AVIAN INFLUENZA VIRUSES IN SHOREBIRD HOSTS AT THE DELAWARE BAY MIGRATORY STOPOVER SITE: INFECTION PATTERNS AND DYNAMICS, HOST ECOLOGY, AND POPULATION EFFECTS

by

ANGELA M. MAXTED

(Under the Direction of David E. Stallknecht)

ABSTRACT

Although waterfowl and gulls are recognized natural hosts to avian influenza viruses (AIV), AIV circulation in shorebirds is poorly understood. Shorebird infections primarily occur during spring in one species (Ruddy Turnstones; *Arenaria interpres morinella*) at one location worldwide (Delaware Bay, USA); close proximity to poultry production areas raises concerns about transmission to poultry. During May–June 2006–2008, we collected samples for virus isolation or serology from 3,233 individuals of 13 Charadriiformes species to better define patterns of AIV infection and exposure at Delaware Bay. Ruddy Turnstones were infected most often and with diverse subtypes. Prevalence and subtypes in Turnstones varied with year; prevalence was highest mid-season and among heavier-than-expected birds. Antibody prevalence increased over the season; together, these results suggested a local epidemic and recovery. Prevalence in Sanderlings (*Calidris alba*) was positively correlated with prevalence in Turnstones and subtypes matched between species, likely representing AIV spillover into Sanderlings. Red Knots (*Calidris canutus rufa*) had rare infections and high antibody prevalence that declined over the season; likely, they were exposed recently prior to arrival at Delaware Bay and partially immune to re-infection. Gulls also had rare infections but some subtypes were not found in other species, indicating partial gene pool separation between shorebirds and gulls. A radiotelemetry study of Ruddy Turnstones and Sanderlings revealed differences in habitat use

that possibly account for variable infection and exposure; Turnstones routinely used salt marsh which might better support AIV transmission than beach habitat shared by all species. Radiotagged birds selected areas farther from agricultural land and poultry operations than expected, suggesting limited AIV transmission risk. Prevalence declined prior to departure, suggesting limited carriage of AIV onto breeding grounds. Analysis of individual Ruddy Turnstone resighting data revealed no difference in 1-year survival rates between AIV-infected and uninfected birds. Due to their susceptibility, temporary abundance in spring, and lack of morbidity or mortality, we propose that Ruddy Turnstones act as a local amplifying host for AIV acquired from resident waterfowl and gulls or from other migratory shorebirds. Additional studies are needed to further define shorebird species' roles in global AIV epidemiology.

INDEX WORDS: AIV, annual survival, *Arenaria interpres*, avian influenza virus, disease ecology, epidemiology, infection dynamics, Ruddy Turnstone, shorebirds, wildlife reservoir

# AVIAN INFLUENZA VIRUSES IN SHOREBIRD HOSTS AT THE DELAWARE BAY MIGRATORY STOPOVER SITE: INFECTION PATTERNS AND DYNAMICS, HOST ECOLOGY, AND POPULATION EFFECTS

by

ANGELA M. MAXTED

BS, University of Wisconsin–Stevens Point, 1998

MS, Purdue University, 2001

DVM, Iowa State University, 2005

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2011

# AVIAN INFLUENZA VIRUSES IN SHOREBIRD HOSTS AT THE DELAWARE BAY MIGRATORY STOPOVER SITE: INFECTION PATTERNS AND DYNAMICS, HOST ECOLOGY, AND POPULATION EFFECTS

by

ANGELA M. MAXTED

| | |
|---|---|
| Major Professor: | David Stallknecht |
| Committee: | Sonia Altizer |
| | Maricarmen Garcia |
| | Elizabeth Howerth |
| | Mark Jackwood |

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2011

# DEDICATION

To shorebird conservationists, for your dedication.

# ACKNOWLEDGMENTS

This research and dissertation would not have been completed without the help of numerous people and organizations. First, I thank my major professor, Dave Stallknecht, and my committee members, Sonia Altizer, Maricarmen Garcia, Buffy Howerth, and Mark Jackwood, for their support and guidance through the entire process. The laboratory expertise and support provided by Ginger Goekjian, Becky Paulson, and Jennifer Smith were extensive and invaluable, and I cannot thank them enough. Field work at Delaware Bay was organized and supported by, in particular, Page Luttrell and Ben Wilcox; I also thank Justin Brown, Erin Casey, Laura Coffee, Michaela Cole, Jay Cumbee, Danielle Downs, Samantha Gibbs, Bill Hamrick, Shamus Keeler, Glenn Martin, Sabrina McGraw, Cara McKinnon, and Jessica Murdock from SCWDS for countless hours of field and laboratory assistance.

