25,

and EZ50 which represent 5, 12, 25, and 50 cluster classes using MODIS channels 3, 7, 8, 14, and 15, and SRTM data (Van Boeckel et al. 2011). For the EZ stratifications, we built prediction maps at the pixel level, using regression coefficients of the EZ with the lowest residual squared error (hereafter referred to as EZ.BestRSE stratification). The CAR stratification, adapted from Verburg and Chen (2000), is a modification of the commonly used China agricultural regionalization by Crook (1993). CAR divides China into 8 regions based on agriculture, economics, environment, and provincial level administrative boundaries. Modifications from Crook (1993) consisted of removing the densely populated Sichuan province from sparsely populated Tibetan Plateau and including it with Yunnan and Guizhou provinces. The final stratification, All.BestRSE, chooses, pixel by pixel, the stratification with the lowest residual squared error from the stratifications described above. Examples of All.BestRSE, EZ.BestRSE, CAR, and LPS stratifications are displayed in Supplementary Fig. S3.3. We set model conditions to perform regressions within each stratification zone, however, if criteria of a minimum of 15 unique dependent estimates per variable pair were not met, coefficients from a single country level model were then used to create predictions within that zone.

GLW distribution models have traditionally been created using anthropogenic variables such as human density, distance to roads, etc., in combination with remotely sensed surrogates of meteorological data (e.g., middle infrared, land surface temperature, etc.) as predictors. We were interested in comparing capabilities of a predictor set using the GLW approach versus one that includes interpreted remote sensing variables such as land use (e.g., cropland, wetland, grassland, etc.). The incentive for using the latter group is the potential to draw more intuitive conclusions between significant predictor variables

and poultry predictions. Thus, we ran models using 3 predictor datasets: GLW, LU, and the combined set GLW+LU (Table 3.2). The main difference between the GLW and LU sets was the inclusion of Fourier transformed MODIS data for GLW (Scharlemann et al. 2008) (Van Boeckel et al. 2011) and land use variables for LU (Liu et al. 2002).

Goodness of fit indicators, RMSE and COR, were compared in an analysis of variance (ANOVA) to determine optimal predictor sets and regional stratification schemes. Data was reviewed for conformity to the assumptions of normality and homogeneity of variance. Histograms of RMSE and COR appeared normal for each of the predictor datasets and stratifications. Since sample sizes between levels were identical in the one-way ANOVA, we assumed the overall F test and multiple comparison tests were robust to departures from the unequal variance assumption (Neter et al. 1996).

Finally, to assess the value of including poultry and agro-ecological relationships from countries surrounding China, we compared goodness of fit scores for China versus those from a related study that models duck distributions across Monsoon Asia (Van Boeckel et al. 2011). The modelling methodology in Van Boeckel et al. 2011 is similar to that used in this study (although overall proposed hypotheses differ) and includes data from 14 countries: China, Cambodia, Bhutan, Thailand, Lao, Vietnam, Myanmar, Bangladesh, India, Nepal, Korea, Malaysia, Philippines, and Indonesia.

Results

We targeted NSB data for model development because of the finer scale at which they are reported (mainly county and prefecture versus provincial level for AHB). Of 3 years of data investigated, year 2004 was most complete (86 percent complete versus 82

and 78 percent for years 2003 and 2005, respectively), and thus was used for model input. We implemented a multi-level methodology for creating complete RESID estimates from the data available (Figure 3.1a). We applied Methods 1 to 4 to approximately $\frac{3}{4}$ of the provinces (22 of 31) that had county and prefecture level data (Table 3.1). The remaining nine provinces had provincial level data; here we applied Methods 5 and 6. Method 6 uses AHB data for those provinces lacking NSB data (based on high correlation between the 2 data sets: r-square value of 99.4%, see Supplemental Fig. S3.1.).

Observed densities (census data), model predictions, and coefficient of variation are shown in Figure 3.2a, 3.2b, and 3.2c, respectively. Observed densities were highest for chickens, and considerably lower for ducks and geese (111.2, 27.4, and 6.7 thousand per km² maximum, respectively). Geographically, maximum densities were higher in southern and eastern China than the remote northern and western regions (northern and western regions defined as CAR zones 5 and 6, see Supplemental Fig. S3.3c.) Duck densities in particular were highest in southeastern China where lowland tropics and rice agriculture is prevalent. Chickens were most ubiquitous, with high densities across most of southern and eastern China, and moderate to low densities across remote regions of the north and west. Model uncertainty (COV) tended to be highest in the remote western regions of China where poultry numbers are lower.

Goodness of fit measures indicate that of the 3 predictor data sets, GLW+LU performed best (Figure 3.3): one-way ANOVAs for RMSE and COR between predicted and observed values were both $P < 0.001$, and Tukey's pairwise comparisons were all $P < 0.005$. Goodness of fit measures for stratification methods were less distinct. We compared RMSE and correlation coefficients for each species, using the best predictor

dataset only (GLW+LU). Of the 6 ANOVAs (RMSE and COR each for chickens, ducks, and geese) all but one (COR for ducks) were significant at $P < 0.05$, however, Tukey's pairwise comparisons did not indicate a single best stratification method for any of the species (Figure 3.4). LPS and All.BestRSE tended to score better for chickens; LPS, All.BestRSE, and CAR for ducks; CAR and All.BestRSE for geese, however, we found that all stratifications chosen for analysis performed significantly better than the country model (Fig. S3.4): one-way ANOVA and Tukey's pairwise comparisons were all $P < 0.001$. Since each stratification method performed significantly better than the global model and without clear statistical difference among stratifications, we chose the stratification with the best mean goodness of fit scores for each species (see Figure 3.4) to present our final output (Figure 3.2b), which was LPS for chickens and ducks, and CAR for geese.

Predictor variables Elevation, Precipitation, and Evapotranspiration were consistently ranked among the top 5 predictors for each species (Table 3.3) based on mean Delta AIC score (the amount by which the AIC score of the best model was increased after removing the predictor). Other top predictors included Area Suitable for Monogastrics, Nighttime Land Surface Temperature, Enhanced Vegetation Index, Daytime Land Surface Temperature, and Middle Infra-red readings. The predicted poultry densities were generally positively associated with Precipitation, Evapotranspiration, Daytime Land Surface Temperature, Middle Infra-red, and Area Suitable for Monogastrics; they were generally negatively associated with Elevation, Nighttime Land Surface Temperature, and Enhanced Vegetation Index. The majority of predictors included in top ranked models by AIC were from the GLW set, however,

important LU predictors included Rice Paddy for ducks and geese; and Elevation, Open Water, Developed Land, and Cropland area for all three species.

We compared the effects of including training data from countries surrounding China (Cambodia, Bhutan, Thailand, Lao, Vietnam, Myanmar, Bangladesh, India, Nepal, Korea, Malaysia, Philippines, and Indonesia) versus restricting the analysis to using training data from within China. Goodness of fit indicators (RMSE and COR) were better for analyses restricted to China (Figure 3.5) suggesting that the relationship between predictor variables and observed poultry densities within China are different from those of surrounding countries.

Discussion

The results of this work indicate that agro-environmental variables can be used to predict spatial poultry distributions in China. The process predicted density patterns that are consistent with known distribution patterns, for example high chicken densities across much of eastern China, particularly the Yellow River Basin and high duck densities in southeastern China and the Sichuan Basin. Geese were least abundant, but exhibited consistent patterns, with highest densities in Sichuan and parts of Guangdong. Validation measures between observed and predicted values indicated good fits based on RMSE and correlations. In comparison to goodness of fit values reported in the related Van Boeckel et al. 2011 paper on duck distribution modelling in Monsoon Asia, goodness of fit scores for ducks within China ranked better than those produced for most other countries.

We observed statistically significant differences in goodness of fit scores among predictor data sets but not among regional stratifications. Each of the regional

stratification methods we compared provided better goodness of fit scores than the country-wide model. However, because a clear best stratification scheme was not statistically evident, we chose the one with the best mean score for each species. This was the Livestock Production Systems approach (LPS) for chickens and ducks, and China agro-ecological approach (CAR) for geese. The combined approach (All.BestRSE) produced the second best mean scores across all species. Van Boeckel et al. (2011) found similar results for their Monsoon Asia duck models with LPS and All.BestRSE showing highest fitness scores. The predicted density maps produced by models in this study and the Monsoon Asia study (Figure 3.2b here and Fig. 4 in Van Boeckel et al. 2011) revealed similar output patterns. Here we conclude that for the China models, either stratification would be appropriate for use, however an advantage of LPS (and CAR, for geese) over the combined approach (All.BestRSE) is the more intuitive interpretation of a single stratification versus the combination of many.

Overall, uncertainty measures were low for each species (COV values ranged from <0.01 to 5). Areas with the highest uncertainties were located in northwestern China where poultry populations are scarce and environmental predictors are variable. In eastern and southern China, where poultry populations are high, uncertainty estimates were low (ranging from <0.01 to 0.08), indicating small standard deviations in relation to mean predicted densities. In general, uncertainty patterns across China were similar among species, and on average, COVs were lowest for chickens, then ducks, and geese.

The use of data external to China for training models produced inferior goodness of fit scores compared to those from models using training data entirely from within China. This exemplifies the fact that relationships between the predictor variables and

poultry distributions differ for China in comparison to neighboring countries. The 13 countries included in this analysis were predominantly located to the south of China. These countries show greater similarity to China's tropical southeastern provinces than to the high-elevation drier provinces in western China and mixed grasslands of north central China, which could account for part of the differences in goodness of fit scores. In addition, China's poultry production system far exceeds those of its neighboring countries, ranking first in egg production and second in meat production (Qing 2002, Wang 2006) on a global scale. For example, in 2004, China's poultry production was more than an order of magnitude higher than those reported by its surrounding countries except Indonesia (5.1 billion versus 1.2 billion for China and Indonesia, respectively). Remaining countries ranged from 500 million (India) to 230 thousand (Bhutan); from UNFAO's Global Livestock Production and Health Atlas (<http://kids.fao.org/glipha/>). Given the observed differences in goodness of fit scores, we do not recommend using external training data to create model predictions for China, nor should results from China be directly extrapolated to other regions in Asia.

The data fill methodology employed in this study (Figure 3.1a) provides a consistent and repeatable method for assembling poultry statistics from multiple sources representing the diverse and expansive regions across China. Despite national efforts to report agricultural statistics in annual yearbooks for each province, the administrative level of reporting varies across regions, ranging from provincial to county level (administrative levels 1 to 3). Figure S3.2 shows the spatial heterogeneity of input data used for our China models, the finest scale data being located in the poultry-rich regions of southeastern China. These differences are reflected in the uncertainty values (Figure

3.2c) with higher COVs in regions in the western and northern regions of China. To accommodate the spatial heterogeneity of input data, we chose to use a mixed random and stratified sampling design that includes a minimum of one point per administrative unit as well as an average density across the country (20 points per decimal degree). Model predictions would likely be improved with finer scale input data for the remote regions of China, however, for the target time frame of our models, we have assembled the best data available to produce distribution predictions which have been qualified with estimates of uncertainty.

Conclusions

Our goal was to produce 1 km resolution population distribution maps each for chickens, ducks, and geese in China for use in HPAI H5N1 epidemiologic modelling. This research indicates that spatial distributions for these species can be modeled using agro-ecological predictors in a regression and disaggregation approach.

We found that a combination of traditional predictors (FAO Gridded Livestock of the World) and land use predictors produced output with the best goodness of fit scores between observed and predicted values. We also learned that of four stratification schemes used to build regression models within different regions of China, the livestock production systems (LPS), China Agro-ecological Regions (CAR), and combined approach (All.BestRSE) produced the best goodness of fit scores.

Obtaining observed population data across China for model training was challenging due to availability of data, however, using a multi-step approach to systematically incorporate the best data available for each region, we produced a

complete and repeatable training set for model development. Should other datasets eventually be released to the public, the modelling process developed above can be used to create updated predictive spatial distribution maps for China.

Our poultry distribution models have been made available to the scientific and public community through the FAO Geonetwork for use in a multitude of applications from disease risk modelling to livestock and environmental management.

Acknowledgements

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Tables

Table 3.1. Data availability and method description for deriving 2004 residual poultry statistics for each of 31 provinces of China. Provinces denoted with asterisk indicates use of Ministry of Agriculture Animal Husbandry data (AHB); all others derived from National Statistics Bureau data (NSB).

Method	Data Availability	Method Description	Applicable Provinces
1	RESID County Level Data	Use county RESID	Beijing, Jiangsu, Zhejiang, Anhui, Fujian, Henan, Hunan, Guangdong, Ningxia
2	RESID Prefecture Level Data	Use prefecture RESID	Hebei, Heilongjiang, Jiangxi, Shandong, Shaanxi
3	SOLD County Level Data	Multiply by conversion for County RESID estimate	Tianjin, Hubei, Chongqing*
4	SOLD Prefecture Level Data	Multiply by conversion for prefecture RESID estimate	Inner Mongolia, Shanghai*, Hainan*, Sichuan*, Qinghai*
5	RESID Provincial Level Data	Use provincial RESID	Shanxi, Gansu
6	No NSB Data at any Level	Use AHB RESID data (provincial scale)	Liaoning, Jilin, Guangxi, Guizhou, Yunnan, Xizang, Xinjiang

Table 3.2. Predictor variables used in China poultry distribution modeling. Three groups were compared: (1) Gridded Livestock of the World predictors (GLW; FAO 2007), (2) a set of land use and anthropogenic predictors (LU), and (3) the GLW and LU predictors combined (GLW+LU).

GLW predictors	
MODIS Channels	TFA Processed Channels 03,07,08,14,15,35: mx,mn,d1,d2,d3,da,a1,a2,a3,p1,p2,p3, produced by SEEG, University of Oxford
1kgrumpdens	Alpha version kilometer resolution human population density for 2000 from GPW GRUMP, at Columbia University
1kgrumpdensb	Beta version kilometer resolution human population density for 2000 from GPW GRUMP at Columbia University
green0301c1rc	MODIS Phenology datasets, Greenup band 1, January 2003, Boston University, Dept Geography (see text)
green0301c2rc	MODIS Phenology datasets, Greenup band 2, January 2003, Boston University, Dept Geography (see text)
senes0301c1rc	MODIS Phenology datasets, Senescence band 1, January 2003, Boston University, Dept Geography (see text)
wd1kslp	Slope, GTOPO30 dataset
1kaglgprc	Length of Growing Period, Derived from FAO LGP layers using statistical modeling by ERGO
1kthlgprc	Length of Growing Period, Derived from LGP layers produced by Thornton, using statistical modeling by ERGO
Rmsuitdeg	Distance in Decimal Degrees to land suitable for Ruminants, derived by ERGO
Mgsuitdeg	Distance in Decimal Degrees to land suitable for Monogastrics, derived by ERGO
1krdsdeg	Distance in Decimal Degrees to Major Roads - using Landscan Roads layer, derived by ERGO
1kwatdeg	Distance in Decimal Degrees to Sea, Major Lakes and Rivers, Derived by ERGO
Glurdeg	Distance in Decimal Degrees to GRUMP alpha urban areas, Derived by ERGO
2kprecyr1k	Annual Precipitation, synoptic period to 2000, produced by Worldclim
acc50k	Travel time to major cities (>50,000) European Commission GEM
V590ELC	MODIS SRTM Elevation product, sea level corrected
V590EL	MODIS SRTM Elevation product
LU predictors	
Land cover	Forest, Grassland, Open Water, Vegetated Wetland, Rice Paddy, Cropland, Developed, Urban
Cropping Intensity	Hua et al. 2009
Human Population	Tian 2005
Elevation	Shuttle Radar Topography Mission
Slope	GTOPO30
*GPW GRUMP = Gridded Population of the World Global Rural Urban Mapping Project	
*ERGO = Environmental Research Group Oxford	
*SEEG = Spatial Epidemiology and Ecology Group	

Table 3.3. Top 5 predictor variables for chicken, duck, and goose distribution modeling regressions. Predictors are listed in decreasing order of mean Delta AIC (amount AIC score was increased after removing variable from the best model). A1=amplitude of annual cycle, DA=combined variance in annual, bi-annual, and tri-annual cycles, D1=variance in annual cycle (see Scharlemann et al. 2008).

Chicken	Ducks	Geese
Annual Precipitation	Elevation	Elevation
Area Suitable for Monogastrics	Annual Precipitation	Annual Precipitation
Elevation	Evapotranspiration (DA)	Daytime Land Surface Temp (D1)
Evapotranspiration (A1)	EVI (mean)	Middle Infra-red (mean)
Nighttime Land Surface Temp (max)	EVI (max)	Evapotranspiration (D1)

Figures

Figure 3.1. (a) Methods used for filling data gaps in total poultry across China, (b) methodology for modeling chicken, duck, and goose distributions for China. RESID = residual poultry at end of year, SOLD = number poultry sold, NSB = National Statistics Bureau, AHB = Animal Husbandry Bureau (see Supplemental Fig. S3.1 for NSB and AHB relationships).

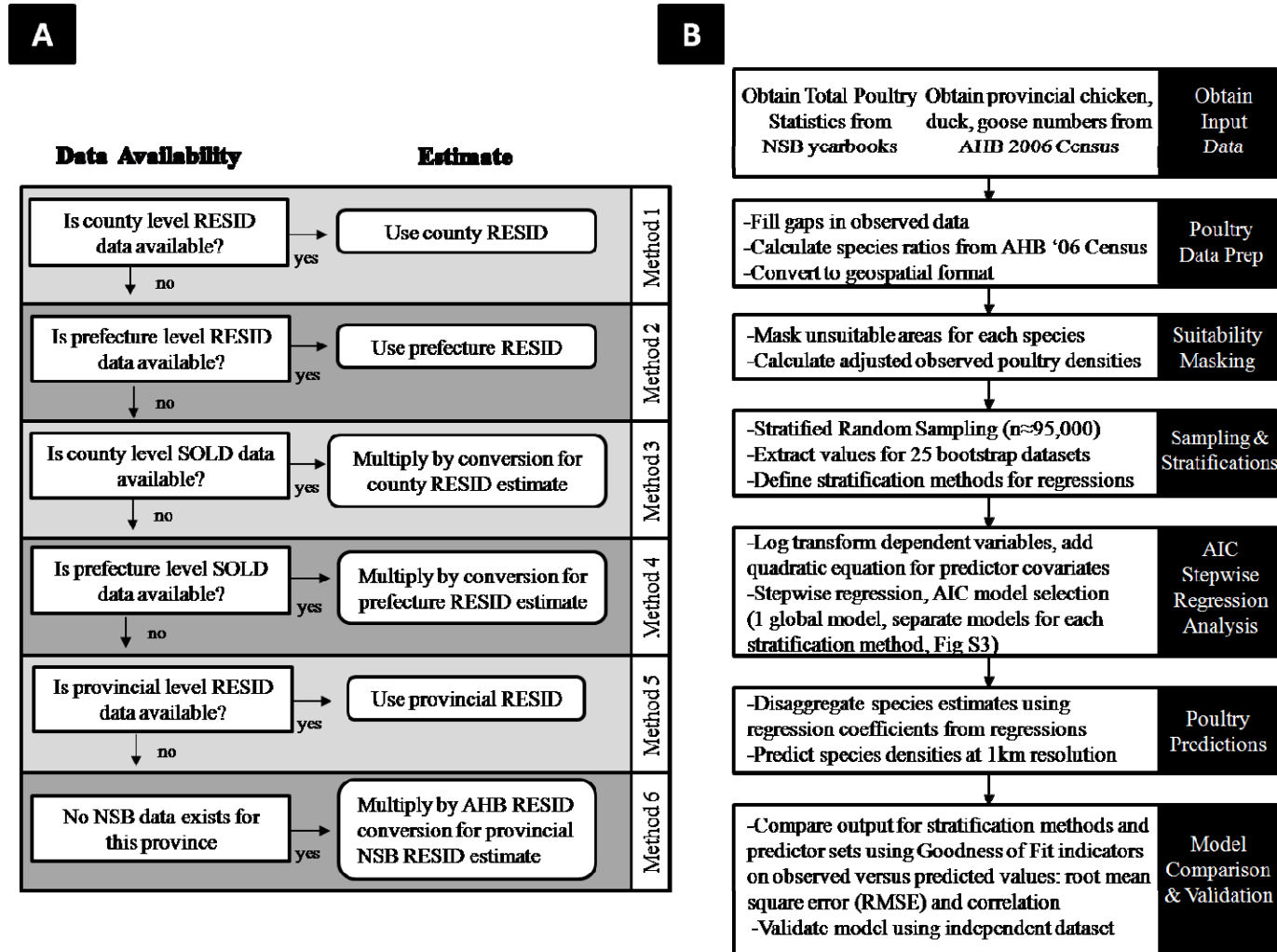


Figure 3.2. (a) Observed densities, (b) model predictions, and (c) coefficient of variation, for chickens, ducks, and geese across China. Mean densities and coefficient of variation represent 25 bootstrapped models. Model output shown for the GLW+LU predictors and LPS (chickens, ducks) or CAR stratification (geese) method (defined by goodness of fit scores).

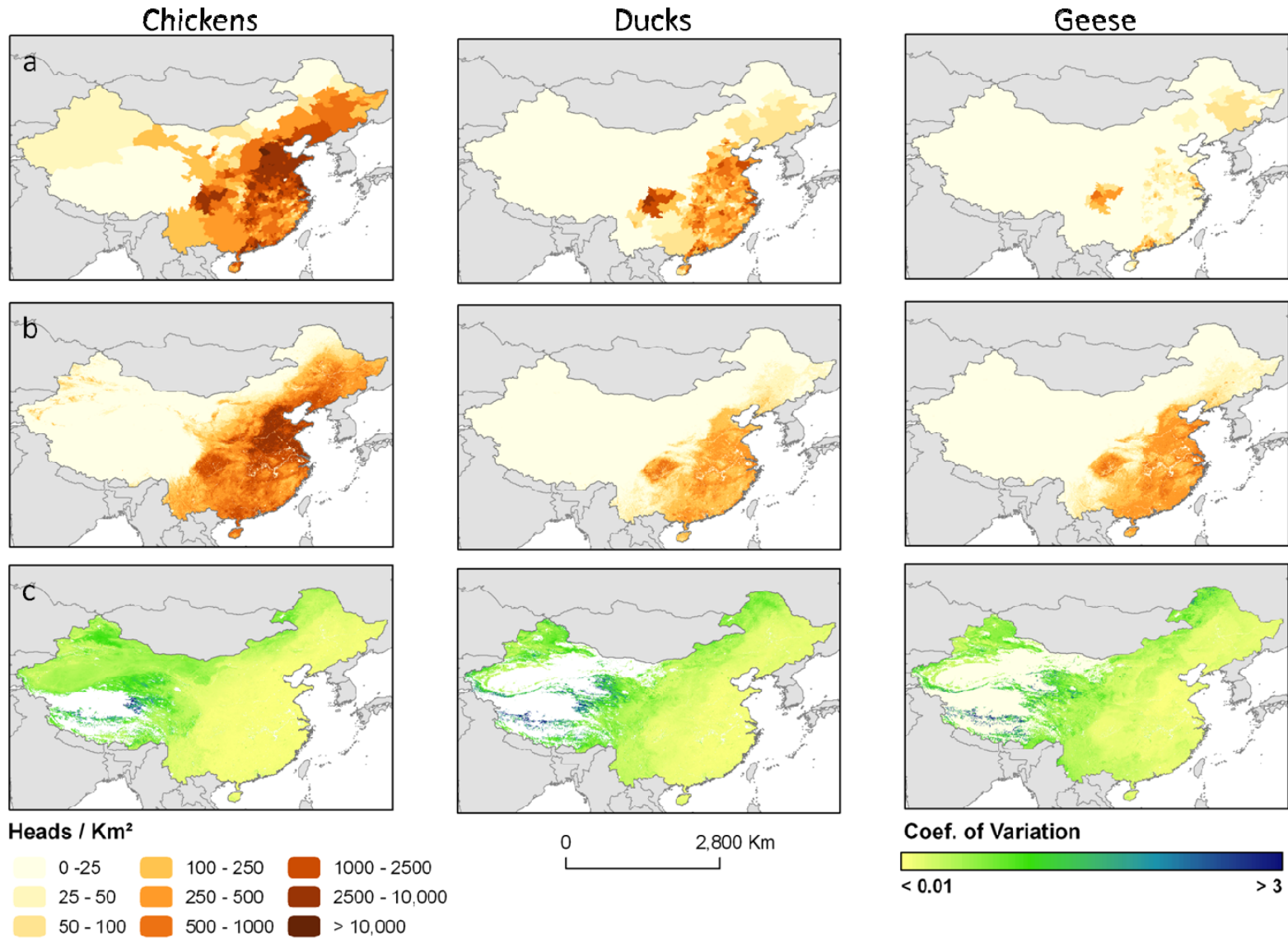


Figure 3.3. Violin plots of (a) Root Mean Square Error (RMSE) and (b) correlation coefficient between predicted and observed chicken, duck, and goose densities (log transformed) for 3 predictor datasets: GLW (traditional Gridded Livestock of the World predictors), LU (landuse and anthropogenic predictors), and GLW+LU (combination of GLW and LU predictors). ANOVA main effects ($P < 0.001$) and Tukey's Pairwise Comparisons (all $P < 0.005$) indicate significant differences among all 3 predictor sets with GLW+LU having lowest mean RMSE and highest mean Correlation between observed and predicted values.

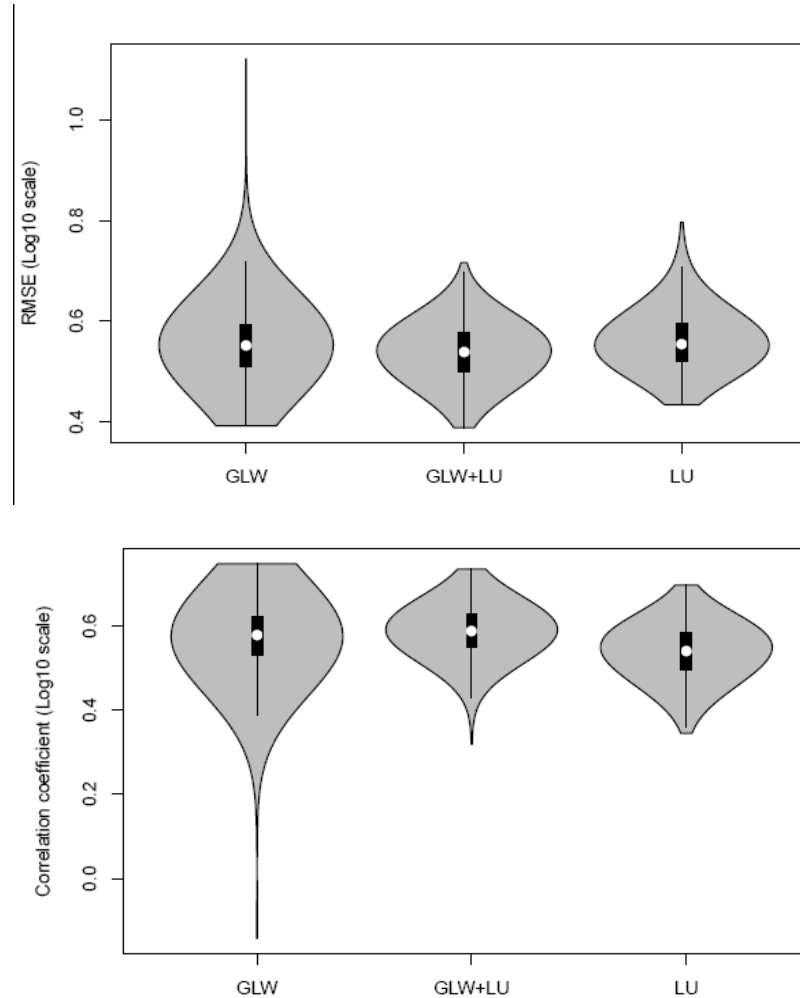


Figure 3.4. Boxplots of Root Mean Square Error (RMSE) and correlation coefficient between predicted and observed chicken, duck, and goose densities (log transformed) for 4 stratification schemes: All.BestRSE (uses prediction from stratification (BestEZ, CAR, or LPS) with the best goodness of fit score on a pixel by pixel basis), EZ.BestRSE (uses prediction from data driven classifications (EZ5, EZ12, EZ25, EZ50) with best goodness of fit score on a pixel by pixel basis), CAR (China Agro-Ecological Regions), and LPS (global livestock production systems). Main effects ANOVA significance values in lower left of each panel; means represented by black circles; Tukey's pairwise comparisons ($p < 0.05$) denoted by letters; grey boxplots represent stratification with best mean GOF, LPS for chickens and ducks and CAR for geese. Although strong differences among stratifications were not evident, all stratifications examined performed better than the global model (i.e., no stratification; $P < 0.001$ see Fig. S3.4).

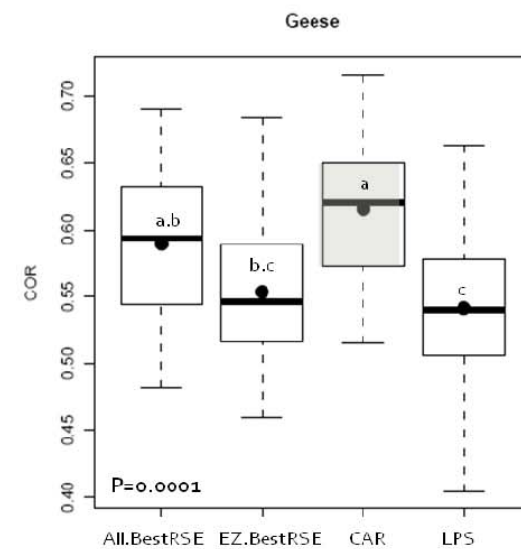
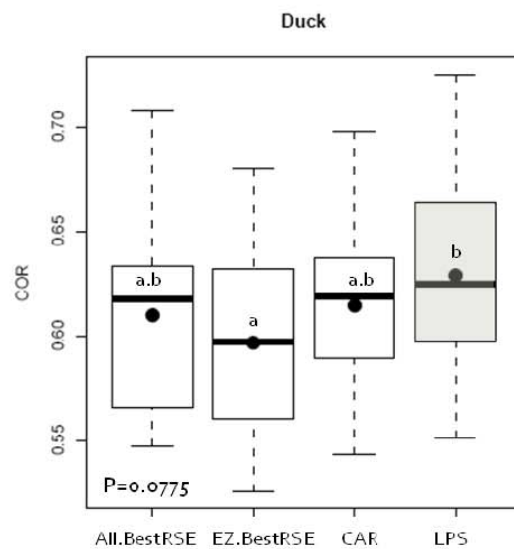
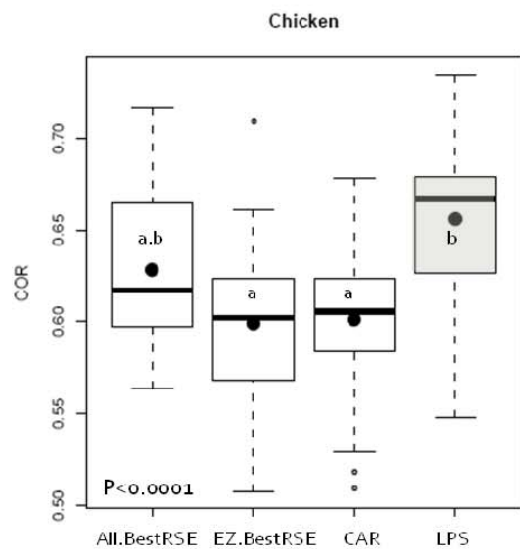
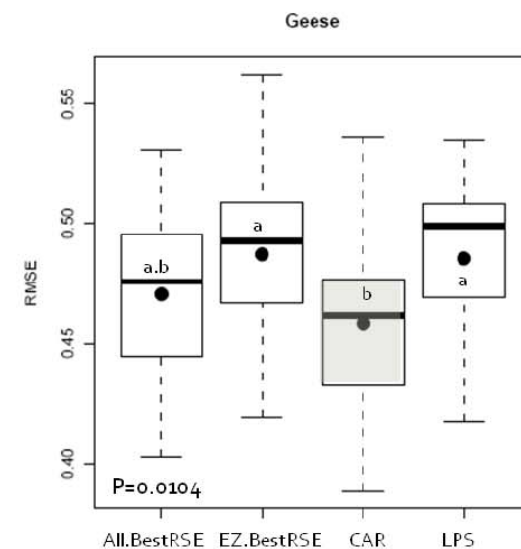
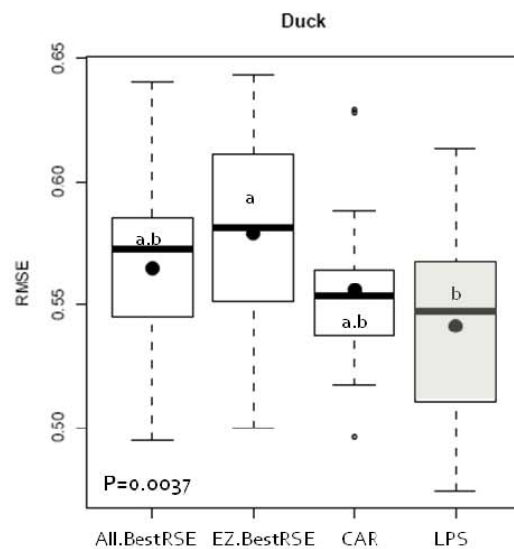
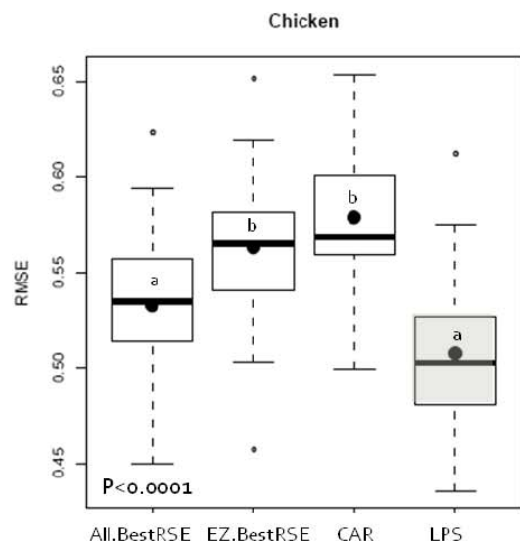
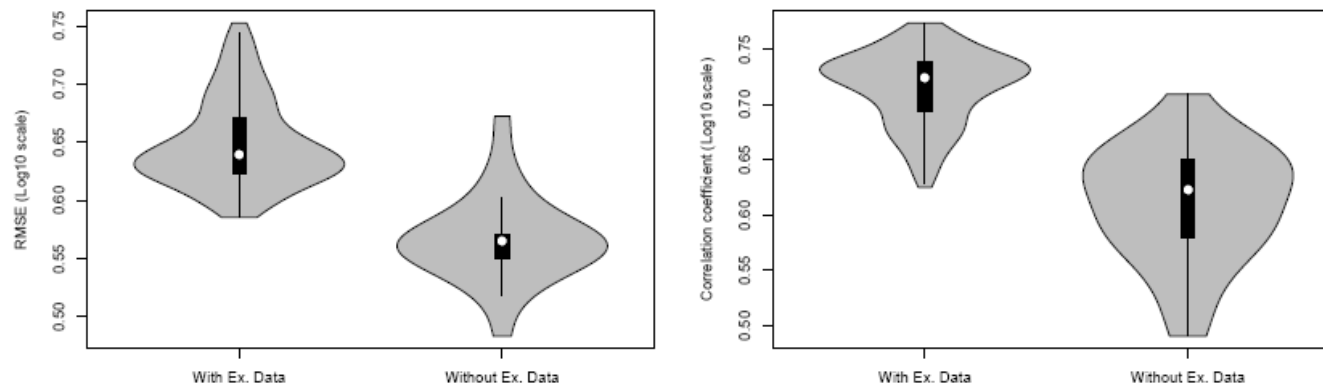


Figure 3.5. (a) Root Mean Square Error (RMSE) and (b) correlation coefficients for ducks (log densities) comparing predictions with and without data from surrounding countries. Data are presented as violin plots, a combination of box and kernel density plots (see Hintze 1998). Higher RMSE and lower correlation coefficients for analyses using data from surrounding countries suggest relationships between poultry densities and predictor variables within China are different from surrounding countries and such additional analyses do not improve predictions within China.



Supplemental Materials

Table S3.1. Publication references for year 2004 sub-provincial level poultry statistics used in poultry population models for this study.

Province (in Chinese)	Province	Sub-provincial Level Poultry Data Source	Sub-provincial Level Poultry Data Source (in Chinese)
北 京	Beijing	Liu Yali. 2005. Beijing Rural statistical Yearbook. Edited by National Bureau of Statistics and Survey Office of Beijing Rural Society and Economy. Beijing. pp.72-75	刘亚力. 2005. 北京农村统计年鉴. 国家统计局和北京市农村社会经济调查队编. 北京. pp.72-75.
天 津	Tianjin	Han Qixiang. 2005. Tianjin statistical Yearbook. Edited by Statistics Bureau of Tianjian Municipality. Beijing. China Statistics Press.	韩启祥. 2005. 天津统计年鉴. 天津市统计局编. 北京. 中国统计出版社.
河 北	Hebei	Cao Zhenguo, Liu Ganghai, Zuo Shaowei. 2005. Hebei Rural statistical Yearbook. Edited by General Office of Hebei Provincial People;s Government and Hebei Provincial Statistics Bureau. Beijing. China Statistics Press. pp.285-569	曹振国, 刘刚海, 左绍伟. 2005. 河北农村统计年鉴. 河北省人民政府办公厅, 河北省统计局编. 北京: 中国统计出版社, pp 285-569
山 西	Shanxi	Zhang Xiaodong. 2005. Shanxi Statistical Yearbook. Edited by Statistics Bureau of Shanxi Province. Beijing. China Statistics Press.	张晓东. 2005. 山西统计年鉴. 山西省统计局编. 北京. 中国统计出版社.
内 蒙 古	Inner Mongolia	Zheng Shicheng. 2005. Inner Mongolia Rural and Pastoral Society and Economy Statistical Yearbook. Edited by Survey Office of Inner Mongolia Rural and Pastoral Society and Economy Statistical Yearbook. Huhehaote. pp.288-310	郑世成. 2005. 内蒙古自治区农村牧区社会经济统计年鉴. 内蒙古自治区农村牧区社会经济调查队编. 呼和浩特. pp.288-310
辽 宁	Liaoning	Fu Yuxiang, Zhang zhongqiu, Yu kuangzhen. 2005. China Animal Husbandry Yearbook. Edited by Editorial Department of China Animal Husbandary Yearbook Beijing. China Agriculture Press.154-169, 200-203.	傅玉祥, 张仲秋, 于康震. 2005. 中国畜牧业年鉴. 国畜牧业年鉴编辑部主编. 北京. 中国农业出版社. 154-169, 200-203.
吉 林	Jilin	Fu Yuxiang, Zhang zhongqiu, Yu kuangzhen. 2005. China Animal Husbandry Yearbook. Edited by Editorial Department of China Animal Husbandary Yearbook Beijing. China Agriculture Press.154-169, 200-203.	傅玉祥, 张仲秋, 于康震. 2005. 中国畜牧业年鉴. 国畜牧业年鉴编辑部主编. 北京. 中国农业出版社. 154-169, 200-203.

Province (in Chinese)	Province	Sub-provincial Level Poultry Data Source	Sub-provincial Level Poultry Data Source (in Chinese)
黑龙江	Heilongjiang	Li Zhifan. 2005. Heilongjiang statistical Yearbook. Edited by Heilongjiang Provincial Statistics Bureau. Beijing. China Statistics Press.	李志范. 2005. 黑龙江统计年鉴. 黑龙江省统计局编. 北京. 中国统计出版社.
上 海	Shanghai	Pan Jiangxin. 2005. Shanghai Statistical Yearbook. Edited by Statistics Bureau of Shanghai Municipality. Beijing. China Statistics Press.	潘建新. 2005. 上海统计年鉴. 上海市统计局编. 北京. 中国统计出版社.
江 苏	Jiangsu	Kuang Changjin. 2005. Jiangsu Rural Statistical Yearbook. Edited by Jiangsu Provincial Statistics Bureau, Jiangsu Provincial Department of Agriculture and Forest, Jiangsu Provincial Department of Ocean and Fishery and Survey Bureau of Rural Economy of Jiangsu Province. Jiangsu. Jiangsu Provincial Statistics Bureau. pp.314-321	康长进. 2005. 江苏省农村统计年鉴. 江苏省统计局等[编]. 江苏: 江苏省统计局, pp 314-321
浙 江	Zhejiang	Hong Yu. Zhang Xinhua etc. 2005. Zhejiang Rural statistical Yearbook. Edited by Zhejiang Provincial Statistics Bureau and Survey Office of Rural Society and Economy of Zhejiang Province. Zhejiang. pp.145-159	洪玉. 张兴华等. 2005. 浙江农村统计年鉴. 浙江省统计局. 浙江省农村社会经济调查队编. 浙江: pp145-159
安 徽	Anhui	Wang Weixiang. 2005. Anhui Rural Economy Statistical Yearbook. Edited by Agriculture Commission of Anhui Province and Anhui Provincial Statistics Bureau. Beijing. China Statistics Press. pp.48-49, 155-158	王维祥. 2005. 安徽农村经济统计年鉴. 安徽省农业委员会, 安徽省统计局编. 北京: 中国统计出版社, pp48-49, 155-158
福 建	Fujian	Chen Jian. 2005. Fujian Economy and Society statistical Yearbook (Rural Fascicule). Edited by Fujian Provincial Statistics Bureau. Fuzhou. Fujian People's Publishing Press pp.220-233. Chen Jian. 2004. Fujian Economy and Society statistical Yearbook (Rural Fascicule). Edited by Fujian Provincial Statistics Bureau. Fuzhou. Fujian People's Publishing Press pp.224-237.	陈建. 2005. 福建经济与社会统计年鉴-农村篇. 福建省统计局编. 福州. 福建人民出版社. Pp: 220-233. 陈建. 2004. 福建经济与社会统计年鉴-农村篇. 福建省统计局编. 福州. 福建人民出版社. Pp: 224-237.
江 西	Jiangxi	Cao Qingyun. 2005. Jiangxi statistical Yearbook. Edited by Jiangxi Provincial Statistics Bureau. Beijing. China Statistics Press.	曹青云. 2005. 江西统计年鉴. 江西省统计局编. 北京: 中国统计出版社.

Province (in Chinese)	Province	Sub-provincial Level Poultry Data Source	Sub-provincial Level Poultry Data Source (in Chinese)
山 东	Shandong	Liu Xinhui. 2005. Shandong Statistical Yearbook. Edited by Shandong Provincial Statistics Bureau. Beijing. China Statistics Press.	刘兴慧. 2005. 山东统计年鉴. 山东省统计局编. 北京. 中国统计出版社.
河 南	Henan	Lu Jie. 2005. Henan Rural statistical Yearbook. Edited by Survey Office of Rural Society and Economy of Henan Province. Beijing. China Statistics Press. pp 106-110; 325-349	陆洁. 2005. 河南农村统计年鉴. 河南省农村社会经济调查队主编. 北京: 中国统计出版社, pp 106-110; 325-349
湖 北	Hubei	Hubei Rural Statistical Yearbook 2005. Edited by Statistics Bureau of Hubei Province and Editor Committee of Hubei Rural Statistical Yearbook. Beijing. China Statistics Press. pp.74-79, 90-91.	湖北农村统计年鉴 2005. 湖北省统计局和《湖北农村统计年鉴》编辑委员会编. 北京. 中国统计出版社.pp.74-79, 90-91
湖 南	Hunan	Survey Office of Rural Economy of Hunan Province. 2005. Hunan Rural Statistical Yearbook. Changsha. Hunan Xiangcai Printing LTD. Pp: 102-110.	湖南省农村经济调查队. 2005. 湖南农村统计年鉴.长沙.湖南湘财印务有限公司. pp:102-110.
广 东	Guangdong	Bu Xinming, Xie Yuexin. 2005. Guangdong Rural Statistical Yearbook. Edited by Edit Committee of Guangdong Rural Statistical Yearbook. Beijing. China Statistics Press. pp.263-280	卜新民, 谢悦新. 2005. 广东农村统计年鉴. 广东农村统计年鉴编纂委员会编. 北京. 中国统计出版社.pp.263-280
广 西	Guangxi	Fu Yuxiang, Zhang zhongqiu, Yu kuangzhen. 2005. China Animal Husbandry Yearbook. Edited by Editorial Department of China Animal Husbandary Yearbook Beijing. China Agriculture Press.154-169, 200-203.	傅玉祥, 张仲秋, 于康震. 2005. 中国畜牧业年鉴. 国畜牧业年鉴编辑部主编. 北京. 中国农业出版社. 154-169, 200-203.
海 南	Hainan	Zhang Heng. 2005. Hainan statistical Yearbook. Edited by Hainan Provincial Statistics Bureau . Beijing. China Statistics Press. pp.265-267	张恒. 2005. 海南统计年鉴. 海南省统计局编. 北京. 中国统计出版社. pp:265-267
重 庆	Chongqing	Zhen Zibin. 2005. Chongqing statistical Yearbook. Edited by Statistics Bureau of Chongqing Municipality. Beijing. China Statistics Press. pp: 231	郑子彬. 2005. 重庆统计年鉴. 重庆市统计局编. 北京. 中国统计出版社.pp: 231
四 川	Sichuan	Hu Pinsheng. etc. 2005. Sichuan Statistical Yearbook. Edited by Statistics Bureau of Sichuan Province. Beijing. China Statistics Press.	胡品生.等. 2005. 四川统计年鉴. 2005. 四川省统计局编. 北京. 中国统计出版社.

Province (in Chinese)	Province	Sub-provincial Level Poultry Data Source	Sub-provincial Level Poultry Data Source (in Chinese)
贵 州	Guizhou	Fu Yuxiang, Zhang zhongqiu, Yu kuangzhen. 2005. China Animal Husbandry Yearbook. Edited by Editorial Department of China Animal Husbandry Yearbook Beijing. China Agriculture Press.154-169, 200-203.	傅玉祥, 张仲秋, 于康震. 2005. 中国畜牧业年鉴. 国畜牧业年鉴编辑部主编. 北京. 中国农业出版社. 154-169, 200-203.
云 南	Yunnan	Fu Yuxiang, Zhang zhongqiu, Yu kuangzhen. 2005. China Animal Husbandry Yearbook. Edited by Editorial Department of China Animal Husbandry Yearbook Beijing. China Agriculture Press.154-169, 200-203.	傅玉祥, 张仲秋, 于康震. 2005. 中国畜牧业年鉴. 国畜牧业年鉴编辑部主编. 北京. 中国农业出版社. 154-169, 200-203.
西 藏	Xizang	Fu Yuxiang, Zhang zhongqiu, Yu kuangzhen. 2005. China Animal Husbandry Yearbook. Edited by Editorial Department of China Animal Husbandry Yearbook Beijing. China Agriculture Press.154-169, 200-203.	傅玉祥, 张仲秋, 于康震. 2005. 中国畜牧业年鉴. 国畜牧业年鉴编辑部主编. 北京. 中国农业出版社. 154-169, 200-203.
陕 西	Shaanxi	Hu Shouxian. 2005. Shaanxi Statistical Yearbook. Edited by Statistics Bureau of Shaanxi Province. Beijing. China Statistics Press. pp.243-244, 266-268.	胡守贤.2005.陕西统计年鉴. 陕西省统计局编. 北京. 中国统计出版社. pp.243-244, 266-268.
甘 肃	Gansu	Fan Huaiyu. 2005. Gansu Rural Yearbook. Edited by Editorial Committee of Gansu Rural Yearbook. Beijing. China Statistics Press. pp.319-321.	樊怀玉. 2005. 甘肃农村年鉴. 甘肃农村年鉴编委会编. 北京. 中国统计出版社. pp: 319-321.
青 海	Qinghai	Kang Ling . 2005. Qinghai statistical Yearbook. Edited by Statistics Bureau of Qinghai Province. Beijing. China Statistics Press.	康玲. 2005. 青海统计年鉴. 青海省统计局编. 北京. 中国统计出版社.
宁 夏	Ningxia	Jia Hongbang. 2005. Ningxia Statistical Yearbook. Edited by Statistics Bureau of Ningxia Autonomous Region. Beijing. China Statistics Press pp.252-253.	贾红邦. 2005. 宁夏统计年鉴. 宁夏回族自治区统计局编. 北京. 中国统计出版社. pp.252-253.
新 疆	Xinjiang	Fu Yuxiang, Zhang zhongqiu, Yu kuangzhen. 2005. China Animal Husbandry Yearbook. Edited by Editorial Department of China Animal Husbandry Yearbook Beijing. China Agriculture Press.154-169, 200-203.	傅玉祥, 张仲秋, 于康震. 2005. 中国畜牧业年鉴. 国畜牧业年鉴编辑部主编. 北京. 中国农业出版社. 154-169, 200-203.