My research partners in Delaware Bay (not only from New Jersey and Delaware but also around the world) were outstanding. In particular, I thank Larry Niles (Conserve Wildlife Foundation of New Jersey), Mandy Dey and Bill Pitts (Nongame and Endangered Species Program, Division of Fish and Wildlife, New Jersey Department of Environmental Protection), Kevin Kalasz (Natural Heritage & Endangered Species Program, Division of Fish and Wildlife, Delaware Department of Natural Resources and Environmental Control), and Humphrey Sitters (International Wader Study Group), as well as Ron Porter, Clive Minton, and many other shorebird experts that descend upon Delaware Bay each spring just as the birds do.

Finally, I could not have completed this work without the support of friends and family: fellow graduate students, staff, and faculty at SCWDS and across the UGA campus who are too numerous to name, the Double Dog Dare Flyball Racing Club, my parents Mike and Mary Maxted, my sister Melody Maxted, and, especially, my husband Mark Nipper.

TABLE OF CONTENTS

# LIST OF TABLES

Page

LIST OF FIGURES

Page

# CHAPTER 1

# INTRODUCTION

## AVIAN INFLUENZA VIRUSES AND SHOREBIRDS

Wild shorebirds (order Charadriiformes, families Charadriidae and Scolopacidae) are frequently cited as a natural wildlife reservoir for avian influenza viruses (AIV) that could infect and cause disease in poultry and humans (Webster et al., 1992; Webster et al., 2007). A thorough understanding of these species' role in influenza ecology is important for the development of strategies to prevent introduction of AI viruses into domestic animal populations, and also for the conservation of shorebird species themselves. With the presence of highly pathogenic avian influenza (HPAI) subtype H5N1 in free-living waterfowl in Eurasia and Africa, there is global concern among waterbird and wetland scientists that aquatic birds and their habitats will be destroyed because of the fear that they could transfer viruses, triggering HPAI epizootics in poultry, or a pandemic in humans (Tracey et al., 2004; Bin Muzaffar et al., 2006; Olsen et al., 2006).

Viruses in the family *Orthomyxoviridae*, genus *Influenzavirus A* can be important pathogens in poultry and a number of animal hosts, including humans. Waterfowl (order Anseriformes), particularly ducks (family Anatidae), are the natural hosts and reservoir for AIV. AIVs have also been isolated from pelagic seabirds, gulls, terns, and shorebirds relatively frequently, leading to speculation regarding these species' abilities to maintain AIV in nature.

Within the Charadriiformes, gulls and terns (family Laridae) seem to be important for the maintenance of at least some AIV in nature. Certain HA subtypes (H13, H16) and lineages of other AIV gene segments are apparently adapted to gulls (Webster et al., 1992; Yamnikova et al., 2003; Fouchier et al., 2005; Obenauer et al., 2006). Low pathogenicity forms of AIV (LPAIV)

are sporadically isolated from shorebird species, but no genetic lineages are uniquely associated with the Charadriidae or Scolopacidae (Spackman et al., 2005).

Very few sampling efforts of shorebird populations have yielded positive isolations, even when they congregate in high numbers at wintering or migratory stopover sites (Fouchier et al., 2003; Hlinak et al., 2006; Langstaff and McKenzie, 2006; Cattoli et al., 2007; D'Amico et al., 2007; Gaidet et al., 2007; Munster et al., 2007; Winker et al., 2007; Escudero et al., 2008; Hanson et al., 2008; Ip et al., 2008; Winker et al., 2008). A notable exception to this pattern is seen at Delaware Bay, Delaware and New Jersey, USA. Every spring, AI viruses infect a substantial proportion of Ruddy Turnstones (*Arenaria interpres morinella*) during their approximately five-week stopover in Delaware Bay. Syntopic shorebird species, such as Red Knots (*Caladris canatus rufa*) and Sanderlings (*Calidris alba*), are infected at much lower rates (Hanson et al., 2008, A. Maxted unpublished data). Approximately 85% of shorebird AIV isolates from which sequences have been submitted to GenBank were collected at Delaware Bay (Bao et al., 2008). Of 292 AIV isolates from Delaware Bay shorebirds during 2000-2005, 90% (*n*=262) were from Ruddy Turnstones (Hanson et al., 2008). Together, these figures suggest that AIV infection in shorebirds is species- or location-specific rather than widespread and generalized across shorebird species.

The reasons for the high prevalence of avian influenza in Ruddy Turnstones in the Delaware Bay ecosystem during spring migration are not understood. Certainly, the interactions between AIV, the many potential waterbird hosts, and the habitats that facilitate host interactions and viral transmission within this ecosystem are complex.

My research addressed the causes of these annual epidemics, focusing on multiple host, agent, and environmental factors at various spatial and temporal scales. It also addressed potential consequences of AIV epidemics, including risk of transmission of disease to domestic poultry and long-term effects of infection on declining shorebird populations.

**The importance of this work**

Disease control and prevention programs are often the goal/outcome of epidemiological studies, and must be based on knowledge of the amount of the disease in a population, the factors associated with its occurrence, the facilities required to control the disease, and the costs and benefits involved. While few AI viruses are capable of direct transmission to people from a wild bird reservoir and even fewer may cause human morbidity or mortality, introduction of AI viruses into domestic bird populations is a major concern for veterinary health, food supply protection, and economic reasons.