Table S3.2. Criteria used for creating unsuitable habitat masks for chicken, duck, and goose distribution models. Second column shows thresholds used for Gridded Livestock of the World (GLW) monogastric livestock (pigs and poultry) models (FAO 2007); columns 3 and 4 show conservative masking thresholds used in this study.

Criteria	Monogastric Livestock (GLW, FAO 2007)	Chickens	Ducks and Geese
Protected areas	Y	no mask	no mask
Population density (Landscan) (km')	1,500 >	no mask	no mask
Lights (Landscan) (%)	> 90	no mask	no mask
Slope (Landscan) (%)		no mask	no mask
Elevation (m)	> 4,750	> 4,750	> 4,750
NDVI max		no mask	< 0.07
Land cover (Landscan) -water	Y	Y	no mask
Land cover (Landscan) -developed	Y	no mask	no mask
Land cover (Landscan) -partly developed	Y	no mask	no mask
Land cover (Landscan) –herbaceous wetlands	Y	no mask	no mask
Land cover (Landscan) -wooded wetlands	Y	no mask	no mask
Land cover (Landscan) -tundra	Y	Y	Y
Land cover (Landscan) -snow and ice	Y	Y	Y

Figure S3.1. Relationship between NSB and AHB poultry data, 2004 for 31 provinces of China. R-square correlation between NSB and AHB reported RESID statistics was 99.4%; 14 of 31 provinces had identical values for the 2 datasets.

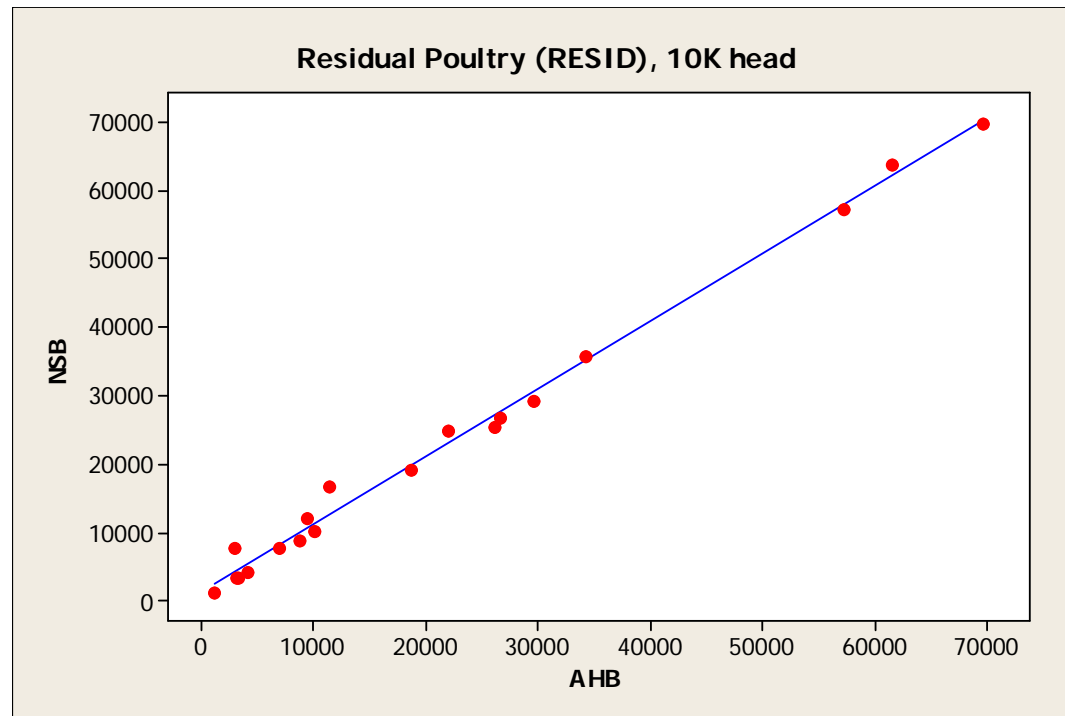


Figure S3.2. Map of China depicting scale of available poultry data for each province and the method for filling in missing data. Red lines show provincial boundaries. Blue lines show sub-provincial boundaries for provinces having sub-provincial data. Method 1: Beijing, Jiangsu, Zhejiang, Anhui, Fujian, Henan, Hunan, Guangdong, Ningxia; Method 2: Hebei, Heilongjiang, Jiangxi, Shandong, Shaanxi; Method 3: Tianjin, Hubei, Chongqing; Method 4: Inner Mongolia, Shanghai, Hainan, Sichuan, Qinghai; Method 5: Shanxi, Gansu; Method 6: Liaoning, Jilin, Guangxi, Guizhou, Yunnan, Xizang, Xinjiang.

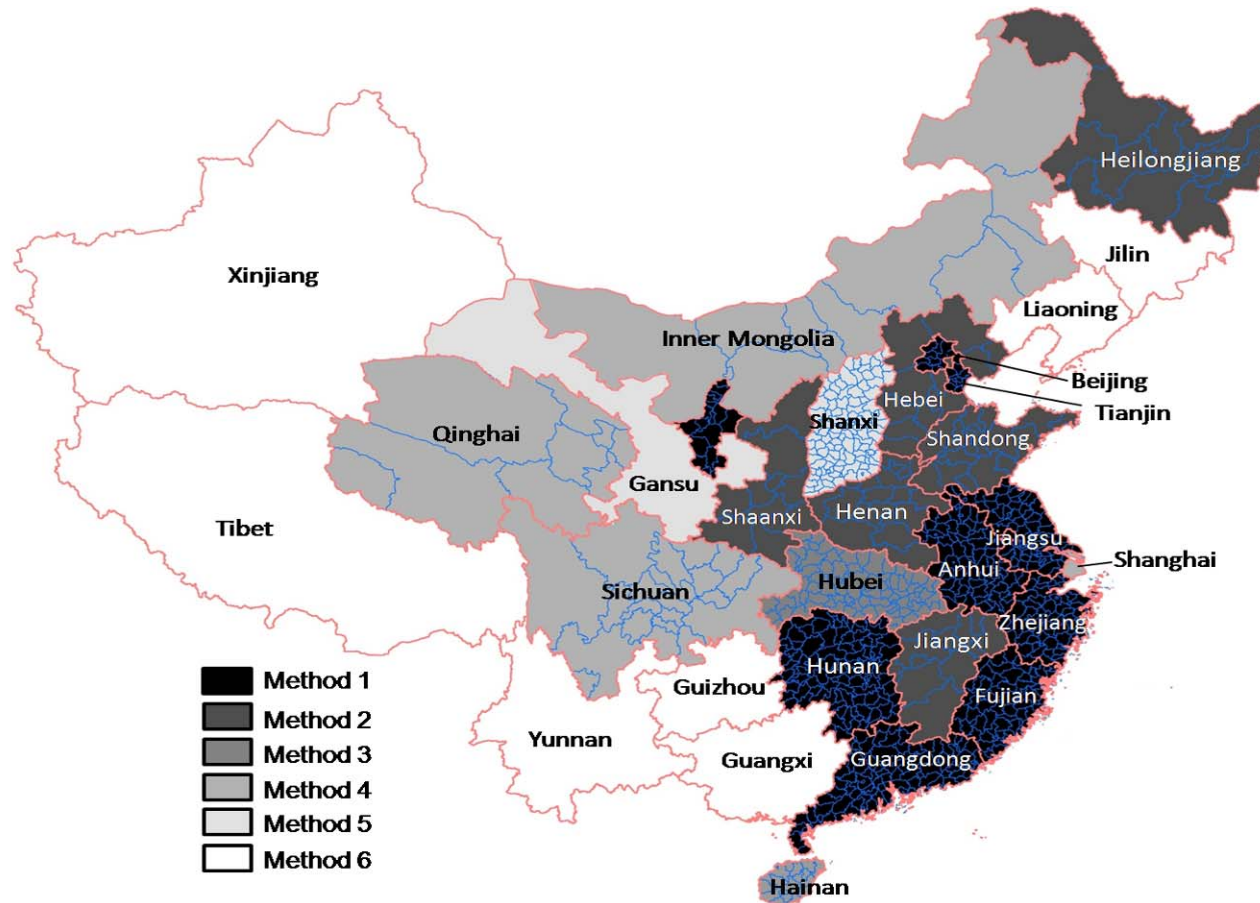


Figure S3.3. Four regional stratification schemes used in the analyses, (a) Sere & Steinfeld (1996) Livestock production systems (LPS), (b) Ecozone 12 isodata clusters (EZ.BestRSE), and (c) China Agro-Ecological Regions (CAR), (d) All.BestRSE.

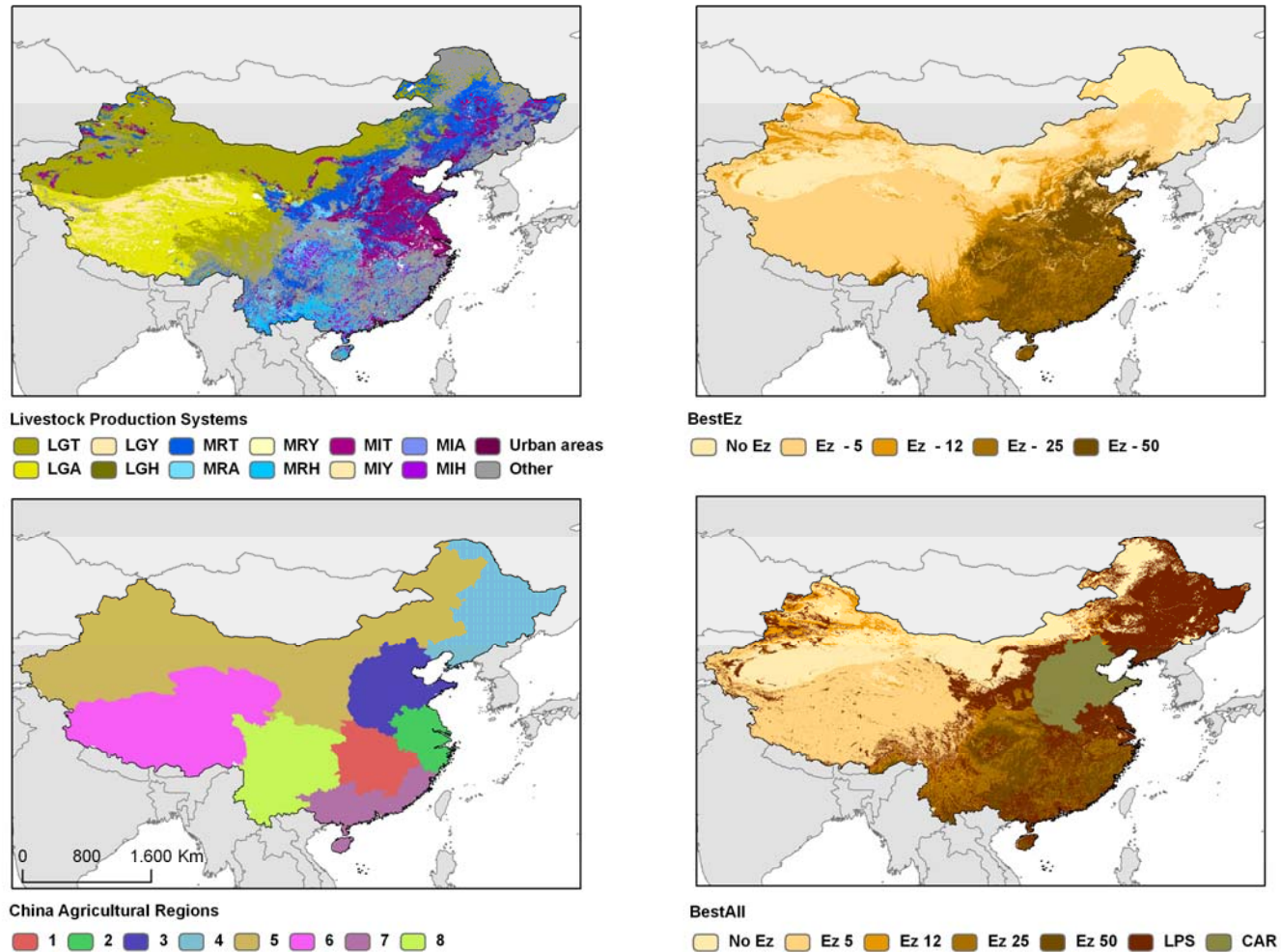
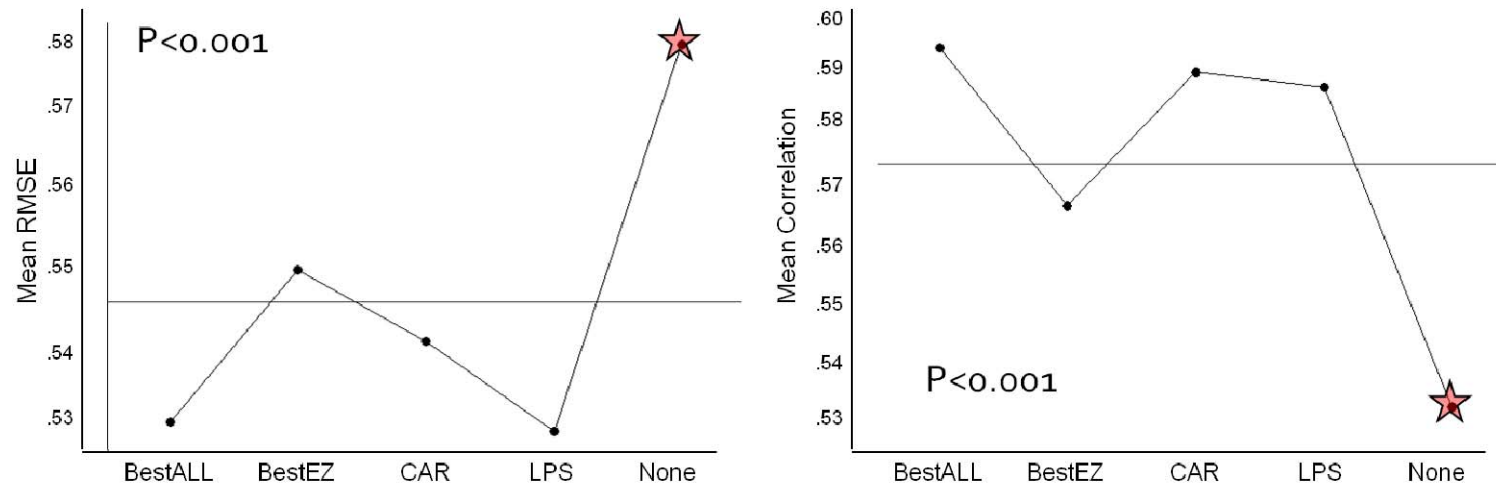


Figure S3.4. Analysis of Variance and Tukey's comparisons ($p < 0.05$) showing that although differences among chosen stratifications were not significant, all performed better than the global model (no stratification).



CHAPTER 4. WATERFOWL MODELS

Manuscript Title: Species Distribution Modeling in a Region of High Need and Limited Data: China's Anatidae Waterfowl

Alternative title: Modeling Distribution and Abundance of China's Anatidae Waterfowl

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Abstract

Understanding the spatial and temporal distribution of species is necessary to manage issues in the fields of conservation science and medicine, yet population data are rarely available at the extent or resolution desired to address targeted research needs. Some regions, such as Asia, are important ecologically but lack long-term or broad scale monitoring programs that more developed regions have been afforded. Species distribution modeling is a rapidly growing field that can provide estimations of species distributions and, depending on the approach taken, can address a multitude of data needs and levels of input data. In this study, we developed species distribution models for China's Anatidae waterfowl as part of a greater effort to identify hotspot regions of disease transfer between wild and domestic bird populations. Although large scale field survey data for China's waterfowl were lacking, the urgency of the disease models

spurred us to move forward using a habitat analysis approach to build baseline presence-absence distribution models. These high-resolution 1 km maps represent the first distribution models for China's waterfowl: 30 breeding and 37 wintering species. Resulting maps varied per species and season. Species diversity maps for the breeding and wintering seasons differed in pattern and richness, with the breeding season showing highest diversity (max=20) in patchy areas across the northeast and high-elevation west, and wintering season showing high densities (max=30) across broad regions of the low-elevation southeastern parts of China. Low omission error rates for individual species indicated strong model performance in predicting species presence. While these models represent an early stage in developing robust spatially explicit Anatidae distributions across China, the modeling process was designed to incorporate new data as it becomes available, enabling it to serve as a framework for long-term efforts to improve our understanding of Anatidae distributions in this region.

Keywords: Anatidae, waterfowl, species distribution modeling, spatial analysis, habitat, disease

Introduction

There has been an increasing demand for species distribution information for the purposes of management and conservation of wild species especially throughout Asia. Knowledge of how species are distributed across the landscape spatially and temporally is critical for a wide range of priority needs including protection of critical habitat, predicting effects of environmental stressors on wildlife, and informing surveillance and prevention measures against zoonotic disease threats. Fine-grained distribution data may be available for some species locally, however, data are rarely available at larger extents

due to the enormous costs of production. Some regions such as North America and parts of Europe have long-term monitoring efforts from which consistent quality data can be drawn upon (Root 1988, Sauer et al. 2003); others including developing regions rarely have similar types of broad-scale programs despite having rich biological resources (Grenyer et al. 2006, Martin et al. 2012). The growing field of species distribution modeling (SDM) offers a wide array of approaches that can support model development for a breadth of applications and data inputs (Morrison et al. 2006, Franklin and Miller 2010). Relevant approaches range from deductive expert knowledge models such as habitat suitability indices (Fish and Wildlife Service 1981) and wildlife-habitat relationship matrices (Verner et al. 1986, Csuti 2000) to inductive models that incorporate regression, Bayesian statistics, maximum entropy, artificial neural networks, genetic algorithms, and other machine learning techniques (Segurado and Araujo 2004, Guisan and Thuiller 2005, Elith et al. 2006, Austin 2007, Elith and Leathwick 2009).

The increasing availability of remote sensing data since the 1980's and development of geographic information systems has provided additional opportunity to model distributions across broad extents (Leyequien et al. 2007). Land cover data, particularly Landsat TM, has been widely used as predictive inputs for SDMs (Venier et al. 2004, Gottschalk et al. 2005) due to its large spatial and temporal extent and easy accessibility (Defries and Belward 2000). This approach, which includes linking species observations or habitat requirements to land characteristics in statistical or spatial format has been applied successfully for a wide array of terrestrial taxa including many avian species (Boyle et al. 2004, Venier et al. 2004, Gottschalk et al. 2005, Prins et al. 2005, Morrison et al. 2006, Franklin and Miller 2010, Toral et al. 2011)).

We turned to SDM as a means to develop input data for disease risk models for the deadly H5N1 avian influenza virus (hereafter H5N1) which has caused considerable damage to the health and economy of more than 60 countries from Asia to Africa since its emergence in 1996 (OIE 2012, World Health Organization 2012a). In particular, the role that wild birds play in the spread of H5N1 has been heavily debated following a rare outbreak in wild populations (Liu et al. 2005) and the subsequent rapid expansion of outbreaks beyond Asia and into Europe and Africa in 2005 and 2006 (Gilbert et al. 2006b, Kilpatrick et al. 2006, Gauthier-Clerc et al. 2007, Feare 2010). Waterbirds of the orders Anseriformes (waterfowl) and Charadriiformes (shorebirds, gulls, and terns) are known reservoirs for low-pathogenic forms of avian influenza (LPAI) which have the potential to mutate into lethal forms following entry into domestic poultry populations (Clark and Hall 2006, Muzaffar et al. 2006, Alexander 2007, Alexander and Capua 2008). Anatidae waterfowl (ducks, geese, and swans) are of particular importance due to their migratory behavior, high abundances, and increased exposure to farmed ducks (Fouchier et al. 2007, Muzaffar et al. 2010, Takekawa et al. 2010b) which can act as silent reservoirs of H5N1 virus (Hulse-Post et al. 2005, Sturm-Ramirez et al. 2005, Chen et al. 2006a). Despite the importance and continuing debate revolving wild birds and H5N1 spread, few studies have explicitly incorporated wild birds in their models (Gilbert and Pfeiffer 2012. *In Press*), in part because obtaining adequate inputs for these populations is difficult, particularly in regions of Asia where the virus continues to persist and reemerge (OIE 2012).

Our focal region of interest is China, the epicenter of H5N1 (Mukhtar et al. 2007), and one of the few locations within H5N1's range that exhibits a diverse combination of

environmental conditions and varying human and wildlife populations. China supports both heavily populated regions where wild birds, poultry, and humans are closely integrated on the landscape such as Poyang Lake in southeastern China (8.8 million people reside among livestock and wintering migratory waterbirds), as well as regions where H5N1 has repeatedly emerged despite a lack of domestic poultry such as along the Qinghai-Tibet Plateau of northwestern China. In addition, China is an important resource for Anatidae populations, as it supports 10 percent of the globe's wetlands (Lu and Jiang 2004) and is positioned at the intersection of multiple migratory flyways (Boere et al. 2006); however, waterfowl in this region are the least studied in the Palearctic and systematic monitoring programs have not yet been developed (Miyabayashi and Mundkur 1999b, Miyabayashi 2003, Kear 2005, Mundkur 2006). Thus, while a strong set of survey data was not available to take advantage of some of the newer advances in SDM (Guisan and Thuiller 2005, Elith et al. 2008, Franklin and Miller 2010, Miller 2010, Elith et al. 2011, Royle et al. In Review), the need to move forward with the disease models drove us to develop entry-level distribution models based on habitat mapping, which could be conducted after a thorough review of the literature. Here we present the first set of spatially explicit distribution models for China's 42 species of Anatidae waterfowl during the breeding and wintering seasons at 1 km spatial resolution. We hypothesized that using a habitat relationship approach (Csuti 2000, Morrison et al. 2006) in combination with local field knowledge would provide useful maps at this resolution and across the extent of China. Validated maps will be made available to the public and scientific community for use in conservation, research, and educational purposes.

Materials and Methods

Model development and validation

Using a habitat suitability approach (Figure 4.1), we created presence-absence predictions for each of China's 30 breeding and 37 wintering Anatidae species. We first developed a database outlining habitat requirements for each species and season supported by a detailed review of the literature (Chinese and English) and communication with local experts. We then derived equations between habitat preferences and predictor variables (Table S4.1) and implemented the equations in a geographic information system (Python (www.python.org) and ArcGIS 10.1, ESRI, Redlands, California) at 1 km resolution. The suitability maps for each species were masked using range boundaries produced by Mackinnon and Phillipps (2000, see Fig. S4.1 for examples), the most comprehensive reference available for China (Meyer De Schauensee 1984, Yan 1996, Mackinnon and Phillipps 2000, Robson 2000, Strange 2000, Kear 2005, Delany and Scott 2006). The range maps are coarse definitions of the extent of a species' range (Hurlbert and Jetz 2007) which we used to restrict the extent of predicted habitat for a given species. We validated the models using presence data available from the literature and local surveys by conducting tests for errors of omission – grid cells where models predict absence of a species but validation data shows presence (Pearce and Boyce 2006, Tsao et al. 2007). Because precision and accuracy of coordinates reported for waterfowl observations varied widely among reference sources (from units of degrees, minutes, seconds recorded for the point where a bird was observed, to a generic centroid within the boundaries of a nature reserve), we validated the models at three scales: immediate (within 1 km pixel), within 5 km and within 10 km of a known observation location.

These designations were based on the average size of China's nature reserves which range between 4 and 10 km² (min and max: 1 km² and 900 km²) (Xie et al. 2004). We then created Anatidae species diversity maps by summing the number of predicted species within each cell for a given season.

Waterfowl Data

The family Anatidae includes all duck, goose, and swan species which can be found across most regions of the globe. These birds are highly adapted to aquatic habitats and exhibit short legs, webbed feet, and wings that are set back on their body. Their bills are generally wide for filtering water. China has 42 species of Anatidae that breed or winter within its borders (Mackinnon and Phillipps 2000), the majority of which are short or long distance migrants. Geese and swans are generally herbivorous and use agricultural settings in winter; ducks vary in their feeding preference ranging from herbivory to piscivory and are more tied to natural habitats in winter than geese and swans.

We conducted a review of the English and Chinese literature for China's waterfowl species. References included peer reviewed journal articles, technical reports, and unpublished surveys from nature reserves. Data collected were used for 2 purposes: to build the waterfowl habitat relationship database and to collate reputable location data for use in validating the models. Habitat information, references, location data, population estimates, and habitat relationship equations were managed in the database.

Environmental Variables

Remotely-sensed land cover data are readily available across large geographic extents (Defries and Belward 2000) and have been used successfully in modeling species

distributions (Seoane et al. 2004, Gottschalk et al. 2005). We used Landsat TM land cover data produced and validated by the Chinese Academy of Sciences (CAS) (Liu et al. 2002) and Shuttle Radar Topography Mission (SRTM) elevation data (Farr et al. 2007) as model predictors. The land cover variables were derived from 30 m Landsat imagery and distributed by CAS at 1 km resolution. Slope and elevation variables were resampled from 90 m SRTM data to 1 km resolution using ArcGIS 10.1 Spatial Analyst. We tested all environmental variables for correlation to avoid issues of multicollinearity (Graham 2003). Significant correlations were not observed (all were below 0.67), however we reduced the data set from 25 variables to 18 (Table S4.2) based on an *a priori* list of relevant cover classes (Burnham and Anderson 2002c).

Results

The Anatidae habitat relationship database was structured in three parts: (a) records outlining seasonal habitat requirements for individual species, (b) population and survey counts, (c) and habitat relationship matrices describing land cover characteristics in relation to habitat requirements. The database holds 9250 records drawn from more than 1000 references. Of the 42 Anatidae species found in China (Mackinnon and Phillipps 2000), 39 are listed as winter residents and 30 as breeders in China. Population data from the literature (Delany and Scott 2006, Cao et al. 2008) indicate that not all of the 42 species in MacKinnon and Phillipps (2000) have been reported in China during surveys conducted within the past decade. Based on this information, we produced distribution maps for 30 breeding and 37 wintering species (Table 4.1). Here we illustrate prediction results and validation points for the bar-headed goose (*Anser indicus*)

(Figure 4.2) a species of cultural value and important to H5N1 transmission (Zhou et al. 2006, Zhang et al. 2008, Prosser et al. 2011a). Resulting presence-absence distribution maps for all species and seasons are included in Figure 4.3.

Omission rates indicated a strong ability for the models to predict areas where a species might be found (omission rate ranged from 0 to 9.5 percent, Table 4.2). The overall number of validation points was low, ranging from 1 to 21 per season for a given species, and we would expect the statistics to broaden as more validation points are added.

Species diversity maps (Figure 4.4) for the breeding season showed highest diversity in the northeast and the high-elevation western regions of China. Hotspots of Anatidae diversity during the wintering season occurred across much of the low-elevation southeastern part of China, and particularly along the Yangtze River basin. Diversity ranged from 0 to 20 for the breeding season and 0 to 31 for the wintering season.

Discussion

The goal of this work was to develop high resolution spatial distribution maps for the suite of waterfowl species that use China's habitats. Because of limitations in availability of survey data for the majority of species, we chose to take a traditional approach of habitat modeling and combine it with control measures to develop and validate our maps. We hypothesized that this approach could produce useful and accurate spatial data layers of predicted presence for China's waterfowl. Visual inspection of the distribution maps shows strong associations with wetland habitats

within the range of a species and validation measures resulted in low model omission rates, indicating a strong capability of the models to predict presence locations.

General distribution patterns across the suite of species differed between the breeding and wintering seasons, as can be seen in the cumulative diversity models (Figure 4.4). Geographically, winter distributions are concentrated in the warmer and lower elevation regions of the southeast while the breeding distributions are more evenly spaced across the landscape and include the northern latitudes and higher elevation regions of western China. These patterns are indicative of differences in waterfowl behavior between these two seasons, in that during winter waterfowl tend to be gregarious, congregating in large flocks of mixed or individual species, and during the breeding season territories are held and females spend much of their time on nest. Because of the concentrated nature of waterfowl populations in winter and the implications for habitat conservation, survey data are more commonly available for this season. Large scale, long term counts such as the Asian Waterbird Census (Li et al. 2009) require a large expert volunteer base and are implemented only for winter counts. Survey data for breeding populations are usually conducted at smaller scales and without cross-study coordination as they are typically employed for individual research projects or by individual nature reserves. These differences in data availability are reflected in the contents of the waterfowl database.

One artifact of our modeling approach is the appearance of a hard transition between predicted presence and absence cells along the outer boundary of each species range (e.g., the artificial circular boundaries evident in the bar-headed goose wintering distribution (Figure 4.2) or spot-billed duck distribution (Figure 4.3)). This is a result of

the masking step (third text box in Figure 4.1) which restricts the predicted presence layer to only include cells that fall within a species range boundary. Range maps are general approximations of regions where a species has been reported in the past. Observations of individuals outside of these boundaries is possible but rare and although a soft transition could have been modeled, we chose to retain the hard boundaries so that results are explicit and easy to interpret, particularly when individual species models are summed to create the cumulative diversity maps. One step for future work as more survey data becomes available is to update the current species boundaries to reflect expansions or reductions in range extents for individual species.

The validation process indicated a strong ability to predict presence locations for individual species, however, because our validation data were presence-only records, we were not able to assess how well the models predicted absence locations. In general, distribution models tend to be better at predicting presence locations than absence locations except for species with very narrow niches (Brotons et al. 2004, Hernandez et al. 2006). Here we expect that our models may be better at predicting presence than absence locations, although until a solid presence-absence data set becomes available, we are not able to confirm this hypothesis. However, the fundamental habitat requirements of a given waterfowl species is not likely to change significantly over the short term, and we designed the modeling process to have multiple levels so that the base maps can be updated as new information becomes available.

Additional challenges exist when using data from outside sources to refine or validate the distribution models. Geographic coordinates associated with public bird survey databases are commonly reported in decimal degrees with two units of precision

which corresponds to a hundredth of a degree, or just over 1 km, depending on the position in relation to the equator (as latitudes reach the poles, they cover more area on the ground). This may pose a problem if the scale of the data being assessed for accuracy is finer than the precision of the truncated location coordinates. In addition, it is not always clear what the reported coordinates represent; e.g., whether a point represents the location from which the bird was observed (such as a bird observation platform), the location of the bird itself (this is unlikely), or the midpoint of the area of a nature reserve within which bird observations were reported. We paid careful attention to these details to resolve these questions where possible and conducted our accuracy assessments at 3 scales (within 1 km, 5 km, and 10 km) to account for such issues.

In this paper we present the first distribution models and species diversity maps for China's Anatidae waterfowl. While we recognize that these models have multiple shortcomings, we hope this example will encourage similar efforts in other regions with limited data but a great need for understanding the distribution of species on the landscape. We also hope this work will stimulate further quality and coordinated efforts to increase the level of input data for these models. At this current stage in a hopefully long and successful process, these high resolution spatial data sets provide a unique and valuable resource to the research and planning communities across many disciplines from wildlife management to conservation medicine and beyond.

Tables

Table 4.1. List of Anatidae waterfowl species for which breeding and/or wintering distribution maps were created for China at 1 km resolution. B=breeding, W=winter, BW=both models produced.

Common Name	Scientific Name	Models	Common Name	Scientific Name	Models
Lesser Whistling Duck	<i>Dendrocygna javanica</i>	B	Northern Pintail	<i>Anas acuta</i>	BW
Mute Swan	<i>Cygnus olor</i>	B	Garganey	<i>Anas querquedula</i>	BW
Whooper Swan	<i>Cygnus cygnus</i>	BW	Baikal Teal	<i>Anas formosa</i>	W
Tundra Swan	<i>Cygnus columbianus</i>	W	Common Teal	<i>Anas crecca</i>	BW
Swan Goose	<i>Anser cygnoides</i>	BW	Marbled Duck	<i>Marmaronetta angustirostris</i>	B
Bean Goose	<i>Anser fabalis</i>	W	Red-crested Pochard	<i>Rhodonessa rufina</i>	B
Greater White-fronted Goose	<i>Anser albifrons</i>	W	Common Pochard	<i>Aythya ferina</i>	BW
Lesser White-fronted Goose	<i>Anser erythropus</i>	W	Ferruginous Pochard	<i>Aythya nyroca</i>	BW
Greylag Goose	<i>Anser anser</i>	BW	Baer's Pochard	<i>Aythya baeri</i>	BW
Bar-headed Goose	<i>Anser indicus</i>	BW	Tufted Duck	<i>Aythya fuligula</i>	BW
Snow Goose	<i>Anser caerulescens</i>	W	Greater Scaup	<i>Aythya marila</i>	W
Brent Goose	<i>Branta bernicla</i>	W	Steller's Eider	<i>Polysticta stelleri</i>	W
Ruddy Shelduck	<i>Tadorna ferruginea</i>	BW	Long-tailed Duck	<i>Clangula hyemalis</i>	W
Common Shelduck	<i>Tadorna tadorna</i>	BW	Black Scoter	<i>Melanitta nigra</i>	W
Cotton Pygmy Goose	<i>Nettapus coromandelianus</i>	B	White-winged Scoter	<i>Melanitta fusca</i>	W
Mandarin Duck	<i>Aix galericulata</i>	BW	Common Goldeneye	<i>Bucephala clangula</i>	BW
Gadwall	<i>Anas strepera</i>	BW	Smew	<i>Mergellus albellus</i>	BW

Common Name	Scientific Name	Models	Common Name	Scientific Name	Models
Falcated Duck	<i>Anas falcata</i>	BW	Red-breasted Merganser	<i>Mergus serrator</i>	BW
Eurasian Wigeon	<i>Anas penelope</i>	BW	Scaly-sided Merganser	<i>Mergus squamatus</i>	BW
Mallard	<i>Anas platyrhynchos</i>	BW	Common Merganser	<i>Mergus merganser</i>	BW
Spot-billed Duck	<i>Anas poecilorhyncha</i>	BW			
Northern Shoveler	<i>Anas clypeata</i>	BW			

Table 4.2. Validation measures for example bar-headed goose and mallard at three scales (1 km, 5 km, and 10 km). Omission rate is calculated by dividing the number of correctly predicted presence locations by the total number of validation (presence) points. For example, of the 21 locations where bar-headed geese were observed during the wintering season (using validation data), two (or 9.5%) of them were incorrectly predicted as “absent” within the grid cell (within 1 km). In this example, increasing the number of neighboring cells for analysis did not improve the error rate (ie. error rates are the same whether we examined the 1 km cell which encompassed the validation point, or within 5 km or 10 km cells in each direction).

Species	Season	Omission Error Rate 1 km	Omission Error Rate 5 km	Omission Error Rate 10 km	Number of Validation Points
Bar-headed goose	Breeding	0	0	0	13
	Winter	0.095	0.095	0.095	21
Mallard	Breeding	0.17	0.17	0.17	6
	Winter	0	0	0	2
	Resident	0	0	0	1

Figures

Figure 4.1. Key steps (top panel) for species distribution modeling of China's 42 species of Anatidae waterfowl. Breeding and wintering season maps were produced for each product. Spatial resolution of grid maps is 1 km.

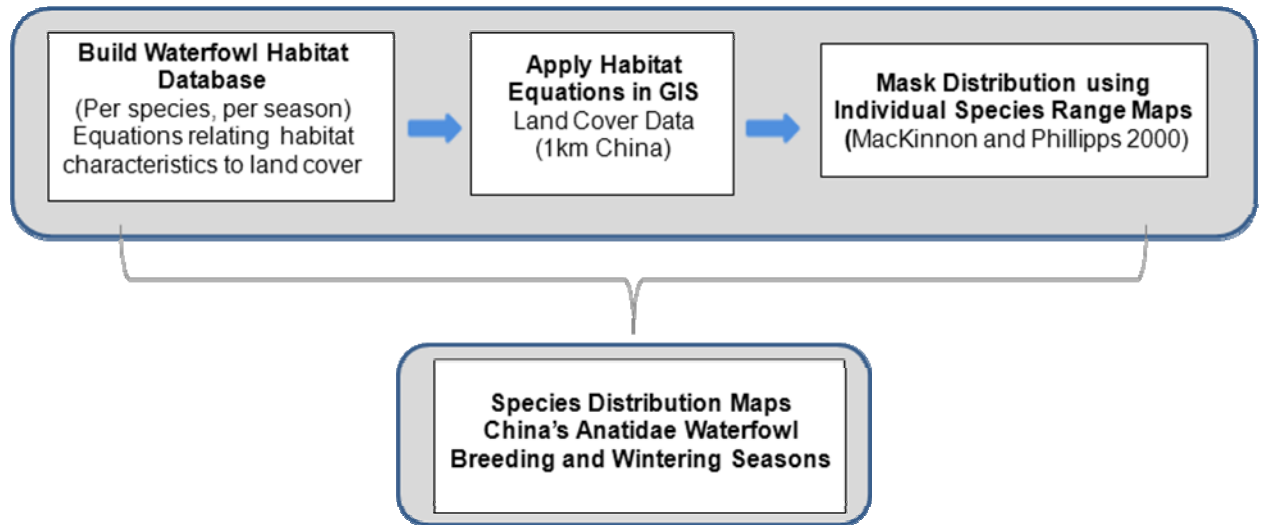


Figure 4.2. Predicted bar-headed goose distributions for breeding (orange) and wintering (purple) seasons across China (A). Locations of survey observations in red circles (validation points). Red frames delimit magnified insets (B) for breeding (left) and wintering (right) areas.

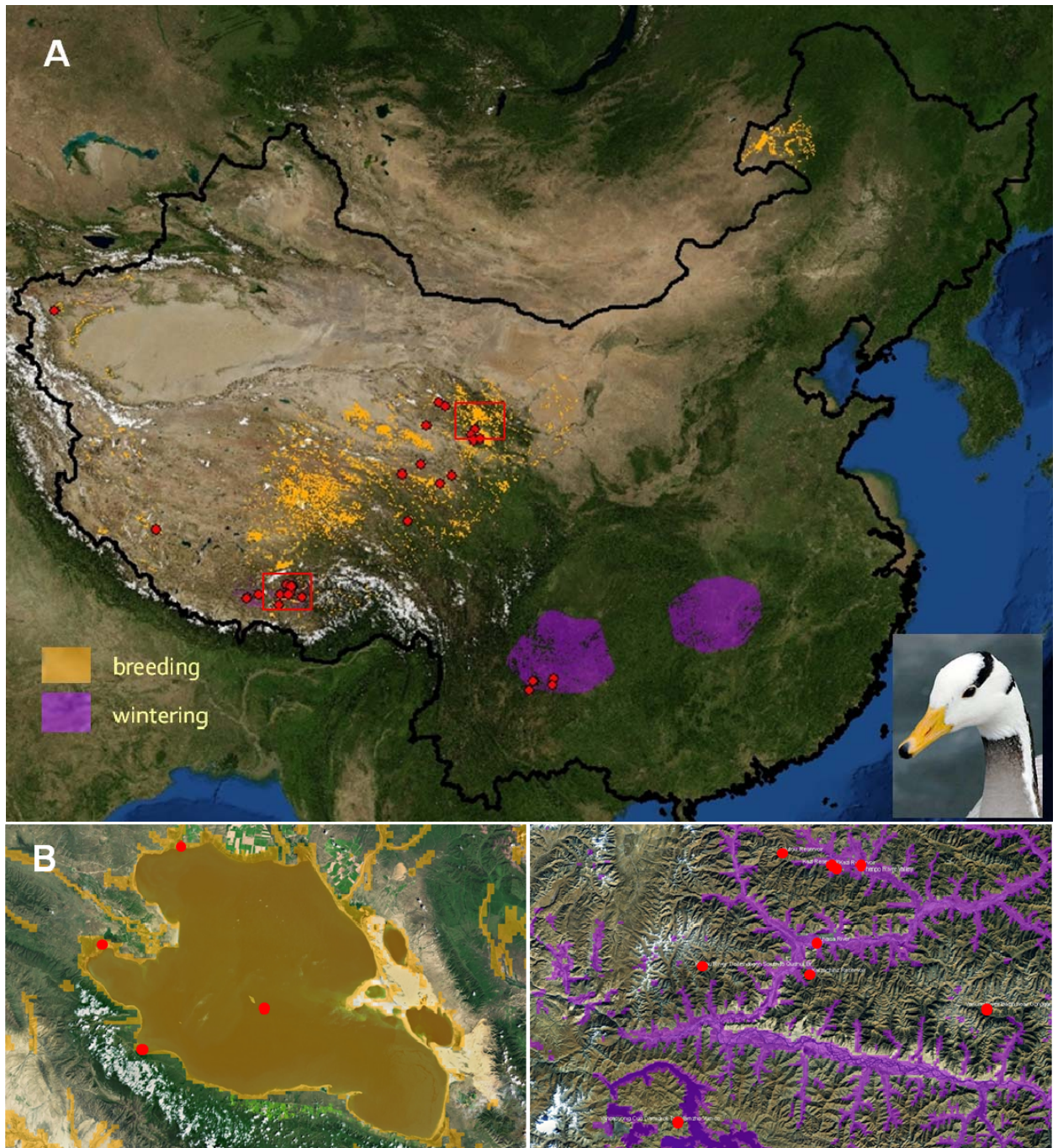


Figure 4.3. Species distribution models for 30 breeding and 37 wintering species of China. Models were developed using a habitat analysis approach at 1 km spatial resolution.

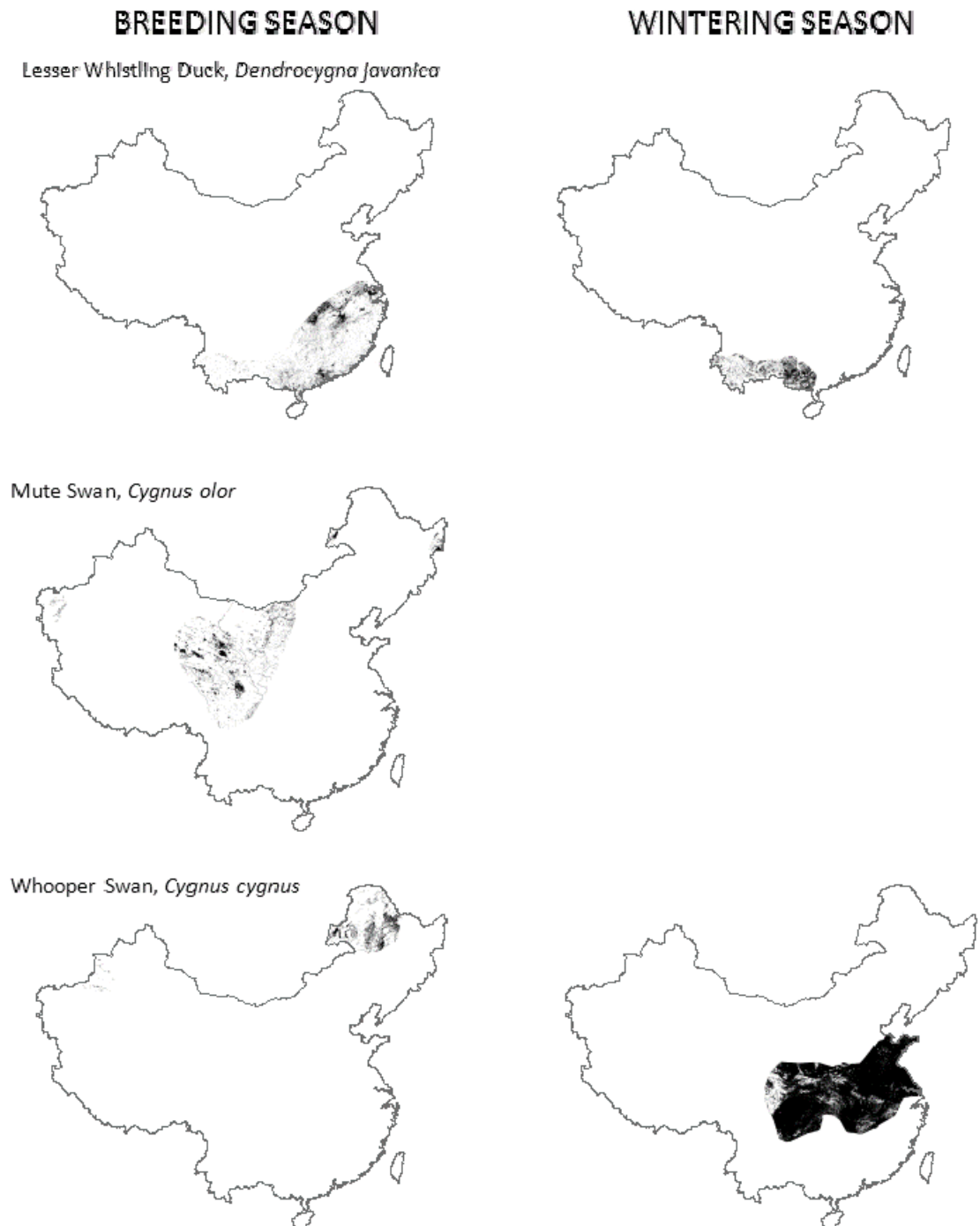


Fig. 4.3 continued.

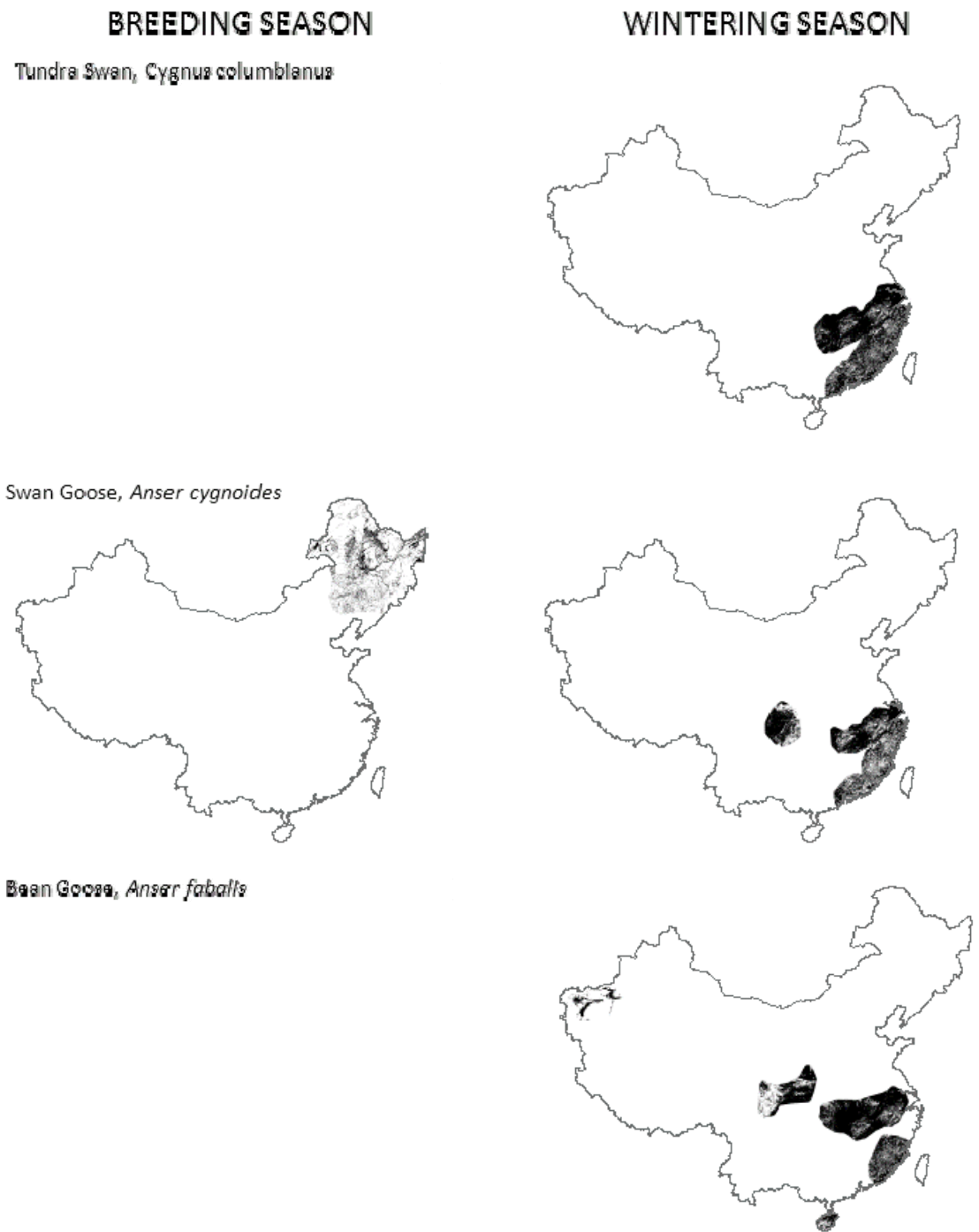


Fig. 4.3 continued.

BREEDING SEASON
Greater White-fronted Goose, *Anser albifrons*

WINTERING SEASON



Lesser White-fronted Goose, *Anser erythropus*



Greylag Goose, *Anser anser*



Fig. 4.3 continued.

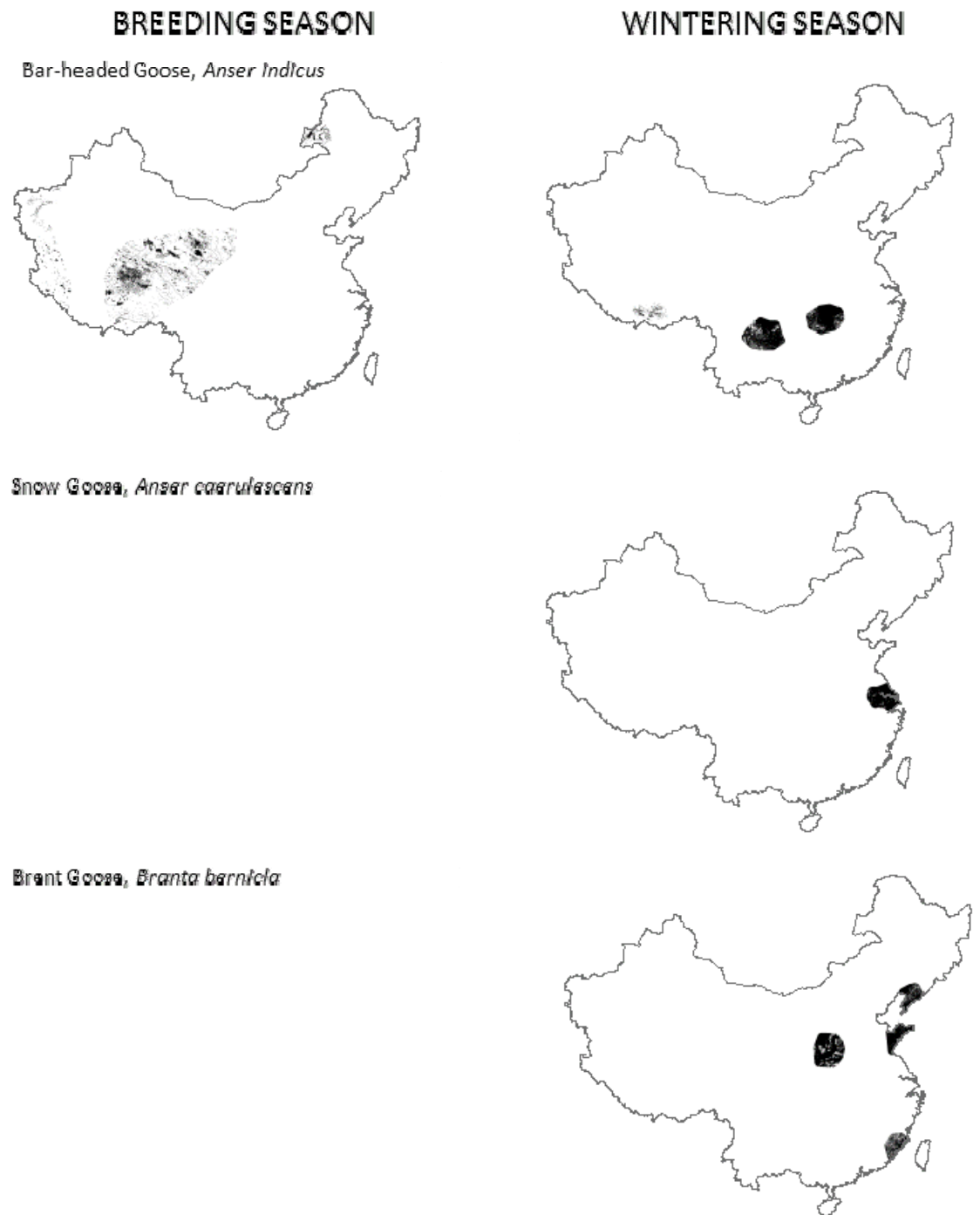


Fig. 4.3 continued.

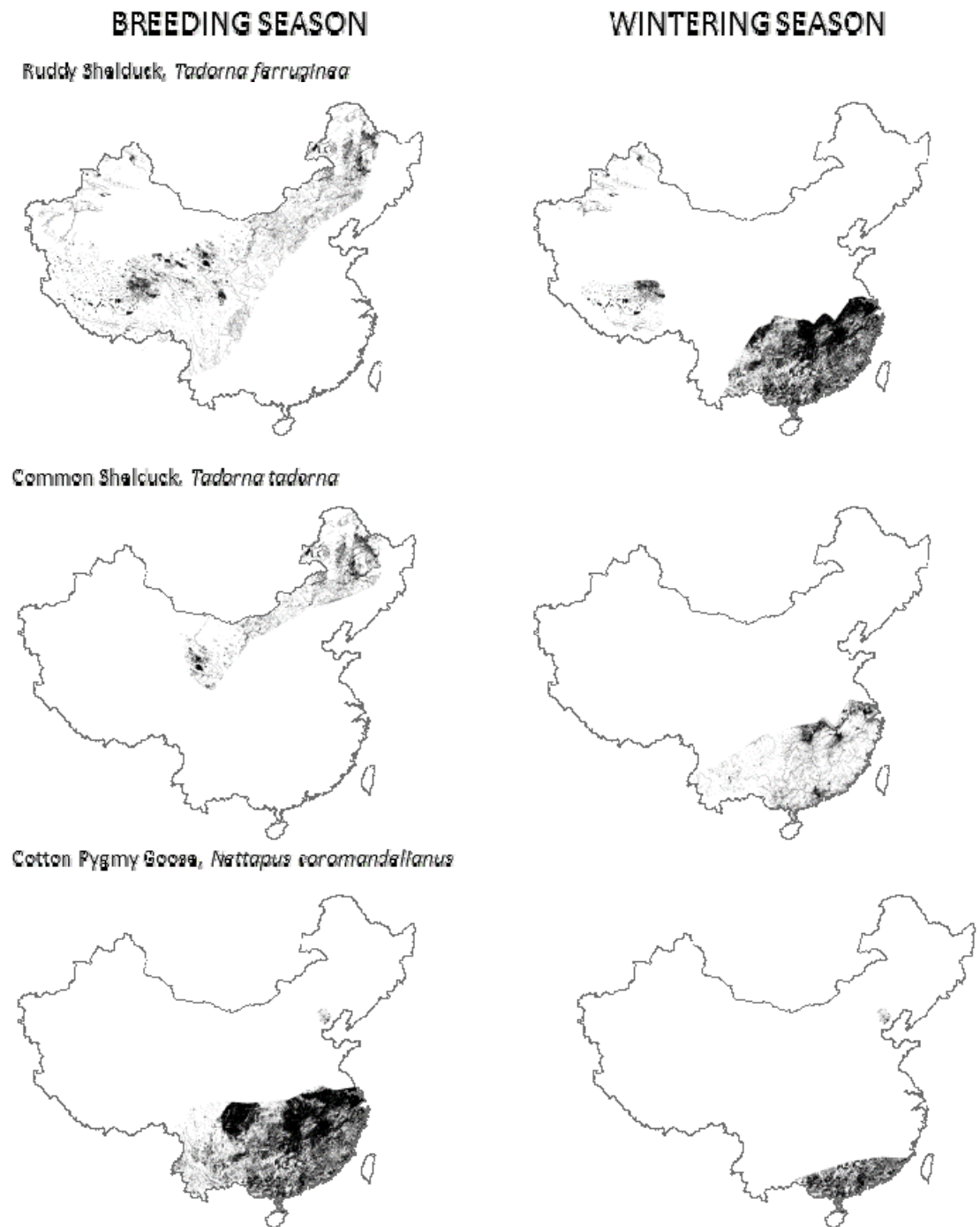


Fig. 4.3 continued.

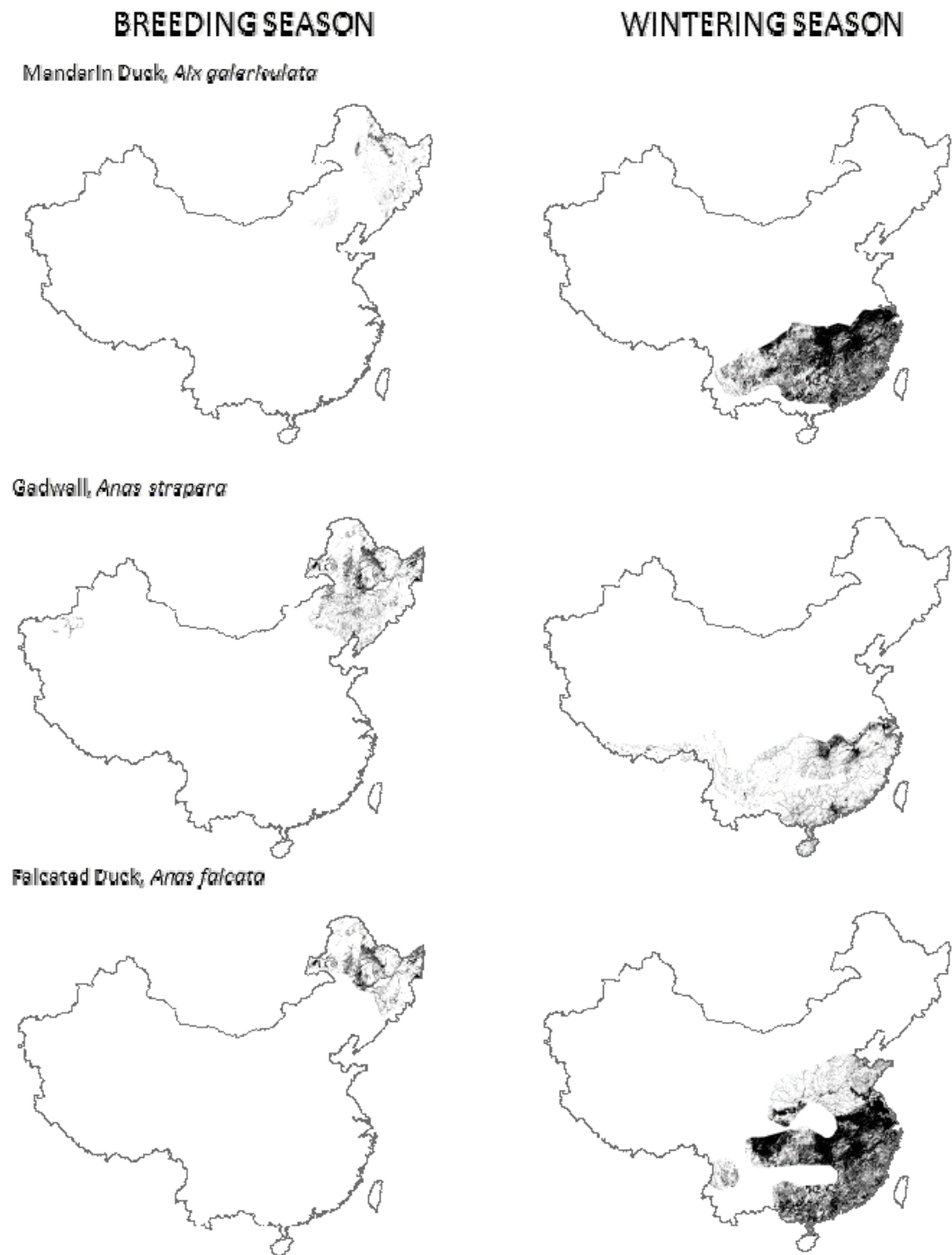


Fig. 4.3 continued.

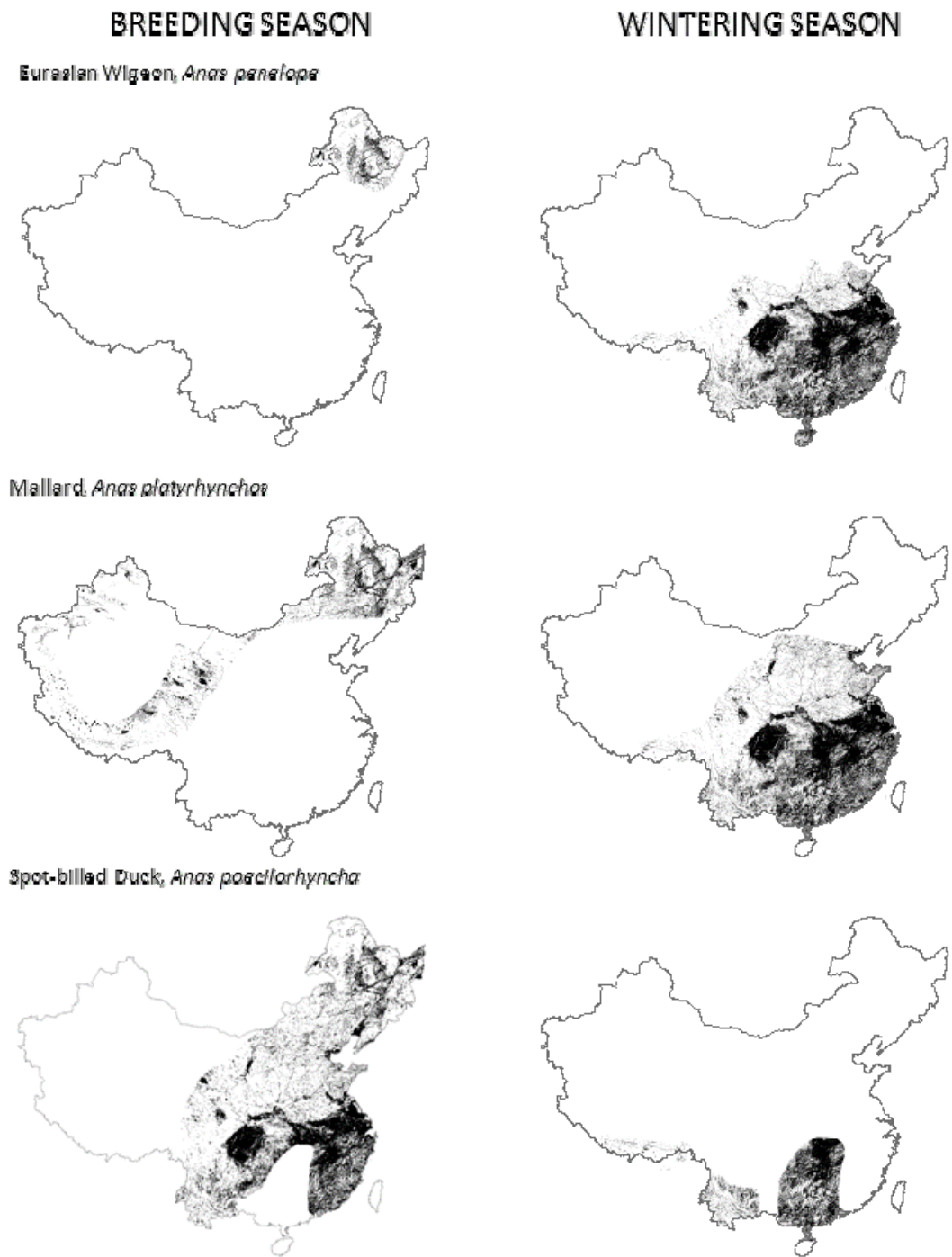


Fig. 4.3 continued.

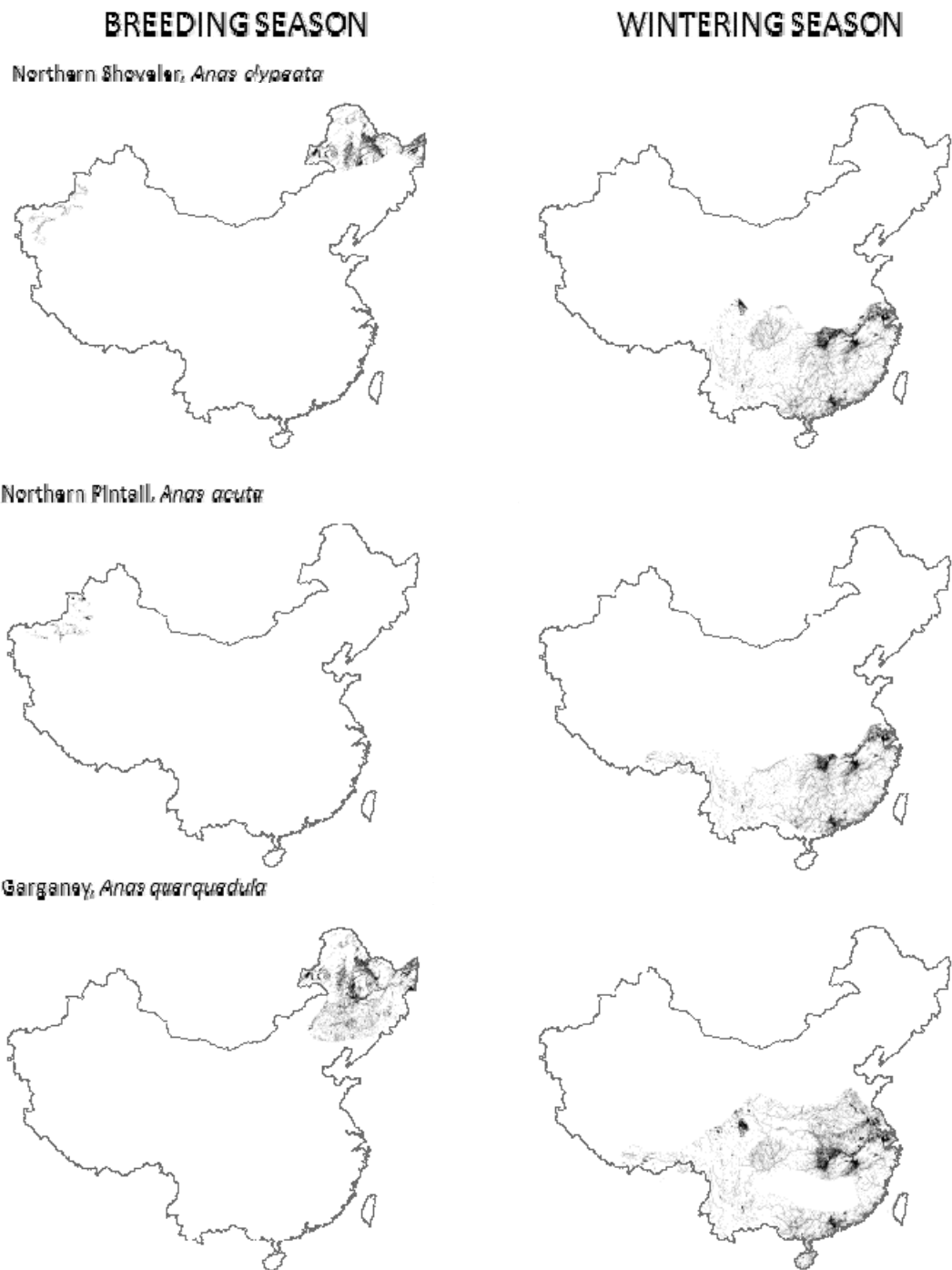


Fig. 4.3 continued.

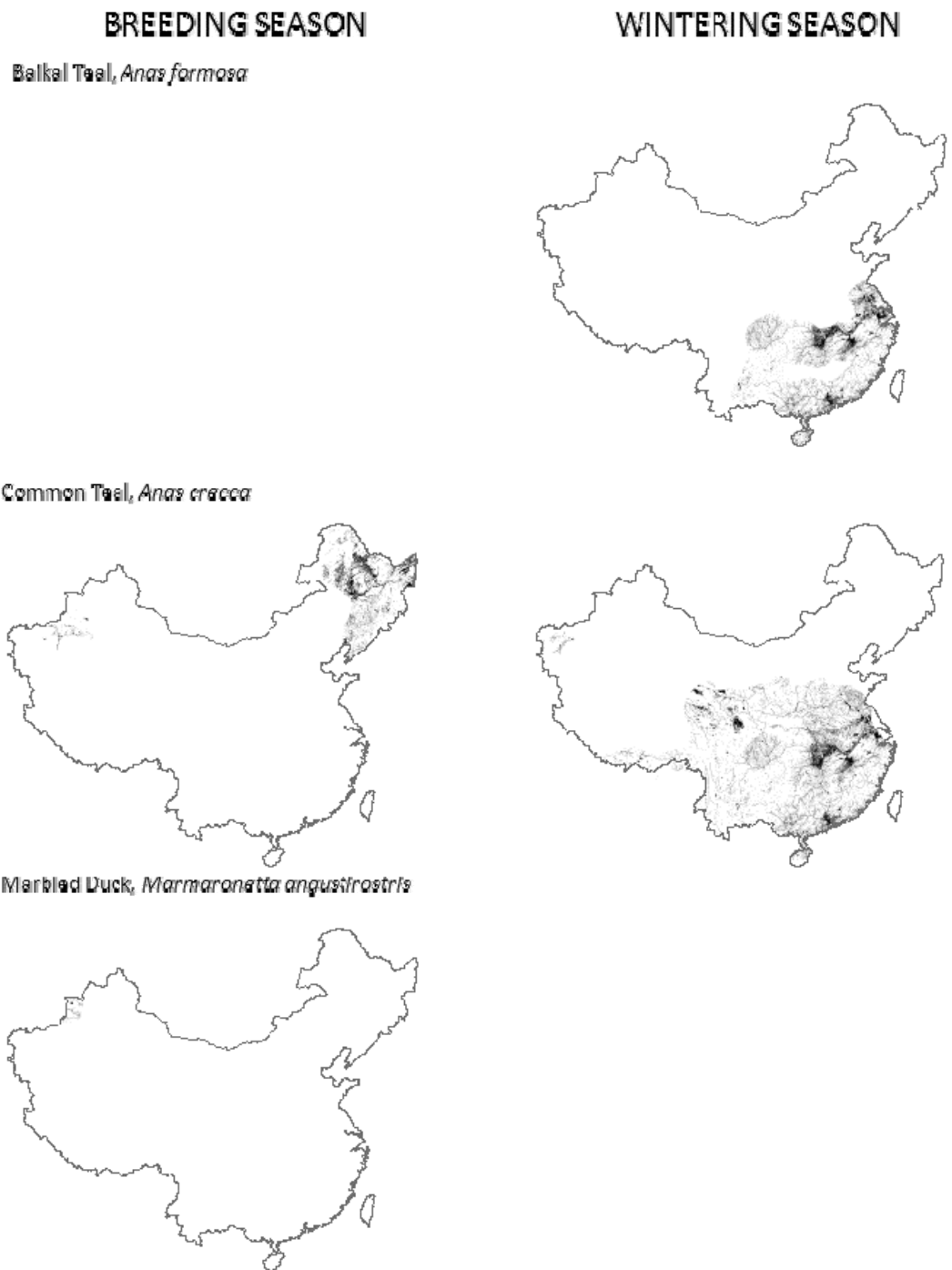


Fig. 4.3 continued.

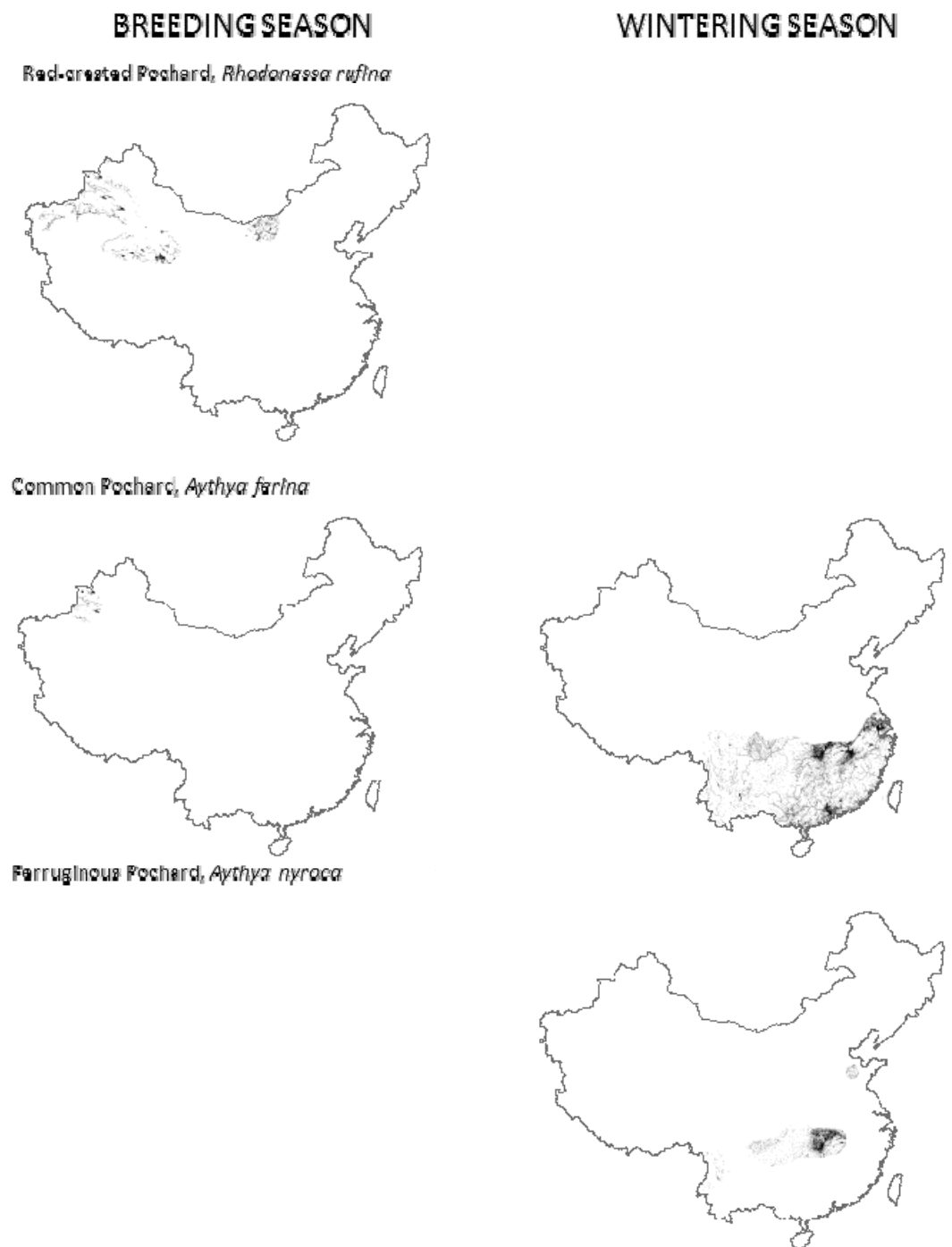


Fig. 4.3 continued.

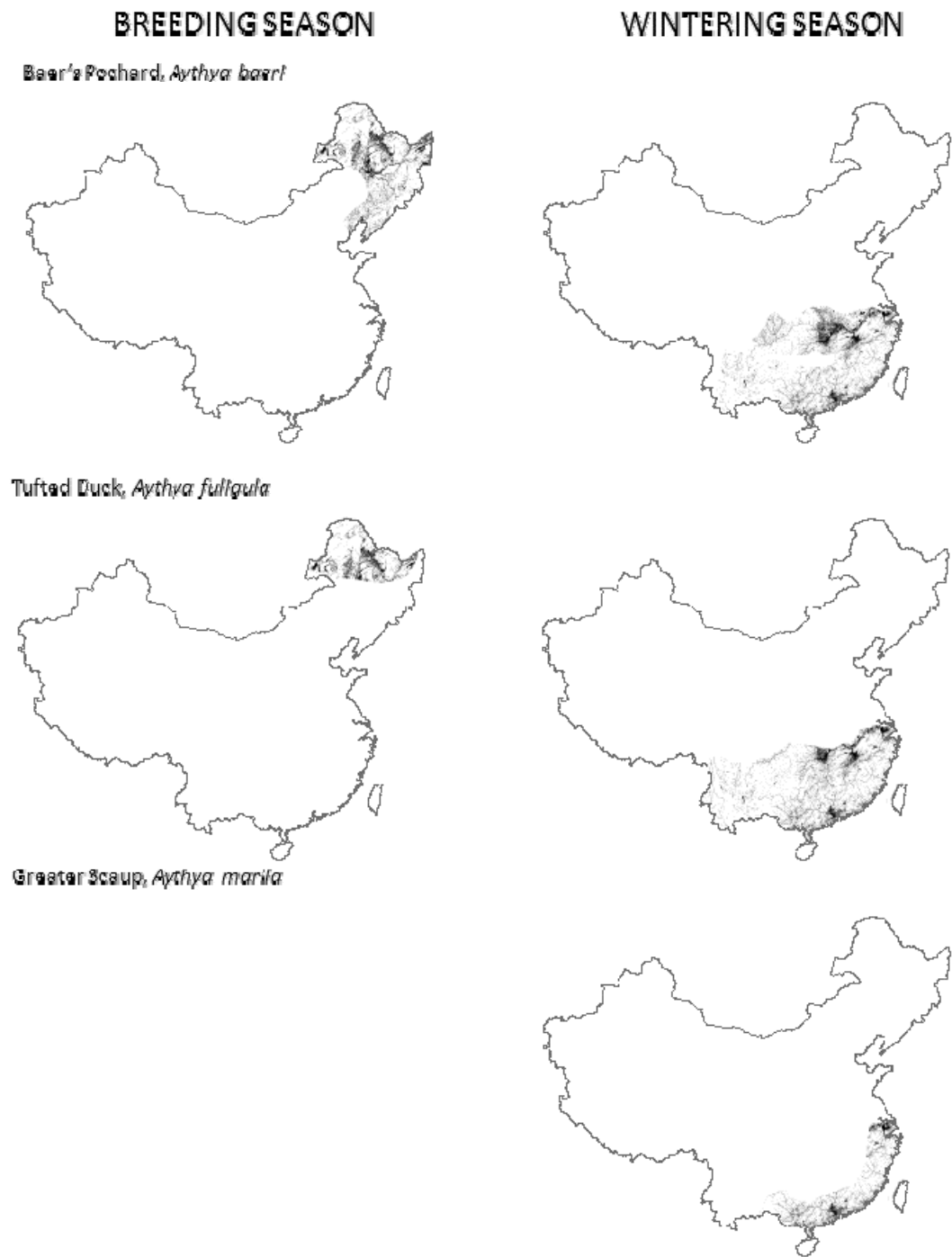


Fig. 4.3 continued.

BREEDING SEASON

Stellar's Eider, *Polysticta stellar*

WINTERING SEASON



Long-tailed Duck, *Clangula hyemalis*



Black Scoter, *Melanitta nigra*



Fig. 4.3 continued.

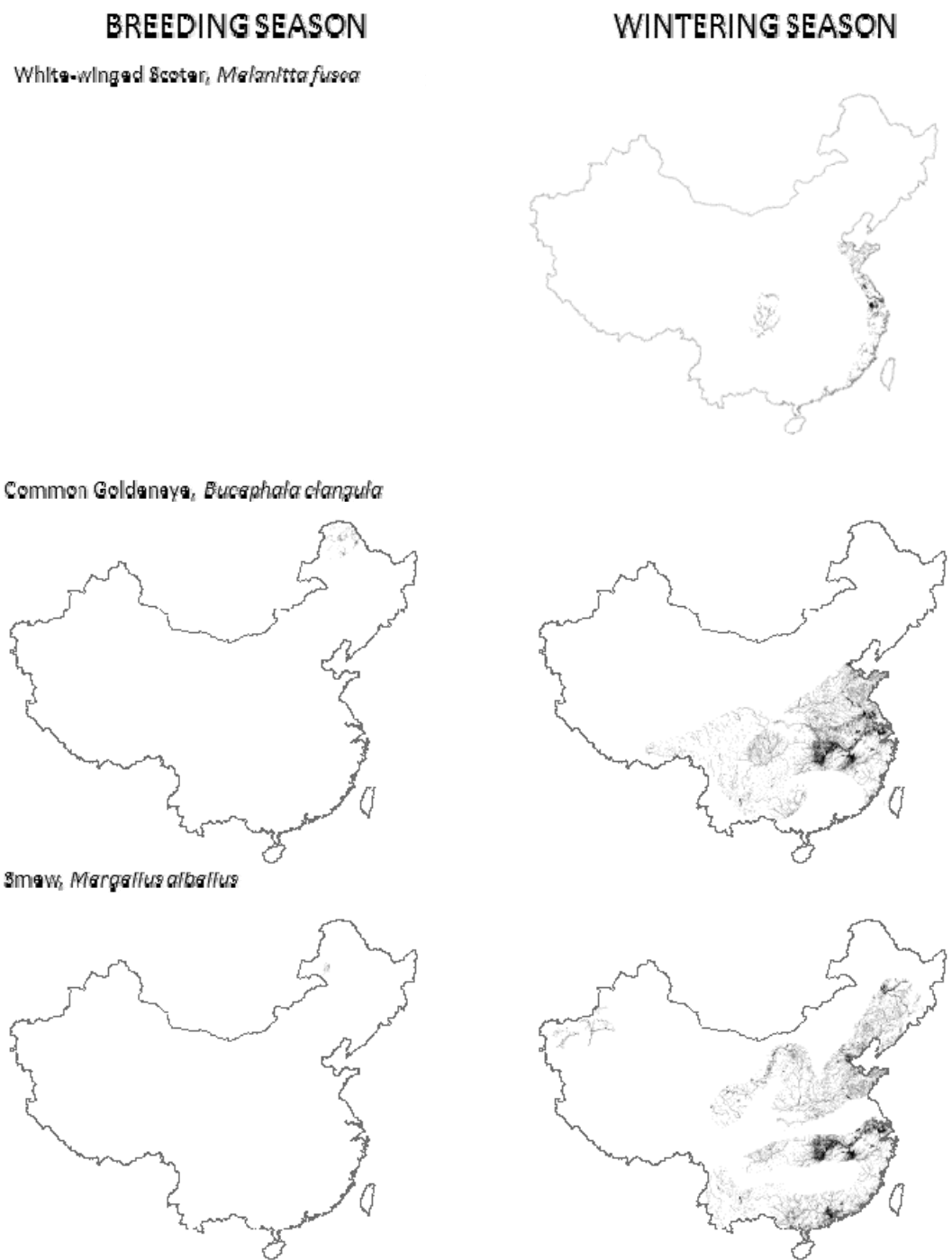


Fig. 4.3 continued.

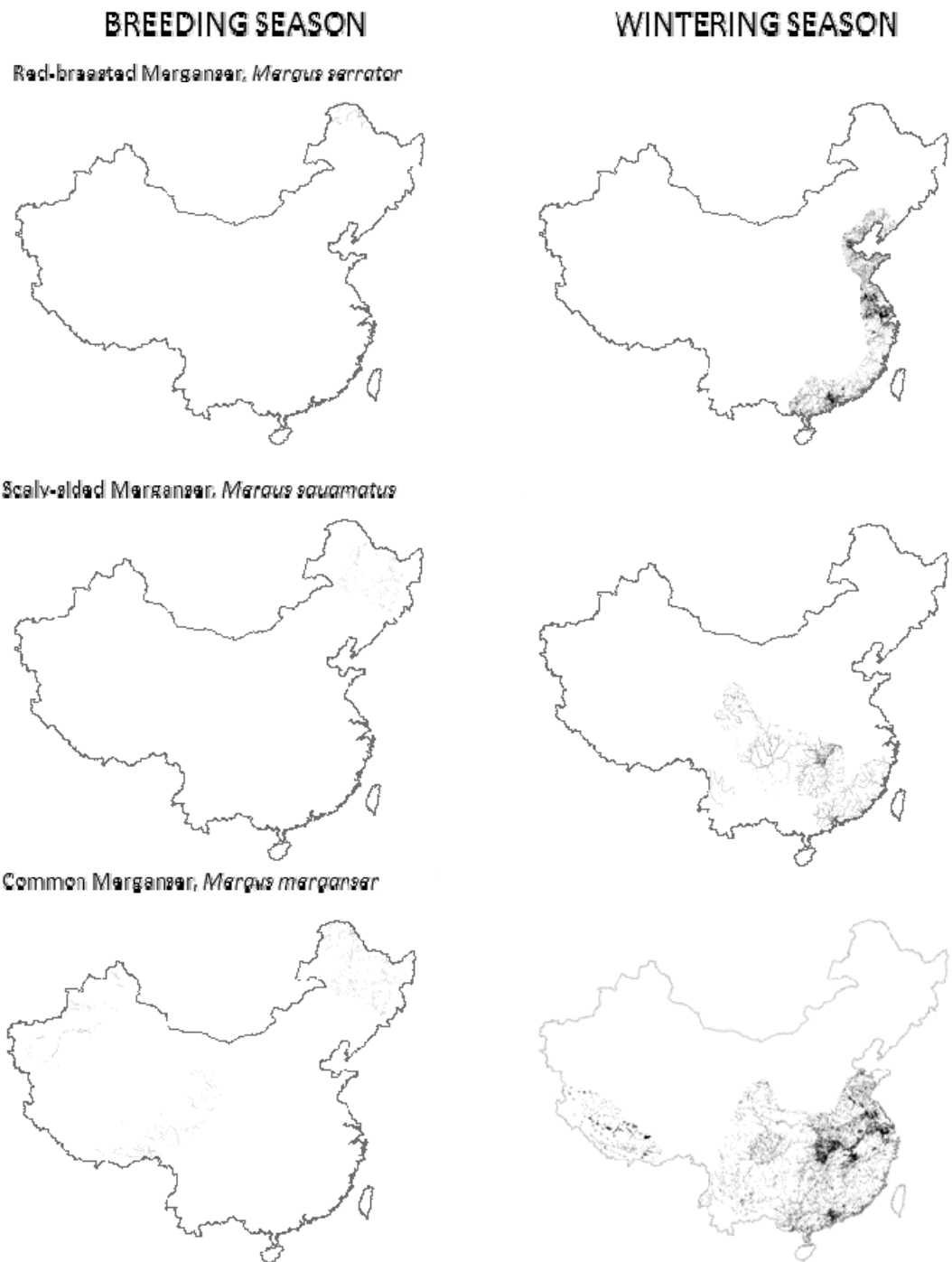
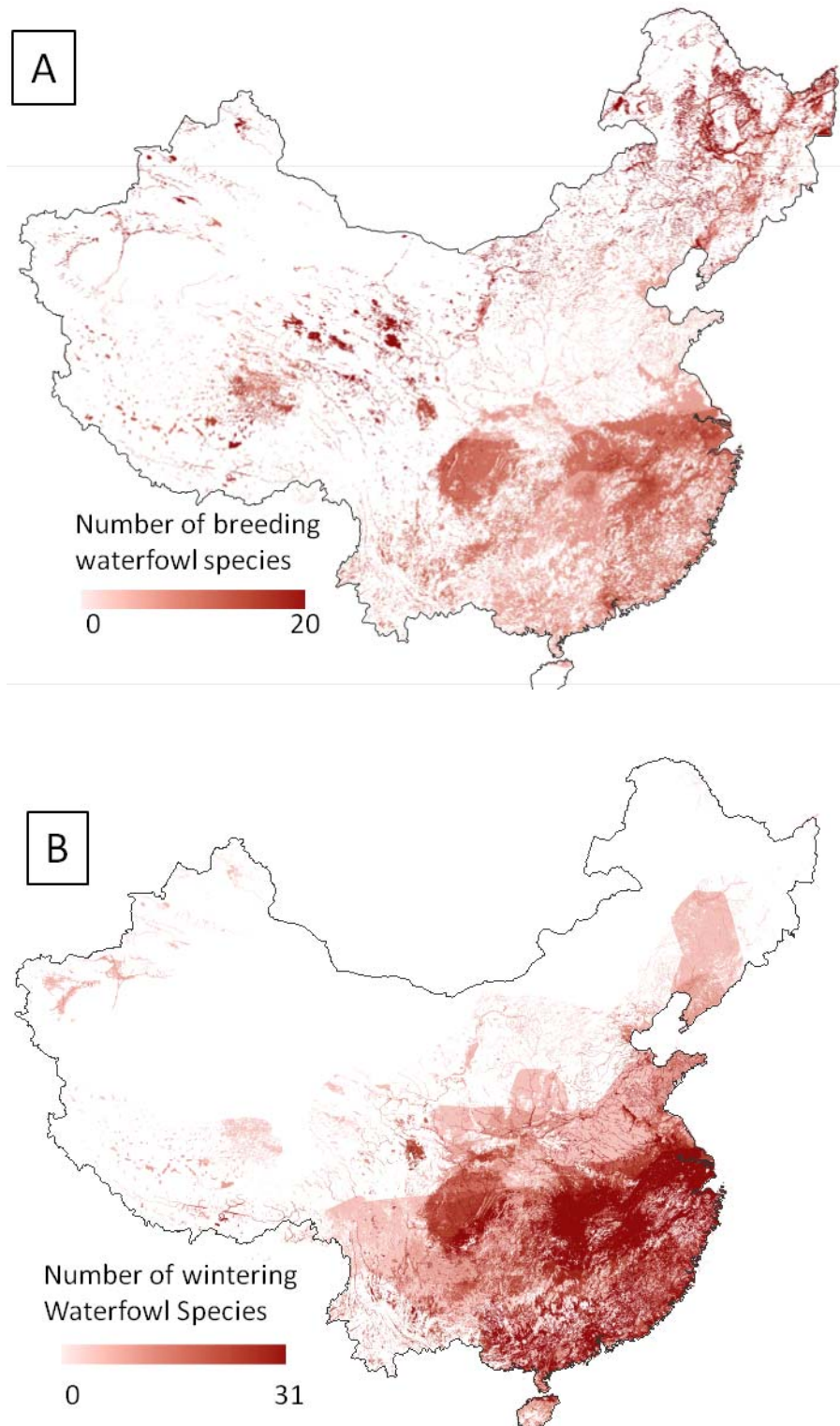


Figure 4.4. Species diversity for Anatidae (A) breeding and (B) wintering seasons. Coarse transitions in map predictions are a result of species range boundaries (e.g., horizontal pattern in southeastern China in panel (B)).



Supplemental Materials

Table S4.1. Habitat relationship equations for example species Bar-headed goose (*Anser indicus*)

Bar-headed Goose (*Anser indicus*)

Habitat Descriptions:

Breeding: Shallow lakes, marshes, lake shores, highland moors, salt lakes

Winter: Natural wetlands, agricultural fields, riverine and lacustrine wetlands, freshwater lakes

Migration: no info

Elevation: Breeds at elevations from 3570 to 5300m; wintering elevations range from lower elevations (Keoladeo Park, India 174m, Yunnan and Sichuan provinces) to higher (Tibetan Plateau).

Regions in China: Winter=Tibet, Sichuan, Yunnan, (India, Pakistan, northern Myanmar)

Descriptive Equations:

Breeding season includes cover types: marsh (64), rivers and irrigation channels (41), lakes (42), reservoir or pond (43) and river or lakeshore (46) in combination >0.

Wintering and resident seasons includes cover types: marsh (64), paddy (11), rainfed (12), rivers and irrigation channels (41), lakes (42), reservoir or pond (43) and river or lakeshore (46) in combination >0.

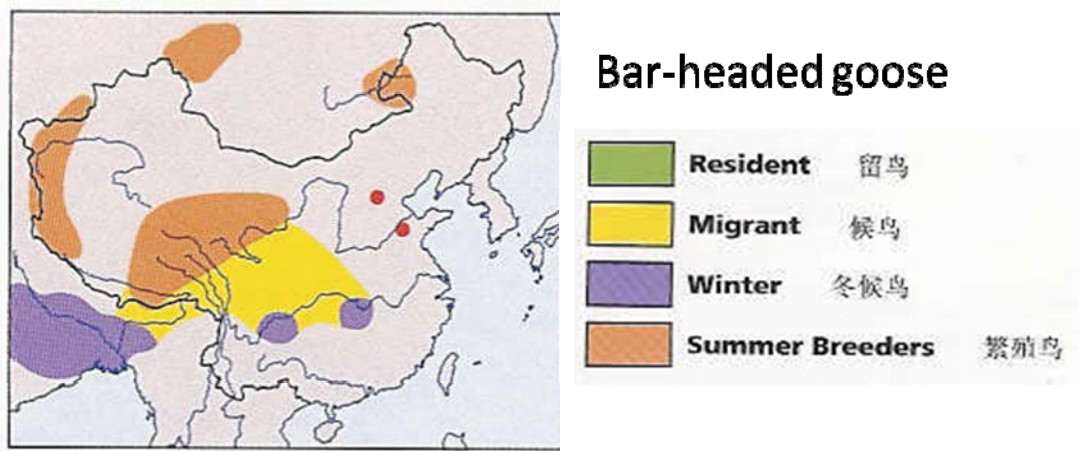
GIS Breeding and Wintering Equations:

- a. $d074_bhgo_br = ([ild64p] + [ild41p] + [ild42p] + [ild43p] + [ild46p]) > 0$
(include any of the above layers that have values greater than zero).
- b. $d074_bhgo_br = ([ild64p] + [ild11p] + [ild12p] + [ild41p] + [ild42p] + [ild43p] + [ild46p]) > 0$ (include any of the above layers that have values greater than zero).