The goal of this work is to provide knowledge of the epidemiology of AIV in species of the avian order Charariiformes, potential wild bird reservoirs, ultimately to inform animal and public health officials of the risk of transmission of AI viruses to human or animal populations that overlap with these species in space and time. While control of AIV in these potential reservoir species is unlikely to be feasible or appropriate, knowledge of the amount of disease in these populations, the timing of infection, potential dispersal of virus by infected birds, and factors that help potential reservoirs to become infected will help organizations concerned with wildlife, domestic animal, and public health understand when and where (and if) preventive strategies are needed and will be most cost-effective. As quoted from Thrusfield (2005):

> *"Infectious diseases maintained in wildlife present complex ecological relationships and even more complex problems relating to their control. Comprehensive epidemiological studies of these diseases help to unravel their life cycles and can indicate suitable methods for their control".*

## HOW THIS DISSERTATION IS ORGANIZED

Chapter 2 is a review of pertinent avian influenza literature, particularly in relation to wild bird reservoirs. Chapter 3 examines patterns of AIV infection and exposure in the broad sense (e.g., what species are infected), between-year variation in AIV prevalence, seroprevalence, and AIV subtypes, and population- and individual bird-level factors that influence infection and

exposure. Temporal patterns of AIV infection and exposure in four key species are examined in Chapter 4. Study into the stopover ecology of two shorebird species, including a discussion about how this related to patterns of AIV exposure and infection, is presented in Chapter 5. Chapter 6 examines possible effects of AIV infection on shorebird populations (specifically, on annual survival). Finally, Chapter 7 includes the main conclusions of this dissertation and its contributions to current knowledge of AIV in shorebird hosts.

## LITERATURE CITED

BAO, Y. M., P. BOLOTOV, D. DERNOVOY, B. KIRYUTIN, L. ZASLAVSKY, T. TATUSOVA, J. OSTELL, and D. LIPMAN. 2008. The influenza virus resource at the National Center for Biotechnology Information. Journal of Virology 82: 596-601.

BIN MUZAFFAR, S., R. C. YDENBERG, and I. L. JONES. 2006. Avian influenza: an ecological and evolutionary perspective for waterbird scientists. Waterbirds 29: 243-257.

CATTOLI, G., C. TERREGINO, V. GUBERTI, R. DE NARDI, A. DRAGO, A. SALVIATO, S. FASSINA, and I. CAPUA. 2007. Influenza virus surveillance in wild birds in Italy: Results of laboratory investigations in 2003-2005. Avian Diseases 51: 414-416.

D'AMICO, V. L., M. BERTELLOTTI, A. J. BAKER, and L. A. DIAZ. 2007. Exposure of Red Knots (*Calidris canutus rufa*) to select avian pathogens; Patagonia, Argentina. Journal of Wildlife Diseases 43: 794-797.

ESCUDERO, G., V. J. MUNSTER, M. BERTELLOTTI, and P. EDELAAR. 2008. Perpetuation of avian influenza in the Americas: Examining the role of shorebirds in Patagonia. Auk 125: 494-495.

FOUCHIER, R. A. M., B. OLSEN, T. M. BESTEBROER, S. HERFST, L. VAN DER KEMP, G. E. RIMMELZWAAN, and A. D. M. E. OSTERHAUS. 2003. Influenza A virus surveillance in wild birds in Northern Europe in 1999 and 2000. Avian Diseases 47: 857-860.

FOUCHIER, R. A. M., V. MUNSTER, A. WALLENSTEN, T. M. BESTEBROER, S. HERFST, D. SMITH, G. F. RIMMELZWAAN, B. OLSEN, and A. D. M. E. OSTERHAUS. 2005. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from Black-headed Gulls. Journal of Virology 79: 2814-2822.

GAIDET, N., T. DODMAN, A. CARON, G. BALANCA, S. DESVAUX, F. GOUTARD, G. CATTOLI, F. LAMARQUE, W. HAGEMEIJER, and F. MONICAT. 2007. Avian influenza viruses in water birds, Africa. Emerging Infectious Diseases 13: 626-629.

HANSON, B. A., M. P. LUTTRELL, V. H. GOEKJIAN, L. NILES, D. E. SWAYNE, D. A. SENNE, and D. E. STALLKNECHT. 2008. Is the occurrence of avian influenza virus in Charadriiformes species and location dependent? Journal of Wildlife Diseases 44: 351-361.

HLINAK, A., R. U. MUHLE, O. WERNER, A. GLOBIG, E. STARICK, H. SCHIRRMEIER, B. HOFFMANN, A. ENGELHARDT, D. HUBNER, F. J. CONRATHS, D. WALLSCHLAGER, H. KRUCKENBERG, and T. MULLER. 2006. A virological survey in migrating waders and other waterfowl in one of the most important resting sites of Germany. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 53: 105-110.

IP, H. S., P. L. FLINT, J. C. FRANSON, R. J. DUSEK, D. V. DERKSEN, R. E. GILL, JR., C. R. ELY, J. M. PEARCE, R. B. LANCTOT, S. M. MATSUOKA, D. B. IRONS, J. B. FISCHER, R. M. OATES, M. R. PETERSEN, T. F. FONDELL, D. A. ROCQUE, J. C. PEDERSEN, and T. C. ROTHE. 2008. Prevalence of Influenza A viruses in wild migratory birds in Alaska: patterns of variation in detection at a crossroads of intercontinental flyways. Virology Journal 5: 71.

LANGSTAFF, I. G., and J. S. MCKENZIE. 2006. Surveillance for highly pathogenic avian influenza in migratory shorebirds at the terminus of the East Asian-Australasian Flyway. Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics, Cairns, Australia: ISVEE 11: 835.

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. D. M. E. 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.

OBENAUER, J. C., J. DENSON, P. K. MEHTA, X. P. SU, S. MUKATIRA, D. B. FINKELSTEIN, X. Q. XU, J. H. WANG, J. MA, Y. P. FAN, K. M. RAKESTRAW, R. G. WEBSTER, E. HOFFMANN, S. KRAUSS, J. ZHENG, Z. W. ZHANG, and C. W. NAEVE. 2006. Large-scale sequence analysis of avian influenza isolates. Science 311: 1576-1580.

OLSEN, B., V. J. MUNSTER, A. WALLENSTEN, J. WALDENSTROM, A. OSTERHAUS, and R. A. M. FOUCHIER. 2006. Global patterns of influenza A virus in wild birds. Science 312: 384-388.

SPACKMAN, E., D. E. STALLKNECHT, R. D. SLEMONS, K. WINKER, D. L. SUAREZ, M. SCOTT, and D. E. SWAYNE. 2005. Phylogenetic analyses of type A influenza genes in natural reservoir species in North America reveals genetic variation. Virus Research 114: 89-100.

THRUSFIELD, M. 2005. Veterinary Epidemiology. 3rd edition. Blackwell Publishing, Oxford, United Kingdom.

TRACEY, J. P., R. WOODS, D. ROSHIER, P. WEST, and G. R. SAUNDERS. 2004. The role of wild birds in the transmission of avian influenza for Australia: an ecological perspective. Emu 104: 109-124.

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., S. KRAUSS, D. HULSE-POST, and K. STURM-RAMIREZ. 2007. Evolution of influenza A viruses in wild birds. Journal of Wildlife Diseases 43: S1-S6.

WINKER, K., K. G. MCCRACKEN, D. D. GIBSON, C. L. PRUETT, R. MEIER, F. HUETTMANN, M. WEGE, I. V. KULIKOVA, Y. N. ZHURAVLEV, M. L. PERDUE, E. SPACKMAN, D. L. SUAREZ, and D. E. SWAYNE. 2007. Movements of birds and avian influenza from Asia into Alaska. Emerging Infectious Diseases 13: 547-552.

WINKER, K., E. SPACKMAN, and D. E. SWAYNE. 2008. Rarity of influenza A virus in spring shorebirds, southern Alaska. Emerging Infectious Diseases 14: 1314-1316.

YAMNIKOVA, S. S., A. S. GAMBARYAN, A. B. TUZIKOV, N. V. BOVIN, M. N. MATROSOVICH, I. T. FEDYAKINA, A. A. GRINEV, V. M. BLINOV, D. K. LVOV, D. L. SUAREZ, and D. E. SWAYNE. 2003. Differences between HA receptor-binding sites of avian influenza viruses isolated from Laridae and Anatidae. Avian Diseases 47: 1164-1168.

# CHAPTER 2

# LITERATURE REVIEW

## AVIAN INFLUENZA VIRUSES AND DISEASE

Avian influenza viruses (AIV) are pleomorphic, spherical to filamentous, enveloped viruses in the family *Orthomyxovirdae,* genus *Influenzavirus A.* Virions normally measure 80-120 nm in diameter, but filamentous forms can be 400-800nm in length. Influenza viruses are classified into one of three types or genera (*Influenzavirus* A, B or C) based on the antigenicity of their internal nucleocapsid (NP) and matrix (M) proteins. Types B and C influenza viruses typically infect humans, but occasionally infect marine mammals and swine. Only type A influenza viruses are established in a number of animal species, infecting humans, horses, swine, mink, poultry, and a variety of wild mammals and birds. Influenza A viruses are further classified into subtypes based on the antigenicity of the hemagglutinin (HA) and neuraminidase (NA) glycoproteins located on the viral envelope. There are currently 16 HA (H1-H16) and nine NA (N1-N9) glycoprotein subtypes recognized. Each influenza A virus contains one combination of HA and NA subtypes, e.g., H3N8. All influenza viruses that infect birds are type A.