Table S4.2. China Land Cover dataset created by the Chinese Academy of Sciences (CAS) Institute of Geographical Science and Natural Resources Research (IGSNRR) from 30m Landsat TM satellite imagery (Liu et al. 2002, Liu et al. 2005). Collapsed fields used for waterfowl distribution modeling.

	Waterfowl Models Land Cover Variables	Collapsed Fields	Original Fields	Land Cover Class Description
Arable Land	Paddy	11	11	Paddy
	Rainfed	12	12	Rainfed
Forest	Forest	21+24	21	Forest
	Scrub-Shrub	22+23	22	Scrub
			23	Shrub
			24	Other Forest
Grassland	Grassland>50%	31	31	Grassland (>50%)
	Grassland20-50%	32	32	Grassland (20-50%)
	Grassland5-20%	33	33	Grassland (5-20%)
Water	River and Irrigation	41	41	River and Irrigation
	Lake	42	42	Lake
	Reservoir, Pool	43	43	Reservoir and Pool
	Snow-capped	44	44	Snow Capped
	Coastal Shores	45	45	Shores (Sea)
	Bank of River/Lake	46	46	Bank/Shoal (River/Lake)
Developed	Urban	51	51	Urban
	Rural/Other	52+53	52	Rural Residence
			53	Other Constructed
Pristine	Sand,Gobi,Salt,Bare,Rock	61-63, 65-67	61	Sand
			62	Gobi
			63	Salt Lick
	Marsh	64	64	Marsh
			65	Bare Ground
			66	Gravel and Rocky Ground
			67	Other

Figure S4.1. Example bar-headed goose range map from MacKinnon and Phillipps (2000) showing breeding (orange), wintering (purple), resident (green), and migration (yellow) range extents. Migration was not included in distribution models because of lack of information. Resident regions were included in both wintering and breeding extents.



CHAPTER 5. RISK MODEL

Manuscript Title: Mapping high risk areas of disease transfer between domestic and wild birds: the case of highly pathogenic avian influenza in China

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Abstract

Highly pathogenic avian influenza (HPAI) H5N1 emerged in southern China in 1996 and has since become the longest persisting influenza virus in history, continuing to evolve and posing threat of a global pandemic. HPAI viruses historically were restricted to domestic poultry populations, however, H5N1 has spilled back to wild birds on multiple occasions, fueling the debate on wild birds and H5N1 transmission. Understanding the spatial and temporal interface between wild and domestic populations is fundamental to taking action against the virus, yet this information is hard to come by and has rarely been included in H5N1 risk models. In this study we aimed to identify areas of high transmission risk between domestic poultry and wild waterfowl in China. We developed unidirectional equations of H5N1 risk between wild and domestic birds across two

seasons in a Monte-Carlo framework which incorporates uncertainty in the modeling process. Patterns differed across seasons with hotspot regions in the northeast, central-east, and western regions of China during the breeding season (spring and summer) and in the central and southeastern regions during the wintering season. Transmission risk from poultry to wild birds was two orders of magnitude greater than risk from wild birds to poultry indicating the importance of parsing the equations in two directions. An intermediary set of models were developed to highlight regions where wild waterfowl and poultry co-occur on the landscape, which have broad utility to public health officials and conservationists alike. Here we present the first set of models to explicitly focus on H5N1 transmission between domestic and wild populations, modeling risk in both a spatial and temporal context, contributing an important piece that had previously been missing from our knowledge base on HPAI.

Keywords: H5N1, avian influenza, spatial modeling, Monte-Carlo, uncertainty, waterfowl, poultry

Introduction

Emerging infectious diseases in wildlife have become a growing concern to human health and biological systems. More than 75 percent of known emerging pathogens are zoonotic, being transmissible from animal to humans (Taylor et al. 2001, Alexander 2007), with the majority (77%) being capable of infecting multiple species (Cleaveland et al. 2001). It has been recognized that the increase in emerging zoonotic diseases is a result of human population expansion and globalization (Morse 1995,

Daszak et al. 2000, Brown 2004). Increasing demand for meat products due to population growth and development has led to rapid intensification of the domestic livestock industry (Delgado 2003), and improvement of transportation and market chains has brought humans and their agricultural systems closer together on a global scale. Coupled with increased threats of climate change and landscape fragmentation (Kovats et al. 2001, McMichael 2003, McMichael et al. 2006), incidence of emerging zoonoses is only likely to continue to rise (Kovats et al. 2001, McMichael 2003, McMichael et al. 2006, Jones et al. 2008).

Highly pathogenic avian influenza virus (HPAIV) H5N1 (hereafter H5N1) is a zoonotic pathogen that first emerged in domestic geese in southern China in 1996 (Xu et al. 1999), and since has become the longest persisting HPAIV in poultry (Smith 2006). While most low pathogenic avian influenza viruses (LPAIV) are found naturally circulating within their wild waterfowl reservoirs (Orders Anseriformes and Charadriiformes) without invoking symptoms (Alexander 2000), deadly HPAIVs result when an LPAIV enters a high density host population, such as a poultry farm, where it can rapidly mutate into a lethal form (Webster and Webster 2001). In late 2003, H5N1 erupted in outbreaks in poultry across 8 Asian countries (xx ref). Less than two years later, the first occurrence of HPAIV “spill-back” occurred in the wild bird community where more than 6,000 wild birds died of H5N1 at Qinghai Lake in the remote plateau region of western China (Liu et al. 2005). The unique characteristics of H5N1, including its ability to (1) spill back from poultry into the wild bird community (Webster et al. 2007b), (2) affect a wide diversity of host species (Cardona et al. 2009), and (3) replicate silently in domestic ducks (Sturm-Ramirez et al. 2005), have allowed this virus to

uniquely persist and reemerge, affecting more than 60 countries across Asia, Europe, and Africa over the past 16 years.

The novelty of this virus has also sparked confusion and debate among the global scientific community regarding the role wild birds play in spread of HPAIV (Yasue et al. 2006, Gauthier-Clerc et al. 2007, Feare 2010). After the spill-back event at Qinghai Lake, subsequent outbreaks were recorded in wild birds in Mongolia, Russia, and eventually Europe (OIE 2012). From this point on, wild birds were commonly implicated with the rapid spread of the virus over long distances; however, little to no data existed regarding movement patterns and disease susceptibility of wild birds to support this argument. Since then, our understanding of H5N1 and wild birds has improved. Multiple studies indicate a range of susceptibility and transmissibility response to H5N1 exposure among wild species (Brown et al. 2007a, Brown et al. 2008, Kalthoff et al. 2008, Keawcharoen et al. 2008), and the advent of a large-scale tracking program has increased our understanding of waterfowl ecology and movement patterns within zones of H5N1 infection.

A recent review of H5N1 risk models (Gilbert and Pfeiffer 2012. *In Press*) notes that few studies explicitly incorporate wild birds in transmission risk models, in part because obtaining adequate inputs for these populations is difficult. In an effort to increase our understanding of how wild birds are involved in the spread of H5N1, we proposed to study H5N1 transmission risk between domestic and wild birds in China, the epicenter of H5N1 (Mukhtar et al. 2007). Centering the study in China has value for multiple reasons. First, the disease originated in southeastern China and continues to persist and emerge in new locations indicating a continued need for research,

surveillance, and control. Secondly, the anthropogenic, wild, and environmental landscapes are each diverse across the country, allowing for varying levels of disease risk, both spatially and temporally. Two focal areas of interest provide contrasting but important situations for H5N1 transmission risk, particularly in regard to wild and domestic interaction. The Poyang Lake region (PYL), located in southeastern China along a feeder river to the Yangtze River basin, is a complex wetland system that supports an integrated mix of 8.8 million people, 14 million ducks, and 100,000 wintering migratory waterbirds including 90 percent of the global population of endangered Siberian Cranes (*Grus leucogeranus*) (Takekawa et al. 2010b). The majority of the human population lives in village settings, well-integrated within the agricultural landscape. Rice-cropping and free range duck farming are prevalent, and the demand for ‘healthy’ wild meat has led to the rise of farmed wild waterfowl, all increasing the potential for wild and domestic populations to exchange virus. In contrast, Qinghai Lake (QHL), where the global debate on wild birds and H5N1 transmission began, is located in a remote arid region on the high-elevation Qinghai-Tibet Plateau. Few poultry are present, and free-ranging duck farming common to the lowlands does not occur. H5N1 outbreaks are common to both regions, and investigating the response of our transmission risk models to these regions is of particular interest.

In this paper, we employ a systematic approach to modeling disease transmission risk between domestic poultry and wild waterfowl populations. We begin by building high resolution (1 km) deterministic models that define areas of wild and domestic bird co-occurrence and H5N1 risk factors from existing SIR disease models. We then follow with coarser resolution models that incorporate uncertainty and produce a measure of

error for each cell of the risk output. These 30 km resolution models match the average county area for China, a more appropriate scale for assessing transmission risk.

Materials and Methods

Poultry Model Inputs

Spatial maps of poultry densities were created by disaggregating census data using regression modeling (Prosser et al. 2011b). Poultry census data was compiled from statistical yearbooks published by China's Ministry of Agriculture and National Bureau of Statistics at provincial or sub-provincial scale. Using land cover and meteorological remote sensing data (Liu et al. 2002, Hay et al. 2006), we identified statistical relationships between the poultry data and environmental variables to predict densities at a 1 km resolution across the extent of China. We applied species level information to produce output maps for chickens, ducks, and geese, and validated models using goodness of fit measures (Prosser et al. 2011b). Since both the farming structure and H5N1 pathogenicity varies between terrestrial (chickens) and aquatic poultry (ducks, geese) (Alexander 2000, Hulse-Post et al. 2005, Sturm-Ramirez et al. 2005, Alexander 2007, Pantin-Jackwood et al. 2007, Kim et al. 2008, Li et al. 2010, Phuong et al. 2011), we then created final inputs for these two categories, (P_{te} , and P_{aq} , respectively).

Wild Anatidae Waterfowl Model Inputs

We developed two indices to characterize densities of wild Anatidae waterfowl on the landscape: waterfowl abundance (W_{ab}), and index of H5N1 prevalence (W_{pr}). Using a habitat analysis approach, we first developed models to predict occurrence (presence/absence) at 1 km resolution for China's 42 waterfowl species (Prosser et al.

2012 *In Prep.*) based on relationships between habitat requirements and environmental predictor variables (Verner et al. 1986, Morrison et al. 2006). Models were built separately for each species and season for a total of 30 breeding and 37 wintering species maps (Prosser et al. 2012 *In Prep.*). Here we expand upon the presence-absence distribution models to create abundance and H5N1 prevalence models (Supplemental Fig. S5.1). Abundance models were created by dividing the seasonal population estimate for China for a given species by the number of presence grid cells and assigning that number to each cell in the distribution. Populations numbers for China were either taken directly or estimated from two leading references: Cao et al. (2008) and Delany and Scott (2006). The resulting 1 km density distributions were summed across all species within a season to develop cumulative abundance indices: W_{abwi} and W_{abbr} for winter and breeding seasons. In a similar fashion, we applied H5N1 prevalence rates for each species (Table 5.1) to the abundance distributions to create an index of prevalence. The prevalence index is the product of a species' abundance and its prevalence rate. Cumulative prevalence indices were developed for the breeding and wintering seasons, W_{prbr} and W_{prwi} , by summing values across species layers for a given season.

We incorporated estimates of uncertainty for the abundance distributions due to the wide range in confidence we had for estimates of given species. We had higher levels of confidence for the population estimates published specifically for China, which were all wintering populations estimates from Cao et al. 2008 (21 of 37 species); for these, we drew a 15% confidence band around the estimates. The remaining wintering estimates (16 species) and all of the breeding population estimates (30 species) were derived from

global population estimates published in Delaney and Scott (2006). Delaney and Scott (2006) list population figures by region for the breeding and wintering seasons and provide country level estimates where sufficient data were available. We derived estimates from the eastern Asia region which included China, Korea, Japan, Mongolia, and northeastern Russia. We drew wider confidence bands around our derived estimates, ranging from 15 percent to 90 percent (the majority ranging between 30 and 60 percent) depending on the size of the population and supporting data. We developed estimates of coefficient of variation (CV) for each species and season using triangular distributions in a Monte Carlo simulation with 10,000 runs. The population estimate was used as the best estimate, and high / low estimates used for the maximum and minimum limits of the triangular distribution (Table 5.1). The coefficient of variation for the cumulative abundance distributions was expressed as the mean CV across all species.

Transmission Risk Equations

We developed three levels of models to predict disease transmission risk between domestic and wild birds in China (Figure 5.1). We first used a deterministic approach to develop and refine the model equations (Level 1 and 2 models) and then we applied Monte-Carlo simulations to the Level 2 equations to incorporate estimates of uncertainty around the parameter inputs. We used two very different approaches between the Level 1 and 2 models – the first predicts where wild and domestic birds may be found together on the landscape; the second incorporates specific H5N1 risk factors for the wild and domestic populations. We chose to take these iterative steps so we can clearly identify the spatial and temporal relationships between the wild and domestic bird populations

before incorporating the H5N1 parameters. The following outlines each of the equations and their associated assumptions.

Equation 1 is the first equation in the Level 1 overlay models. It produces a binary transmission map (risk / no risk) working under the hypothesis that (a) transmission is bi-directional (equal probability) between poultry and wild birds, such that within a cell, wild birds can spread virus to domestic birds and vice versa, and (b) both domestic and wild birds must be “present” for transmission to occur:

$$1. [P_{01}] * [W_{01}] = T_{risk},$$

where P_{01} is the presence of poultry (either terrestrial or aquatic) and W_{01} is presence of wild Anatidae waterfowl (subscripts 01 stand for absence/presence). T_{risk1} value of 0 indicates no transmission risk and value of 1 indicates risk.

Equation 2 is the second equation of the Level 1 models. It differentiates between the presence of one or both types of domestic poultry, where Pt_{01} is presence of terrestrial poultry, and Pa_{01} is presence of aquatic poultry:

$$2. [Pt_{01} + Pa_{01}] * [W_{01}] = T_{risk2},$$

Equations 1 and 2 define the spatial and seasonal distribution of where potential disease transfer may occur between domestic and wild (Anatidae) populations in China.

The third equation incorporates risk factors for H5N1 transmission between domestic and wild populations based on our current state of knowledge on H5N1 in these

populations. Here we do not assume that virus transfer is equal in both directions and instead develop unique equations for the risk of H5N1 transmission from poultry to waterfowl (T_{PtoW} , equation 3a), and waterfowl to poultry (T_{WtoP} , equation 3b). The model is a hybrid between a density dependent (McCallum et al. 2001) and environmental transmission model where direct transmission is defined by the fecal to oral route facilitated by transmission through the water environment (Alexander 2007):

$$3a. ([P_{te} * C_{te} * V_{te}] + [P_{aq} * V_{aq}]) * ([W_{pr} * U]) = T_{PtoW}$$

$$3b. ([W_{pr} * V_{wf}]) * ([P_{te} * B_{te}] + [P_{aq}]) * U = T_{WtoP},$$

where P_{te} and P_{aq} represent the density (birds/km²) of terrestrial and aquatic poultry; C_{te} is the contaminant containment rate for terrestrial poultry (the rate at which virus enters the system from terrestrial poultry farms); V_{te} , V_{aq} , and V_{wf} are viral shedding rates for terrestrial poultry, aquatic poultry, and Anatidae waterfowl, respectively; W_{pr} is the wild Anatidae waterfowl cumulative prevalence rate (summed prevalences across all species distributions within a season, W_{prbr} and W_{prwi} for breeding and wintering seasons); U is the virus uptake rate through water (drinking rate / minimum load for infection); and B_{te} is a biosecurity scalar that removes terrestrial poultry within biosecure farms from the at-risk population. The equation is run separately for each of the W_{pr} indices.

Equation 3a works under the hypothesis that (a) transmission risk from poultry to wild waterfowl occurs through the environment (fecal to oral route) where (b) the amount of virus shed is proportional to the density of terrestrial and aquatic poultry, (c) that the

shedding rates may differ between terrestrial and aquatic poultry, (d) and the risk to wild waterfowl is dependent upon the density of wild waterfowl which (e) uptake virus at a constant rate. Equation 3b works under the hypothesis that (a) transmission risk from wild waterfowl to poultry occurs through the environment (fecal to oral route), (b) is dependent on the density of wild waterfowl present on the landscape, (c) their viral shedding rate, (d) the density of aquatic poultry available to come in contact with infected waters, and (e) the uptake rate of virus from water.

Estimating Contaminant Containment and Backyard Poultry from Poultry Densities

Analysis of poultry systems in Asia has shown a pattern of increased biosecurity in relation to the scale of farming (e.g., higher biosecurity for industrial and integrated systems in comparison to smaller commercial farms or back-yard production) (FAO and OIE 2005). The contaminant containment (C_{te}) and biosecurity (B_{te}) parameters were developed as scalars to reduce the effective terrestrial poultry populations within the equations. The parameters are based on the assumption that more biosecure farms (a) control the flow of potential pathogens that leave the farm and enter the environment, and (b) protect themselves from incoming pathogens from the environment and other farms (cleansing vehicles before entering the farm, housing animals in structures secure from wild species, etc.). The C_{te} scalar is included in equation 3a that encompasses risk in the direction of poultry to wild birds. We first draw a relationship with poultry density under the assumption that cells having the highest poultry densities also contain the largest-scale and presumably most biosecure farms. Based on a frequency analysis of 1 km cells of poultry densities across China (Prosser et al. 2011b), we developed a threshold of 5000 head/km² of terrestrial poultry (and two others for the sensitivity analysis) above which

were considered as biosecure (Supplemental Fig. S5.2). Cells with this designation were applied the C_{te} scalar in the risk equation (3a). For equation 3b, where virus flow is in the direction of wild to domestic birds, we used the B_{te} scalar to predict the portion of total terrestrial poultry that may be backyard animals (i.e. ones with exposure to virus thru the environment). This was accomplished by sectioning total terrestrial poultry into three groups and applying functions to each to estimate the number of backyard birds. Here we considered terrestrial poultry densities of less than 50 to be all backyard birds; densities between 50 and 1000 to be a mix of backyard poultry and small to mid-scale commercial farms, and densities of greater 1000 to be a mix of backyard poultry and commercial farms of any size. The B_{te} scalar for the first group is 1, for the second group is 0.5, and the third is replaced by a constant of 1000. For example, the number of terrestrial poultry contributing virus to the environment is $[P_{te} * B_{te}]$; if $P_{te} = 25$, $[P_{te} * B_{te}] = 25$; if $P_{te} = 100$, $[P_{te} * B_{te}] = 50$; and if $P_{te} = 2000$, $[P_{te} * B_{te}] = 1000$ (Supplemental Fig. S5.3). We did not include biosecurity factors (C_{te} or B_{te}) for aquatic poultry due to the overall smaller scale of farming in relation to chickens (83 versus 15 and 2 percent for chickens, ducks, and geese, respectively (China National Bureau of Statistics 2008)), and more importantly, the general nature for ducks to be farmed in a setting that is open to the environment providing a pathway for transmission to wild birds (Gilbert et al. 2006a, Muzaffar et al. 2010). Due to the uncertain nature of the biosecurity parameters, assessment in the sensitivity analyses (see below) is important.

Sensitivity Analysis

We conducted a sensitivity analysis to determine the response in model output given a range of values for a particular input parameter (Morgan and Henrion 1990,

Kroese et al. 2010). Sensitivity analyses were run for the 1 km deterministic equations using high and low estimates for a given parameter while keeping all other model parameters constant. The effect was assessed by examining the raw differences as well as calculating the percent difference between the high and low estimates. Parameter ranges and descriptions of the model inputs are outlined in Table 5.2. For the biosecurity terms C_{te} and B_{te} we tested both effects of high and low inputs as well as the complete effect of the term on the model output. Results from these analyses were used to determine which parameters should be modeled with uncertainty in the Level 3 Monte-Carlo simulations (Figure 5.1).

Spatial and Temporal Scale of Analysis

Our first approach was to model the deterministic equations at 1 km resolution in a geographic framework using ArcGIS 10.0 (ESRI, Redlands California) and Python (www.python.org). We then ran the Monte-Carlo uncertainty analyses (Morgan and Henrion 1990, Kroese et al. 2010) at a coarser resolution of 30 km, approximately the average county size for China, and a resolution more realistic to our ability to model transmission risk. Both the deterministic models and Monte-Carlo analyses were run for two temporal seasons that relate the annual chronology of wild waterfowl to transmission risk. The breeding season occurs during the spring and summer months, generally from April to July, and the wintering season from November to March.

Assessing Uncertainty using Monte Carlo Simulation

In each step of the data production phase of this research, we included estimates of uncertainty with our model predictions in the form of coefficient of variation (standard

deviation divided by the mean). For the poultry models, these were conducted using a bootstrap procedure (Prosser et al. 2011b) and for the waterfowl indices we used a Monte-Carlo approach. We also examined the effects of resampling the waterfowl abundance inputs from 1 km to 30 km using a standard bilinear technique (de Smith et al. 2007). We mapped the differences by subtracting the 30 km grid cell values from the 1 km values and symbolizing in units of: no change, change within 1 standard deviation of the mean, and change greater than 1 standard deviation of the mean.

After the deterministic equations were set, we applied to these equations a quantitative risk analysis using Monte Carlo simulations to (1) incorporate uncertainty in the model predictions and (2) to develop a complimentary map of estimates of error on a spatial basis (Morgan and Henrion 1990, Kroese et al. 2010). Uncertainty for the poultry variables, P_{te} and P_{aq} , were described using a normal distribution (Table 5.3). This was determined by fitting a random sample of poultry estimates across 25 bootstrapped layers for 100 spatial locations using the “fitdistrplus” package for R (R Core Development Team 2012). We used best estimates and minimum – maximum limits within triangular distributions for the remaining variables for which we had neither variability nor uncertainty measures (Table 5.3). We used the “mc2d” package written for R to perform the triangular and truncated normal distributions. Each simulation was run for 10,000 iterations to ensure model convergence (Supplemental Fig. S5.4) and we plotted tornado charts based on Spearman’s rank correlations between the model risk and input parameters to determine which variables were most influential to the model output.

Results

Input Parameters

A total of ten input variables were created for the transmission risk models, six of which varied across the nearly ten million cell extent of China (Figure 5.2, Table 5.2) and four of which were model level coefficients (Table 5.2). General patterns for terrestrial and aquatic poultry were similar with highest densities in the east and south of China (Figure 5.2). Overall, terrestrial poultry (chickens) densities were higher than the aquatic species densities (379 versus 86 mean birds per km²), and although the two groups overlap in most places, terrestrial poultry were located in some regions such as the extreme west where aquatic poultry were not. The C_{te} and B_{te} variables were composite indicators of the potential of virus to flow in or out of terrestrial poultry farms. The C_{te} distribution was limited to discrete regions in northeastern China, as a function of the grid cells with highest chicken densities (Figure 5.2). The B_{te} distribution attempted to differentiate backyard from commercial poultry by splitting poultry densities into three sectors and applying individual equations to each. The resulting distribution showed highest levels in eastern-central China, mid-levels across the east, and low levels across the west (Figure 5.2).

Waterfowl abundance maps were created as an interim step towards development of H5N1 prevalence indices. Supplemental Fig. S5.5 illustrates differences in abundance, prevalence, coefficient of variation, and spatial distribution for two example species important in H5N1 transmission: the bar-headed goose (*Anser indicus*) and mallard (*Anas platyrhynchos*). The cumulative waterfowl abundance model values ranged from zero to 5.0 birds per cell for the breeding season and zero to 7.9 for the wintering season with

mean values of 0.07 and 0.08 across China (Supplemental Fig. S5.6). Mean cumulative H5N1 prevalence rates were higher for the breeding versus wintering season (average of 0.01 versus 0.006 EID₅₀ across all grid cells of China), and maximum values were 0.32 and 0.39, respectively. Mean coefficient of variation was 0.19 and 0.11 for the breeding and wintering seasons. Investigation of the effect of resampling the abundance and prevalence distributions from 1 to 30 km indicated greatest differences in the northeast for the breeding season and in the southeast for the wintering season (Supplemental Fig. S5.7). The average difference across all grid cells was 0.155 and 0.152 for the breeding and wintering abundance indices, and 0.01 and 0.006, respectively for the prevalence indices.

Risk Models

The first group of models (Level 1 and 2) was based on implementation of the deterministic equations at 1 km resolution across China. Overlay models (Level 1) between poultry and wild waterfowl presence showed distinct patterns of transmission risk across China (Figure 5.3). In both the breeding and wintering seasons, dense concentrations of positive risk grid cells were found across much of southeastern China. The regions of the northeast and west of China showed more localized patterns of transmission risk, with wider extent for the breeding season than the wintering season. The spatial pattern was similar between equation 1 models (total poultry and wild birds) and equation 2 models (presence of one or both poultry groups in combination with wild waterfowl). The equation 2 models also showed localized regions mainly in the west where only one poultry type (usually chickens) was present in combination with wild waterfowl (Figure 5.3, lower panel).

Level 2 models incorporated H5N1 transmission factors into the equations, with one equation representing risk of disease spread from domestic to wild birds (Figure 5.4a), and a second representing risk from wild to domestic birds (Figure 5.4b). The distribution of grid cells of the Level 2 models having risk values greater than zero matched the distribution of transmission risk in the Level 1 models, however, the Level 2 models also predicted the quantity of transmission risk within each cell. We symbolized the Level 2 results (as well as Level 3) using quantiles, which has been shown to be a straight-forward and effective method for visualizing disease risk (Brewer and Pickle 2002, Brewer 2006). Spatial patterns in the levels of disease risk between the two unidirectional equations (3a and 3b) were similar across the broad scale of China, however, the level of risk was greater for the poultry to waterfowl models (equation 3a) by approximately two orders of magnitude (Table 5.4). The output maps showed distinct spatial patterns between seasons. Within the breeding season, highest levels of risk (in both directions) were found in localized patches in northeastern China as well as along the Yangtze River plain of south-central China. For the wintering season, the higher levels of risk were confined to the south and east of China particularly along the major river basins. Within each set of equations, the winter models had higher means than the breeding season models by 46 and 53 percent, for equation 3a and 3b, respectively. Predictions from the deterministic equations were summarized in Figures 5.3 and 5.4.

The Level 3 models incorporated estimates of uncertainty in the model equations and applied a spatial resolution of 30 km (Figure 5.5) using the same unidirectional equations (3a and 3b) as the deterministic equations in Levels 1 and 2 described above. A comparison of the means indicated higher risk for these versus the deterministic

models, and for the wintering season versus the breeding season. The spatial patterns of risk in the Monte-Carlo models generally followed those of the deterministic equation models (Figure 5.4 and 5.5) although some difference was observed for the wintering models where higher risk predictions occurred in a portion of the northeast. In both seasons, the central region along the east coast also showed higher risk in the Monte-Carlo versus deterministic models.

Patterns in uncertainty associated with the Monte-Carlo model predictions were similar across seasons and unidirectional equations on a broad scale. The most uncertain areas of prediction were located in the western part of the country and the least uncertain areas were located in the south and east (Figure 5.5). There was a 40 percent difference in the overall uncertainty measures between equations 3a and 3b, with the latter having higher coefficient of variation.

Investigation of model predictions for two important subregions of China for H5N1 transmission showed very different results between regions depending on the season. Figure 5.6 illustrates low transmission risk for the Qinghai Lake (QHL) region during the winter season and high transmission potential for the Poyang Lake (PYL) region. In contrast, inset (C) illustrates an increased pattern of risk in the QHL region during the breeding season.

Sensitivity and Uncertainty

Our level of confidence varied widely among input parameters which we addressed in three ways: (1) by conducting a sensitivity analysis of high and low estimates to determine the effect the range of uncertainty had on model outputs, (2) by including matching estimates of coefficient of variation for each cell in our model results

(described above and in Figure 5.5), and (3) by determining which parameters had the most influence on the model results (using tornado graphs). The sensitivity analysis showed a wide range in parameter effect on the model results (Figure 5.7) with viral shedding and uptake rates having the highest effect followed by terrestrial biosecurity, waterfowl H5N1 prevalence, and contamination containment, in decreasing order. The effect of virus shedding rate and viral uptake rate on the model output resulted in a large percent difference (200 percent) between high and low estimates. Sensitivity analysis for the cumulative waterfowl prevalence rates was based on high and low estimates of waterfowl populations during the breeding and wintering seasons, while prevalence rates for each species was fixed at a single value from the literature (most species had only one estimate, if any). Analysis showed a much higher percent difference in mean values for the breeding season in comparison to the wintering season (72 percent versus 22 percent). For the contamination containment parameter, we investigated two aspects: the threshold for determining cells considered to have a “secure” containment designation (3000, 5000, or 7000 P_{te}), and the scalar for the percent of the population contributing virus to the environment (0.25, 0.5, and 0.75). The threshold showed no difference in mean model output and the scalar showed a minor difference of four percent.

Using tornado graphs to plot the correlation of input variables with the model output, we were able to determine which variables contributed most to each model output (Figure 5.8). For the poultry to wild transmission risk models (equation 3a), the contribution of input parameters in decreasing order were: terrestrial poultry, aquatic poultry, wild Anatidae waterfowl, and contamination containment. For the wild to poultry transmission risk models (equation 3b), contributions of the input parameters

were similar: (in decreasing order) wild Anatidae waterfowl, terrestrial backyard poultry, and aquatic poultry. The remaining variables (virus shedding rates per group and virus uptake rate) were not modeled for uncertainty and therefore were not included in the tornado plots.

Discussion

Model Summary and Interpretation

The main objective of this study was to provide a systematic approach to modeling spatial and temporal patterns of disease transmission risk between poultry and wild waterfowl populations in China and to quantify the amount of uncertainty associated with our predictions. We employed an iterative approach to first model where wild and domestic birds are likely to co-occur (Level 1) and subsequently to incorporate H5N1 specific parameters into the model (Level 2 and 3) so that the key relationships between the wild and domestic bird populations could be clearly observed. The models showed consistency in spatial and temporal patterns across the deterministic equations and between the deterministic and Monte-Carlo approaches. High risk hotspots during the wintering season were observed in the southern and eastern lowland regions of China. These areas have high poultry populations, particularly free-grazing ducks in association with rice farming, and are important wintering areas for many migratory waterfowl species. Hotspot regions of risk during the breeding season were observed in the northeast and mid-eastern regions of China. High risk hotspots during the breeding season were observed across a greater extent but in a more localized pattern in comparison to the winter risk models (Figure 5.3 and 5.4). This difference in pattern can

be explained by examining the breeding waterfowl distributions (Figure 5.2). The majority of waterfowl species in China tend to breed in the north and high-elevation western regions where wetland habitat is distributed in a patchier, more localized pattern than the extensive lowland wetlands and rice paddies of the southeast. Uncertainty measures from the Monte-Carlo simulations showed consistent and interesting patterns between seasons and uni-directional equations (Figure 5.5). The highest predicted errors were located in the western regions and lowest in the southeast. This pattern can be explained in part by the lower error variation for poultry densities in the southeast where the highest poultry densities occur (Prosser et al. 2011b), but also by the more localized and sparse distributions of waterfowl as described above. The results tell us that we can have higher confidence in our predictions of transmission risk in the eastern part of China compared to predictions made for the west. It is important to note the difference in scale of risk between the two unidirectional equations (3a and 3b, Table 5.4). Results from models describing transmission risk from poultry to waterfowl (3a) were three orders of magnitude higher than those from waterfowl to poultry (3b). Concurrently, the coefficient of variation estimates were 40 percent higher for the 3b equations. The difference in magnitude of the risk predictions is expected as we hypothesize that the amount of virus flow from wild to domestic birds is less than the amount of flow from domestic to wild, and subsequently our confidence in predicting these values is lower.

Returning to the effects of model inputs on the spatial distribution of risk output, we observe an interesting pattern in northeastern China during the wintering season, which is most observable in the Level 1 maps (Figure 5.3, top and bottom right panels). An artifact can be seen where a portion of the northeastern tip of China shows a densely

concentrated section of risk amongst an otherwise sparse region. This concentrated section is due to the presence of a single species, the greater white-fronted goose (*Anser albifrons*), that winters in parts of southern China but also winters in a subsection of the northeast (Supplemental Fig. S5.8). The portion of the wintering distribution in northeastern China is rare among waterfowl species, and appears especially concentrated due to use of agricultural fields which tend to have denser distribution than natural wetlands. The artifact remains to a lesser degree in the Level 2 models that use the cumulative H5N1 prevalence estimates for the waterfowl parameter (Figure 5.4, top and bottom right panels). Prevalence of the greater white-fronted goose was reported at a rate of 2.2 percent (low in comparison to some other species) and the model output shows a low but measurable risk in this region. In the Monte-Carlo model, the same region shows transmission risk, but without a discernable edge between risk and non-risk areas, due to averaging across the simulation runs. The artifact is most observable in the Level 1 risk models because input values from the poultry and wild bird populations are given equal weight (as presence or absence) and not a quantitative value. We illustrated the above example to explain the response of the varying levels of models to different parameter factors within.

An interesting pattern was also observed in the uncertainty maps developed for the waterfowl abundance models. Regions with high mean CV's tended to be concentrated in the southeastern part of China, for both the breeding and wintering models (Supplemental Fig. S5.6, lower panel). This pattern was expected for the wintering species, since the majority of China's wintering Anatidae population is found in this region; however, it was a surprising result for the breeding species which are

generally located in the north and high elevation western parts of China. In the case of the breeding models, CV's were high (> 0.5) for two groups of waterfowl: (1) uncommon breeders within China including two swan species (mute and whooper swans: *Cygnus olor* and *C. cygnus*), and diving ducks (pochards, goldeneyes, and mergansers: *Rhodonessa*, *Aythya*, *Bucephala*, and *Mergus* spp.), and (2) two tropical breeding duck species (lesser whistling duck and cotton-pygmy goose: *Dendrocygna javanica* and *Nettapus coromandelianus*). The concentration of high CV values in southeastern China (Figure 5.4, bottom left panel) was driven by the tropical species which tend to have wide distributions and large confidence intervals surrounding the population estimates. As expected, the mean CV (across all grid cells of China) was higher for the breeding season than the wintering season because of the associated less-certain population estimates (Table 5.1). Including a measure of uncertainty in the abundance models gives us a mechanism to address the species-level differences in confidence in population estimates.

Sensitivity Analyses

The sensitivity analysis (Figure 5.7) was conducted on the deterministic equations to illustrate how the model results would change when varying input values for a given parameter. We observed a wide range in effect across the ten input parameters with virus shedding rate and virus uptake rate having the largest (200%) difference in model results. The virus shedding and uptake parameters were obtained from the literature (Table 5.2) and the rates we compiled were either similar (in the case of viral shedding rates) or taken directly (in the case of the viral uptake rate: (Roche et al. 2009)) from those used in existing SIR (susceptible, infected, recovered) disease transmission models (Liu et al. 2008, Roche et al. 2009, Rohani et al. 2009). The range between high and low inputs

spanned from four to ten orders of magnitude (Table 5.2), which explains the large effect on sensitivity. Since the range in inputs had such an overwhelming effect on model outputs, and they were directly taken from the literature, we chose to keep these rates fixed in the Monte-Carlo models so we could more clearly assess the effects of our modeled input parameters (wild bird and poultry distributions, and biosecurity and contaminant containment parameters).

Sensitivity results for the remaining variables ranged from 104 to zero percent. The biosecurity term was designed to reduce the total terrestrial population to an estimate of the density of backyard poultry for use in equation 3b. The equation was complex and one we had the least confidence in. We therefore tested the overall effect of removing the term ($P_{te} * B_{te}$) completely from the equation, which resulted in a 104 and 100 percent difference for the breeding and wintering seasons, respectively. We conclude that the term has a significant effect on the model results, and until a better estimate can be derived, we allow the reader to decide which set of models to use (a set of models with the ($P_{te} * B_{te}$) term removed is illustrated in Supplemental Fig. S5.9). Sensitivity estimates for the waterfowl H5N1 prevalence parameter had a high effect on model output for the breeding season (72%) and moderate for the wintering season models (22%). This difference can be attributed to the lower confidence (high-low estimates) surrounding the Anatidae breeding population figures versus wintering figures (Table 5.1). The final variable, C_{te} (contaminant containment), showed very low differences in the sensitivity analysis, ranging from zero to four percent. This was due to the low number of grid cells that the scalar was applied to (0.01 to 2 percent of the 10 million grid cells of China). Finally, we also tested the effect of removing the C_{te} term entirely,

under the hypothesis that even the most biosecure farms might not prohibit virus from leaving their farms (during field interviews we learned that even large scale farms sell chicken feces to other farmers for fertilizer or fish food). The analysis showed little effect on the mean output (0.2 percent for breeding and wintering seasons).

Tornado Plots

We also assessed the relative contribution of each parameter to the model output for the Monte-Carlo simulations (Figure 5.8). The tornado plots show us that for the poultry to wild transmission models (equation 3a), the domestic birds dominate the equation. This is an interesting observation that fits with the directionality of the equation whereby virus flows from domestic bird to wild bird; also that the effect of the waterfowl densities is tempered by applying prevalence rates for each species. In equation 3b (wild to domestic transmission), of the three populations, waterfowl contributes the most to the model output, but terrestrial and aquatic poultry have only slightly lower correlations. With similar reasoning, we expect wild birds to drive the equation in this direction, and we observe that the contribution is tempered by the prevalence rates that were applied to each waterfowl species.

Qinghai and Poyang Lake Focal Regions

Examination of our two focal areas, Qinghai Lake (QHL) and Poyang Lake (PYL) indicated contrasting seasonal patterns between the two regions (Figure 5.5). During the wintering season, risk was high in the PYL region and almost non-existent in the QHL region. The differences were driven by the addition of hundreds of thousands of migratory waterfowl that return to the PYL region in the winter and reside amongst some

of the highest poultry densities in the country. In the QHL region, waterfowl migrate away from the cold and arid plateau for the winter months, plus risk is lower there year-round due to the low poultry densities in the area to begin with. The risk for QHL changes during the breeding season (Figure 5.6c) with the return of tens of thousands of waterfowl that nest in the region. The differences in risk could not be predicted without explicitly incorporating the ecology of the wild bird populations, which is one of the main strengths of our approach.

Utility and Limits of the Models

We explicitly took a multi-level approach towards modeling transmission risk between wild and domestic waterfowl in China. The Level 1 and 2 deterministic models allow us to observe, at a fine resolution, the patterns of wild and domestic waterfowl distributions independent of the effects of incorporating H5N1 risk factors. While the Level 1 models are simplistic and not designed to quantify different levels of H5N1 transmission risk, they provide value as a coarse filter to targeting areas where wild and domestic waterfowl are most likely to co-occur. This type of information alone has broad application towards disease and conservation questions that go well beyond H5N1, and we suspect that they would be useful to a wide range of practitioners including wildlife managers, researchers, and disease specialists alike.

The Level 2 models were used to define the H5N1 transmission risk equations for use in the Monte-Carlo simulations, as well as for observing patterns at a high spatial resolution (1 km). As the number of simulations increase in a Monte-Carlo analysis, the mean values should converge towards results of the deterministic models (Kroese et al. 2010). We carefully investigated mean output for the models as well as for each input

variable between the deterministic and Monte-Carlo simulations (Burmaster and Anderson 1994). First, the mean values were nearly identical for the 1 km and 30 km deterministic models, indicating a lack of bias in the resampling process (Supplemental Table S5.1). Second, we noted higher means for the input parameters that were modeled using the triangular distribution of the Monte-Carlo models (Supplemental Table S5.1, Section B). These models initially used a global (fixed) minimum and maximum value for all cells within the China grid which is less computationally intensive but has the effect of increasing the mean values. We then reran the models using individual minimum and maximum values for each cell (Supplemental Fig. S5.10) which reduced the mean values to match the 30 km deterministic models; however, because we could not have negative values for input parameters such as waterfowl abundance and prevalence, we truncated the triangular distributions to fit within each parameter's input range (Table 5.2). Truncating the distributions increased the mean values for each parameter (Supplemental Table S5.1, Section B), however, the values here were closer to the 30 km deterministic models than the models using a global min/max, and this is the approach used in the final models reported (Figure 5.5).

We recommend use of the Level 3 models for informing surveillance and prevention measures against H5N1 threats as this set is modeled at a coarser scale targeted at the county or district level which is more realistic for predicting disease risk (as opposed to the Level 2, 1 km resolution models). More importantly, instead of basing models on a single point estimate at each grid cell for each input parameter, the Monte-Carlo models randomly sample inputs for a given cell using the assigned distribution for each parameter, which allows estimates of uncertainty to be incorporated within the

model results. Thus, within each grid cell of the output map, a probabilistic estimate of risk is given. In addition, an accompanying map of the coefficient of variation across all grid cells informs the user of which regions have stable results and which regions could use better input data. On a broad scale, the Level 3 models can be used to help target focal areas for improving surveillance and prevention efforts particularly for the question of transmission between wild and domestic birds. For example, health experts and wildlife officials may both be interested in using the poultry to wild risk models (Figure 5.5a) to identify regions where wild migratory birds are at higher risk of exposure to new and evolving virus strains from poultry. Poultry farmers and health officials alike may use the wild to poultry risk models to identify areas where farming practices or vaccination programs should be enhanced to protect poultry from exposure to wild birds (albeit as the models show, this route may be less likely). As the models take a combined density-dependent and environmental transmission approach, the results may also help target environmental surveillance programs. The models are not meant to be used as a final dictation of transmission risk, rather to be a guiding tool for practitioners from multiple disciplines to join together on-the-ground to address questions and issues related to disease transmission between wild and domestic birds.

Finally, we have put considerable thought towards how the models could be improved and validated. Our approach towards modeling transmission risk took a spatial analysis approach as opposed to a data-driven statistical approach. For example, a number of studies have identified drivers of H5N1 transmission by drawing statistical relationships between outbreak events and environmental or anthropogenic risk factors (Pfeiffer et al. 2007, Fang et al. 2008, Gilbert et al. 2008, Tiensin et al. 2009, Martin et al.

2011, Gilbert and Pfeiffer 2012. *In Press*). We considered using avian surveillance and outbreak data to train and validate our models, and indeed, a strong match exists between outbreak locations and our predicted risk areas. However, use of the outbreak data in this case would be misleading since we cannot determine the source of infection (be it wild birds, poultry, or other) for each outbreak case. The ideal training and validation data set would consist of geographic and temporal data on infections in wild and domestic birds including information on the type of host that caused the infection. Deriving the infecting population from the virus isolates is the difficult part - even the use of phylogenetic analyses may not definitively answer this question as intermediary transmissions may occur between outbreak events. Thus, we use a forward course by understanding the spatial and temporal relationships between the wild and domestic waterfowl distributions and developing the risk models based on this concept.

Conclusions

Here we present a structured approach to predicting transmission risk between domestic poultry and wild waterfowl in China. Our approach allows us to separate the spatial relationships between poultry and waterfowl from the disease-specific factors to better understand the contributions of each to transmission risk. We explicitly incorporate uncertainty measures with our risk predictions and conduct sensitivity analyses to understand the effects of uncertainty on the model outputs. It is the first analysis of its kind and one of the few that focuses specifically on interactions between the wild and domestic bird populations, providing a unique contribution to our growing knowledge on the topic of wild birds and H5N1 transmission.

Tables

Table 5.1. Species name, code, population estimates, and H5N1 prevalence rates for China's 42 Anatidae waterfowl species. Mcode refers to the reference map code in MacKinnon and Phillipps (2000). Population estimates were based on ^aCao et al. 2008 and ^bDelaney and Scott 2006. Prevalence rates were taken from ^cKou et al. (2009); ^daverage of Olsen et al. (2006), Munster et al. (2007), Gaidet et al. (2007b), and Hesterberg (2009); and ^eaverage for swans, geese, or ducks from Kou et al. (2009).

Mcode	Common Name	Scientific Name	Winter Population	Winter Low	Winter High	Summer Population ^a	Summer Low ^a	Summer High ^a	H5N1 Prevalance Rate
M064	Lesser Whistling Duck	<i>Dendrocygna javanica</i>	1,500 ^a	1,000	2,000	15,000	5,000	25,000	5.3 ^c
M066	Mute Swan	<i>Cygnus olor</i>	0	0	0	650	300	1,000	3.4 ^c
M067	Whooper Swan	<i>Cygnus cygnus</i>	5,900 ^b	5,015	6,785	300	100	500	4.0 ^d
M068	Tundra Swan	<i>Cygnus columbianus</i>	81,000 ^b	68,850	93,150	0	0	0	2.8 ^d
M069	Swan Goose	<i>Anser cygnoides</i>	78,000 ^b	66,300	89,700	40,000	30,000	50,000	1.4 ^c
M070	Bean Goose	<i>Anser fabalis</i>	150,000 ^b	127,500	172,500	0	0	0	0.0 ^d
M071	Greater White-fronted Goose	<i>Anser albifrons</i>	33,000 ^b	28,050	37,950	0	0	0	2.2 ^c
M072	Lesser White-fronted Goose	<i>Anser erythropus</i>	21,000 ^b	17,850	24,150	0	0	0	2.1 ^d
M073	Greylag Goose	<i>Anser anser</i>	40,000 ^a	15,000	65,000	40,000	15,000	65,000	0.8 ^c
M074	Bar-headed Goose	<i>Anser indicus</i>	15,000 ^a	10,000	20,000	56,000	52,000	60,000	2.3 ^d
M075	Snow Goose	<i>Anser caerulescens</i>	50 ^a	25	75	0	0	0	1.4 ^c
M077	Brent Goose	<i>Branta bernicla</i>	0	0	0	0	0	0	1.0 ^c
M079	Ruddy Shelduck	<i>Tadorna ferruginea</i>	15,000 ^a	10,000	20,000	19,000	13,000	25,000	2.2 ^d
M081	Common Shelduck	<i>Tadorna tadorna</i>	18,000 ^b	15,300	20,700	12,000	9,000	15,000	3.6 ^d
M083	Cotton Pygmy Goose	<i>Nettapus coromandelianus</i>	200 ^a	100	300	15,000	5,000	25,000	5.3 ^c
M084	Mandarin Duck	<i>Aix galericulata</i>	20,000 ^a	10,000	30,000	6,000	4,000	8,000	5.3 ^c

Mcode	Common Name	Scientific Name	Winter Population	Winter Low	Winter High	Summer Population ^a	Summer Low ^a	Summer High ^a	H5N1 Prevalance Rate
M085	Gadwall	<i>Anas strepera</i>	7,700 ^b	6,545	8,855	14,000	10,000	18,000	2.1 ^d
M086	Falcated Duck	<i>Anas falcate</i>	78,000 ^b	66,300	89,700	17,000	10,000	24,000	5.3 ^e
M087	Eurasian Wigeon	<i>Anas penelope</i>	50,000 ^b	42,500	57,500	37,000	25,000	50,000	1.9 ^c
M089	Mallard	<i>Anas platyrhynchos</i>	73,000 ^b	62,050	83,950	575,000	375,000	750,000	11.2 ^d
M090	Spot-billed Duck	<i>Anas poecilorhyncha</i>	100,000 ^b	85,000	115,000	450,000	300,000	600,000	3.7 ^d
M092	Northern Shoveler	<i>Anas clypeata</i>	27,000 ^b	22,950	31,050	40,000	30,000	50,000	10.2 ^c
M093	Northern Pintail	<i>Anas acuta</i>	46000 ^b	39,100	52,900	2,000	1,000	3,000	9.8 ^c
M094	Garganey	<i>Anas querquedula</i>	50000 ^a	30,000	70,000	30,000	20,000	40,000	5.3 ^e
M095	Baikal Teal	<i>Anas formosa</i>	91,000 ^b	77,350	104,650	0	0	0	5.3 ^e
M096	Common Teal	<i>Anas crecca</i>	146,000 ^b	124,100	167,900	80,000	60,000	100,000	3.1 ^c
M097	Marbled Duck	<i>Marmaronetta angustirostris</i>	0	0	0	1,500	100	2,900	5.0 ^e
M098	Red-crested Pochard	<i>Rhodonessa rufina</i>	0	0	0	1,000	500	1,500	2.9 ^c
M099	Common Pochard	<i>Aythya ferina</i>	18,000 ^b	15,300	20,700	500	100	900	5.0 ^e
M101	Ferruginous Pochard	<i>Aythya nyroca</i>	5,000 ^a	2,000	8,000	5,000	2,000	8,000	5.0 ^e
M102	Baer's Pochard	<i>Aythya baeri</i>	850 ^b	723	978	1,500	1,000	2,000	5.0 ^e
M103	Tufted Duck	<i>Aythya fuligula</i>	11,000 ^b	9,350	12,650	1,000	500	1,500	7.1 ^d
M104	Greater Scaup	<i>Aythya marila</i>	80,000 ^a	60,000	100,000	0	0	0	5.0 ^e
M105	Steller's Eider	<i>Polysticta stelleri</i>	0	0	0	0	0	0	5.0 ^e
M107	Long-tailed Duck	<i>Clangula hyemalis</i>	30,000 ^a	20,000	40,000	0	0	0	5.0 ^e
M108	Black Scoter	<i>Melanitta nigra</i>	40,000 ^a	20,000	60,000	0	0	0	5.0 ^e
M109	White-winged Scoter	<i>Melanitta fusca</i>	40,000 ^a	20,000	60,000	0	0	0	5.0 ^e
M110	Common Goldeneye	<i>Bucephala clangula</i>	20,000 ^a	10,000	30,000	1,000	500	1,500	5.0 ^e
M111	Smew	<i>Mergellus albellus</i>	15,000 ^b	12,750	17,250	200	100	300	5.0 ^e

Mcode	Common Name	Scientific Name	Winter Population	Winter Low	Winter High	Summer Population ^a	Summer Low ^a	Summer High ^a	H5N1 Prevalance Rate
M112	Red-breasted Merganser	<i>Mergus serrator</i>	3,500 ^a	2,000	5,000	200	100	300	5.0 ^e
M113	Scaly-sided Merganser	<i>Mergus squamatus</i>	200 ^a	100	300	100	50	150	5.0 ^e
M114	Common Merganser	<i>Mergus merganser</i>	29,000 ^b	24,650	33,350	10,000	7,000	13,000	5.0 ^e

Table 5.2. Parameters of 1 km resolution transmission risk equations including the range of values, approach for sensitivity analyses, and reference for each.