The genome of influenza A viruses consists of eight negative-sense, single-stranded RNA segments coding for eight structural and three nonstructural proteins. The three largest gene segments encode for the subunits of the viral polymerase and are named PB2, PB1, and PA, respectively, according to their basic (PB1, PB2) or acidic (PA) characteristics on isoelectric gels. The viral polymerase is responsible for transcribing messenger RNA (mRNAs), for synthesizing positive-sense template RNAs (cRNAs) from the viral genome, and for transcribing the cRNAs into viral gene segments (vRNAs) that are packaged into progeny viruses. The fourth gene segment encodes the hemagglutinin glycoprotein (HA), which serves to bind host

cell membrane sialic acid receptors and facilitate membrane fusion during entry into the host cell. HA is synthesized as a precursor protein, H0, which must be cleaved by host proteases into H1 and H2 glycoproteins before the virus can gain entry into the cell. The cleavability of the HA by host cell proteases is the most important determinant of viral pathogenicity. The HA is also the major target of host neutralizing antibodies. Segment five encodes the nucleoprotein (NP), an internal protein that surrounds the vRNA and cRNA to form the ribonucleoprotein complex, RNP. RNP is the gene template recognized by the viral polymerase. The neuraminidase (NA) protein is encoded by the sixth segment. NA is a surface protein that cleaves sialic acid at the virion-host cell interface to allow the release of mature virions. The NA is also a major antigenic target of host antibodies. The seventh segment encodes for two proteins by alternative splicing, the matrix (M1) and M2 proteins. The M1 is the most abundant protein in the mature virion and is primarily a structural protein. It also plays a role in virus assembly. The M2 is a small transmembrane envelope protein that acts as a proton channel during viral uncoating. Segment eight also encodes two proteins, originally named NS1 and NS2 because they were thought to be non-structural. The NS2 mediates the export of newly formed RNPs from the cell nucleus and is also called the nuclear export protein (NEP). NS2 is present in small amounts in virions in association with M1 and the RNP. The truly non-structural NS1 is a regulator of mRNA splicing and translation, and modulates host interferon responses to viral infection. Thus, NS1 is directly related to the pathogenicity of the virus strain. Recently, an additional protein encoded by the +1 open reading frame (ORF) of PB1 (PB1-F2) was found (Chen et al., 2001). The C-terminal domain of this protein can trigger apoptosis in immune-related cells and increase comorbidity to secondary bacterial infections in mice (Zamarin et al., 2006). However, the role of this protein in AIV pathogenesis in birds is not clear.

Disease caused by AIV infection in birds can range from asymptomatic infection to peracute, fatal disease. AIV can be subdivided into low pathogenicity avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) based on *in vivo* testing of an isolate's virulence in

experimentally infected chickens or *in vitro* testing that identifies the amino acid sequence defining the cleavage domain of the isolate's HA protein. The U.S. Animal Health Association defines HPAI as any AIV that: 1) kills 75% or greater of 4- to 6-week-old naïve chickens within 10 days of intravenous inoculation; 2) is of the H5 or H7 subtype with multiple basic amino acids at the proteolytic cleavage site of HA; or 3) kills one of five chickens and can grow in cell culture without supplemental trypsin (U.S. Animal Health Association, 1994). Any AIV that do not meet established criteria for classification as HPAI are, by default, LPAI. The World Organization for Animal Health (OIE) defines notifiable forms of avian influenza (NAI) as an infection of poultry caused by any virus of the H5 or H7 subtypes or by any AIV with an intravenous pathogenicity index (IVPI) greater than 1.2. NAI viruses may be HPAI or LPNAI (low pathogenicity notifiable avian influenza) viruses (OIE, 2008).

Influenza A viruses are renowned for their rapid evolution. Frequent point mutations in the viral genome are attributable to the high error rate of the RNA polymerase (i.e., the lack of proofreading ability during RNA transcription, Suarez, 2000), and to evolutionary selection due to immune pressure of the infected host (Webster et al., 1992b). Genes encoding for the envelope glycoproteins HA and NA, not surprisingly, show the highest evolutionary rate because these glycoproteins are the primary targets of host neutralizing antibodies and other immune processes and are under the greatest selection pressure. Such changes in the antigenic properties of AIV are termed antigenic drift. In contrast, antigenic shift results from reassortment of gene segments from two or more parent viruses coinfecting a host cell. Progeny virions may contain genes descended from distantly related lineages or different HA or NA antigenic subtypes, resulting in viruses with substantially different antigenic properties and fitness in the host. New HA-NA subtype combinations frequently occur via reassortment when multiple AIV are cocirculating in a susceptible host population (Hinshaw et al., 1980; Hatchette et al., 2004; Wallensten et al., 2005).

All AIV gene segments are divided into North American and Eurasian lineages based on the relatedness of their gene sequences. These lineages most likely result from long-term geographical and ecological separations of natural host species between the Eastern and Western Hemispheres (Webster et al., 1992a; Olsen et al., 2006) However, reassortant viruses containing gene segments from both North American and Eurasian lineages are occasionally isolated, indicating that some interhemispheric movement of viruses occurs (Makarova et al., 1999; Wallensten et al., 2005; Krauss et al., 2007; Koehler et al., 2008).

## AVIAN INFLUENZA IN DOMESTIC BIRDS

The known history of avian influenza spans only the last 130 years (Lupiani and Reddy, 2009). The first described outbreak of clinical avian influenza, or “fowl plague”, in domestic poultry occurred during 1878 in Italy. The disease spread rapidly to eastern Austria and Germany, and later to Belgium and France, with the stock of an itinerant poultry merchant. HPAI became endemic in central Europe during the early 1900’s and had been reported in most of Europe, Russia, the Middle East, Asia, and North and South America by the mid-1900’s. In the United States, the first HPAI outbreak occurred during fall-winter of 1924-1925, originating in the live bird markets of New York City. Disease rapidly spread through New Jersey, Connecticut, and Pennsylvania, and was recorded in Indiana, Michigan, West Virginia, Missouri, and Illinois within months. Contaminated rail cars originating from the East coast were the most likely source of disease spread.

The first HPAI epizootic confirmed by virus isolation occurred in Scotland in 1959. Since then, at least 28 HPAI epizootics have been recorded worldwide, half of which have occurred during the past 10 years (Alexander, 2007; Lupiani and Reddy, 2009). The majority of outbreaks had limited geographical spread, in many cases limited to a single farm or flock of birds. Some of these outbreaks were self-limiting, while immediate government- or self-imposed culling of infected flocks and disinfection of premises undoubtedly prevented disease spread in many other cases. However, many other HPAI outbreaks have become geographically widespread,

resulting in devastating economic losses. For example, the 1983-1984 H5N2 outbreak among chickens and turkeys in Pennsylvania resulted in the destruction of >17 million birds at a direct cost of $62 million and indirect costs estimated at >$250 million (Fitchner, 1987). Other recent HPAI outbreaks that incurred devastating losses occurred in Mexico (H5N2; 1994), Pakistan (H7N3; 1994), Hong Kong (H5N1; 1997), Italy (H7N1; 1999), Chile (H7N3; 2002), The Netherlands (H7N7; 2003), Canada (H7N3; 2004), and the ongoing (since 2002) H5N1 panzootic affecting areas of Asia, Europe, Africa, and the Middle East.

In domestic poultry, LPAI causes negligible to moderate clinical disease and corresponding economic losses, e.g., through reduced egg production. LPAIV of subtypes H5 and H7, when circulating in poultry, can become highly pathogenic through mutation (Ito et al., 2001), recombination (Suarez et al., 2004), or oligonucleotide deletion (Spackman et al., 2003) or insertion (Suarez et al., 2004). Once these viruses mutate into highly pathogenic form, they can cause rapid mortality and severe economic losses like those described above.

Phylogenetic and epidemiological evidence suggests that LPAIV are occasionally transferred from wild avian reservoirs to domestic birds, in which they can, rarely, mutate into pathogenic forms (Garcia et al., 1997; Capua and Alexander, 2007). Initial transmission can occur when domestic birds come into contact with fecal material from infected waterfowl or gulls, or aquatic environments containing infective virus. For example, shared water sources were deemed responsible for LPAI virus transmission between gulls and ranged domestic turkeys (Sivanandan et al., 1991); virus isolation from the water preceded virus or antibody detection in the turkeys. In some cases, viruses isolated from wild waterfowl near to and shortly before AI outbreaks in poultry shared a recent genetic ancestor (Campitelli et al., 2004; Spackman et al., 2006), indicating potential introduction from waterfowl into poultry. Phylogenetically, viral RNA sequences from poultry outbreaks generally cluster with those from waterfowl and gulls and indicate gene spillover from these aquatic bird reservoirs (Garcia et al., 1997; Widjaja et al., 2004; Spackman et al., 2005).

## AVIAN INFLUENZA IN WILD BIRDS

Influenza infection in wild birds was first recognized during 1961, when a HPAIV of subtype H5N3 was implicated in the mortality of at least 1,300 Common Terns (*Sterna hirundo*) from a colony in South Africa (Becker, 1966). Subsequently, extensive surveillance has revealed asymptomatic LPAIV infection in at least 105 wild bird species of 13 orders (Stallknecht and Shane, 1988a; Olsen et al., 2006), and revealed that aquatic birds are the natural hosts of AIV (Webster et al., 1992b). All 16 HA and nine NA subtypes have been isolated from wild aquatic birds, in at least 103 of the 144 possible combinations (Bao et al., 2008). Currently, AIV has a known worldwide distribution; virus has been isolated from wild birds in Africa, Australia, Asia, Europe, North America, and South America (Stallknecht and Shane, 1988a; Olsen et al., 2006; Spackman et al., 2007; Pereda et al., 2008). Serologic evidence of exposure to AIV has been documented in Antarctic birds (Morgan and Westbury, 1981; Austin and Webster, 1993; Wallensten et al., 2006b; Miller et al., 2008).