Parameter	Description	Value Range (Mean, StDev)	Sensitivity Analysis	Notes
P_{te}	Terrestrial poultry density	0 to 9418 (379.4, 745.7) Chickens/km ²	Fixed	Chicken densities for China (Prosser et al. 2011b)
P_{aq}	Aquatic poultry density	0 to 2796 (86.2, 164.7) Ducks and geese/km ²	Fixed	Duck and goose densities for China (Prosser et al. 2011b)
W_{pr}	Waterfowl Prevalence index, breeding: W_{prbr} Waterfowl Prevalence index, winter: W_{prwi}	W_{prbr} : 0 to 0.32 (0.01, 0.04) W_{prwi} : 0 to 0.39 (0.006, 0.025)	Low/High Low/High	Distributions from (Prosser et al. 2012 <i>In Prep.</i>) Population estimates from (Delany and Scott 2006, Cao et al. 2008) Prevalence rates from (Olsen et al. 2006, Gaidet et al. 2007b, Munster et al. 2007, Hesterberg et al. 2009, Kou et al. 2009)
C_{te}	Contaminant containment, terrestrial poultry (Biosecure threshold $P_{te} = 5000$)	Biosecure=0.75 and 0.25 Non-biosecure=1	Low/High	Biosecure threshold of 5000 chickens per km ² . Reduction of population by 0.25 or 0.75 given biosecure designation (Fig. S5.2)
B_{te}	Biosecurity, terrestrial poultry thresholds: (a) $P_{te} \geq 50$: $P_{backyard} = P_{te} * 1.0$ (b) $50 < P_{te} \leq 1000$, $P_{backyard} = P_{te} * 0.5$ (c) $P_{te} > 1000$, $P_{backyard} = 1000$	0 to 1000	Low/High	Tri-part equation (Fig. S5.3): (a) At densities ≤ 50 , 100% of population is backyard poultry (b) From 50 to 1000, half are backyard poultry (c) At greater than 1000, backyard poultry is limited to 1000
V_{te}	Viral shedding rate, terrestrial poultry	$10^{1.4}$ and $10^{9.8}$ EID ₅₀	Low/High	Viral shedding rates per individual per day from (Shortridge et al. 1998, Yu et al. 2007, Jeong et al. 2009)
V_{aq}	Viral shedding rate, aquatic poultry	10^1 and $10^{5.7}$ EID ₅₀	Low/High	Viral shedding rates per individual per day from (Perkins and Swayne 2002a, Chen et al. 2004, Sturm-Ramirez et al. 2004, Sturm-Ramirez et al. 2005, Phuong et al. 2011)

V_{wf}	Viral shedding rate, wild waterfowl	$10^{2.5}$ and $10^{6.5}$ EID ₅₀	Low/High	Viral shedding rates per individual per day from (Brown et al. 2008)
U	Viral uptake= Consumption rate of virus in the environment / minimum load for infection	$(10^{-15} / (10^{4.7} \text{ to } 10^{1.8}) \text{ EID}_{50})$	Low/High	Consumption rate of virus in environment 10^{-15} (Liu et al. 2008) Minimum viral load of $10^{4.7}$ EID ₅₀ (and $10^{1.8}$ EID ₅₀) to initiate infection with low pathogenic AIV (Lu and Castro 2004, Ito et al. 1995, from Roche et al. 2009)

Table 5.3. Parameter descriptions, value ranges, and Monte Carlo distributions used for the 30 km resolution uncertainty models. Parameters of truncated normal distribution are mean and standard deviation. Parameters of the triangular distribution are minimum, best estimate, and maximum.

Parameter	Description	Value Range	Distribution	Level
P _{te}	Terrestrial poultry density (chickens)	0 to 5871 Chickens/km ²	Truncated normal	Grid cell
P _{aq}	Aquatic poultry density (ducks and geese)	0 to 2796 Ducks and geese/km ²	Truncated normal	Grid cell
W _{pr}	H5N1 prevalence index Breeding Season: W _{prbr} Wintering Season: W _{prwi}	W _{prbr} 0 to 0.29 W _{prwi} 0 to 0.39	Triangular	Grid cell
C _{te}	Contaminant containment, terrestrial poultry	0.5 to 1	Triangular	Grid cell
B _{te}	Biosecurity, terrestrial poultry (for MC use pte*bte term)	0 to 1000	Triangular	Grid cell
V _{te}	Viral shedding rate, terrestrial poultry	10 ⁰ , 10 ^{9.8} , 10 ^{6.8} EID ₅₀ ^a	Triangular	Model
V _{aq}	Viral shedding rate, aquatic poultry	0, 10 ^{6.5} , 10 ^{2.98} EID ₅₀ ^a	Triangular	Model
V _{wf}	Viral shedding rate, waterfowl	10 ^{2.5} , 10 ^{6.5} , 10 ^{4.77} EID ₅₀ ^a	Triangular	Model
U	Viral uptake: Consumption rate of virus in the environment / minimum load for infection	1.58e-17, 1.99e-20, 1.99e-20 EID ₅₀ ^a	Triangular	Model

^a Minimum, maximum, and best estimate for the model-level triangular distribution. Best estimate for V terms are mean shedding rates. Best estimate for U term takes a conservative value of the higher estimate for minimum load for infection. See Supplemental Table S5.2 for estimates and references.

Table 5.4. Differences in mean values across all cells for two modeling approaches (Level 2 and 3) and four transmission scenarios.

Model	Equation 3a Breeding Season	Equation 3a Wintering Season	Equation 3b Breeding Season	Equation 3b Wintering Season
Level 2 (deterministic)	3.82E-10	7.13E-10	1.48E-13	3.13E-13
Level 3 (Monte-Carlo)	1.18E-09	1.66E-09	6.03E-13	8.39E-13
Coefficient of Variation	144	147	219	223

Figures

Figure 5.1. Three levels of spatial models implemented for assessing H5N1 transmission risk between wild and domestic birds in China. The deterministic Level 1 and 2 models were developed to refine the transmission equations. Level 1 models are overlay models that predict where wild and domestic birds may come in contact. Level 2 models incorporate uni-directional equations for H5N1 transmission risk between poultry and wild birds. Level 3 models incorporate uncertainty using Monte-Carlo simulations at 30km resolution, the average county size of China and a scale deemed more appropriate towards risk modeling.

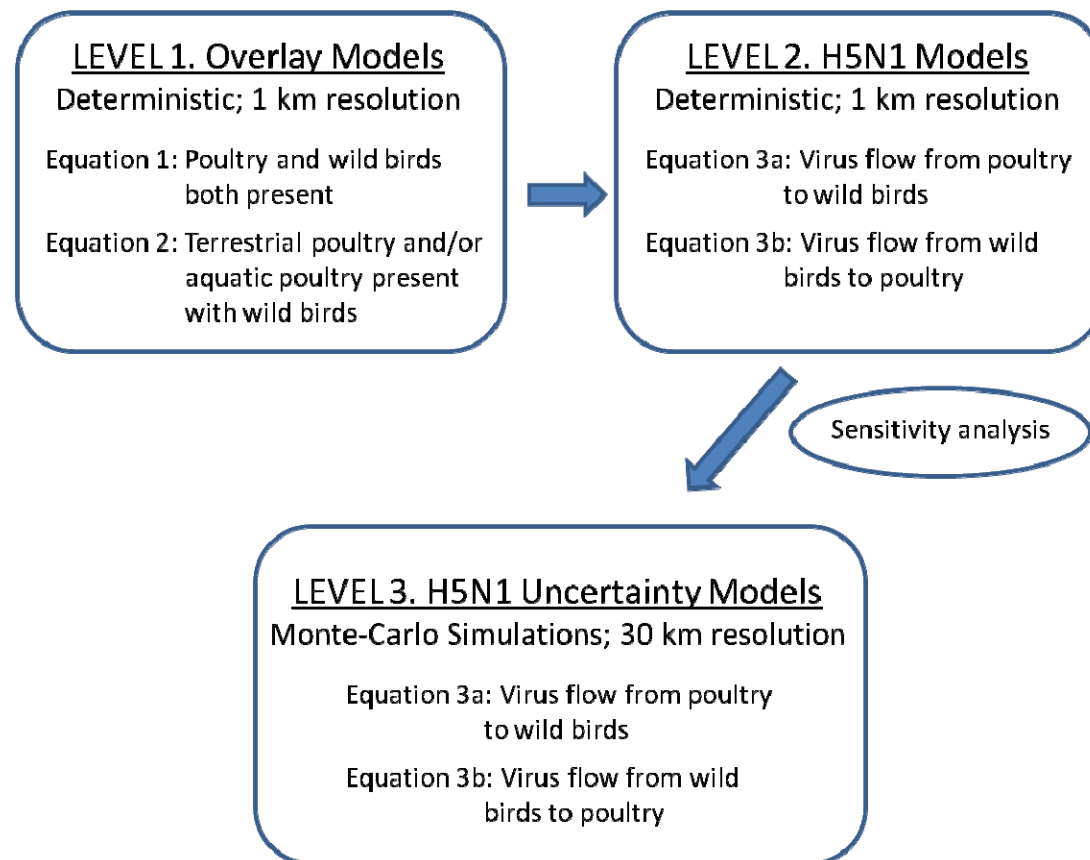


Figure 5.2. Input data for models of transmission risk between domestic poultry and wild Anatidae waterfowl for China at 1 km resolution. W_{prbr} and W_{prwi} are cumulative H5N1 prevalence for Anatidae species during the breeding and wintering seasons, respectively; P_{te} and P_{aq} are terrestrial and aquatic poultry densities, respectively, C_{te} is the contamination containment rate for terrestrial poultry, and B_{te} is the biosecurity rate for terrestrial poultry. The remaining terms (V_{te} , V_{aq} , V_{wf} , and U) are model level coefficients where all grid cells have the same value (see Table 5.1 and Methods).

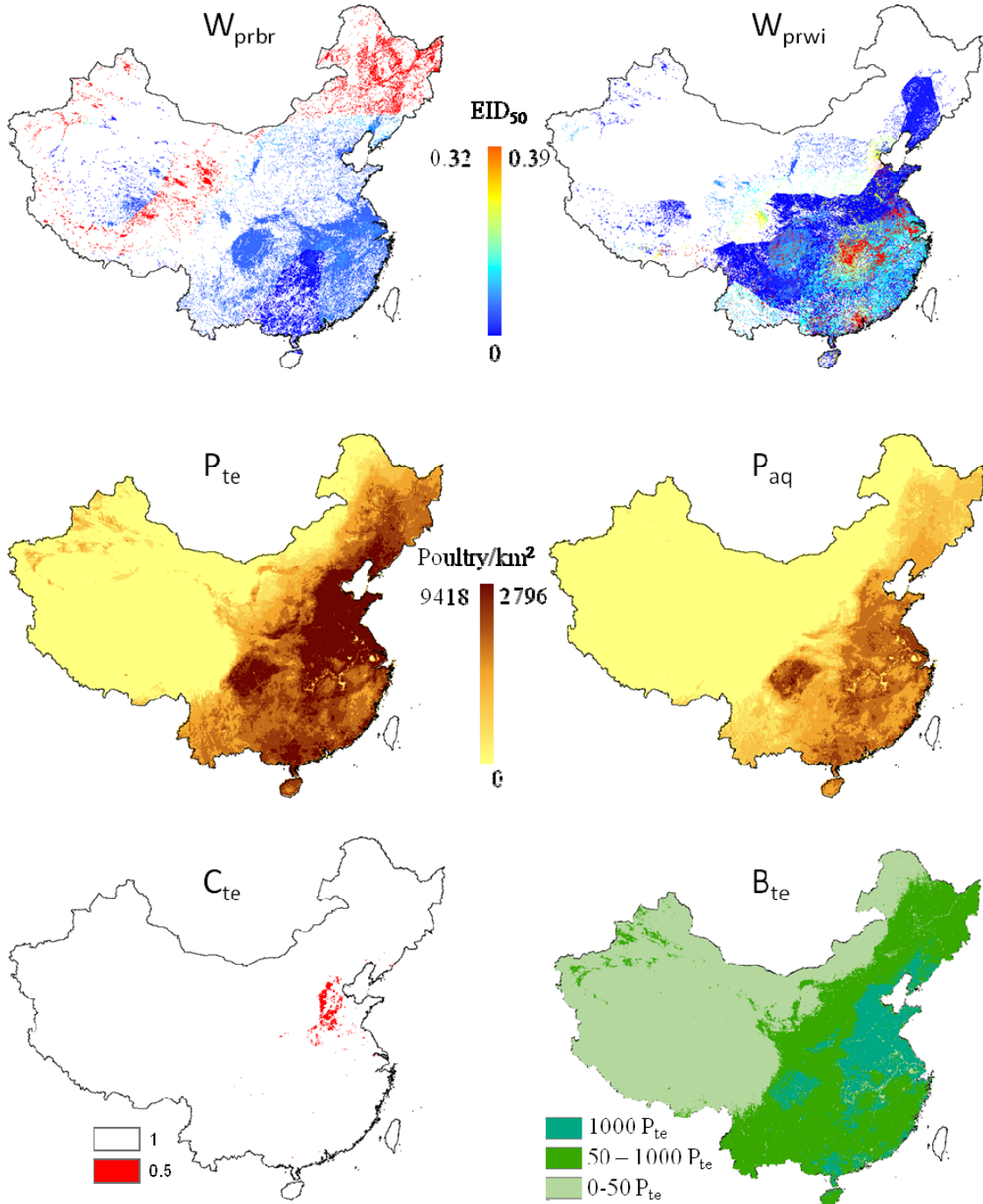


Figure 5.3. Highly pathogenic H5N1 transmission risk between domestic poultry and wild Anatidae waterfowl at 1 km resolution for China. Level 1 models include (A) grid cells where domestic poultry and wild Anatidae are present, $T_{risk1} = [P_{01}] * [W_{01}]$; and (B) grid cells where both terrestrial and aquatic poultry are present in combination with wild Anatidae (red) versus where only one poultry group (blue) shares a grid cell with wild Anatidae, $T_{risk2} = [Pt_{01} + Pa_{01}] * [W_{01}]$. Pt_{01} = presence of terrestrial poultry, Pa_{01} = presence of aquatic poultry, and W_{01} = presence of wild Anatidae waterfowl.

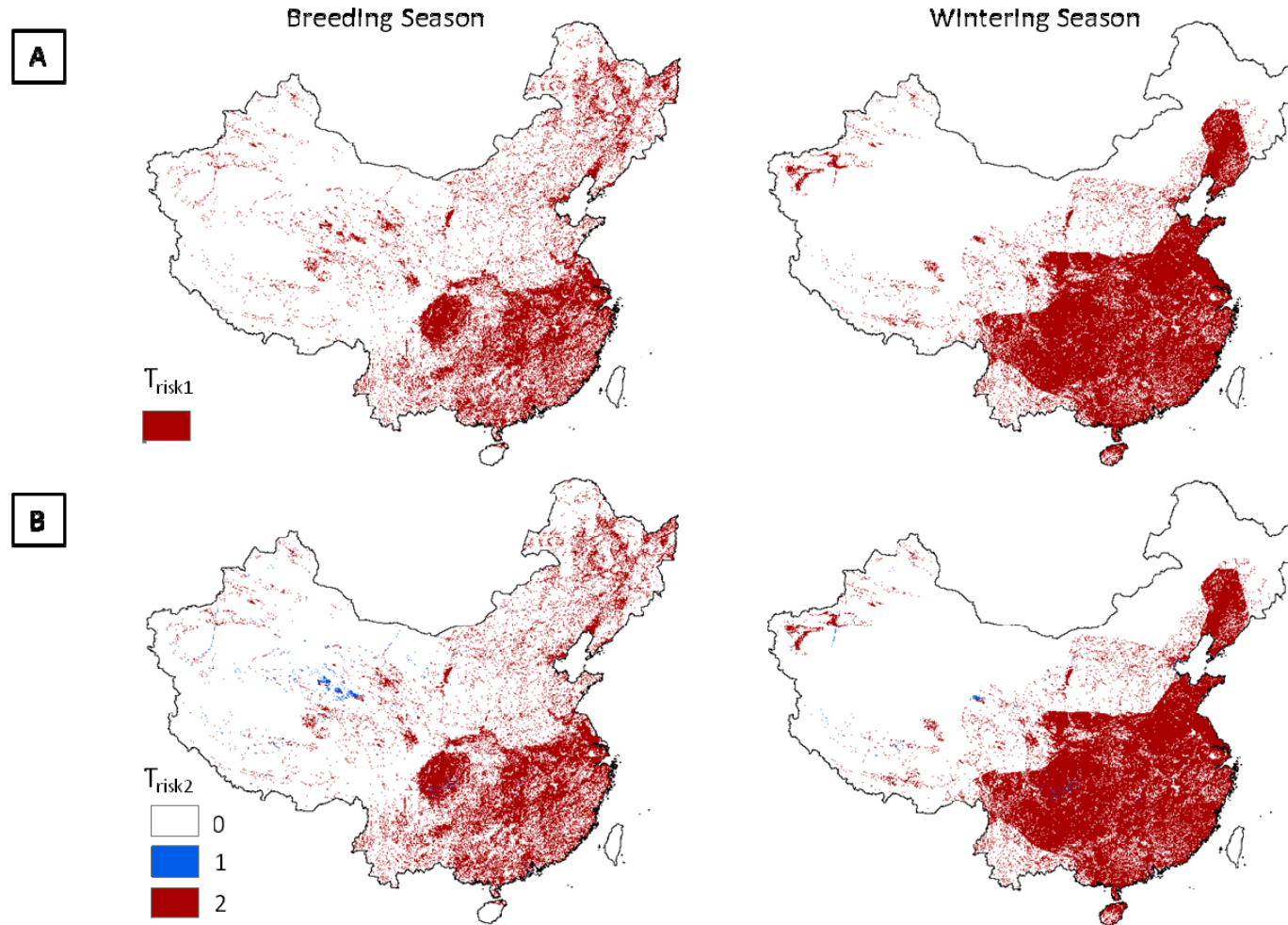


Figure 5.4. Highly pathogenic H5N1 transmission risk between domestic poultry and wild Anatidae waterfowl at 1 km resolution for China. Level 2 models include H5N1-specific transmission factors and are unidirectional with (A) representing transmission risk from domestic to wild birds, and (B) from wild birds to domestic. Equation 3a: $T_{PtoW} = ([P_{te} * C_{te} * V_{te}] + [P_{aq} * V_{aq}]) * ([W_{pr} * U])$ and 3b: $T_{WtoP} = ([W_{pr} * V_{wf}]) * ([P_{te} * B_{te}] + [P_{aq}]) * U$, where P_{te} and P_{aq} are terrestrial and aquatic poultry density, C_{te} is the terrestrial poultry contamination containment rate, V_{te} and V_{aq} are terrestrial and aquatic poultry virus shedding rates, W_{pr} is the wild Anatidae cumulative H5N1 prevalence index, and U is the viral uptake rate. Maps are symbolized using quantiles.

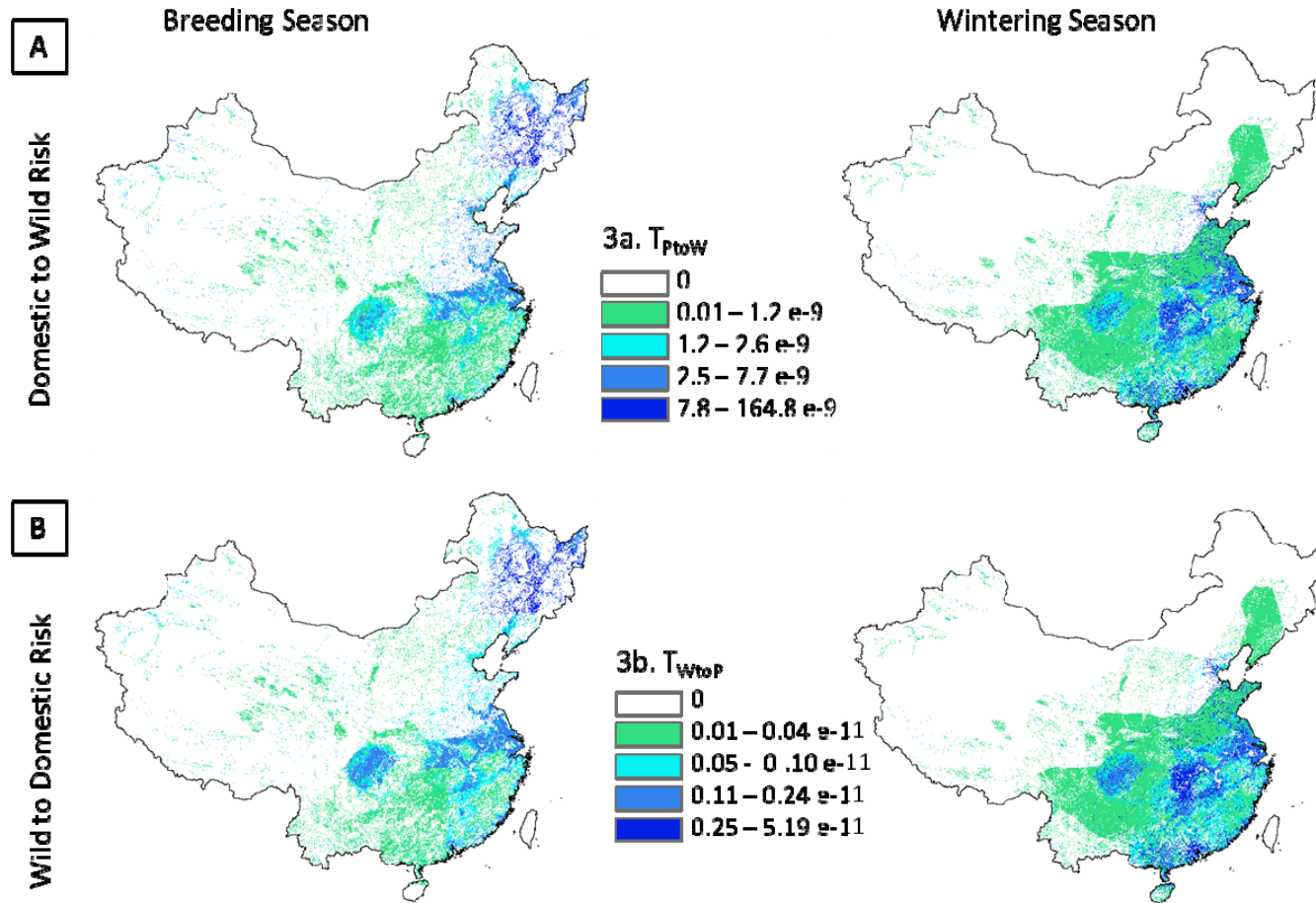


Figure 5.5. H5N1 transmission risk between wild and domestic birds in China and associated uncertainty predictions. Spatial resolution is 30 km. Risk maps represented as mean and coefficient of variation (left and right in each pair of maps, respectively). (A) Top panel represents transmission risk from poultry to wild waterfowl; (B) bottom panel represents transmission risk from wild waterfowl to poultry. Maps are symbolized using quantiles. Grey boxes correspond to the Qinghai Lake and Poyang Lake Regions outlined in Fig.5.6.

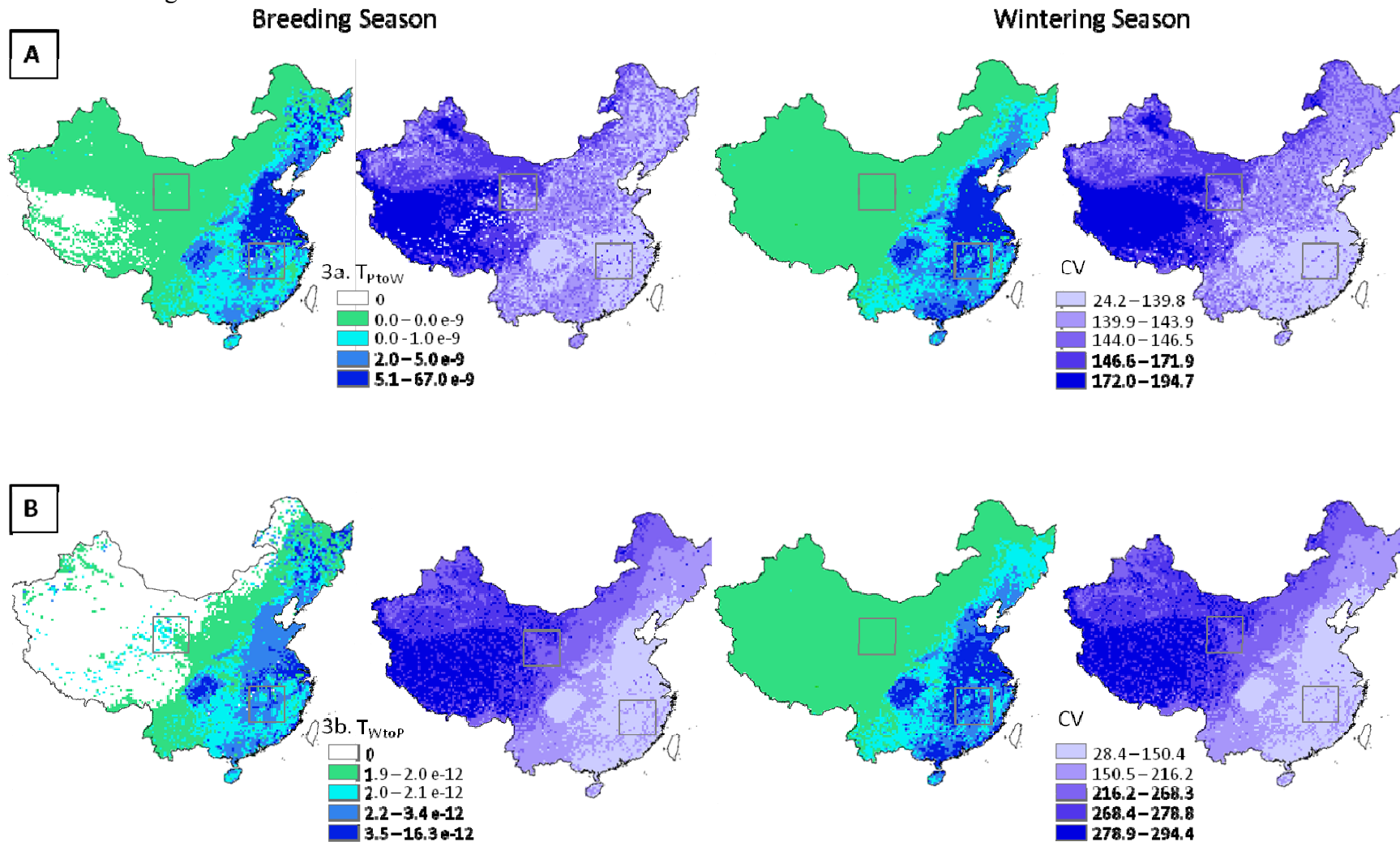


Figure 5.6. Comparison of model outputs for Qinghai Lake (QHL) and Poyang Lake (PYL) subregions for (A) 1 km deterministic and (B) 30 km Monte-Carlo model outputs using equation 3a (poultry to wild transmission risk) and winter season. Insets (C) and (D) show comparisons for the breeding season 3b (wild to poultry transmission).

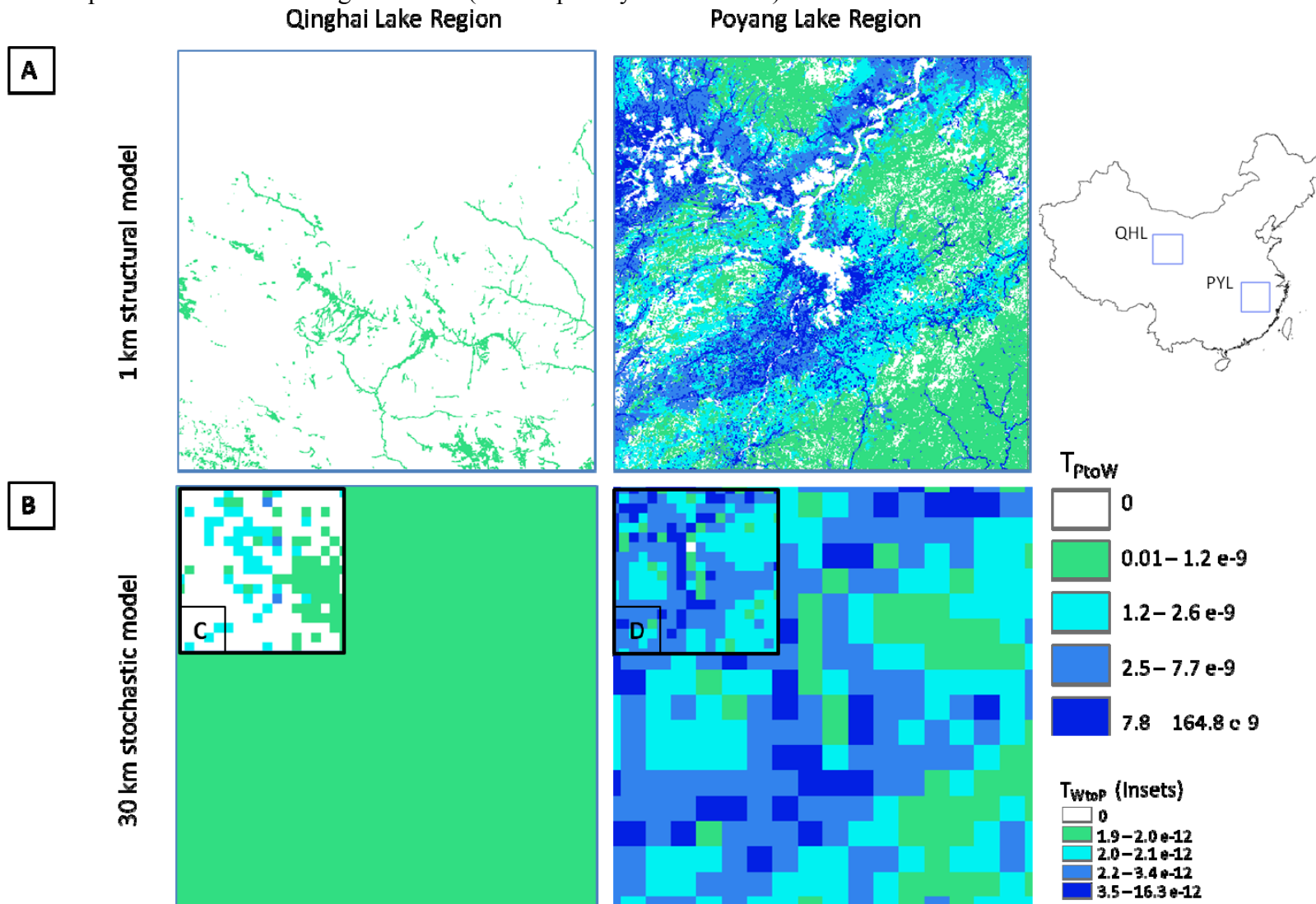


Figure 5.7. Percent difference scores for each variable in a sensitivity analysis for transmission risk between domestic and wild waterfowl in China. High-low estimates were tested for each variable while keeping all others in the equation constant. Equation 3a: $T_{PtoW} = ([P_{te} * C_{te} * V_{te}] + [P_{aq} * V_{aq}]) * ([W_{pr} * U])$ and 3b: $T_{WtoP} = ([W_{pr} * V_{wf}]) * ([P_{te} * B_{te}] + [P_{aq}] * U)$, where P_{te} and P_{aq} are terrestrial and aquatic poultry density, C_{te} is the terrestrial poultry contamination containment rate, V_{te} and V_{aq} are terrestrial and aquatic poultry virus shedding rates, W_{pr} is the wild Anatidae cumulative H5N1 prevalence index, and U is the viral uptake rate. C_{te} and B_{te} were also tested for the effect of removing each completely from the model.

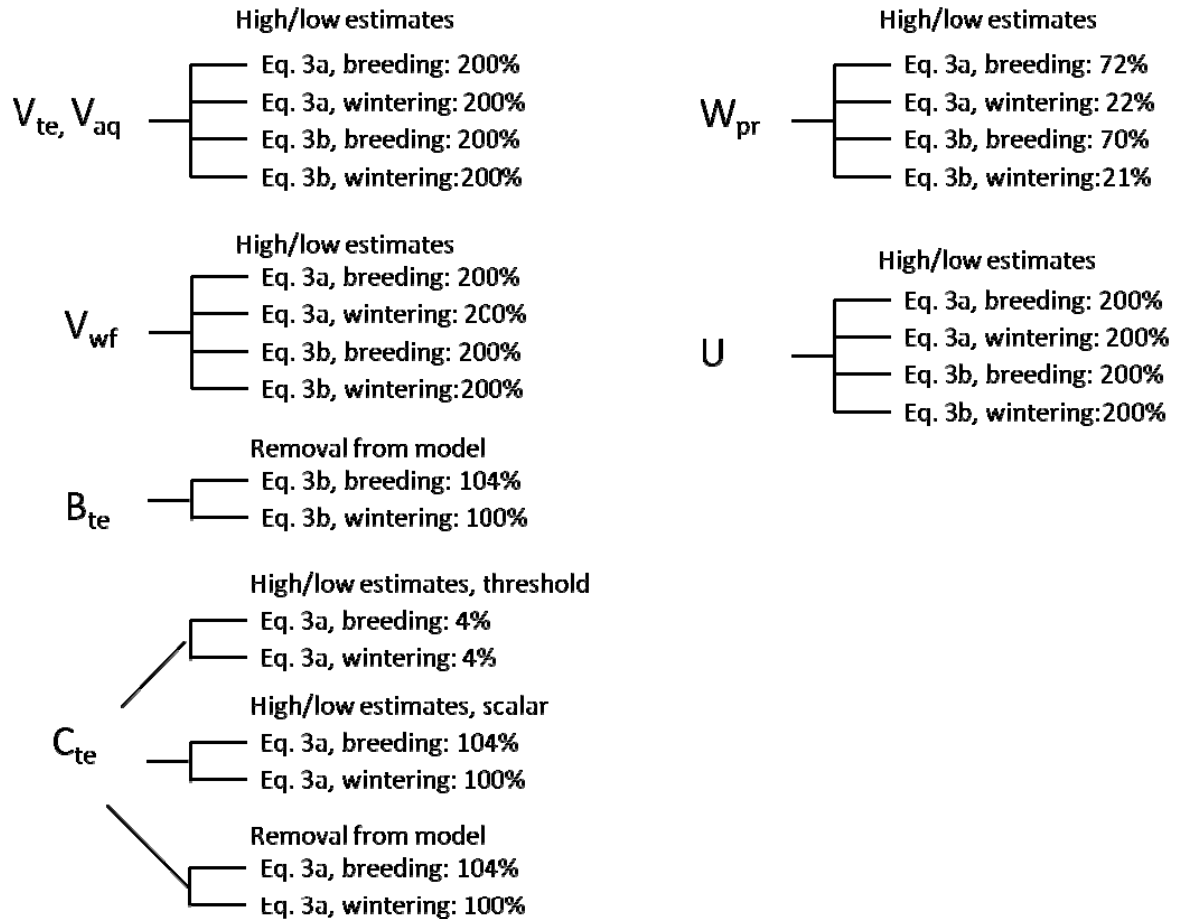
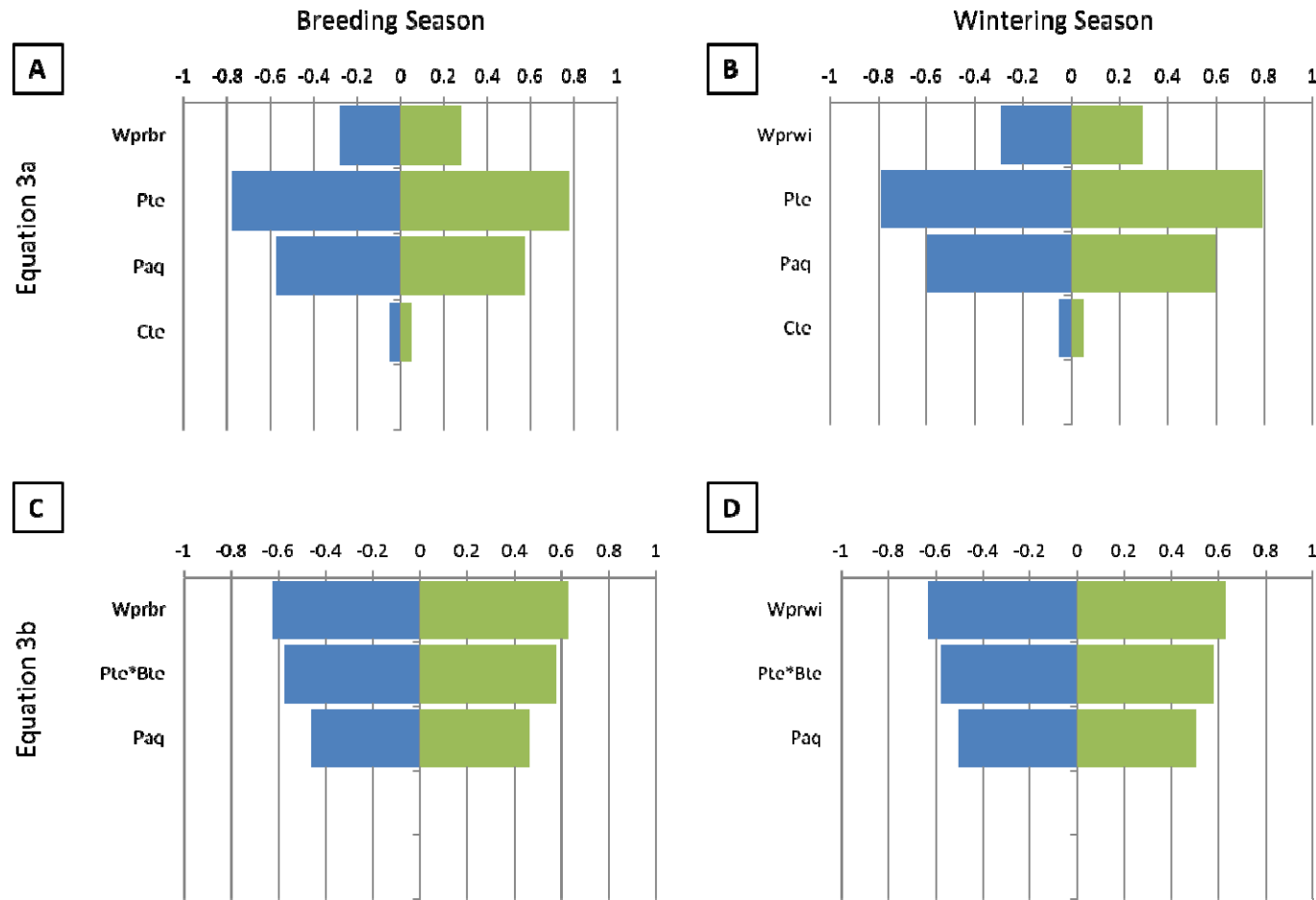


Figure 5.8. Tornado plots representing correlation between transmission risk and model inputs for (A) Equation 3a breeding season, (B) Equation 3a wintering season, (C) Equation 3b breeding season, and (D) Equation 3b wintering season. Equation 3a: $T_{PtoW} = ([P_{te} * C_{te} * V_{te}] + [P_{aq} * V_{aq}]) * ([W_{pr} * U])$ and 3b: $T_{WtoP} = ([W_{pr} * V_{wf}]) * ([P_{te} * B_{te}] + [P_{aq}]) * U$, where P_{te} and P_{aq} are terrestrial and aquatic poultry density, C_{te} is the terrestrial poultry contamination containment rate, V_{te} and V_{aq} are terrestrial and aquatic poultry virus shedding rates, W_{pr} is the wild Anatidae cumulative H5N1 prevalence index, and U is the viral uptake rate. Only Monte-Carlo variables are plotted in the tornado graphs.



Supplemental Materials

Table S5.1. Comparisons of mean model outputs for 1 km deterministic, 30 km deterministic, and 30 km Monte-Carlo models of H5N1 transmission risk between wild and domestic birds in China. Section A notes values for parameters using truncated normal distributions for the Monte-Carlo simulations. Section B notes values for parameters using triangular distributions for the Monte-Carlo simulations. *The 30 km Monte-Carlo Individual Min/Max Truncated values were used in the final models, and were closer to the 30 km deterministic means than the models that used a global min/max value for the distributions.

A.	1 km	30 km	30 km	
Parameter	Deterministic	Deterministic	Monte-Carlo	
<i>Truncated Normal Distributions</i>				
Pte	379	378	379	
Paq	86	86	86	
			30 km	
			Monte-Carlo	
			Individual	
	1 km	30 km	Min/Max	
B. Parameter	Deterministic	Deterministic	Global	
			Min/Max	
			Truncated*	
<i>Triangular Distributions</i>				
Wprbr	0.01	0.01	0.13	0.03
Wprwi	0.006	0.006	0.099	0.037
Pte*Bte	183	184	395	227
Cte	1.00	1.00	0.83	0.83

Table S5.2. Highly pathogenic avian influenza H5N1 viral shedding and uptake rates for poultry and wild waterfowl.

Parameter	Tracheal / Oropharyngeal	Cloacal	Units	Reference	Values used in Uncertainty Model
V_{te}	$10^{6.45}$	$10^{5.95}$	EID ₅₀ Log10/ml	Forrest et al. 2010	Triangular parameters ^a : Min= 10^1 Max= $10^{9.8}$ Best Estimate= $10^{6.8}$ EID ₅₀
	$10^{3.6}$	$10^{1.4}$	TCID ₅₀ Log10/ml	Jeong et al. 2009	
		$10^{6.2}$ to $10^{9.8}$	EID ₅₀ Log10/ml	Yu et al. 2007	
	$10^{4.5}$	$10^{3.5}$	EID ₅₀ Log10/ml	Shortridge et al. 1998	
V_{aq}	$10^{0.2}$ to 10^6	$10^{1.5}$ to $10^{4.5}$	TCID ₅₀ Log10/ml	Phuong et al. 2011	Triangular parameters ^b : Min= 10^0 Max= $10^{6.5}$ Best Estimate= $10^{3.7}$ EID ₅₀
	10^0 to $10^{6.5}$	10^0 to $10^{4.75}$	EID ₅₀ Log10/ml	Sturm-Ramirez et al. 2005	
	$10^{4.0}$ to $10^{6.0}$	$10^{3.5}$ to $10^{4.0}$	EID ₅₀ Log10/ml	Sturm-Ramirez et al. 2004	
	$10^{2.0}$ to $10^{4.3}$	$10^{2.0}$ to $10^{4.3}$	EID ₅₀ Log10/ml	Chen et al. 2004	
	$10^{1.6}$	10^0	EID ₅₀ Log10/ml	Perkins and Swayne 2002	
V_{wf}	$10^{5.10}$ to $10^{6.46}$	$10^{2.5}$ to $10^{4.9}$	EID ₅₀ Log10/ml	Brown et al. 2008	Triangular parameters ^c : Min= $10^{2.5}$ Max= $10^{6.5}$ Best Estimate= $10^{4.77}$ EID ₅₀
	$10^{1.75}$ to $10^{2.63}$	0 to $10^{1.75}$	TCID ₅₀ Log10/ml	Kalthoff et al. 2008	
	10^1 to 10^3	0 to $10^{6.2}$	TCID ₅₀ Log10/ml	Keawcharoen et al. 2008	
	27.4 to 44.6	31.6 to 44.6	CT value	Kwon et al. 2010	
U		$(10^{-15} / 10^{4.7} \text{EID}_{50})$			$(10^{-15} / 10^{4.7} \text{EID}_{50})$

^a V_{te} triangular parameters derived from all studies including isolates from 2003 or later and units in EID₅₀ (Forrest et al. 2010, Yu et al. 2007). Best estimate taken as mean shedding rate across the two references.

^b V_{aq} triangular parameters derived from all studies including isolates from 2003 or later (Phuong et al. 2011, Sturm-Ramirez 2004 and 2005, Chen et al. 2004). Best estimate taken as mean shedding rate of the oropharyngeal and cloacal samples from each reference.

^c V_{wf} triangular parameters derived from Brown et al. 2008 because other studies in the table used units other than EID₅₀. Best estimate taken as mean shedding rate of the six challenged waterfowl species (oropharyngeal and cloacal samples).

Figure S5.1. Key steps (top panel) and three main products: (A) presence-absence distribution maps, (B) abundance maps, and (C) prevalence maps for China's 42 species of Anatidae waterfowl. Breeding and wintering season maps were produced for each product. Spatial resolution of original grid maps is 1 km and abundance and prevalence maps were resampled to 30 km resolution for the disease models.