AIV are primarily transmitted by an indirect fecal-oral route through virus-contaminated water (Webster et al., 1978; Hinshaw et al., 1979). Infection in the virus's natural hosts is usually asymptomatic and produces no gross or microscopic pathology, but occasionally produces inflammation in the enteric or respiratory tracts which is often associated with secondary bacterial infections (Swayne and Halvorson, 2003).

### Avian influenza in the Anseriformes

Waterfowl (order Anseriformes) are considered the natural host and most important reservoir of LPAIV based on global high prevalence, large viral subtype and genetic diversity, prolonged shedding of large amounts of virus, and asymptomatic infection within these species (Stallknecht and Shane, 1988a; Webster et al., 1992b).

AIV prevalence varies considerably among the different families, subfamilies, and even individual species of waterfowl. In the review by Olsen et al. (2006), overall AIV isolation rates in swans and geese (subfamily Anserinae) were 1.9% and 1.0%, respectively. Prevalence was

9.5% among ducks (subfamily Anatinae), but when dabbling ducks (tribe Anatini) and diving ducks (tribe Aythyini) were distinguished, prevalences were 10% and 1.6%, respectively. Geese and swans often graze on land, dabbling ducks feed primarily on surface waters in freshwater wetlands, and diving ducks feed at greater depths and often in marine environments. Differences in these species' ecologies determine their exposure to, and infection with, AIV present in the environment (Stallknecht and Shane, 1988a; Sivanandan et al., 1991; Garamszegi and Moller, 2007).

AIV prevalence in waterfowl is highest during late summer- fall, when large numbers of immunologically naïve juvenile birds aggregate with adults before southward migration (Hinshaw et al., 1985). Many studies conducted in North America and northern Europe have found AIV prevalence in juvenile dabbling ducks, especially Mallards (*Anas platyrhynchos)*, exceeding 40% during late summer-fall (e.g., Deibel et al., 1985; Sivanandan et al., 1991; Parmley et al., 2008). AIV prevalence drops as southward migration occurs, and generally is low during winter (Stallknecht and Shane, 1988b; Fouchier et al., 2003; Hanson et al., 2005; Gaidet et al., 2007). However, several recent studies have documented relatively high AIV prevalence in certain species during winter and northward migration (Sivanandan et al., 1991; De Marco et al., 2003; Hanson et al., 2005; Wallensten et al., 2006a; Gaidet et al., 2007; Terregino et al., 2007; Jahangir et al., 2008).

Phylogenetic analyses of North American waterfowl AIV do not show significant clustering by species or geographical location, indicating that viruses circulate freely across flyways and populations (Spackman et al., 2005; Chen and Holmes, 2009).

**Avian influenza in the Charadriiformes**

Birds in the order Charadriiformes are repeatedly cited as a natural wildlife reservoir for avian influenza viruses (Webster et al., 1992a; Webster et al., 2007). Three groups of Charadriiformes species are considered important in the ecology of AIV: gulls (family Laridae, subfamily Larinae), terns (family Laridae, subfamily Sterninae), and shorebirds (strictly, families

Charadriidae [plovers] and Scolopacidae [sandpipers and allies]). AIV have also been detected in Common Murres (*Uria aalge*) in the Alcidae (Auk) family (Wallensten et al., 2005), skuas in the Stercorariidae family (Austin and Webster, 1993; Miller et al., 2008), and oystercatchers in the Haematopodidae family (Ludwig et al., 1994; Ghersi et al., 2009).

Larid species (gulls and terns) are probably the most important Charadriiformes hosts for LPAIV in nature. AIV frequently have been detected in gulls and/or terns on every continent except Antarctica (e.g., Graves, 1992; Süss et al., 1994; Lvov et al., 2001; Olsen et al., 2006; Gaidet et al., 2007; Lebarbenchon et al., 2007; Ip et al., 2008; Kishida et al., 2008; Pereda et al., 2008) In fact, the HPAI virus that caused the 1961 Common Tern mortality event in South Africa (Becker, 1966) was the first AIV detected in wild birds. AIV prevalence in the Laridae generally is highest during summer-early fall (Olsen et al., 2006), possibly because they are then breeding in dense colonies. While AIV transmission in the Laridae is not understood, colonial breeding may facilitate virus spread through direct bird-to-bird contact.

In contrast, AIV are detected in shorebirds very rarely, with one location and species exception. Each spring, AIV infect a substantial proportion of Ruddy Turnstones (*Arenaria interpres*) during their approximately five-week stopover in Delaware Bay. Syntopic shorebird species such as Red Knots (*Calidris canutus rufa*) and Sanderlings (*Calidris alba*) are infected at much lower rates; prevalence in these species is proportional to prevalence in Ruddy Turnstones in a given year. Of 292 AIV isolated from Delaware Bay shorebirds between 2000 and 2005, 90% ($n$=262) were from Ruddy Turnstone samples (Hanson et al., 2008).