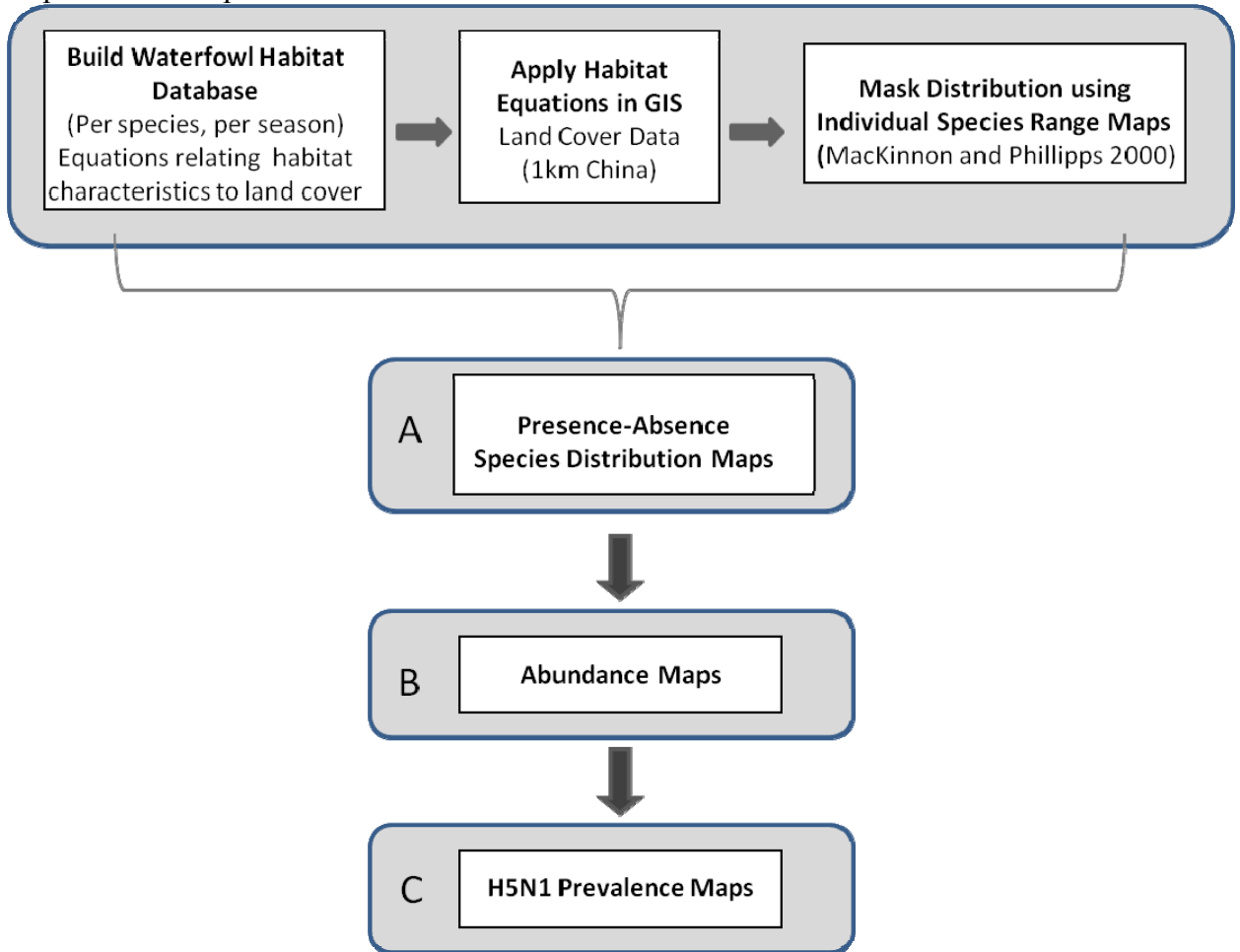


Figure S5.2. Relationship between C_{te} scalar and terrestrial poultry (P_{te}) term for deterministic equation 3a (H5N1 transmission risk from poultry to wild waterfowl). Biosecurity threshold (contaminant containment) is drawn at $P_{te} = 5000$. Sensitivity analysis include thresholds at 3000 and 5000. The C_{te} scalar is 0.5; high and low scalars for the sensitivity analysis are 0.75 and 0.25. An additional sensitivity analysis also test the effect of removing the $P_{te} * C_{te}$ term form the equation.

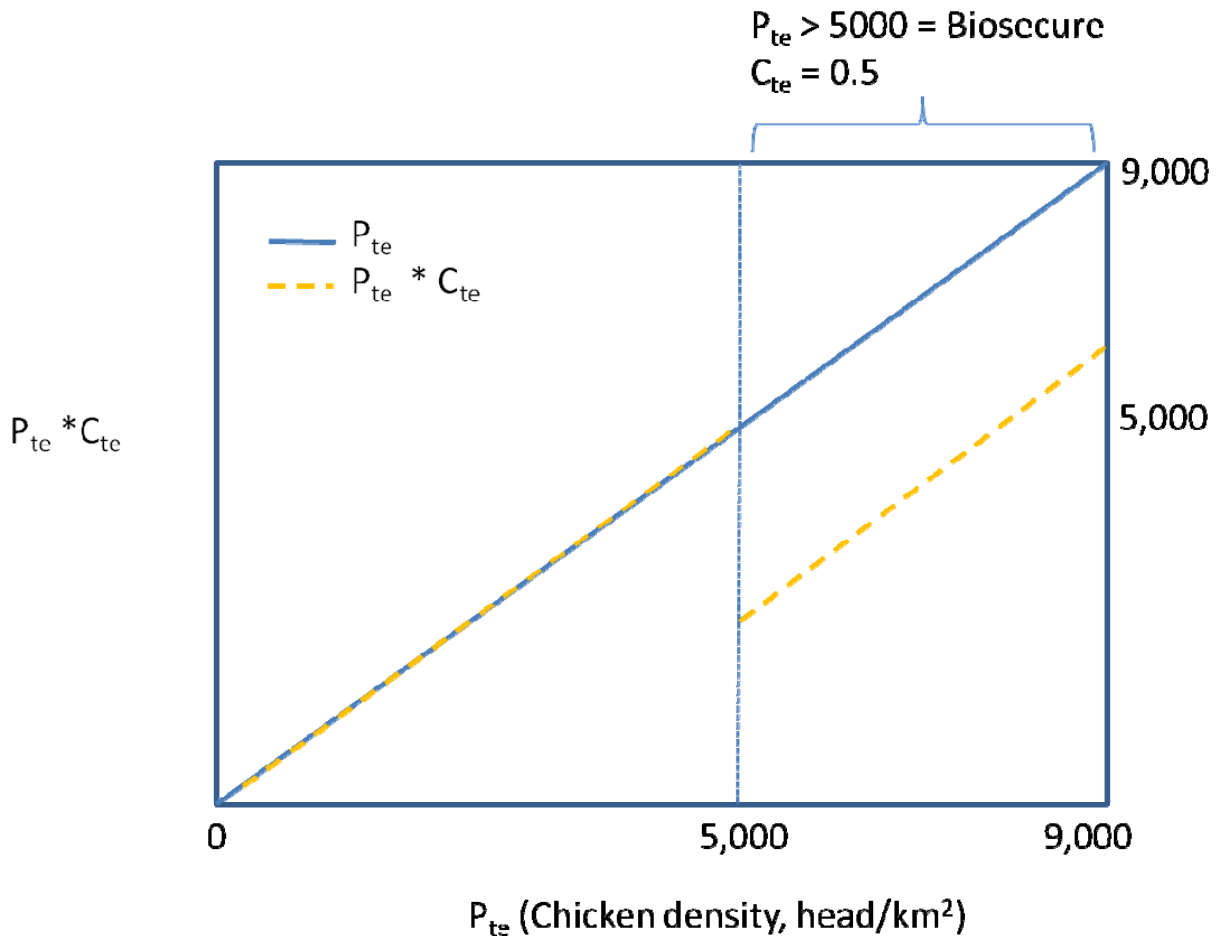


Figure S5.3. Relationship between B_{te} scalar and terrestrial poultry (P_{te}) term for deterministic equation 3b (H5N1 transmission risk from wild waterfowl to poultry). P_{te} is grouped into three sections where (a) $P_{te} \leq 50$, (b) $50 < P_{te} < 1000$, and (c) $P_{te} \geq 1000$. B_{te} scalars are (a) $B_{te} = 1$, (b) $B_{te} = 0.5$, and (c) $B_{te} = a$ constant where $P_{te} * B_{te} = 1000$. The sensitivity analysis for the B_{te} term tested the effect of removing it from the equation.

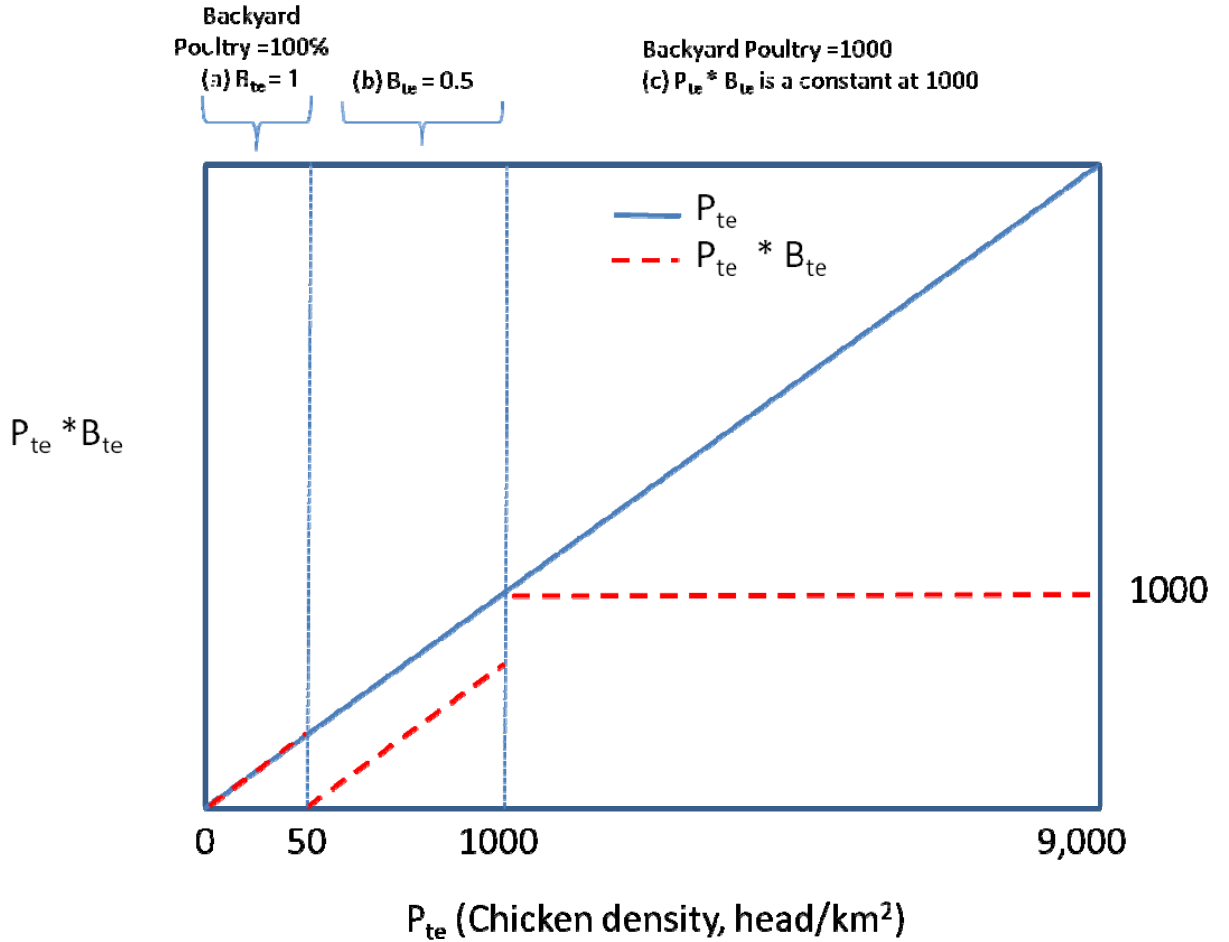


Figure S5.4. Monte Carlo convergence tests for five model variables (a-e) comparing LHS and random sampling for 10, 100, 500, 1000, and 10000 simulations. Three MC runs were conducted for each scenario (combination of sampling type and number of simulations). Data is for 30km resolution variables.

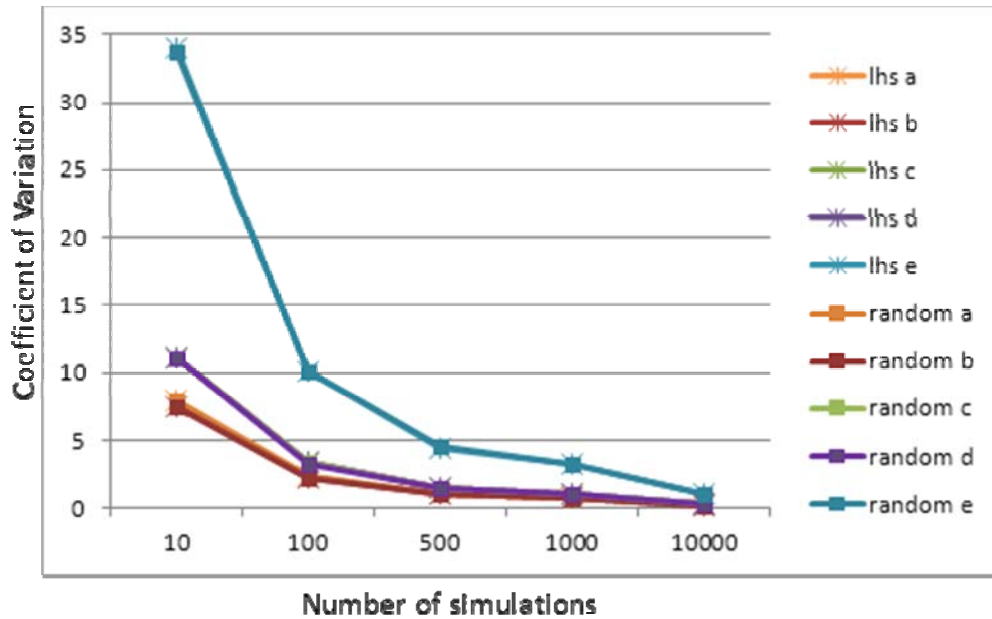


Figure S5.5. Species level distribution maps for two example waterfowl species (bar-headed goose and mallard). Upper panels represent breeding season, lower panels represent wintering season. Legends represent values for four separate outputs: (1) presence-absence distributions, (2) abundance estimates (birds per cell), (3) prevalence (cumulative sum of species abundances multiplied by species prevalence rate), and (4) coefficient of variation (standard deviation divided by mean) for the abundance estimate.

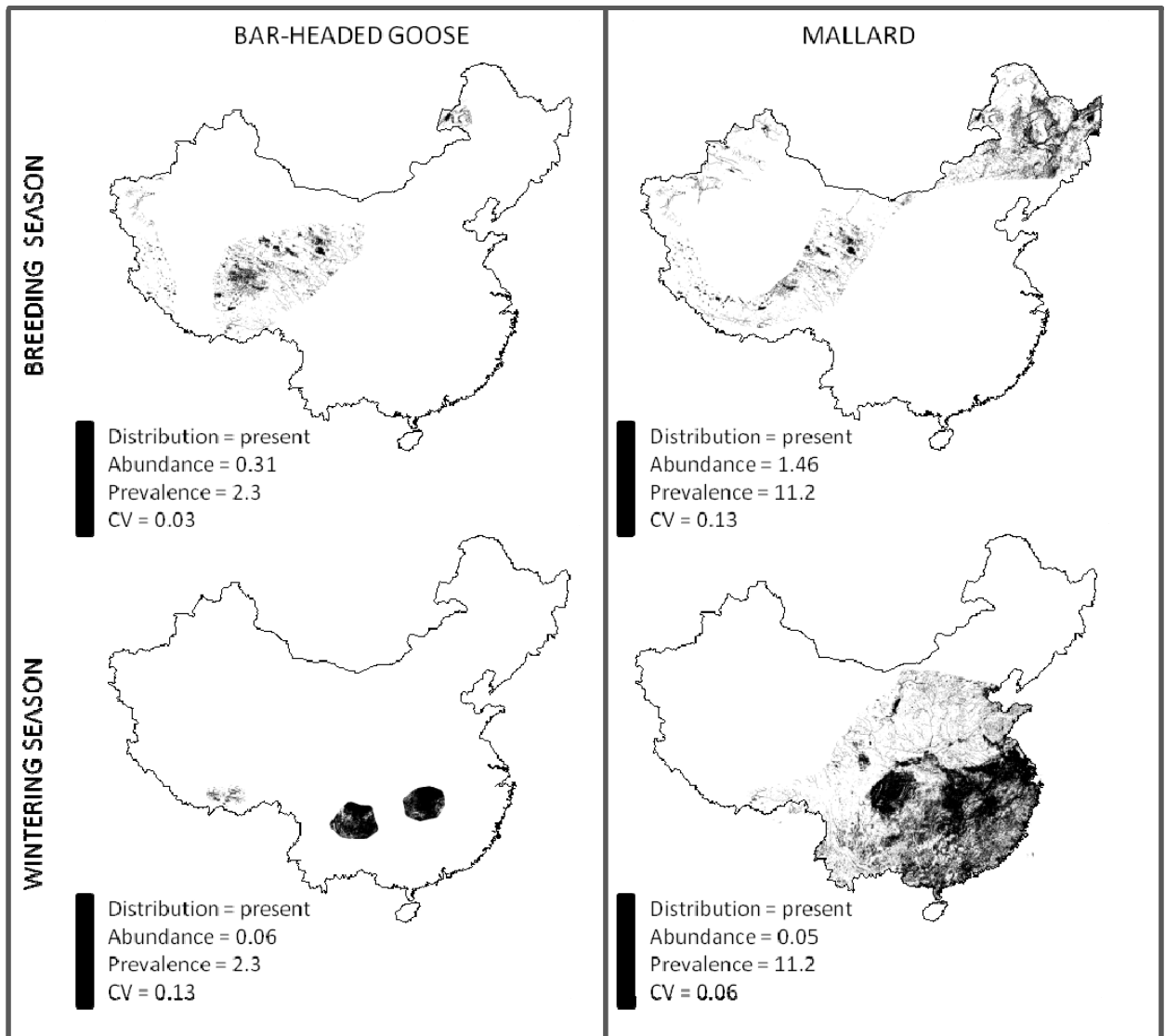


Figure S5.6. Abundance, prevalence index and coefficient of variation for China's Anatidae waterfowl species based on habitat modeling. Left panel represents breeding season (approximately April through July); right panel represents wintering season (approximately November through March). Coarse transitions in predictions are a result of species range boundaries (eg. convex pattern in southeastern China in the breeding season panel). Coefficient of variation (standard deviation divided by mean) calculated for abundance.

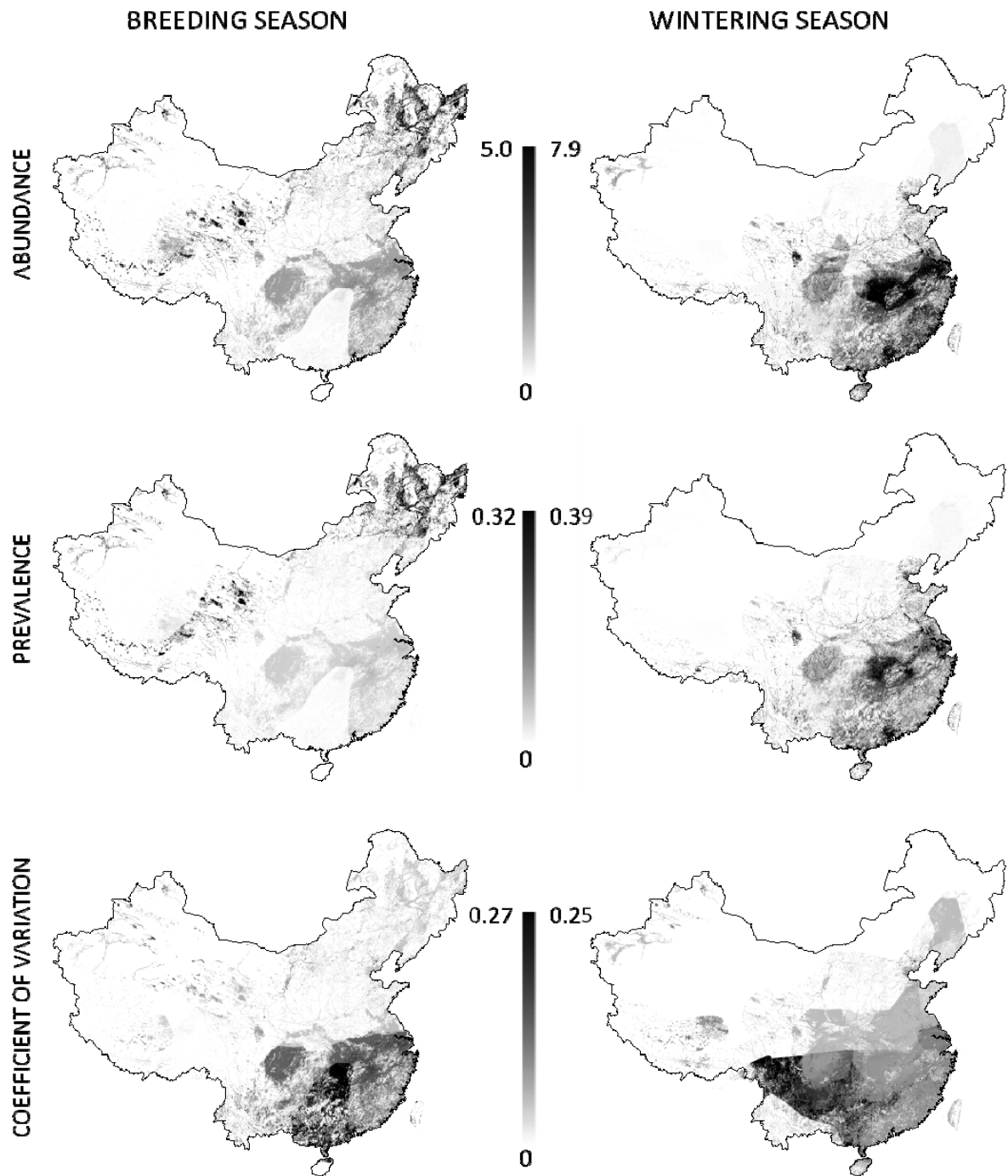


Figure S5.7. Map difference between 1 km and 30 km resolution maps visualized in standard deviations from the mean for waterfowl abundance (top panel) and H5N1 prevalence estimates. Left panel is breeding season, right panel is wintering season.

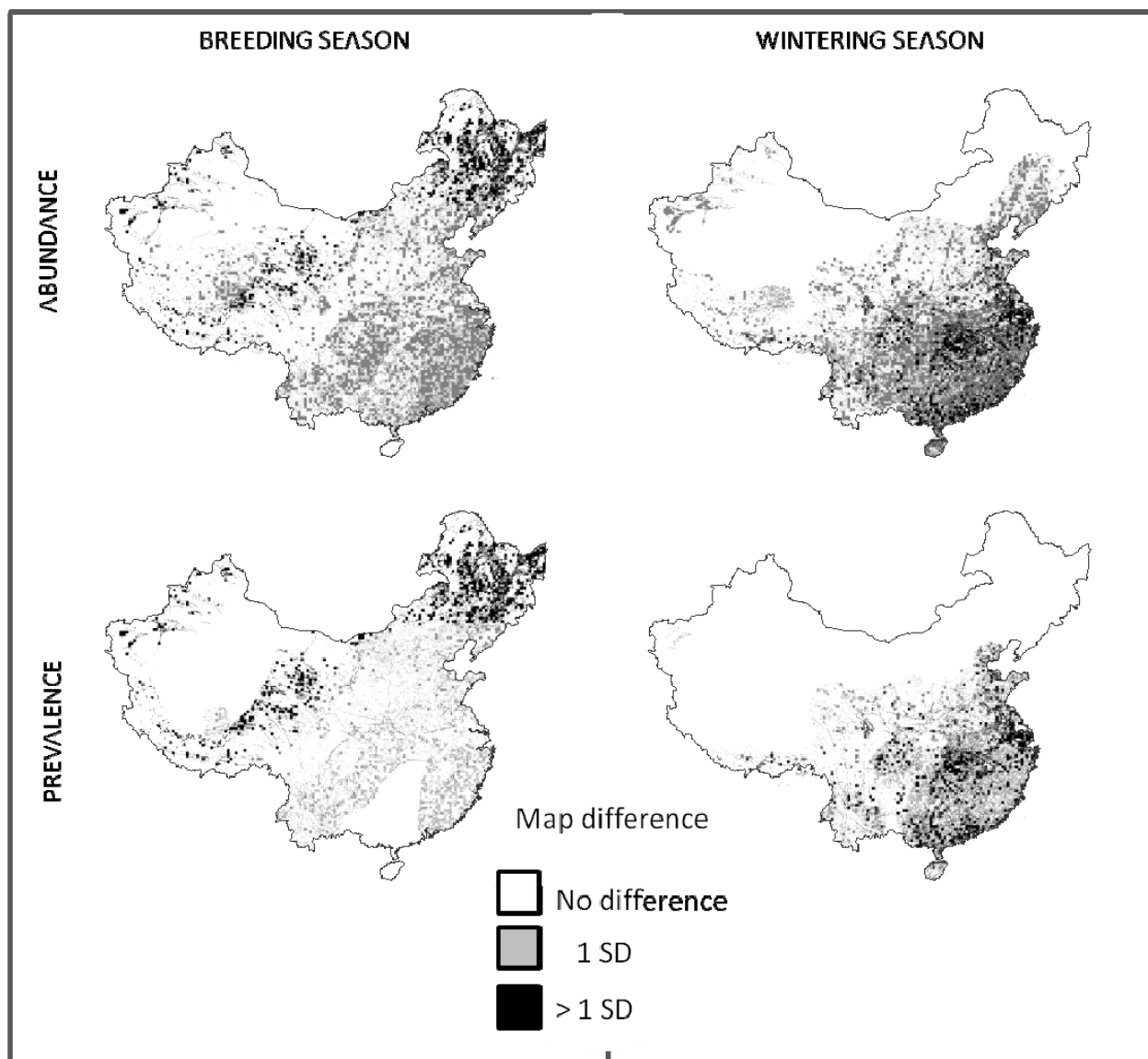


Figure S5.8. Wintering season distribution maps for greater white-fronted goose (*Anser albifrons*) in China. Discussion in text regarding the uncommon subsection of wintering range in the northeast.

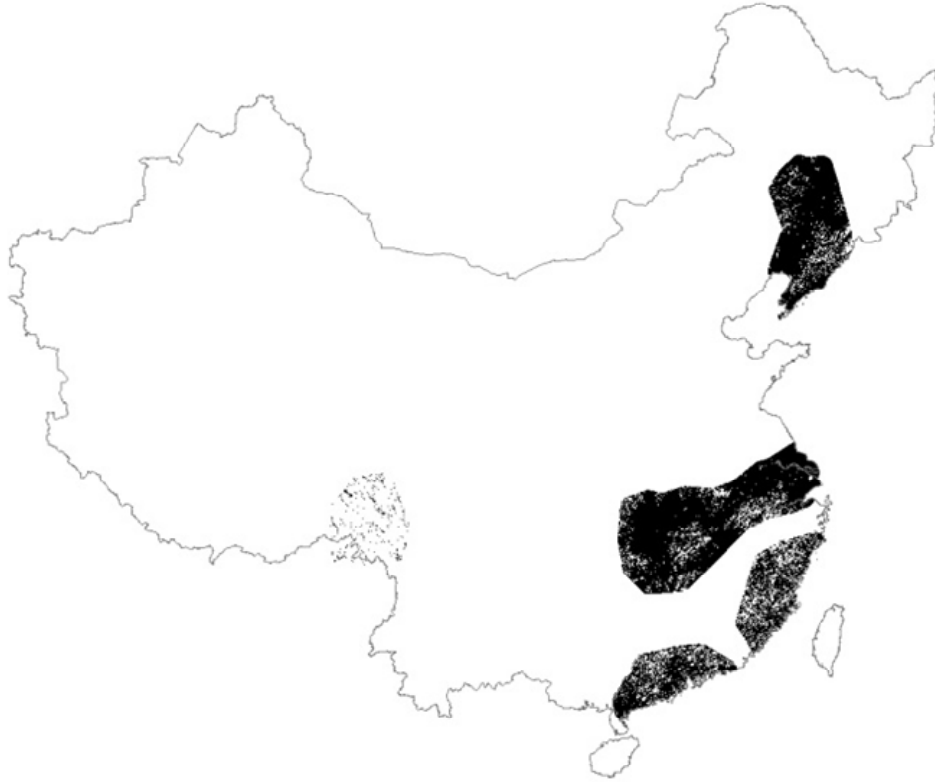


Figure S5.9. Risk output for 1 km deterministic equation 3b with the $(P_{te} * B_{te})$ term removed (see Figure 3a in main text for comparison). The resulting formula is: $T_{WtoP} = (W_{pr} * V_{wf}) * (P_{aq} * U)$, which predicts the risk of virus transmission from wild Anatidae waterfowl to domestic aquatic poultry (and not including terrestrial poultry).

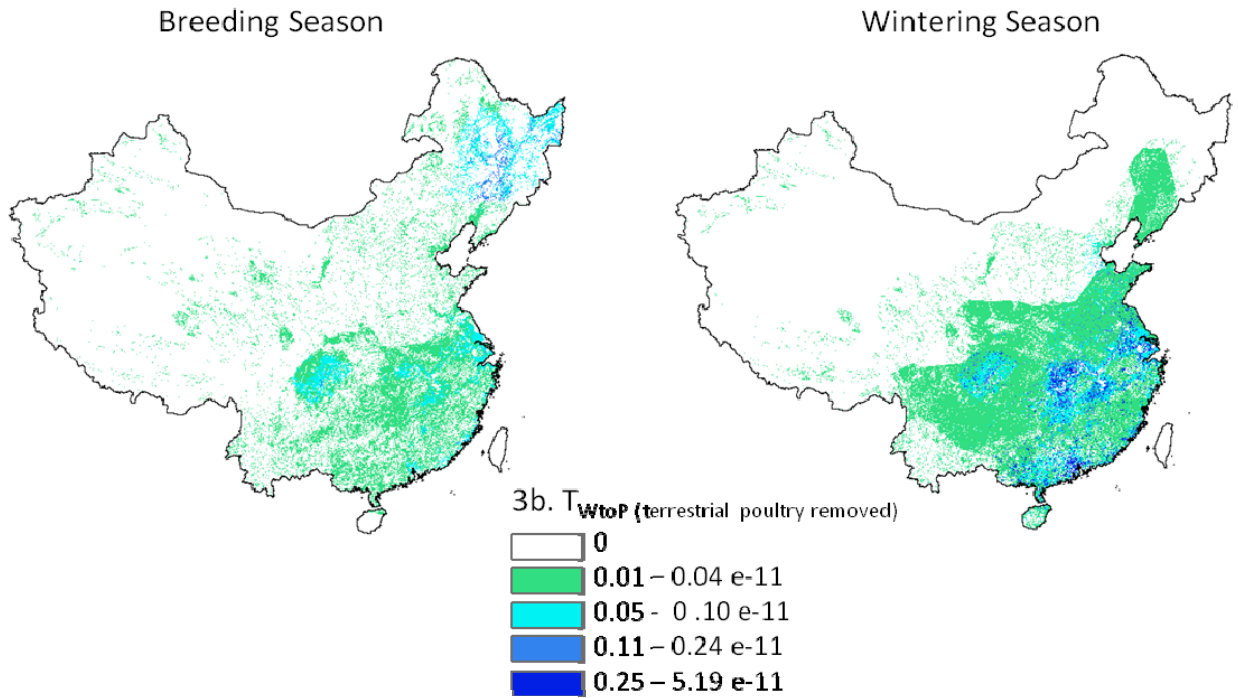
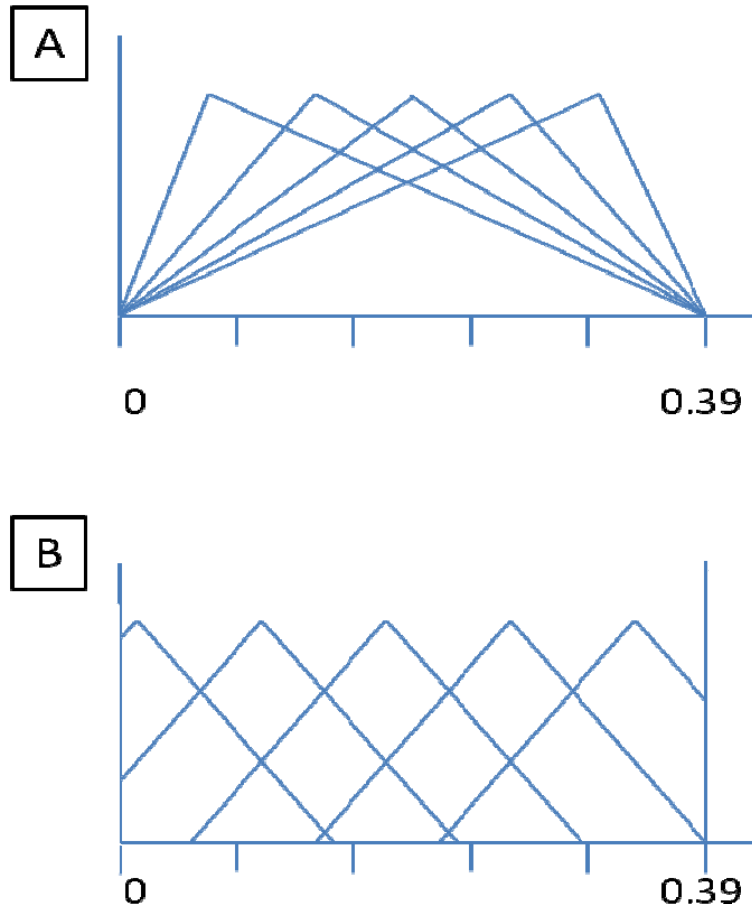


Figure S5.10. Illustrating the difference between using (A) global (fixed) minimum and maximum value versus (b) individual values for minimum and maximum parameters for the triangular distribution of the Monte-Carlo simulations. Parameters of a triangular distribution are: minimum, best estimate, and maximum values (represented visually by the left point, apex, and right point of the triangles in panel A). For each simulation, the Monte-Carlo algorithm draws a value from within the defined parameters of the distribution. Using a global minimum and maximum is less computationally intensive than setting the values individually for each best estimate value. In this example, the winter waterfowl prevalence index (Wprwi) values range from 0 to 0.39. Triangular distributions using global min/max values are fixed at 0 and 0.39 (panel A). Triangular distributions using individual min/max values for each of the 10,495 30 km grid cells across China are calculated by taking the best estimate \pm half the range (eg., if the best estimate for a given cell is 0.24, the minimum and maximum values used in the triangular distribution for that cell would be 0.045 and 0.435. The final estimate for each simulation is truncated to remain within the upper and lower bounds of the distribution. Truncating the distributions will affect the output by driving the mean output up if more values fall outside the lower range limit and driving the mean down if more values fall outside the upper range limit.



CHAPTER 6. SYNTHESIS

Problem Statement

Emerging infectious diseases in wildlife have become a growing concern to human health and biological systems with more than 75 percent of known emerging pathogens being transmissible from animal to humans (Taylor et al. 2001). With the intensification of our agricultural systems, improvement of transportation and market chains, increased globalization, and addition of environmental stressors such as climate change and landscape fragmentation, the incidence of emerging zoonoses is likely to increase (Jones et al. 2008). Emergence of the deadly zoonotic disease, highly pathogenic avian influenza (HPAI) H5N1 (hereafter H5N1) has caused major global concern over a potential pandemic (Russell and Webster 2005, Lu 2006, 2006) and since its emergence in 1996 (Xu et al. 1999) H5N1 has become the longest persisting HPAIV in history (Smith 2006). Despite a wealth of research that has been conducted over the past 16 years, there are a number of outstanding questions related to this disease that remain unanswered, one of which centers around the role wild birds play in the spread of H5N1.

Dissertation in a Nutshell

A recent review of H5N1 risk models (Gilbert and Pfeiffer 2012. *In Press*) indicates a persistent omission of wild birds in transmission risk models, in part because obtaining adequate inputs for these populations is difficult. In an effort to increase our understanding of how wild birds are involved in the spread of H5N1, I proposed to study H5N1 transmission risk between domestic and wild birds in China, the epicenter of H5N1

(Mukhtar et al. 2007). I chose China as the focal area for study for two main reasons. Firstly, the disease originated in southeastern China and continues to persist and emerge in new locations indicating a continued need for research, surveillance, and control. Secondly, the anthropogenic, wild, and environmental landscapes are each diverse across the country, allowing for varying levels of disease risk, both spatially and temporally, conditions which are found in few other places where the virus is endemic.

I took a combined approach of using field studies and modeling to accomplish the goal of assessing H5N1 transmission risk between domestic and wild bird populations. Chapters 1 through 5 build upon each other and integrate key pieces of information and model inputs that were previously lacking (Figure 6.1). Chapter 1 builds background on the problem and summarizes our current state of knowledge regarding wild bird migration and transmission risk modeling. Chapter 2 uses satellite telemetry to study migratory connections in comparison to hypothesized pathways of virus movement in published phylogenetic studies. In addition to providing new evidence on migratory connectivity between outbreak regions, the satellite data provides evidence that wild birds do come in contact with domestic species on the landscape – an important link for the transmission risk models. Chapter 3 outlines the difficult steps taken to collate and integrate poultry census data from multiple sources and the modeling steps taken to predict species-level poultry for China. It also examines differences in stratification methods for the regression modeling and differences among three remotely-sensed predictor sets. The final product is 1 km resolution models for chickens, ducks, and geese across China, plus an assessment of uncertainty. Chapter 4 models wintering and breeding season waterfowl distributions for each of China's Anatidae species and

includes high-resolution species diversity maps. In Chapter 5, results of the satellite telemetry field studies are integrated with the poultry and waterfowl models to provides H5N1 transmission risk models at multiple scales and with uncertainty measures of the risk predictions. An important interim step was to model hotspot regions of wild and domestic bird contact. These models have great utility beyond H5N1 modeling and towards other potential emerging disease and conservation issues.

The following text is a synthesis of the work completed in each chapter - a narrative of what we learned through this process, how we might improve our efforts, and a catch-all for important topics specific to each chapter that were not included in the manuscripts. I first begin by summarizing our current state of knowledge on the key factors of H5N1 transmission and maintenance, then follow with a section for each chapter, and finally, a concluding statement.

Key factors of H5N1 transmission and maintenance

Here I provide a short description of our current state of knowledge regarding key factors of H5N1 maintenance and spread. This collated information is meant to be an abbreviated and concise summary of the pertinent factors used to formulation of the transmission models.

Highly pathogenic H5N1 is predominantly a disease of poultry. The virus has become endemic in poultry populations in five countries including China, Vietnam, Egypt, Indonesia, and Bangladesh (FAO 2011a). Wild birds, particularly waterfowl (Anatidae) and shorebirds (Charadriidae) are reservoirs of low pathogenic avian influenzas and although they are rarely infected with HPAI's, H5N1 has been reported in

a number of wild bird deaths since its emergence in 1996 (OIE 2012). Anatidae waterfowl are susceptible to H5N1, and have been suspected to be involved in the spread of H5N1 across long distances under certain conditions and settings (Gaidet et al. 2010, Prosser et al. 2011a, Newman et al. 2012a). In general, however, intensive surveillance programs across Asia, Africa, Europe, and North America (including more than 750,000 samples) have found very few healthy wild birds positive for H5N1 and wild birds are not considered to be reservoirs of this virus.

HPAI H5N1 has no long-term reservoir outside of live animals (FAO 2008). The primary source of spread is through live infected birds, generally through the fecal-oral route (Shortridge et al. 1998, Alexander 2007) although H5N1 has evolved to replicate in the respiratory tract (Webster et al. 2007a) providing an additional route of transmission from bird to bird under high density situations such as poultry farms. Pathogenicity and viral shedding varies among host species (Alexander 2000). Of the three domestic poultry species commonly farmed in China (chickens, ducks, and geese), chickens show the highest rates of mortality, shedding virus for approximately four days before 100% mortality of the infected flock (Alexander and Capua 2008). Pathogenicity in domestic ducks is less clear with some studies showing high to mixed rates of mortality and length of viral shedding (Sturm-Ramirez et al. 2004, Pantin-Jackwood et al. 2007, Kim et al. 2008, Li et al. 2010) and others showing a clear evolution of domestic ducks as silent reservoirs, shedding virus for multiple weeks without exhibiting clinical signs of infection (Hulse-Post et al. 2005, Sturm-Ramirez et al. 2005, Phuong et al. 2011). The situation for domestic geese is not as well studied, although it appears they are a less

productive host for virus evolution and persistence than asymptomatic domestic ducks (Alexander 2007).

In China and across much of Asia, the farming production structure differs for aquatic (duck, goose) and terrestrial poultry (chicken). Chickens are farmed on land either in housing structures or in the outside environment generally depending on scale of the farm (see below). In contrast, domestic ducks are commonly farmed in association with rice production systems where duck production is timed to match the planting or harvesting of rice (Muzaffar et al. 2010). Young ducks are released to feed in rice paddies during the day and return to penned housing for safety from predators during the night. This integrated farming system reduces the cost of food for duck farmers while providing benefits to the rice farmer by reducing the insect load on growing rice and removing waste grains after harvesting (FAO 2010). The free-range nature of this type of duck production provides opportunity for wild and domestic ducks to share aquatic habitats (Li 2001b). Additionally, in parts of southeastern China, an increasing demand for ‘healthy’ wild meat has led to the establishment of dozens of wild bird farms that are managed under a similar model as the duck-rice systems which also provides opportunity for wild migratory populations to mix with the farmed wild populations (Xiao et al. unpublished data http://csa.ou.edu/NIH/Xiao_Newman.pdf).

China is the global leader in poultry egg and meat production, supporting 44 and 18 percent of the world’s total production (Wang 2006). In 2005, the national flock was 5.3 billion birds (end of year standing population), and nearly 10 billion birds were marketed across the year. Chickens are the largest sector, followed by ducks and geese at 83, 15, and 2 percent, respectively (China National Bureau of Statistics 2008). The scale

of farming has implications for disease transmission risk in regard to multiple factors including the source for incoming birds, the farm's position in the market chain, and the level of biosecurity that is implemented. FAO (2004) defined four poultry sectors which relate the scale of farming to levels of biosecurity. The largest industrial farms (Sector 1) have higher levels of biosecurity and commercially marketed birds; here birds are confined to sealed houses often with an all-in all-out production system (birds raised and sold as a single cohort) that allows for cleansing of the housing between production cycles. Sector 2 commercial poultry farms have moderate to high number of birds and biosecurity levels; birds are marketed commercially and are housed indoors. Smaller scale commercial farms (Sector 3) have low to minimal biosecurity controls and marketing at live bird markets; birds are housed in open structures with potential access to the environment. Small-scale backyard production (Sector 4) does not employ biosecurity measures and birds are consumed locally. Although these patterns are found to be generally true under field investigations, FAO (2008) also notes that even Sector 1 farms, purported to be most biosecure, can have inadequate biosecurity and some smaller farms may have biosecurity sufficient for the level of risk that they face.

An important pathway for transmission of AIVs is through contaminated water or moist environments (Webster et al. 1978, Markwell and Shortridge 1982, Ito et al. 1995). Alexander (2007) reported that the greatest threat of spread of avian influenza is by mechanical transfer of infective feces in which virus may be present at concentrations as high as 10^7 infectious particles per gram and may survive for longer than 44 days. The length of time an influenza virus can survive in wet conditions varies depending on the strain of virus and water conditions such as pH, temperature, and salinity. Some strains

of avian influenza have been known to survive in water at 17° C for up to 207 days, and even longer at 4° C (Stallknecht et al. 1990). Stallknecht et al. (1990a) found that AIVs survived longer in water at lower temperatures (17 versus 28° C), and lower salinities (0 ppt versus 20 ppt). They also found an interaction effect between pH and salinity. Shortridge et al. (1998) found that AIV survived for 4 days in wet feces at 25° C. Brown et al. (2007b) were the first to study persistence of H5 and H7 AIVs in water (LPAI viruses from wild birds and HPAI H5N1 isolated from whooper swans in Mongolia and duck meat in Anyang). They determined that these viruses can persist for extended periods of time in water, that the persistence of these viruses is inversely proportional to temperature and salinity of water, and that a significant interaction exists between the effects of temperature and salinity on the persistence of these viruses (the effect of salinity is more prominent at lower temperatures). Some studies suggest that avian influenza viruses can survive in ice, allowing for persistence of the virus over winter (Zhang et al. 2006), although others discredit these findings due to laboratory contamination (Worobey 2008). Lang (2008) successfully extracted a wide diversity of AIVs from sediments of ponds used heavily by waterfowl during spring, fall, and winter (under ice), although they did not test whether the viruses were viable and able to replicate. Despite the relatively small number of studies conducted on AIV persistence in water and environment, transmission of virus through this medium is likely an important factor in the spread of AIV.

Chapter 1 – Literature Review

The literature review needed to cover a broad spectrum of topics pertinent to the goal of modeling H5N1 transmission risk between wild and domestic birds including: (1)

avian influenza structure, classification, hosts, transmission pathways, H5N1, and the debate on wild birds; (2) wild Anatidae waterfowl global characteristics, population monitoring programs, and distribution mapping approaches; (3) gridded population modeling; and (4) disease risk modeling. Conducting the review on each of the topics was inherently informative, particularly the influenza and modeling sections which were less familiar to me. One of the most interesting aspects was discovering the gaps in knowledge and how these have changed (or not) over the course of this work. For example, early in the process, virtually no information existed on the movement patterns of wild birds in H5N1 endemic regions nor regarding how the virus affects different species. At this point in time, data may still be limited, but there have been a number of tracking studies in Asia and Africa – many related to this work (see USGS-UNFAO partnership: <http://www.pwrc.usgs.gov/resshow/prosser/USGS-FAOWildBirdAIProgram.pdf>, (Muzaffar et al. 2008, Newman et al. 2009, Prosser et al. 2009, Gaidet et al. 2010, Batbayar et al. 2011, Gilbert et al. 2011, Prosser et al. 2011a, Newman et al. 2012b)); and there have been valuable H5N1 challenge studies in wild species such as the work completed by the Southeastern Cooperative Wildlife Disease Study at University of Georgia and others (Brown et al. 2008, Kalthoff et al. 2008, Keawcharoen et al. 2008, Lebarbenchon et al. 2009). Numerous H5N1 risk modeling papers have also been published, particularly over the last few years. Gilbert and Pfeiffer (2012. *In Press*) have summarized risk models that focus on the spatio-temporal patterns of H5N1 – within they note that the number of published studies on HPAIV H5N1 increased from 40 prior to the first large wave of outbreaks (2003-2004) to nearly 2000 in 2010. Three common risk factors identified within the published modeling studies

included domestic waterfowl, anthropogenic variables, and indicators of water. They also note that very few studies include wild birds in their risk models. The work in this dissertation will be one of the first to address this gap in knowledge.

Chapter 2 – Satellite Telemetry

The telemetry paper is one of the first to examine movement ecology of wild host species in relation to H5N1 epidemiology. Prior to this work, fine scale movement data of wild waterfowl from outbreak locations did not exist. One of the most prominent lessons learned from this part of the study is that relying on phylogenetics alone to explain movement patterns of the virus is incomplete; having an understanding of the ecology of the host species is critical as well. Understanding the ecology of host species can help support or refute hypotheses put forward by the phylogenic studies and aid in explaining the mechanisms by which H5N1 spread to different geographic regions. From an ecological or conservation perspective, the findings from this study are also valuable towards learning about the migratory patterns, timing, and habitat use of the waterfowl species studied – a field that is in great need of more information.

Chapter 3 – Poultry Models

Aside from the need for this project, the poultry models were sought after by others in the H5N1 risk modeling community, including partners of the renowned Gridded Livestock of the World (GLW) producers. The main reason for the high demand was that prior to this work, species-level poultry data was not available – domestic ducks, in particular, were the target due to their importance in H5N1 transmission as potential silent reservoirs. China is one of Asia's largest producers of domestic poultry, and is

central to the origin and persistence of H5N1 virus. The connection was made between this project and the GLW partners as I had the data for China's poultry and had created distribution maps for chickens, ducks, and geese. All parties benefitted from this collaboration as I was able to work with the world's experts on modeling poultry distributions (which improved my original models and also provided output consistent with GLW methods) and they received access to China data to complete efforts towards modeling duck distributions across all of Monsoon Asia. Artifacts of the original work being followed by the partnership can be seen in certain aspects of this manuscript, and help to explain some of the decisions made within. For example, I examined the effects of three predictor data sets and three stratification methods for the regression modeling. The three predictor datasets were borne of my original predictors for China, the traditional GLW predictors, and a combination of the two. My original predictors included remote sensing data and models specifically developed for China: LandSat land cover (produced and validated by the Chinese Academy of Sciences), cropping intensity (Yan et al. 2010, Yan et al. In Review), and human population (Tian et al. 2005). The GLW predictors included a combination of MODIS data developed for epidemiological modeling (Hay et al. 2000, Hay et al. 2006), agro-anthropogenic variables developed by the Environmental Research Group of Oxford (for example distance to different types of livestock, length of growing period, etc.), and human factors such as human population and distance to urban areas from the Global Rural Urban Mapping Project (GRUMP). The GLW models are global models and therefore rely on global datasets for spatial consistency. In some cases, such as the global GRUMP models which are purported to be less accurate in predicting human factors such as population, accuracy was sacrificed

for global consistency. For this reason, in the final manuscript, I retained my original predictor sets and compared their capacity to model poultry distributions in comparison to the GLW and combined data sets. As it turns out, the combined set performed the best. In similar fashion, I retained my China-focused stratification scheme and compared it with two others from the GLW group. The best stratification differed depending on the species, with geese being best modeled using my original stratifications and chickens and ducks using one of the GLW stratification schemes.

Unfortunately, there are no suitable independent datasets available to validate the poultry models. To address this issue, I took an approach that is commonly accepted as a validation procedure by peer-reviewed studies which includes reserving a portion of the available data for assessing model fit. Here I reserved 75 percent of the available census data for training the models and used 25 percent for validation which was measured by goodness of fit tests correlation and root mean square error.

Obtaining the census data and developing a method for filling data gaps was equally challenging if not more so than the modeling aspect of the study. Talking to poultry experts and scouring the multitudes of agricultural yearbooks to find data and develop the best approach towards quantifying poultry populations across all of China at a sub-provincial level took much effort. Here one of the valuable lessons learned was how to integrate data from different sources and develop a methodology that is repeatable and transparent.

Chapter 4 – Waterfowl Models

A lack of survey data required us to take a different approach towards creating species distribution models for China's waterfowl. Survey data was not sufficient to

employ data driven models (such as the regression modeling in the poultry paper) and therefore I turned to habitat modeling to develop occurrence models for each species. However, each seasonal distribution map (30 breeding and 37 wintering) is a contribution to the field of waterfowl ecology, and the manuscript for this chapter was written to focus on the presence-absence models and accompanying species diversity maps. These are the first distribution models created for China's waterfowl species and at this point in time represent our best state of knowledge on distributions across the suite of species. I would like to make several improvements to the models, some of which are obtainable in the near future, and some of which may not be possible for many years to come. I hope publication of these entry-level models will stimulate further study on these species, and eventually the development of a coordinated monitoring program for China's waterfowl. The China Anatidae Network was established in 2008, marking a move towards coordinated research and better communication among parties that study and monitor waterfowl in China.

Chapter 5 – Transmission Risk Models

In a recent review of spatial risk modeling of H5N1, Gilbert and Pfeiffer (2012. *In Press*) indicate a continued absence of studies that incorporate wild birds in the modeling process. They attribute the difficulty in obtaining pertinent spatial data on wild birds as one of the main reasons for this gap. Thus, the work outlined in this chapter and Chapter 4 present a much needed contribution towards improving our understanding of spatial and temporal risk of wild birds and H5N1 transmission. It will be one of the first papers to explicitly model H5N1 transmission risk between domestic and wild birds.

One of the challenges in this process was making the jump from the waterfowl distribution models of Chapter 4 to models of abundance and prevalence. Cao et al. (2010) has provided population estimates for China's wintering species, but conducting surveys for breeding waterfowl is much more difficult as their behavior turns from gregarious in the winter to secretive and dispersed during the nesting season. At a certain point in the process, I questioned whether I could create the abundance maps for the breeding season given the lack of published data. However, not being able to account for differences between wintering and breeding seasons for the waterfowl component of the risk models would have greatly reduced the value of the risk models. Having a tool such as the Monte-Carlo simulations that allow one to quantify the uncertainty associated with model inputs is what helped me to move forward. Here I could derive the best estimates possible but also quantify the level of confidence associated with these estimates, and incorporate it explicitly in the final risk models.

One of the main limits of these models was not being able to take a data-driven approach during model development because there is no good set of outbreak data that can be used to draw statistical relationships with predictor variables. In order to do so, we would have to know what type of host initiated the infection in the outbreak group (for example, to be able to decipher relationships between predictors and poultry outbreaks caused by wild bird infections and vice-versa). This is difficult and perhaps impossible to determine without having genetic samples of many types of populations surrounding an outbreak. Even the few large scale active surveillance efforts within China in wild birds and wet markets (Kou et al. 2009, Lei et al. 2011, Martin et al. 2011) do not indicate the infecting population. However, even if we could derive this

information from the existing outbreak data, the approach has its drawbacks, as well. The fault with using outbreak data to train the models is the potential bias in outbreak reporting – that the distribution of outbreaks reported may not adequately represent the true distribution of infections. In addition, underreporting has been purported to be high in China. In the end, taking the approach of modeling the poultry and waterfowl populations and developing risk models from these frees us of potential biases in an outbreak predictor data set.

Concluding Remarks

When I began this project, the debate on wild birds and their role in transmission of H5N1 was a new and hotly debated topic, with little solid data to support arguments on either side. Since then, multiple key publications have advanced our knowledge on the topic of H5N1 transmission. Notably, strong associations have been drawn between duck farming and H5N1 prevalence (Gilbert et al. 2006a, Songserm et al. 2006, Gilbert et al. 2007, Gilbert et al. 2008), challenge studies indicate varying levels of pathogenicity among wild and domestic host species (Hulse-Post et al. 2005, Sturm-Ramirez et al. 2005, Brown et al. 2008, Keawcharoen et al. 2008), and a wide scale telemetry project concluding differing levels of involvement of wild birds in H5N1 transmission in different regions (Newman et al. 2009, Prosser et al. 2009, Gaidet et al. 2010, Takekawa et al. 2010a, Gilbert et al. 2011, Prosser et al. 2011a, Newman et al. 2012a). The topic of wild birds and H5N1 continues to be debated and likely will never be fully resolved, as the situation is complicated and factors that apply in one region may not in others. Despite our advances, there still are a number of topics regarding wild birds and H5N1 that could use further research including how H5N1 affects individual wild species –

from the length of the asymptomatic period post-exposure to pathogenicity and transmissibility rates. It would also be helpful to have information on whether wild birds can truly fly if infected with H5N1, including possibly a response curve of their reduced capability to migrate through the incubation, infection, and recovery stages.

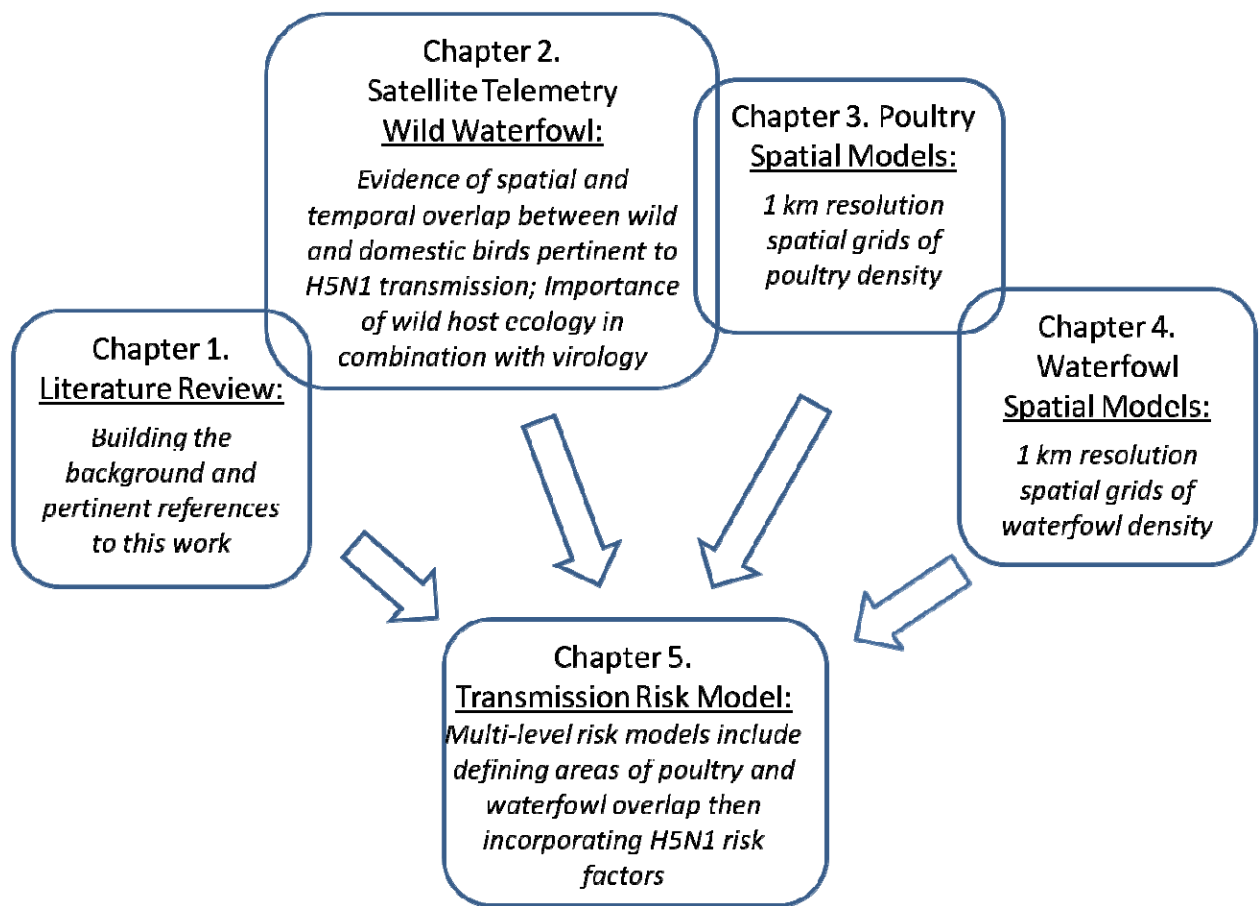
I was asked during the defense if this dissertation answers the question: Are wild birds implicated in transmission of H5N1, and if not, how would we answer this question? Firstly, the models in this dissertation were not designed to answer this question – they were designed to model where wild and domestic birds have the potential to interchange virus material. They work under the assumption that wild birds have the potential to become infected and transmit virus to the environment and thereby other populations. However, wild birds *were* implicated by much of the political community as H5N1 spread beyond Qinghai Lake in 2005 and on to new countries and continents – without any scientific data to support these claims. The implication without information on movement ecology or disease ecology in these populations is one a main reason we began investigating migratory movements in relation to H5N1 (Chapter 2). The results from Chapter 2 and some related papers (Prosser et al. 2009, Prosser et al. 2011a, Newman et al. 2012b) indicate that yes, in certain cases, wild birds appear to contribute to the long-distance transport of H5N1 (but don't in other regions (Newman et al. 2009, Takekawa et al. 2010a)). For example, in the Central Asian Flyway, it appears that the bar-headed goose may have acted as a vector of H5N1 transmission. We observed spatial and temporal overlap between marked geese and captive bar-headed goose farms on the wintering grounds (showing the potential for virus transfer from infected poultry to wild migratory geese) and, (b) H5N1 outbreaks. Also, although it was not common, the fastest

goose migrated from the Lhasa wintering grounds (where H5N1 outbreaks occurred in poultry) to Qinghai Lake in just over 5 days. Challenge studies have shown bar-headed geese to be asymptomatic for approximately 5 days post exposure to H5N1 (Brown et al. 2008) indicating that there is the potential for this species to move long distances (1200km; the distance from Lhasa to Qinghai Lake) before being crippled by the effects of H5N1 infection.

The need to truly work in an interdisciplinary manner to accomplish this work has been both challenging and rewarding. A critical benefit of this project was the opportunity to work with experts from fields very different from my own, including remote sensing, modeling, and disease experts. In particular, very close cooperation was conducted with the epidemiologist in this group, making the final transmission models a more rigorous product. Also, the guidance provided by the GLW partners has greatly improved the original poultry distribution models. Finally, the multiple collaborations revolving around the waterfowl models have been slower to manage, but I expect will be extremely beneficial to the field of waterfowl ecology.

As a result of this work, we now have the first models that focus specifically on H5N1 transmission between wild and domestic birds. We also have the first species level poultry maps for China, and the first waterfowl maps for China. The adage that all models are wrong (by statistician George E.P. Box, 1987) is true, and certainly apply to the models herein. However, I do believe these models are useful to a broad spectrum of researchers and practitioners from the fields of epidemiology and public health to wildlife conservation. I hope that this work will stimulate additional papers on this topic, and the development of better models.

Figure 6.1. Dissertation chapters in a nutshell. Interconnected boxes list the main results of each chapter and illustrate how they each support the main transmission risk models of Chapter 5.



Personnel and collaborators

Personnel

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Collaborators

John Takekawa, *USGS Western Ecological Research Center, Vallejo, California*. Dr. Takekawa is one of the world's leading experts on use of satellite telemetry to study wild bird movements. He is the co-principal investigator for the USGS wild bird avian influenza program.

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Zhi Xing and Yuansheng Hou, *Qinghai Lake National Nature Reserve, Qinghai, P.R. China*. Mr. Xing is the Director of Qinghai Lake National Nature Reserve (QLNNR); Mr. Hou is the lead ornithologist at QLNNR. All work conducted on the reserve is coordinated jointly through CAS and QLNNR.

Scott Newman, *UN FAO International Wildlife Coordinator for Avian Influenza, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, Rome, ITALY 00100, phone: 039-06-570-5353, scott.newman@fao.org*. Scott is the wildlife coordinator for the United Nations FAO and is a lead cooperator for our USGS wild bird avian influenza program.

Changqing Ding, *Beijing Forestry University, Beijing, P.R. China*. Dr. Ding is an expert in ornithology in China; he is currently preparing a field guide of waterfowl of China. Dr. Ding will help with the development and review of the waterfowl population maps.

APPENDIX A.

Table A.1. Prevalence of influenza A virus in live wild birds from three review papers covering North America (Olsen et al. 2006), Europe (Munster et al. 2007), and Africa (Gaidet et al. 2006).

Order	Family	Common Name	Species	North America		Europe		Africa	
				Olsen et al. 2006		Munster et al. 2007		Gaidet et al. 2006	
				Sampled (n)	Percent Positive	Sampled (n)	Percent Positive	Sampled (n)	Percent Positive
Anseriformes	Ducks	36 species (Olsen et al. 2006)		34,503	9.5				
		9 species (Munster et al. 2007)				13751	6.9		
		19 species (Gaidet et al. 2006)						2864	9.4
		Mallard	<i>Anas platyrhynchos</i>	15250	12.9	8938	7.3		
		Northern Pintail	<i>Anas acuta</i>	3036	11.2	448	2.9	24	8.3
		Blue-winged Teal	<i>Anas discors</i>	1914	11.5				
		Common Teal	<i>Anas crecca</i>	1314	4	940	6.4	24	12.5
		Eurasian Wigeon	<i>Anas penelope</i>	1023	0.8	2538	3		
		Wood Duck	<i>Aix sponsa</i>	926	2.2				
		Common Shelduck	<i>Tadorna tadorna</i>	881	6.5	355	0.6		
		American Black Duck	<i>Anas rubripes</i>	717	18.1				
		Green-winged Teal	<i>Anas carolinensis</i>	707	4				
		Gadwall	<i>Anas strepara</i>	687	1.5	298	2.7		
		Spot-billed Duck	<i>Anas poecilorhyncha</i>	574	3.7				
		Northern Shoveler	<i>Anas clypeata</i>			135	3.7	6	16.7
		Tufted Duck	<i>Aythya fuligula</i>			62	3.2		
		Common Eider	<i>Somateria mollissima</i>			37	5.4		
	Geese	8 species (Olsen et al. 2006)		4806	1				
		6 species (Munster et al. 2007)				6428	1.8		
		Canada Goose	<i>Branta canadensis</i>	2273	0.8				
		Greylag Goose	<i>Anser anser</i>	977	1.1				
		White-fronted Goose	<i>Anser albifrons</i>	596	2.2	3821	2.1		

Order	Family	Common Name	Species	North America		Europe		Africa	
				Olsen et al. 2006		Munster et al. 2007		Gaidet et al. 2006	
				Sampled (n)	Percent Positive	Sampled (n)	Percent Positive	Sampled (n)	Percent Positive
Charadriiformes	Swans	Barnacle Goose	<i>Branta leucopsis</i>			1139	0.7		
		Brent Goose	<i>Branta bernida</i>			413	1		
		Bean Goose	<i>Anser fabalis</i>			315	0.6		
		Pink-footed Goose	<i>Anser brachyrhynchus</i>			285	2.1		
		3 species (Olsen et al. 2006)		5009	1.9				
		2 species (Munster et al. 2007)				200	2		
		Tundra Swan	<i>Cygnus columbianus</i>	2137	2.8				
		Bewick's Swan	<i>Cygnus columbianus bewickii</i>			153	2		
		Mute Swan	<i>Cygnus olor</i>	1597	1.3	47	2.1		
		Whooping Swan	<i>Cygnus cygnus</i>	930	1.5				
	Gulls	9 species (Olsen et al. 2006)		14505	1.4				
		4 species (Munster et al. 2007)				2602	0.8		
		3 species (Gaidet et al. 2006)						366	3.8
		Ring-billed Gull	<i>Larus delawarensis</i>	6966	2				
		Black-tailed Gull	<i>Larus crassirostris</i>	1726	1				
		Black-headed Gull	<i>Larus ridibundus</i>	770	2.2	1583	0.9		
		Herring Gull	<i>Larus argentatus</i>	768	1.4	753	0.7		
		Mew Gull	<i>Larus canus</i>	595	0	226	0.9		
		Greater Black-backed Gull	<i>Larus marinus</i>			41	4.9		
	Terns	9 species (Olsen et al. 2006)		2521	0.9				
		Common tern	<i>Sterna hirundo</i>	961	1.7				
	Shorebirds	10 species (Olsen et al. 2006)		2637	0.8				
		2 species (Munster et al. 2007)							
		13 species (Gaidet et al. 2006)						409	1.5
		Red Knot	<i>Calidris canutus</i>			230	0.4		
		Red-necked Stint	<i>Calidris ruficolis</i>			5	20		
Gruiformes	Auks	Guillemot	<i>Uria aalge</i>			817	0.4		
	Rails	3 species (Olsen et al. 2006)		1962	1.4				
		1 species (Munster et al. 2007)				237	0.4		

Order	Family	Common Name	Species	North America		Europe		Africa	
				Olsen et al. 2006		Munster et al. 2007		Gaidet et al. 2006	
				Sampled (n)	Percent Positive	Sampled (n)	Percent Positive	Sampled (n)	Percent Positive
Procellariiformes	Petrels	8 species (Gaidet et al. 2006)						438	0.7
		Eurasian Coot	<i>Fulica atra</i>	1861	1.2	237	0.4		
		5 species		1416	0.3				
		Wedge-tailed Shearwater	<i>Puffinus pacificus</i>	794	0.5				
Gaviiformes	Cormorants	1 species (Olsen et al. 2006)		4500	0.4				
		2 species (Gaidet et al. 2006)						148	0
Other	Other	Great Cormorant	<i>Phalacrocorax carbo</i>	4500	0.4			130	0
		36 species (Gaidet et al. 2006)						196	0

Table A.2. Summary of global HPAI outbreaks in domestic poultry (Alexander 2000, United Nations Food and Agriculture Organization 2004, Swayne 2008).

Year	Country	Species	Virus Subtype
1959	Scotland	Chicken	H5N1
1963	England	Turkey	H7N3
1966	Ontario	Turkey	H5N9
1976	Victoria (Australia)	Chicken	H7N7
1979	Germany	Chicken	H7N7
1979	England	Turkey	H7N7
1983-1984	Pennsylvania	Chicken	H5N2
1983	Ireland	Turkey	H5N8
1985	Victoria (Australia)	Turkey	H7N3
1991	England	Turkey	H5N1
1992	Victoria (Australia)	Chicken	H7N3
1994	Queensland (Australia)	Chicken	H7N3
1994	Mexico	Chicken	H7N2
1994	New South Wales	Chicken	H7N4
1994-1995	Pakistan	Chicken	H7N3
1996	Guangdong (China)	Goose	H5N1
1997-2008	Hong Kong	Chicken	H5N1
1997	Italy	Turkey	H5N2
1997	New South Wales	Chicken	H7N4
1999-2000	Italy	Turkey	H7N1
2002	Chile	Chicken	H7N3
2003	Pakistan	Chicken	H7N3
2003	Netherlands, Belgium, Germany	Chicken	H7N7
2004	Canada	Chicken	H7N3
2004	Texas	Chicken	H5N2
2004, 2006	South Africa	Ostrich	H5N2
2005	Korea	Chicken	H7N7
2007	Canada	Chicken	H7N3

APPENDIX B

Table B.1. Examples of data sources used for sub-provincial level poultry statistics: Xinjiang province (Tibet).

Province (in Chinese)	Province	Year (Data)	Sub-provincial Level Poultry Data Source	Sub-provincial Level Poultry Data Source (in Chinese)
新疆	Xinjiang (Tibet)	2005	Chen Hong. 2006. Xinjiang Statistical Yearbook. Edited by Statistics Bureau of Xinjiang Uygur Autonomous Region. Beijing. China Statistics Press.	陈虹. 2006. 新疆统计年鉴. 新疆维吾尔自治区统计局编. 北京. 中国统计出版社.
		2004	Wang Guirong. 2005. Xinjiang Statistical Yearbook. Edited by Statistics Bureau of Xinjiang Uygur Autonomous Region. Beijing. China Statistics Press. pp.393-395	王贵荣. 2005. 新疆统计年鉴. 新疆维吾尔自治区统计局编. 北京. 中国统计出版社. pp.393-395
		2003	Wang Guirong. 2004. Xinjiang Statistical Yearbook. Edited by Statistics Bureau of Xinjiang Uygur Autonomous Region. Beijing. China Statistics Press. pp. 331-333	王贵荣. 2004. 新疆统计年鉴. 新疆维吾尔自治区统计局编. 北京. 中国统计出版社. pp. 331-333
		2005	Sun Fachen. 2006. Xinjiang Production &Construction Group Statistical Yearbook. Edited by Statistics Bureau of Xinjiang Production & Construction Group. Beijing. China Statistics Press. pp. 258-269.	孙法臣. 2006. 新疆生产建设兵团统计年鉴. 新疆生产建设兵团统计局编. 北京. 中国统计出版社.pp. 258-269
		2004	Shen Weizhen. 2005. Xinjiang Production &Construction Group Statistical Yearbook. Edited by editorial committee of Xinjiang Production &Construction Group Statistical Yearbook. Beijing. China Statistics Press. pp. 260-282.	沈炜珍. 2005. 新疆生产建设兵团统计年鉴. 新疆生产建设兵团统计年鉴编辑委员会编. 北京. 中国统计出版社. pp. 260-282.
		2003	Shen Weizhen. 2004. Xinjiang Production &Construction Group Statistical Yearbook. Edited by editorial committee of Xinjiang Production &Construction Group Statistical Yearbook. Beijing. China Statistics Press.	沈炜珍. 2004. 新疆生产建设兵团统计年鉴. 新疆生产建设兵团统计年鉴编辑委员会编. 北京. 中国统计出版社

Table B.2. Provincial level poultry statistics for chickens, ducks, and geese from China statistical yearbooks.

Province Name		Number of farms					Number of animals present at end of year.				Total number of poultry sold per year			
		TOTAL Poultry Farms	Chicken Egg Farms	Chicken Meat Farms	Duck Farms	Goose Farms	Chickens (egg layers)	Chickens (broilers)	Ducks	Geese	Egg type grand-parent stock stations(pair)	Meat type grand-parent stock stations(pair)	Breeder duck stations (piece)	Breeder goose stations (piece)
全 国	China	4012	1 243	1 361	793	615	17727387	39421036	8185024	1 369798	17639244	39004091	166149019	6957729
北 京	Beijing	49	21	21	7		1182500	779000	114800		2118000	360000	1360000	
天 津	Tianjin	15	11	2	2		148200	43000	24000			940000		
河 北	Hebei	169	123	37	7	2	1907906	846300	130000	26000	800000	2900000	2000000	75000
山 西	Shanxi	45	42	2	1		1108100	52000	2200					
蒙 古	Neimenggu	8	5	3										
辽 宁	Liaoning	188	60	104	11	13	652600	2078900	71470	134900	4080000		5717600	4047000
吉 林	Jilin	106	44	55		7	980000	3160000		76000				
龙 江	Heilongjiang	111	34	72		5	281710	1192536		29600		500000		500
上 海	Shanghai	35	10	17	8		221200	278740	116527		4400000	1791000	29005200	
江 苏	Jiangsu	673	243	206	74	150	2045062	3425429	441536	423220	1840000	4940000	920000	1405000
浙 江	Zhejiang	91	18	34	23	16	187000	1154370	388550	38500	100000	269091	12594932	252600
安 徽	Anhui	279	59	79	63	78	1154580	2678412	1250900	91040			36500	7500
福 建	Fujian	64	4	28	28	4	113200	745453	381360	4200			5038500	50830
江 西	Jiangxi	30	9	15	3	3	25401	494913	65818	5788		1510000	268000	272330
山 东	Shandong	579	223	272	78	6	2497852	6710953	1678062	19121	2300414	1764000	23697416	8020

Province Name		Number of farms					Number of animals present at end of year.				Total number of poultry sold per year			
		TOTAL Poultry Farms	Chicken Egg Farms	Chicken Meat Farms	Duck Farms	Goose Farms	Chickens (egg layers)	Chickens (broilers)	Ducks	Geese	Egg type grand-parent stock stations(pair)	Meat type grand-parent stock stations(pair)	Breeder duck stations (piece)	Breeder goose stations (piece)
河 南	Henan	150	99	37	10	4	2033541	3476156	546000	14100	1910700	6700000		
湖 北	Hubei	65	23	13	14	15	254950	392200	131828	100462			1105110	297180
湖 南	Hunan	15		10	2	3		96000	50000	2100	2130	11520000	8653846	63000
广 东	Guangdong	631	11	122	226	272	486850	6842307	918150	330521				
广 西	Guangxi	91	2	83	3	3	39000	2919792	900862	4790			669005	36548
海 南	Hainan	79	2	43	33	1	11200	262500	126100	2000		3000000	10581200	15000
重 庆	Chongqing	97	24	15	47	11	228343	284010	199603	8251	60500		12562210	85406
四 川	Sichuan	253	36	49	147	21	909050	926459	630188	46205		2710000	51373500	341815
贵 州	Guizhou	8	2	5	1		6000	14500	2600			100000	468000	
云 南	Yunnan	17	7	7	2	1	203000	152735	2670	13000				
西 藏	Xizang	2	1	1			4723	21450						
陕 西	Shanxi	110	95	12	3		562744	268200	11800		27500		98000	
甘 肃	Gansu	20	18	2			38225	36221						
青 海	Qinghai	1	1											
宁 夏	Ningxia	11	8	3			341650	29000						
新 疆	Xinjiang	20	8	12			102800	59500						

Table B.3. Number of counties and prefectures for which poultry statistics were available from China yearbooks for 2003, 2004, and 2005.

Province	County Data													Prefecture Data												
	#counties	#sold			#residual			poultry meat (ton)			poultry egg (ton)			#prefecture	#sold			#residual			poultry meat (ton)			poultry egg (ton)		
		2005	2004	2003	2005	2004	2003	2005	2004	2003	2005	2004	2003		2005	2004	2003	2005	2004	2003	2005	2004	2003	2005	2004	2003
Beijing	11	0	11	0	11	11	0	11	11	0	11	11	0	1	0	1	1	1	1	1	1	1	1	1	1	1
Tianjin	13	13	13	13	13	13	13	13	13	13	13	13	13	0	0	0	0	0	0	0	0	0	0	0	0	0
Hebei	144	0	0	0	0	0	0	0	0	0	140	140	140	11	11	11	11	11	11	11	11	11	11	11	11	11
Shanxi North (山西)	107	0	0	0	0	0	0	0	0	0	107	107	107	0	0	0	0	0	0	0	0	0	0	0	0	0
Neimenggu (Inner Mongolia)	89	0	88	85	0	0	0	0	87	85	0	87	86	12	12	12	12	0	0	0	12	12	12	12	12	12
Liaoning	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	14	14	14
Jilin	46	0	0	0	0	0	0	0	0	0	46	46	46	0	0	0	0	0	0	0	0	0	0	0	0	0
Heilongjiang	0	0	0	0	0	0	0	0	0	0	0	0	0	13	13	13	13	0	0	0	13	13	13	13	13	13
Shanghai	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1	1
Jiangsu	72	72	72	72	72	72	72	72	72	72	0	0	0	13	13	13	13	13	13	13	13	13	13	0	0	0
Zhejiang	69	67	67	68	67	67	68	67	67	66	63	67	66	0	0	0	0	0	0	0	0	0	0	0	0	0
Anhui	78	78	78	78	78	78	0	78	78	0	78	78	78	17	17	17	17	17	17	17	17	17	0	17	17	17
Fujian	70	70	70	70	70	70	70	70	70	70	70	70	70	0	0	0	0	0	0	0	0	0	0	0	0	0
Jiangxi	88	0	0	88	0	0	88	0	0	88	79	79	88	11	11	11	11	11	11	11	11	11	11	11	11	11
Shandong	0	0	0	0	0	0	0	0	0	0	0	0	0	17	17	17	17	17	17	17	17	17	17	17	17	17
Henan	125	0	0	0	0	124	124	0	108	124	124	124	124	18	18	18	18	18	18	18	0	18	18	18	18	18
Hubei	78	78	78	78	0	0	0	78	78	78	78	78	78	0	0	0	0	0	0	0	0	0	0	0	0	0
Hunan	102	102	102	102	102	102	102	102	102	102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Guangdong	94	94	94	94	94	94	94	94	94	94	94	94	94	0	0	0	0	0	0	0	0	0	0	0	0	0
Guangxi	91	0	0	0	0	0	0	0	0	0	84	85	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hainan	18	18	18	18	0	0	0	18	18	18	18	18	18	0	0	0	0	0	0	0	0	0	0	0	0	0
Chongqing	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	1	1	1	0	0	0
Sichuan	0	0	0	0	0	0	0	0	0	0	0	0	0	21	21	21	21	0	0	0	21	21	21	21	21	21
Guizhou	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	9	9	9
Yunnan	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	16	16	16
Xizang	0	0	0	0	0	0	0	0	0	0	0	0	0	7	7	7	7	0	0	0	0	0	0	7	7	7
Shanxi West (陕西)	96	0	0	0	96	0	0	0	0	0	96	96	96	10	0	0	0	10	10	10	0	0	0	10	10	10
Gansu	81	0	0	0	0	0	0	81	81	81	81	81	81	0	0	0	0	0	0	0	0	0	0	0	0	0
Qinghai	41	0	0	0	0	0	0	0	0	0	23	23	0	8	5	6	5	0	0	0	0	0	0	7	7	6
Ningxia	18	0	0	0	18	18	18	0	0	0	18	18	18	0	0	0	0	0	0	0	0	0	0	0	0	0
Xinjiang	87	0	0	0	0	0	0	86	85	81	0	0	0	15	0	0	0	0	0	0	15	15	15	15	15	15

APPENDIX C.

Table C.1. China Land Cover dataset created by the Chinese Academy of Sciences (CAS) Institute of Geographical Science and Natural Resources Research (IGSNRR) from 30m Landsat TM satellite imagery (Liu et al. 2002, Liu et al. 2005).

Broad Category	Code	Land Cover Class
Arable Land	11	Paddy
	12	Rainfed
Forest	21	Forest
	22	Scrub
	23	Shrub
	24	Other Forest
Grassland	31	Grassland (>50%)
	32	Grassland (20-50%)
	33	Grassland (5-20%)
Water	41	River and Irrigation
	42	Lake
	43	Reservoir and Pool
	44	Snow Capped
	45	Shores (Sea)
	46	Bank/Shoal (River/Lake)
Developed	51	Urban
	52	Rural Residence
	53	Other Constructed
Pristine	61	Sand
	62	Gobi
	63	Salt Lick
	64	Marsh
	65	Bare Ground
	66	Gravel and Rocky Ground
	67	Other

Literature Cited

- Alexander, D. J. 2000. A review of avian influenza in different bird species. *Veterinary Microbiology* 74:3-13.
- _____. 2003. Report on avian influenza in the Eastern Hemisphere during 1997-2002. *Avian Diseases* 47:792-797.
- _____. 2007. An overview of the epidemiology of avian influenza. *Vaccine* 25:5637-5644.
- Alexander, D. J., and I. Capua. 2008. Avian influenza in poultry. *Worlds Poultry Science Journal* 64:513-531.
- Augustin, N. H., M. A. Muggleston, and S. T. Buckland. 1996. An autologistic model for the spatial distribution of wildlife. *Journal Of Applied Ecology* 33:339-347.
- Austin, M. 2007. Species distribution models and ecological theory: A critical assessment and some possible new approaches. *Ecological Modelling* 200:1-19.
- Batbayar, N., J. Y. Takekawa, S. H. Newman, D. J. Prosser, T. Natsagdorj, and X. Xiao. 2011. Migration Strategies of Swan Geese *Anser cygnoides* from Northeast Mongolia. *Wildfowl* 61:90-109.
- Becker, W. B. 1966. Isolation and classification of tern virus - Influenza virus A/tern/South Africa/1961. *Journal of Hygiene-Cambridge* 64:309-320.
- Boender, G. J., T. J. Hagenaars, A. Bouma, G. Nodelijk, A. R. W. Elbers, M. C. M. de Jong, and M. van Boven. 2007. Risk Maps for the Spread of Highly Pathogenic Avian Influenza in Poultry. *PLoS Computational Biology* 3:e71.
- Boere, G. C., C. A. Galbraith, and D. A. Stroud. 2006. *Waterbirds Around the World*. The Stationary Office, Edinburgh, UK.
- Bonn, D. 2006. Wild birds, poultry, and avian influenza. *Lancet Infectious Diseases* 6:262-262.
- Boyle, T. P., S. M. Caziani, and R. G. Waltermire. 2004. Landsat TM inventory and assessment of waterbird habitat in the southern altiplano of South America. *Wetlands Ecology and Management* 12:563-573.
- Brewer, C. A. 2006. Basic Mapping Principles for Visualizing Cancer Data Using Geographic Information Systems (GIS). *American journal of preventive medicine* 30:S25-S36.
- Brewer, C. A., and L. Pickle. 2002. Evaluation of Methods for Classifying Epidemiological Data on Choropleth Maps in Series. *Annals of the Association of American Geographers* 92:662-681.
- Brotons, L., W. Thuiller, M. B. Araujo, and A. H. Hirzel. 2004. Presence-absence versus presence-only modelling methods for predicting bird habitat suitability. *Ecography* 27:437-448.
- Brown, C. 2004. Emerging Zoonoses and Pathogens of Public Health Significance -- An Overview. *Rev Sci Tech* 23:435-442.
- Brown, J. D., D. E. Stallknecht, and D. E. Swayne. 2008. Experimental infection of swans and geese with highly pathogenic avian influenza virus (H5N1) of Asian lineage. *Emerging Infectious Diseases* 14:136-142.
- Brown, J. D., D. E. Stallknecht, S. Valeika, and D. E. Swayne. 2007a. Susceptibility of wood ducks to H5N1 highly pathogenic avian influenza virus. *Journal of Wildlife Diseases* 43:660-667.

- Brown, J. D., D. E. Swayne, R. J. Cooper, R. E. Burns, and D. E. Stallknecht. 2007b. Persistence of H5 and H7 avian influenza viruses in water. *Avian Diseases* 51:285-289.
- Buranathai, C., A. Amonsin, A. Chaisigh, A. Theamboonlers, N. Pariyothorn, and Y. Poovorawan. 2007. Surveillance activities and molecular analysis of H5N1 highly pathogenic avian influenza viruses from Thailand, 2004-2005. *Avian Diseases* 51:194-200.
- Burmaster, D. E., and P. D. Anderson. 1994. Principles of Good Practice for the Use of Monte Carlo Techniques in Human Health and Ecological Risk Assessments. *Risk Analysis* 14:477-481.
- Burnham, K. P., and D. R. Anderson. 2002a. Information and likelihood theory: A basis for model selection and inference. Pages 49-79 *in* Model selection and multimodel inference: A practical approach. Springer-Verlag, New York, New York.
- _____. 2002b. Model Selection and Multimodel Inference: A Practical-Theoretic Approach. 2nd edition. Springer-Verlag.
- _____. 2002c. Model selection and multimodel inference: a practical information-theoretic approach. Volume Second Edition. Springer Science and Business Media, Inc., New York.
- Butler, D. 2006. Doubts hang over source of bird flu spread. *Nature* 439:772.
- Cao, L., M. Barter, and G. Lei. 2008. New Anatidae population estimates for eastern China: Implications for current flyway estimates. *Biological Conservation* 141:2301-2309.
- Cao, L., Y. Zhang, M. Barter, and G. Lei. 2010. Anatidae in eastern China during the non-breeding season: Geographical distributions and protection status. *Biological Conservation* 143:650-659.
- Cardona, C. J., Z. Xing, C. E. Sandrock, and C. E. Davis. 2009. Avian influenza in birds and mammals. *Comparative Immunology Microbiology and Infectious Diseases* 32:255-273.
- Chen, H., G. Deng, Z. Li, G. Tian, Y. Li, P. Jiao, L. Zhang, Z. Liu, R. G. Webster, and K. Yu. 2004. The evolution of H5N1 influenza viruses in ducks in southern China. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 101:10452-10457.
- Chen, H., G. J. Smith, S. Y. Zhang, K. Qin, J. Wang, K. S. Li, R. G. Webster, J. S. Peiris, and Y. Guan. 2005. Avian flu: H5N1 virus outbreak in migratory waterfowl. *Nature* 436:191-192.
- Chen, H., G. J. D. Smith, K. S. Li, J. Wang, X. H. Fan, J. M. Rayner, D. Vijaykrishna, J. X. Zhang, L. J. Zhang, C. T. Guo, C. L. Cheung, K. M. Xu, L. Duan, K. Huang, K. Qin, Y. H. C. Leung, W. L. Wu, H. R. Lu, Y. Chen, N. S. Xia, T. S. P. Naipospos, K. Y. Yuen, S. S. Hassan, S. Bahri, T. D. Nguyen, R. G. Webster, J. S. M. Peiris, and Y. Guan. 2006a. Establishment of multiple sublineages of H5N1 influenza virus in Asia: Implications for pandemic control. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 103:2845-2850.
- Chen, H. L., Y. B. Li, Z. J. Li, J. Z. Shi, K. Shinya, G. H. Deng, Q. L. Qi, G. B. Tian, S. F. Fan, H. D. Zhao, Y. X. Sun, and Y. Kawaoka. 2006b. Properties and

- dissemination of H5N1 viruses isolated during an influenza outbreak in migratory waterfowl in western China. *Journal Of Virology* 80:5976-5983.
- China National Bureau of Statistics. 2007. Rural Statistical Yearbook. China Statistics Press, Beijing.
- _____. 2008. China 2006 National Agricultural Census data synthesis summary. China Statistics Press, Beijing.
- Claas, E. C. J., A. Osterhaus, R. van Beek, J. C. De Jong, G. F. Rimmelzwaan, D. A. Senne, S. Krauss, K. F. Shortridge, and R. G. Webster. 1998. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. *Lancet* 351:472-477.
- Clark, L., and J. Hall. 2006. Avian influenza in wild birds: Status as reservoirs, and risks to humans and agriculture. *Ornithological Monographs*:3-29.
- Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 356:991-999.
- Colwell, R., P. Epstein, D. Gubler, M. Hall, P. Reiter, J. Shukla, W. Sprigg, E. Takafuji, and J. Trtanj. 1998. Global climate change and infectious diseases. *Emerging Infectious Diseases* 4:451-452.
- Crook, F. W. 1993. China's grain production economy: a review by regions. *in* E. R. S. US Department of Agriculture, editor. China, International Agriculture and Trade Reports. Washington, DC.
- Csuti, B., and P. Crist. 2000. Methods for developing terrestrial vertebrate distribution maps for Gap Analysis. Pages <http://www.gap.uidaho.edu/handbook/VertebrateDistributionModeling/history.htm> *in* A handbook for conducting Gap analysis. USGS Gap Analysis Program, Moscow, ID.
- Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Wildlife ecology - Emerging infectious diseases of wildlife - Threats to biodiversity and human health. *Science* 287:443-449.
- de Smith, M. J., M. F. Goodchild, and P. A. Longley. 2007. Geospatial Analysis: A Comprehensive Guide to Principles, Techniques, and Software Tools. Matador, Leicester, United Kingdom.
- Defries, R. S., and A. S. Belward. 2000. Global and regional land cover characterization from satellite data: an introduction to the Special Issue. *International Journal Of Remote Sensing* 21:1083-1092.
- Deichmann, U., D. Balk, and G. Yetman. 2001. Transforming population data for interdisciplinary usages: From census to grid.
- Delany, S., and D. Scott. 2002. Waterbird Population Estimates: Third Edition. Third edition. Wetlands International.
- _____. 2006. Waterbird Population Estimates: Fourth Edition. Fourth edition. Wetlands International, Wageningen, The Netherlands.
- Delgado, C. 2003. Rising Consumption of Meat and Milk in Developing Countries Has Created a New Food Revolution. *American Society for Nutritional Sciences* 133:3907S-3910S.

- Dobson, J. E., E. A. Bright, P. R. Coleman, R. C. Durfee, and B. A. Worley. 2000. LandScan: A global population database for estimating populations at risk. *Photogrammetric Engineering And Remote Sensing* 66:849-857.
- Edwards, T. C., E. T. Deshler, D. Foster, and G. G. Moisen. 1996. Adequacy of wildlife habitat relation models for estimating spatial distributions of terrestrial vertebrates. *Conservation Biology* 10:263-270.
- Elith, J., C. H. Graham, R. P. Anderson, M. Dudik, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, J. R. Leathwick, A. Lehmann, J. Li, L. G. Lohmann, B. A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, J. M. Overton, A. T. Peterson, S. J. Phillips, K. Richardson, R. Scachetti-Pereira, R. E. Schapire, J. Soberon, S. Williams, M. S. Wisz, and N. E. Zimmermann. 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* 29:129-151.
- Elith, J., and J. Leathwick. 2009. The Contribution of Species Distribution Modelling to Conservation Prioritization. Pages 70-93 in A. Moilanen, K. A. Wilson, and H. P. Possingham, editors. *Spatial Conservation Prioritization: Quantitative methods and computational tools*. Oxford University.
- Elith, J., J. R. Leathwick, and T. Hastie. 2008. A working guide to boosted regression trees. *Journal Of Animal Ecology* 77:802-813.
- Elith, J., S. J. Phillips, T. Hastie, M. Dudik, Y. E. Chee, and C. J. Yates. 2011. A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions* 17:43-57.
- Ellis, T. M., R. B. Bousfield, L. A. Bissett, K. C. Dyrting, G. S. Luk, S. T. Tsim, K. Sturm-Ramirez, R. G. Webster, Y. Guan, and J. S. Malik Peiris. 2004. Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. *Avian Pathology* 33:492-505.
- Fang, L.-Q., S. J. de Vlas, S. Liang, C. W. N. Looman, P. Gong, B. Xu, L. Yan, H. Yang, J. H. Richardus, and W.-C. Cao. 2008. Environmental Factors Contributing to the Spread of H5N1 Avian Influenza in Mainland China. *PLoS ONE* 3:e2268.
- FAO. 2004. Recommendations on the Prevention, Control, and Eradication of Highly Pathogenic Avian Influenza (HPAI) in Asia, September 2004. 85 pp.
- _____. 2007a. Gridded Livestock of the World 2007.
- _____. 2007b. Strategy for prevention and preparedness for avian influenza pandemic in Mongolia 2007 - 2009: Animal health component.
- _____. 2007c. Wild Birds and Avian Influenza: an introduction to applied field research and disease sampling techniques.
- _____. 2008. Biosecurity for Highly Pathogenic Avian Influenza. *FAO Animal Production and Health* (165):80pp.
- _____. 2010. Distribution and Characteristics of Duck-Fish Farming Systems in Eastern China, by Miao Weimin. *FAO Smallholder Poultry Production Paper No. 2*. Rome.
- _____. 2011a. Current evolution of avian influenza H5N1 viruses.
- _____. 2011b. Fourth Report on the Global Programme for the Prevention and Control of HPAI (January-December 2010).

- FAO, and OIE. 2005. A Global Strategy for the Progressive Control of Highly Pathogenic Avian Influenza (HPAI). 85 pp.
- Farr, T. G., P. A. Rosen, E. Caro, R. Crippen, R. Duren, S. Hensley, M. Kobrick, M. Paller, E. Rodriguez, L. Roth, D. Seal, S. Shaffer, J. Shimada, J. Umland, M. Werner, M. Oskin, D. Burbank, and D. Alsdorf. 2007. The shuttle radar topography mission. *Reviews of Geophysics* 45:33.
- Feare, C. J. 2006. Fish Farming and the Risk of Spread of Avian Influenza. Unpublished Report, BirdLife International. 13pp.
- _____. 2007. The role of wild birds in the spread of HPAI H5N1. *Avian Diseases* 51:440-447.
- _____. 2010. Role of Wild Birds in the Spread of Highly Pathogenic Avian Influenza Virus H5N1 and Implications for Global Surveillance. *Avian Diseases* 54:201-212.
- Fergus, R., M. Fry, W. B. Karesh, P. P. Marra, S. Newman, and E. Paul. 2006. Migratory birds and avian flu. *Science* 312:845-846.
- Ferguson, N. M., D. A. T. Cummings, C. Fraser, J. C. Cajka, P. C. Cooley, and D. S. Burke. 2006. Strategies for mitigating an influenza pandemic. *Nature* 442:448-452.
- Fouchier, R. A. M., T. M. Bestebroer, S. Herfst, L. Van der Kemp, G. F. Rimmelzwaan, and A. Osterhaus. 2000. Detection of influenza A viruses from different species by PCR amplification of conserved sequences in the matrix gene. *Journal of Clinical Microbiology* 38:4096-4101.
- Fouchier, R. A. M., V. J. Munster, J. Keawcharoen, A. Osterhaus, and T. Kuiken. 2007. Virology of avian influenza in relation to wild birds. *Journal of Wildlife Diseases* 43:S7-S14.
- Franklin, J., and J. A. Miller. 2010. Mapping Species Distributions: Spatial Inference and Prediction. Cambridge University Press.
- Gaidet, N., J. Cappelle, J. Y. Takekawa, D. J. Prosser, S. A. Iverson, D. C. Douglas, W. M. Perry, T. Mundkur, and S. Newman. 2010. Potential spread of highly pathogenic avian influenza H5N1 by wildfowl: Dispersal ranges and rates determined from large-scale satellite telemetry. *Journal Of Applied Ecology*:1147-1157.
- Gaidet, N., G. Cattoli, S. Hammoui, S. Newman, W. Hagemeijer, J. Y. Takekawa, J. Cappelle, T. Dodman, T. Joannis, P. Gil, I. Monne, A. Fusaro, I. Capua, S. Manu, P. Micheloni, U. Ottosson, J. H. Mshelbwala, J. Lubroth, J. Domenech, and F. Monicat. 2008. Evidence of infection by H5N2 highly pathogenic avian influenza viruses in healthy wild waterfowl. *Plos Pathogens* 4:e1000127.
- Gaidet, N., T. Dodman, A. Caron, G. Balanca, S. Desvaux, F. Goutard, G. Cattoli, F. Lamarque, W. Hagemeijer, and F. Monicat. 2007a. Avian influenza viruses in water birds, Africa. *Emerging Infectious Diseases* 13:626-629.
- Gaidet, N., T. Dodman, A. Caron, G. Balanca, S. Desvaux, F. Goutard, G. Cattoli, V. Martin, A. Tripodi, F. Lamarque, W. Hagemeijer, and F. Monicat. 2007b. Influenza surveillance in wild birds in eastern europe, the middle east, and africa: Preliminary results from an ongoing FAO-led survey. *Journal of Wildlife Diseases* 43:S22-S28.

- Gauthier-Clerc, M., C. Lebarbenchon, and F. Thomas. 2007. Recent expansion of highly pathogenic avian influenza H5N1: a critical review. *Ibis* 149:202-214.
- Gilbert, M., P. Chaitaweesub, T. Parakarnawongsa, S. Premashthira, T. Tiensin, W. Kalpravidh, H. Wagner, and J. Slingenbergh. 2006a. Free-grazing ducks and highly pathogenic avian influenza, Thailand. *Emerging Infectious Diseases* 12:227-234.
- Gilbert, M., S. Newman, J. Y. Takekawa, L. Loth, C. Biradar, D. J. Prosser, S. Balachandran, M. V. Subba Rao, T. Mundkur, B. P. Yan, Z. Xing, Y. S. Hou, N. Batbayar, T. Natsagdorj, J. B. Hogerwerf, J. Slingenbergh, and X. Xiao. 2011. Flying Over an Infected Landscape: Distribution of Highly Pathogenic Avian Influenza H5N1 Risk in South Asia and Satellite Tracking of Wild Waterfowl. *EcoHealth*:1-11.
- Gilbert, M., and D. U. Pfeiffer. 2012. *In Press*. Risk factor modelling of the spatio-temporal patterns of highly pathogenic avian influenza (HPAIV) H5N1: A review. *Spatial and Spatio-temporal Epidemiology*.
- Gilbert, M., X. M. Xiao, P. Chaitaweesub, W. Kalpravidh, S. Premashthira, S. Boles, and J. Slingenbergh. 2007. Avian influenza, domestic ducks and rice agriculture in Thailand. *Agriculture Ecosystems & Environment* 119:409-415.
- Gilbert, M., X. M. Xiao, J. Domenech, J. Lubroth, V. Martin, and J. Slingenbergh. 2006b. Anatidae migration in the western palearctic and spread of highly pathogenic avian influenza H5N1 virus. *Emerging Infectious Diseases* 12:1650-1656.
- Gilbert, M., X. M. Xiao, D. U. Pfeiffer, M. Epprecht, S. Boles, C. Czarnecki, P. Chaitaweesub, W. Kalpravidh, P. Q. Minh, M. J. Otte, V. Martin, and J. Slingenbergh. 2008. Mapping H5N1 highly pathogenic avian influenza risk in Southeast Asia. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 105:4769-4774.
- Globig, A., E. Starick, and O. Werner. 2006. Isolation of avian influenza from migratory waterfowl in Germany. Results of a two year study. *Berliner Und Munchener Tierarztliche Wochenschrift* 119:132-139.
- Gordis, L. 2004. *Epidemiology*. Third Edition edition. Elsevier Saunders, Philadelphia.
- Gottschalk, T. K., F. Huettmann, and M. Ehlers. 2005. Thirty years of analysing and modelling avian habitat relationships using satellite imagery data: a review. *International Journal Of Remote Sensing* 26:2631-2656.
- Goutard, F., F. Roger, J. Guitian, G. Balanca, K. Argaw, A. Demissie, V. Soti, V. Martin, and D. Pfeiffer. 2007. Conceptual framework for avian influenza risk assessment in Africa: The case of Ethiopia. *Avian Diseases* 51:504-506.
- Grenyer, R., C. D. L. Orme, S. F. Jackson, G. H. Thomas, R. G. Davies, T. J. Davies, K. E. Jones, V. A. Olson, R. S. Ridgely, P. C. Rasmussen, T.-S. Ding, P. M. Bennett, T. M. Blackburn, K. J. Gaston, J. L. Gittleman, and I. P. F. Owens. 2006. Global distribution and conservation of rare and threatened vertebrates. *Nature* 444:93-96.
- Guan, Y., J. S. M. Peiris, A. S. Lipatov, T. M. Ellis, K. C. Dyrting, S. Krauss, L. J. Zhang, R. G. Webster, and K. F. Shortridge. 2002. Emergence of multiple genotypes of H5N1 avian influenza viruses in Hong Kong SAR. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 99:8950-8955.

- Guisan, A., and W. Thuiller. 2005. Predicting species distribution: offering more than simple habitat models. *Ecology Letters* 8:993-1009.
- Guo, Y., X. Xu, and X. Wan. 1998. Genetic characterization of an avian influenza A (H5N1) virus isolated from a sick goose in China. *Zhonghua Shi Xan He Lin Chaung Bing Du Xue Za Zhi* 12:322-325 (in Chinese).
- Hay, S. I., S. E. Randolph, and D. J. Rogers. 2000. Remote sensing and geographical information systems for epidemiology. Academic Press, San Diego.
- Hay, S. I., A. J. Tatem, A. J. Graham, S. J. Goetz, and D. J. Rogers. 2006. Global environmental data for mapping infectious disease distribution. *Adv Parasit* 62:37-77.
- Hernandez, P. A., C. H. Graham, L. L. Master, and D. L. Albert. 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography* 29:773-785.
- Hesterberg, U., K. Harris, D. Stroud, V. Guberti, L. Busani, M. Pittman, V. Piazza, A. Cook, and I. Brown. 2009. Avian influenza surveillance in wild birds in the European Union in 2006. *Influenza and Other Respiratory Viruses* 3:1-14.
- Horimoto, T., E. Rivera, J. Pearson, D. Senne, S. Krauss, Y. Kawaoka, and R. G. Webster. 1995. Origin and Molecular Changes Associated with Emergence of a Highly Pathogenic H5N2 Influenza Virus in Mexico. *Virology* 213:223-230.
- Hou, Y. Qinghai Lake National Nature Reserve ornithologist.
- Hulse-Post, D. J., K. M. Sturm-Ramirez, J. Humberd, P. Seiler, E. A. Govorkova, S. Krauss, C. Scholtissek, P. Puthavathana, C. Buranathai, T. D. Nguyen, H. T. Long, T. S. Naipospos, H. Chen, T. M. Ellis, Y. Guan, J. S. Peiris, and R. G. Webster. 2005. Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 102:10682-10687.
- Hurlbert, A. H., and W. Jetz. 2007. Species richness, hotspots, and the scale dependence of range maps in ecology and conservation. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 104:13384-13389.
- Janies, D., A. W. Hill, R. Guralnick, F. Habib, E. Waltari, and W. C. Wheeler. 2007. Genomic analysis and geographic visualization of the spread of avian influenza (H5N1). *Systematic Biology* 56:321-329.
- Jeong, O. M., M. C. Kim, M. J. Kim, H. M. Kang, H. R. Kim, Y. J. Kim, S. J. Joh, J. H. Kwon, and Y. J. Lee. 2009. Experimental infection of chickens, ducks and quails with the highly pathogenic H5N1 avian influenza virus. *Journal of Veterinary Science* 10:53-60.
- Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. *Nature* 451:990-U994.
- Kalthoff, D., A. Breithaupt, J. P. Teifke, A. Globig, T. Harder, T. C. Mettenleiter, and M. Beer. 2008. Highly pathogenic avian influenza virus (H5N1) in experimentally infected adult mute swans. *Emerging Infectious Diseases* 14:1267-1270.
- Kear, J. 2005. Ducks, Geese, and Swans. Volume 1: General Chapters and Species Accounts (*Anhima to Salvadorina*). Volume Volume 1: General Chapters and Species Accounts (*Anhima to Salvadorina*). Oxford University Press, New York.

- Keawcharoen, J., D. van Riel, G. van Amerongen, T. Bestebroer, W. E. Beyer, R. van Lavieren, A. Osterhaus, R. A. M. Fouchier, and T. Kuiken. 2008. Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). *Emerging Infectious Diseases* 14:600-607.
- Kilpatrick, A. M., A. A. Chmura, D. W. Gibbons, R. C. Fleischer, P. P. Marra, and P. Daszak. 2006. Predicting the global spread of H5N1 avian influenza. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 103:19368-19373.
- Kim, J. K., P. Seiler, H. L. Forrest, A. M. Khalenkov, J. Franks, M. Kumar, W. B. Karesh, M. Gilbert, R. Sodnomdarjaa, B. Douangngeun, E. A. Govorkova, and R. G. Webster. 2008. Pathogenicity and Vaccine Efficacy of Different Clades of Asian H5N1 Avian Influenza A Viruses in Domestic Ducks. *Journal Of Virology* 82:11374-11382.
- Kou, Z., Y. Li, Z. Yin, S. Guo, M. Wang, X. Gao, P. Li, L. Tang, P. Jiang, Z. Luo, Z. Xin, C. Ding, Y. He, Z. Ren, P. Cui, H. Zhao, Z. Zhang, S. Tang, B. Yan, F. Lei, and T. Li. 2009. The survey of H5N1 flu virus in wild birds in 14 Provinces of China from 2004 to 2007. *PLoS ONE* 4:e6926.
- Kovats, R. S., D. H. Campbell-Lendrum, A. J. McMichel, A. Woodward, and J. S. H. Cox. 2001. Early effects of climate change: do they include changes in vector-borne disease? *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 356:1057-1068.
- Kroese, D. P., T. Taimre, and Z. I. Botev. 2010. *Handbook of Monte Carlo Methods*. John Wiley & Sons, Inc., Hoboken, New Jersey.
- L'Vov D, K., M. Shchelkanov, P. G. Deriabin, T. V. Grebennikova, A. G. Prilipov, E. A. Nepoklonov, G. G. Onishchenko, N. A. Vlasov, T. I. Aliper, A. D. Zaberezhnyi, D. E. Kireev, O. P. Krasheninnikov, S. T. Kiriukhin, E. I. Burtseva, and A. N. Slepishkin. 2006. Isolation of influenza A/H5N1 virus strains from poultry and wild birds in West Siberia during epizooty (July 2005) and their depositing to the state collection of viruses (August 8, 2005). *Vopr Virusol* 51:11-14.
- Lang, A. S., A. Kelly, and J. A. Runstadler. 2008. Prevalence and diversity of avian influenza viruses in environmental reservoirs. *Journal Of General Virology* 89:509-519.
- Lebarbenchon, C., F. Albespy, A.-L. Brochet, V. Grandhomme, F. o. Renaud, H. Fritz, A. J. Green, F. Thomas, S. van der Werf, P. Aubry, M. Guillemain, and M. Gauthier-Clerc. 2009. Spread of Avian Influenza Viruses by Common Teal (*Anas crecca*) in Europe. *PLoS ONE* 4:e7289.
- Lei, F. M., P. Cui, and C. L. Zhang. 2011. Avian Influenza H5N1 Threats Wild Bird Conservation and Eco-health - Qinghai Lake Case Report. *EcoHealth* 7:S131-S131.
- Li, K. S., Y. Guan, J. Wang, G. J. Smith, K. M. Xu, L. Duan, A. P. Rahardjo, P. Puthavathana, C. Buranathai, T. D. Nguyen, A. T. Estoepangestie, A. Chaisingh, P. Auewarakul, H. T. Long, N. T. Hanh, R. J. Webby, L. L. Poon, H. Chen, K. F. Shortridge, K. Y. Yuen, R. G. Webster, and J. S. Peiris. 2004. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature* 430:209-213.

- Li, L. 2001a. Survey results and new records of waterfowl at Qinghai Lake. *China Crane News* 5:36-37.
- Li, W. 2001b. *Agro-Ecological Farming Systems in China*. Man and the Biosphere Series 26. UNESCO. 454p.
- Li, Y. B., J. Z. Shi, G. X. Zhong, G. H. Deng, G. B. Tian, J. Y. Ge, X. Y. Zeng, J. S. Song, D. M. Zhao, L. L. Liu, Y. P. Jiang, Y. T. Guan, Z. G. Bu, and H. L. Chen. 2010. Continued Evolution of H5N1 Influenza Viruses in Wild Birds, Domestic Poultry, and Humans in China from 2004 to 2009. *Journal Of Virology* 84:8389-8397.
- Li, Z. W. D., A. Bloem, S. Delany, G. Martakis, and J. O. Quintero. 2009. Status of waterbirds in Asia - Results of the Asian Waterbird Census: 1987-2007. Wetlands International, Kuala Lumpur, Malaysia.
- Liu, J., M. Liu, X. Deng, D. Zhuang, Z. Zhang, and D. Luo. 2002. The land use and land cover change database and its relative studies in China. *Journal of Geographical Sciences* 12:275-282.
- Liu, J., H. Xiao, F. Lei, Q. Zhu, K. Qin, X. W. Zhang, X. L. Zhang, D. Zhao, G. Wang, Y. Feng, J. Ma, W. Liu, J. Wang, and G. F. Gao. 2005. Highly pathogenic H5N1 influenza virus infection in migratory birds. *Science* 309:1206.
- Liu, R., V. R. S. K. Duvvuri, and J. Wu. 2008. Spread Pattern Formation of H5N1 Avian Influenza and its Implications for Control Strategies. *Mathematical Modelling of Natural Phenomena* 3:161-179.
- Lopez, A., and T. Mundkur. 1997. Asian Waterbird Census 1994-1996: Results of the Coordinated Waterbird Census and an Overview of the Status of Wetlands in Asia. Wetlands International, Kuala Lumpur.
- Lu, P. S. 2006. Early diagnosis of avian influenza. *Science* 312:337.
- Lu, X., and M. Jiang. 2004. Progress and prospect of wetland research in China. *Journal of Geographical Sciences* 14:45-51.
- Macdonald, D. W., and M. K. Laurenson. 2006. Infectious disease: Inextricable linkages between human and ecosystem health. *Biological Conservation* 131:143-150.
- Mackinnon, J., and K. Phillipps. 2000. *A Field Guide to the Birds of China*. Oxford University Press Inc., New York.
- Makarova, N. V., H. Ozaki, H. Kida, R. G. Webster, and D. R. Perez. 2003. Replication and transmission of influenza viruses in Japanese quail. *Virology* 310:8-15.
- Martin, L. J., B. Blossey, and E. Ellis. 2012. Mapping where ecologists work: biases in the global distribution of terrestrial ecological observations. *Frontiers in Ecology and the Environment* 10:195-201.
- Martin, V., D. Pfeiffer, X. Zhou, X. Xiao, D. J. Prosser, F. Guo, and M. Gilbert. 2011. Spatial Distribution and Risk Factors of Highly Pathogenic Avian Influenza (HPAI) H5N1 in China. *Plos Pathogens* 7:e1001308.
- McCallum, H., N. Barlow, and J. Hone. 2001. How should pathogen transmission be modelled? *Trends In Ecology & Evolution* 16:295-300.
- McMichael, A. J. 2003. Global climate change: will it affect vector-borne infectious diseases? *Internal Medicine Journal* 33:554-U552.
- McMichael, A. J., R. E. Woodruff, and S. Hales. 2006. Climate change and human health: present and future risks. *The Lancet* 367:859-869.

- Melville, D. S., and K. F. Shortridge. 2006. Spread of H5N1 avian influenza virus: an ecological conundrum. *Letters In Applied Microbiology* 42:435-437.
- Meyer De Schauensee, R. 1984. *The Birds of China*. Smithsonian Institution Press, Washington, DC.
- Miller, J. 2010. Species Distribution Modeling. *Geography Compass* 4:490-509.
- Miyabayashi, Y. 2003. Conservation efforts under the Anatidae Action Plan in the East Asian Flyway: a progress report for 1999-2003. *in* *Proceedings of First Meeting of AWC Coordinators*. DOC 8.
- Miyabayashi, Y., and T. Mundkur. 1999a. Atlas of key sites for Anatidae in the East Asian flyway. *in* *Wetlands International - Japan, Tokyo, and Wetlands International - Asia Pacific*, Kuala Lumpur. 148pp. Web publication: <http://www.jawgp.org/anet/aaa1999>.
- _____. 1999b. Atlas of Key Sites for Anatidae in the East Asian Flyway. *Wetlands International*.
- Morgan, M. G., and M. Henrion. 1990. *Uncertainty: A guide to dealing with uncertainty in quantitative risk and policy analysis*. Cambridge University Press, New York, New York.
- Morrison, M. L., B. G. Marcot, and R. W. Mannan. 2006. *Wildlife-habitat Relationships: Concepts and Applications*. Third Edition. University of Wisconsin Press, Madison, Wisconsin.
- Morse, S. S. 1995. Factors in the Emergence of Infectious Diseases. *Emerging Infectious Diseases* 1:7 - 15.
- Mukhtar, M. M., S. T. Rasool, D. G. Song, C. L. Zhu, Q. Hao, Y. Zhu, and J. G. Wu. 2007. Origin of highly pathogenic H5N1 avian influenza virus in China and genetic characterization of donor and recipient viruses. *Journal Of General Virology* 88:3094-3099.
- Mundkur, T. 2006. East Asia-Pacific Flyway: Workshop Introduction. *in* G. C. Boere, C. A. Galbraith, and D. A. Stroud, editors. *Waterbirds around the world*. The Stationary Office, Edinburgh, UK. p 315.
- Munster, V. J., C. Baas, P. Lexmond, J. Waldenstrom, A. Wallensten, T. Fransson, G. F. Rimmelzwaan, W. E. P. Beyer, M. Schutten, B. Olsen, A. Osterhaus, and R. A. M. Fouchier. 2007. Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. *Plos Pathogens* 3:630-638.
- Muzaffar, S. B., J. Y. Takekawa, D. J. Prosser, D. C. Douglas, B. P. Yan, Z. Xing, Y. S. Hou, E. C. Palm, and S. H. Newman. 2008. Seasonal movements and migration of Palla's Gulls *Larus ichthyaetus* from Qinghai Lake, China. *Forktail* 24:100-107.
- Muzaffar, S. B., J. Y. Takekawa, D. J. Prosser, S. H. Newman, and X. Xiao. 2010. Rice production systems and avian influenza: Interactions between rice, poultry and wild birds. *Waterbirds* 33:219-230.
- Muzaffar, S. B., R. C. Ydenberg, and I. L. Jones. 2006. Avian influenza: An ecological and evolutionary perspective for waterbird scientists. *Waterbirds* 29:243-257.
- Nagy, A., J. Machova, J. Hornickova, M. Tomci, I. Nagl, B. Horyna, and I. Holka. 2007. Highly pathogenic avian influenza virus subtype H5N1 in Mute swans in the Czech Republic. *Veterinary Microbiology* 120:9-16.
- Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman. 1996. *Applied Linear Statistical Models*. 4th edition. McGraw-Hill, New York.

- Neumann, K., B. Elbersen, P. Verburg, I. Staritsky, M. Pérez-Soba, W. de Vries, and W. Rienks. 2009. Modelling the spatial distribution of livestock in Europe. *Landscape Ecology* 24:1207-1222.
- Newman, S., N. J. Hill, K. A. Spragens, D. J. Prosser, B. Yan, F. Lei, N. Batbayar, T. Natsagdorj, C. M. Bishop, P. J. Butler, S. Balachandran, L. Loth, T. Mundkur, D. C. Douglas, and J. Y. Takekawa. 2012a. Central Asia as a thoroughfare for Highly Pathogenic Avian Influenza Virus H5N1 in wild migratory birds: An eco-virological approach. *PLoS ONE* 7:e30636.
- Newman, S. H., N. J. Hill, K. A. Spragens, D. Janies, I. O. Voronkin, D. J. Prosser, B. Yan, F. Lei, N. Batbayar, T. Natsagdorj, C. M. Bishop, P. J. Butler, M. Wikelski, S. Balachandran, T. Mundkur, D. C. Douglas, and J. Y. Takekawa. 2012b. Eco-Virological Approach for Assessing the Role of Wild Birds in the Spread of Avian Influenza H5N1 along the Central Asian Flyway. *PLoS ONE* 7:e30636.
- Newman, S. H., S. A. Iverson, J. Y. Takekawa, M. Gilbert, D. J. Prosser, N. Batbayar, T. Natsagdorj, and D. C. Douglas. 2009. Migration of whooper swans and outbreaks of highly pathogenic avian influenza H5N1 virus in eastern Asia. *PLoS ONE* 4:e5729.
- Normile, D. 2006a. Avian influenza - Wild birds only partly to blame in spreading H5N1. *Science* 312:1451-1451.
- _____. 2006b. Avian influenza. Evidence points to migratory birds in H5N1 spread. *Science* 311:1225.
- OIE. 2004a. Manual of Diagnostics Tests and Vaccines for Terrestrial Animals, 5th Edition. Chapter 2.7.12. Pages 465-481 in World Organization of Animal Health, editor. Avian Influenza. Updated May 2005, Paris, France.
- _____. 2004b. Update on highly pathogenic avian influenza in animals: Type H5 and H7. in http://www.oie.int/downld/AVIAN%20INFLUENZA/A_AI-Asia.htm.
- _____. 2005. Highly pathologic avian influenza in Mongolia Follow-up Report No. 2 in.
- _____. 2010. Update on highly pathogenic avian influenza in animals: Type H5 and H7. http://www.oie.int/downld/AVIAN%20INFLUENZA/A_AI-Asia.htm. in http://www.oie.int/downld/AVIAN%20INFLUENZA/A_AI-Asia.htm.
- _____. 2012. Update on highly pathogenic avian influenza in animals: Type H5 and H7. in <http://www.oie.int/animal-health-in-the-world/update-on-avian-influenza/2012/>.
- Olsen, B., V. J. Munster, A. Wallensten, J. Waldenstrom, A. D. Osterhaus, and R. A. Fouchier. 2006. Global patterns of influenza a virus in wild birds. *Science* 312:384-388.
- Palese, P., and M. L. Shaw. 2007. Orthomyxoviridae: The Viruses and Their Replication. Pages 1647-1689 in D. M. Knipe, and P. M. Howley, editors. *Fields Virology* 5th Edition. Lippincott Williams & Wilkins, Philadelphia.
- Pantin-Jackwood, M. J., D. L. Suarez, E. Spackman, and D. E. Swayne. 2007. Age at infection affects the pathogenicity of Asian highly pathogenic avian influenza H5N1 viruses in ducks. *Virus Research* 130:151-161.
- Patz, J. A., P. R. Epstein, T. A. Burke, and J. M. Balbus. 1996. Global climate change and emerging infectious diseases. *Jama-Journal of the American Medical Association* 275:217-223.

- Pearce, J., and M. S. Boyce. 2006. Modelling distribution and abundance with presence-only data. *Journal Of Applied Ecology* 43:405-412.
- Perdue, M. L. 2008. Molecular Determinants of Pathogenicity for Avian Influenza Viruses. Pages 23-41 *in* D. Swayne, editor. *Avian Influenza*. Blackwell Publishing, Ames, Iowa.
- Perez, D. R., W. Lim, J. P. Seiler, G. Yi, M. Peiris, K. F. Shortridge, and R. G. Webster. 2003. Role of quail in the interspecies transmission of H9 influenza A viruses: Molecular changes on HA that correspond to adaptation from ducks to chickens. *Journal Of Virology* 77:3148-3156.
- Perkins, L. E., and D. E. Swayne. 2002a. Pathogenicity of a Hong Kong-origin H5N1 highly pathogenic avian influenza virus for emus, geese, ducks, and pigeons. *Avian Diseases* 46:53-63.
- _____. 2002b. Susceptibility of laughing gulls (*Larus atricilla*) to H5N1 and H5N3 highly pathogenic avian influenza viruses. *Avian Diseases* 46:877-885.
- _____. 2003. Varied pathogenicity of a Hong Kong-origin H5N1 avian influenza virus in four passerine species and budgerigars. *Veterinary Pathology* 40:14-24.
- Perroncito, E. 1878. Epizootia tifoide nei gallina-cei. *Annali della Reale Accademia d' Agricoltura di Torino* 21:87-126.
- Pfeiffer, D. U., P. Q. Minh, V. Martin, M. Epprecht, and M. J. Otte. 2007. An analysis of the spatial and temporal patterns of highly pathogenic avian influenza occurrence in Vietnam using national surveillance data. *The Veterinary Journal* 174:302-309.
- Phuong, D. Q., N. T. Dung, P. H. Jorgensen, K. J. Handberg, N. T. Vinh, and J. P. Christensen. 2011. Susceptibility of Muscovy (*Cairina Moschata*) and mallard ducks (*Anas Platyrhynchos*) to experimental infections by different genotypes of H5N1 avian influenza viruses. *Veterinary Microbiology* 148:168-174.
- Popovkina, A. B. 2006. Conflicting trends in ruddy shelduck *Tadorna ferruginea* populations: a myth or reality? Pages 480-481 *in* G. C. Boere, C. A. Galbraith, and D. A. Stroud, editors. *Waterbirds around the world*. The Stationary Office, Edinburgh, UK. .
- Prins, E., B. S. Petersen, A. Aunins, and J. Priednieks. 2005. Using Landsat TM and field data to produce maps of predicted bird densities in Latvian farmland. *International Journal Of Remote Sensing* 26:1881-1891.
- Prosser, D. J., P. Cui, J. Y. Takekawa, M. J. Tang, Y. S. Hou, B. M. Collins, B. P. Yan, N. J. Hill, T. X. Li, Y. D. Li, F. M. Lei, S. Guo, Z. Xing, Y. B. He, Y. C. Zhou, D. C. Douglas, W. M. Perry, and S. H. Newman. 2011a. Wild Bird Migration across the Qinghai-Tibetan Plateau: A Transmission Route for Highly Pathogenic H5N1. *PLoS ONE* 6.
- Prosser, D. J., D. Lee, C. Ding, R. M. Erwin, R. S. DeFries, and E. C. Ellis. 2012 *In Prep*. Species Distribution Modeling in a Region of High Need and Limited Data: China's Anatidae Waterfowl. *Frontiers in Ecology and the Environment OR Ecological Modelling OR Biological Conservation*.
- Prosser, D. J., J. Y. Takekawa, S. H. Newman, B. P. Yan, D. C. Douglas, Y. S. Hou, Z. Xing, T. X. Li, L. Y. D., D. Zhao, W. M. Perry, E. C. Palm, and D. H. Zhang. 2009. Satellite-marked waterfowl reveal migratory connection between H5N1 outbreak areas in China and Mongolia. *Ibis* 151:568-576.

- Prosser, D. J., J. X. Wu, E. C. Ellis, F. Gale, T. P. Van Boeckel, G. R. W. Wint, T. Robinson, X. Xiao, and M. Gilbert. 2011b. Modelling the distribution of chickens, ducks, and geese in China. *Agriculture Ecosystems & Environment* 141:381-389.
- Qing, F. S. 2002. China's Poultry Industry Affected by WTO. Pages 12-14 in *World Poultry*. www.worldpoultry.net, www.worldpoultry.net.
- R Core Development Team. 2012. The R Project for Statistical Computing. <http://www.R-project.org>.
- Ramsar Convention Secretariat. 2007. Designating Ramsar sites: The strategic framework and guidelines for the future development of the List of Wetlands of International Importance. 3rd edition. Volume 14. Ramsar Convention Secretariat, Gland, Switzerland.
- Rawlings, J. O., S. G. Pantula, and D. A. Dickey. 1998. *Applied Regression Analysis: A Research Tool*. 2nd edition. Springer-Verlag, New York.
- Robbins, C. S., and E. A. T. Blom. 1996. *Atlas of the breeding birds of Maryland and the District of Columbia*. University of Pittsburgh Press, Pittsburgh.
- Robinson, T. P., G. Franceschini, and W. Wint. 2007. The Food and Agriculture Organization's Gridded Livestock of the World. *Veterinaria Italiana* 43:745-751.
- Robson, C. 2000. *A Guide to the Birds of Southeast Asia*. Princeton University Press, Princeton, New Jersey.
- Roche, B., C. Lebarbenchon, M. Gauthier-Clerc, C. M. Chang, F. Thomas, F. Renaud, S. van der Werf, and J. F. Guegan. 2009. Water-borne transmission drives avian influenza dynamics in wild birds: The case of the 2005-2006 epidemics in the Camargue area. *Infection Genetics and Evolution* 9:800-805.
- Rohani, P., R. Breban, D. E. Stallknecht, and J. M. Drake. 2009. Environmental transmission of low pathogenicity avian influenza viruses and its implications for pathogen invasion. *Proceedings Of The National Academy Of Sciences Of The United States Of America*.
- Root, T. 1988. *Atlas of wintering North American birds: An analysis of Christmas Bird Count Data*. University of Chicago Press, Chicago, Illinois.
- Rowan, M. K. 1962. Mass mortality among European common terns in South Africa in April-May 1961. *British Birds* 55:103-114.
- Royle, J. A., R. B. Chandler, C. Yackulick, and J. D. Nichols. In Review. Likelihood Analysis of Species Occurrence Probability from Presence-Only Data for Modeling Species Distributions.
- Russell, C. J., and R. G. Webster. 2005. The genesis of a pandemic influenza virus. *Cell* 123:368-371.
- Saad, M. D., L. S. Ahmed, M. A. Gamal-Eldein, M. K. Fouda, F. M. Khalil, S. L. Yingst, M. A. Parker, and M. R. Monteville. 2007. Possible avian influenza (H5N1) from migratory bird, Egypt. *Emerging Infectious Diseases* 13:1120-1121.
- Sauer, J. R., J. E. Fallon, and R. Johnson. 2003. Use of North American Breeding Bird Survey Data to Estimate Population Change for Bird Conservation Regions. *The Journal of Wildlife Management* 67:372-389.
- Schafer, W. 1955. Vergleichende sero-immunologische Untersuchungen über die Viren der Influenza und der klassischen Geflügelpest. *Zeitschrift für Naturforschung* 10b:81-91.

- Scharlemann, J. P. W., D. Benz, S. I. Hay, B. V. Purse, A. J. Tatem, G. R. W. Wint, and D. J. Rogers. 2008. Global Data for Ecology and Epidemiology: A Novel Algorithm for Temporal Fourier Processing MODIS Data. *PLoS ONE* 3:e1408.
- Scott, J. M., F. Davis, B. Csuti, R. Noss, B. Butterfield, C. Groves, H. Anderson, S. Caicco, F. Derchia, T. C. Edwards, J. Ulliman, and R. G. Wright. 1993. GAP analysis - A geographic approach to the protection of biological diversity *Wildlife Monographs*:1-41.
- Segurado, P., and M. B. Araujo. 2004. An evaluation of methods for modelling species distributions. *Journal Of Biogeography* 31:1555-1568.
- Seoane, J., J. Bustamante, and R. Diaz-Delgado. 2004. Are existing vegetation maps adequate to predict bird distributions? *Ecological Modelling* 175:137-149.
- Sere, C., and H. Steinfeld. 1996. World livestock production systems: Current status, issues and trends.
- Shortridge, K. F., N. N. Zhou, Y. Guan, P. Gao, T. Ito, Y. Kawaoka, S. Kodihalli, S. Krauss, D. Markwell, K. G. Murti, M. Norwood, D. Senne, L. Sims, A. Takada, and R. G. Webster. 1998. Characterization of avian H5N1 influenza viruses from poultry in Hong Kong. *Virology* 252:331-342.
- Sims, L. D., and I. H. Brown. 2008. Multicontinental epidemic of H5N1 HPAI virus (1996-2007). Pages 251-286 in D. E. Swayne, editor. *Avian Influenza*. Blackwell Publishing, Ames, Iowa, USA.
- Sims, L. D., J. Domenech, C. Benigno, S. Kahn, A. Kamata, J. Lubroth, V. Martin, and R. Roeder. 2005. Origin and evolution of highly pathogenic H5N1 avian influenza in Asia. *Veterinary Record* 157:159-+.
- Sims, L. D., T. M. Ellis, K. K. Liu, K. Dyrting, H. Wong, M. Peiris, Y. Guan, and K. E. Shortridge. 2003. Avian influenza in Hong Kong 1997-2002. *Avian Diseases* 47:832-838.
- Smith, D. J. 2006. Predictability and preparedness in influenza control. *Science* 312:392-394.
- Songserm, T., R. Jam-on, N. Sae-Heng, N. Meemak, D. J. Hulse-Post, K. M. Sturm-Ramirez, and R. G. Webster. 2006. Domestic ducks and H5N1 influenza epidemic, Thailand. *Emerging Infectious Diseases* 12:575-581.
- Stallknecht, D. E., and S. M. Shane. 1988. Host Range Of Avian Influenza-Virus In Free-Living Birds. *Veterinary Research Communications* 12:125-141.
- Stallknecht, D. E., S. M. Shane, M. T. Kearney, and P. J. Zwank. 1990. Persistence of avian influenza viruses in water. *Avian Diseases* 34:406-411.
- Strange, M. 2000. *A Photographic Guide to the Birds of Southeast Asia*. Princeton University Press, Singapore.
- Sturm-Ramirez, K. M., T. Ellis, B. Bousfield, L. Bissett, K. Dyrting, J. E. Rehg, L. Poon, Y. Guan, M. Peiris, and R. G. Webster. 2004. Reemerging H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to ducks. *Journal Of Virology* 78:4892-4901.
- Sturm-Ramirez, K. M., D. J. Hulse-Post, E. A. Govorkova, J. Humberd, P. Seiler, P. Puthavathana, C. Buranathai, T. D. Nguyen, A. Chaisingh, H. T. Long, T. S. Naipospos, H. Chen, T. M. Ellis, Y. Guan, J. S. Peiris, and R. G. Webster. 2005. Are ducks contributing to the endemicity of highly pathogenic H5N1 influenza virus in Asia? *Journal Of Virology* 79:11269-11279.

- Swayne, D. 2008. The global nature of avian influenza. Pages 123-144 *in* D. Swayne, editor. Avian Influenza. Blackwell Publishing, Ames, Iowa.
- Takekawa, J. Y., S. Newman, X. Xiao, D. J. Prosser, K. A. Spragens, E. C. Palm, B. Yan, T. Li, F. Lei, D. Zhao, D. C. Douglas, S. B. Muzaffar, and W. Ji. 2010a. Migration of waterfowl in the East Asian Flyway and spatial relationship to HPAI H5N1 outbreaks. *Avian Diseases* 54:466-476.
- Takekawa, J. Y., D. J. Prosser, S. Newman, S. B. Muzaffar, N. J. Hill, B. P. Yan, X. Xiao, F. Lei, T. Li, S. Schwarzbach, and J. A. Howell. 2010b. Victims and Vectors: highly pathogenic H5N1 and the ecology of water birds. *Avian Biology Research* 3:51-73.
- Taylor, L. H., S. M. Latham, and M. E. J. Woolhouse. 2001. Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 356:983-989.
- Thornton, P. K., R. L. Kruska, N. Henninger, P. M. Kristjanson, R. S. Reid, F. Atieno, A. Odero, and T. Ndegwa. 2002. Mapping poverty and livestock in the developing world. International Livestock Research Institute, Nairobi.
- Tian, Y. Z., T. X. Yue, L. F. Zhu, and N. Clinton. 2005. Modeling population density using land cover data. *Ecological Modelling* 189:72-88.
- Tiensin, T., S. S. U. Ahmed, S. Rojanasthien, T. Songserm, P. Ratanakorn, K. Chaichoun, W. Kalpravidh, S. Wongkasemjit, T. Patchimasiri, K. Chanachai, W. Thanapongtham, S. Chotinan, A. Stegeman, and M. Nielsen. 2009. Ecologic Risk Factor Investigation of Clusters of Avian Influenza A (H5N1) Virus Infection in Thailand. *Journal of Infectious Diseases* 199:1735-1743.
- Toral, G. M., D. Aragones, J. Bustamante, and J. Figuerola. 2011. Using Landsat images to map habitat availability for waterbirds in rice fields. *Ibis* 153:684-694.
- Tsoar, A., O. Allouche, O. Steinitz, D. Rotem, and R. Kadmon. 2007. A comparative evaluation of presence-only methods for modelling species distribution. *Diversity and Distributions* 13:397-405.
- Tumpey, T. M., D. L. Suarez, L. E. L. Perkins, D. A. Senne, J. G. Lee, Y. J. Lee, I. P. Mo, H. W. Sung, and D. E. Swayne. 2002. Characterization of a highly pathogenic H5N1 avian influenza virus isolated from duck meat. *Journal Of Virology* 76:6344-6355.
- U.S. Fish and Wildlife Service. 2007. Waterfowl population status, 2007. Department of the Interior, Washington, D.C. USA.
- United Nations Food and Agriculture Organization. 2004. EMPRES transboundary animal diseases bulletin. *EMPRES Bulletin* 25:1-56.
- _____. 2007. Gridded Livestock of the World 2007. Rome.
- USGS National Wildlife Health Center. 2012. List of species affected by H5N1 (Highly Pathogenic Avian Influenza). *in* http://www.nwhc.usgs.gov/disease_information/avian_influenza/affected_species_chart.jsp.
- Van Boeckel, T. P., D. J. Prosser, G. Franceschini, C. Biradar, W. Wint, T. Robinson, and M. Gilbert. 2011. Modelling the distribution of domestic ducks in Monsoon Asia. *Agriculture Ecosystems & Environment* 141:373-380.

- Van Boeckel, T. P., D. J. Prosser, G. R. W. Wint, T. Robinson, and M. Gilbert. 2010 (*in review*). Modelling the distribution of domestic ducks in Monsoon Asia. *Agriculture Ecosystems & Environment*.
- van der Zijpp, A. J. 1999. Animal food production: the perspective of human consumption, production, trade and disease control. *Livestock Production Science* 59:199-206.
- Venier, L. A., J. Pearce, J. E. McKee, D. W. McKenney, and G. J. Niemi. 2004. Climate and satellite-derived land cover for predicting breeding bird distribution in the Great Lakes Basin. *Journal Of Biogeography* 31:315-331.
- Verburg, P. H., and Y. Q. Chen. 2000. Multiscale characterization of land-use patterns in China. *Ecosystems* 3:369-385.
- Verner, J., M. L. Morrison, and C. J. Ralph, editors. 1986. *Wildlife 2000: Modeling Habitat Relationships of Terrestrial Vertebrates*. The University of Wisconsin Press, Madison, WI.
- Vijaykrishna, D., J. Bahl, S. Riley, L. Duan, J. X. Zhang, H. Chen, J. S. M. Peiris, G. J. D. Smith, and Y. Guan. 2008. Evolutionary Dynamics and Emergence of Panzootic H5N1 Influenza Viruses. *PLoS Pathog* 4:e1000161.
- Wallace, R. G., H. Hodac, R. H. Lathrop, and W. M. Fitch. 2007. A statistical phylogeography of influenza A H5N1. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 104:4473-4478.
- Wallensten, A., V. J. Munster, N. Latorre-Margalef, M. Brytting, J. Elmberg, R. A. M. Fouchier, T. Fransson, P. D. Haemig, M. Karlsson, A. Lundkvist, A. Osterhaus, M. Stervander, J. Waldenstrom, and B. Olsen. 2007. Surveillance of influenza A virus in migratory waterfowl in northern Europe. *Emerging Infectious Diseases* 13:404-411.
- Wang, H. 2006. The Chinese Poultry Industry at a Glance. Pages 10-11 *in* *World Poultry*. www.worldpoultry.net.
- Wang, J., D. Vijaykrishna, L. Duan, J. Bahl, J. X. Zhang, R. G. Webster, J. S. M. Peiris, H. Chen, G. J. D. Smith, and Y. Guan. 2008. Identification of the progenitors of Indonesian and Vietnamese avian influenza A (H5N1) viruses from southern China. *Journal Of Virology* 82:3405-3414.
- Webby, R. J., and R. G. Webster. 2001. Emergence of influenza A viruses. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 356:1817-1828.
- Weber, T. P., and N. I. Stilianakis. 2007. Ecologic immunology of avian influenza (H5N1) in migratory birds. *Emerging Infectious Diseases* 13:1139-1143.
- _____. 2008. Migratory birds, the H5N1 influenza virus and the scientific method. *Virology Journal* 5.
- Webster, R. G., W. J. Bean, O. T. Gorman, T. M. Chambers, and Y. Kawaoka. 1992. Evolution and ecology of influenza-A viruses. *Microbiological Reviews* 56:152-179.
- Webster, R. G., and E. A. Govorkova. 2006. Focus on research: H5N1 influenza - Continuing evolution and spread. *New England Journal Of Medicine* 355:2174-2177.

- Webster, R. G., D. J. Hulse-Post, K. A. Sturm-Ramirez, Y. Guan, A. Peiris, G. Smith, and H. Chen. 2007a. Changing epidemiology and ecology of highly pathogenic avian H5N1 influenza viruses. *Avian Diseases* 51:269-272.
- Webster, R. G., S. Krauss, D. Hulse-Post, and K. Sturm-Ramirez. 2007b. Evolution of influenza A viruses in wild birds. *Journal of Wildlife Diseases* 43:S1-S6.
- Webster, R. G., M. Peiris, H. L. Chen, and Y. Guan. 2006. H5N1 outbreaks and enzootic influenza. *Emerging Infectious Diseases* 12:3-8.
- Whittingham, M. J., P. A. Stephens, R. B. Bradbury, and R. P. Freckleton. 2006. Why do we still use stepwise modeling in ecology and behaviour? *Journal Of Animal Ecology* 75:1182-1189.
- Wildlife Conservation Society. 2005. Joint wildlife conservation - Mongolia avian influenza survey: Final report to the United Nations Food and Agriculture Organization. Wildlife Conservation Society.
- _____. 2007. Disease Surveillance: Avian Influenza in Migratory Birds in Mongolia, Results of Extensive Surveys 2006. Wildlife Conservation Society
- World Health Organization. 2010. Cumulative number of confirmed human cases of avian influenza A/(H5N1) reported to WHO as of 3 August 2010. *in* http://www.who.int/csr/disease/avian_influenza/country/en/index.html.
- _____. 2012a. Cumulative number of confirmed human cases of avian influenza A/(H5N1) reported to WHO as of 12 March 2012. *in* http://www.who.int/csr/disease/avian_influenza/country/en/index.html.
- _____. 2012b. Current WHO phase of pandemic alert for avian influenza H5N1. *in* http://apps.who.int/csr/disease/avian_influenza/phase/en/index.html.
- World Organization of Animal Health. 2005. Manual of Diagnostics Tests and Vaccines for Terrestrial Animals. Chapter 2.7.12. *in* World Organization of Animal Health, editor. Avian Influenza. Paris, France.
- Worobey, M. 2008. Phylogenetic evidence against evolutionary stasis and natural abiotic reservoirs of influenza A virus. *Journal Of Virology* 82:3769-3774.
- Xiao, X. M., S. Boles, S. Frolking, C. S. Li, J. Y. Babu, W. Salas, and B. Moore. 2006. Mapping paddy rice agriculture in South and Southeast Asia using multi-temporal MODIS images. *Remote Sensing of Environment* 100:95-113.
- Xie, Y., S. Wang, and P. Schei, editors. 2004. China's Protected Areas. Tsinghua University Press, Beijing.
- Xu, H., Z. H. Hou, L. Ai, and L. C. Tan. 2007. Precipitation at Lake Qinghai, NE Qinghai-Tibet Plateau, and its relation to Asian summer monsoons on decadal/interdecadal scales during the past 500 years. *Palaeogeography Palaeoclimatology Palaeoecology* 254:541-549.
- Xu, H., X. Y. Liu, and Z. H. Hou. 2008. Temperature variations at Lake Qinghai on decadal scales and the possible relation to solar activities. *Journal of Atmospheric and Solar-Terrestrial Physics* 70:138-144.
- Xu, X. Y., K. Subbarao, N. J. Cox, and Y. J. Guo. 1999. Genetic characterization of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: Similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. *Virology* 261:15-19.
- Yan. 1996. The Birds of China (*in Chinese*). 521p.

- Yan, H., X. Xiao, and H. Huang. 2010. Satellite observed crop calendar and its spatial-temporal characteristics in multiple cropping area of Huang-Huai-Hai Plain, China. *Acta Ecologica Sinica* 30:2416-2423.
- Yan, H., X. Xiao, H. Q. Huang, J. Y. Liu, and J. B. Wang. In Review. Mapping multiple cropping index in China from agro-meteorological observations and time series MODIS imagery. *Agricultural Systems*.
- Yasue, M., C. J. Feare, L. Bennun, and W. Fiedler. 2006. The epidemiology of H5N1 avian influenza in wild birds: Why we need better ecological data. *Bioscience* 56:923-929.
- Yu, Z., *1 Yunfeng Song,*1 Hongbo Zhou,* Q. H. Xiaojuan Xu, * Haiya Wu,* , Y. Z. Anding Zhang, * Jianfeng Chen,* , Q. L. Hanbing Dan, * Xiangmin Li,* , and a. M. J. Huanchun Chen. 2007. Avian Influenza (H5N1) Virus in Waterfowl and Chickens, Central China *Emerging Infectious Diseases* 13:772-775.
- Zell, R. 2004. Global climate change and the emergence/re-emergence of infectious diseases. *International Journal of Medical Microbiology* 293:16-26.
- Zhang, G.-G., D.-P. Liu, H.-X. Jiang, Y.-Q. Hou, M. Dai, and G.-Z. Chu. 2008. The current status of waterbirds after Avian Influenza outbreak at Qinghai Lake, China. *Chinese Journal of Zoology* 43:51-56.
- Zhang, G., D. Shoham, D. Gilichinsky, S. Davydov, J. D. Castello, and S. O. Rogers. 2006. Evidence of influenza A virus RNA in Siberian lake ice. *Journal Of Virology* 80:12229-12235.
- Zhao, Z. M., K. F. Shortridge, M. Garcia, Y. Guan, and X. F. Wan. 2008. Genotypic diversity of H5N1 highly pathogenic avian influenza viruses. *Journal Of General Virology* 89:2182-2193.
- Zhou, J. Y., H. G. Shen, H. X. Chen, G. Z. Tong, M. Liao, H. C. Yang, and J. X. Liu. 2006. Characterization of a highly pathogenic H5N1 influenza virus derived from bar-headed geese in China. *Journal Of General Virology* 87:1823-1833.