AIV isolations are rarely made from shorebirds outside of Delaware Bay, despite recent intensive surveillance inspired by outbreaks of HPAI H5N1 in Eurasia (Sivanandan et al., 1991; Ito et al., 1995; Fouchier et al., 2003; Hlinak et al., 2006; Langstaff and McKenzie, 2006; Cattoli et al., 2007; D'Amico et al., 2007; Gaidet et al., 2007; Winker et al., 2007; Escudero et al., 2008; Hanson et al., 2008; Ip et al., 2008; Winker et al., 2008). Approximately 85% of shorebird AIV

isolates from which sequences have been submitted to GenBank were sampled at Delaware Bay, with the remainder from Australia, Alaska, Hong Kong, and Georgia (Bao et al., 2008).

Similarly, attempts to detect AIV specifically in Ruddy Turnstones in Alaska (Winker et al., 2007; Ip et al., 2008), Florida, Georgia, and Bermuda (Hanson et al., 2008), northern Europe (Sivanandan et al., 1991), and Germany (Hlinak et al., 2006) have failed. Only two studies report positive isolations in Ruddy Turnstones outside of Delaware Bay, both located in South America. The first is Diamante Bronco saline in northeastern Brazil (Secretaria de Vigilância em Saúde, 2004b). During March-April 2003, 61 cloacal swab samples from Ruddy Turnstones were combined into three pools; two pools were positive for AIV subtype H3 by virus isolation. Additionally, H3 viruses were detected in single-species sample pools from Semipalmated Sandpipers (*Calidris pusilla*), White-rumped Sandpipers (*Calidris fusicollis*), Black-bellied Plovers (*Pluvialis squatarola*), and Semipalmated Plovers (*Charadrius semipalmatus*), and one pool containing a mixture of samples from a Red Knot, a Sanderling, a Gull-billed Tern (*Gelochelidon nilotica*), three Least Sandpipers (*Calidris minutila*), and a Whimbrel (*Numenius phaeopus*). No other subtypes were isolated. Most of the birds sampled at this location, including Ruddy Turnstones, belong to the same populations that migrate through Delaware Bay each spring (Myers et al., 1990; Perkins et al., 2007; Piersma, 2007). Interestingly, no H3 AIVs were detected at Delaware Bay in May 2003 (Hanson et al., 2008). In November 2003, H2 and H4 AIVs were isolated from mixed-Charadriiformes-species sample pools at the same location in Brazil (Secretaria de Vigilância em Saúde, 2004a). Samples from Ruddy Turnstones were present in two of four positive pools, and in two of three negative pools. Similarly, no H2 or H4 AIV were isolated in Delaware Bay during the previous spring. These isolation data reveal that different AIV strains circulated within the same population of shorebirds at two consecutive migratory stopover locations, with no transfer of virus between sites.

A second study isolated two H10N9 viruses from Ruddy Turnstones in October 2006 at Puerto Viejo along the coast of central Peru (Ghersi et al., 2009). Prevalence was 3.4% at this

site (*n*=61), but all samples were negative at a nearby site (*n*=20). A genetically identical H10N9 virus was isolated from an American Oystercatcher (*Haematopus palliates*) at the Puerto Viejo site two weeks later; no waterfowl were infected with this strain. No H10 viruses were isolated at Delaware Bay during the springs immediately prior to or following the above study (2006 and 2007), but were present in 2000, 2001, 2004, and 2008.

Shorebirds, including Ruddy Turnstones, may be unable to carry a given AIV over long migration distances. Alternatively, infected birds may be unable to fly the long distances between stopovers, an idea supported by the reduced migratory performance observed in LPAI-infected Bewick's Swans (*Cygnus columbianus bewickii*) (van Gils et al., 2007). Although few AIV have been isolated and sequenced from South America, their internal proteins form monophyletic branches suggesting that there is limited movement of AIV between the South and North American continents (Pereda et al., 2008).

Kawaoka et al. (1988) first proposed that shorebirds and gulls maintain a different set of AIV than do waterfowl. Shorebird and Laridae isolates were mostly H9 and H13 subtypes, which were rare or absent in ducks previously sampled in Alberta and New York. More than 50% of these viruses did not replicate in experimentally infected ducks. Likewise, Sharp et al. (1993) proposed that waterfowl do not maintain all AIV subtypes in nature, since a large proportion of HA and NA subtypes were rare in Alberta ducks. Recent phylogenetic analyses confirm that gulls harbor unique AIV subtypes (H13 and H16) and genetic lineages of internal gene segments that are typically not found in waterfowl (Gorman et al., 1990; Obenauer et al., 2006). Also, the structure of the hemagglutinin binding site differs between "gull"- and "duck"-lineage viruses (Yamnikova et al., 2003; Fouchier et al., 2005), suggesting specific virus adaptation between these species groups. AIV from shorebirds are genetically similar to those from either ducks or gulls; no distinct lineages have been associated with shorebirds (Widjaja et al., 2004; Spackman et al., 2005; Jackwood and Stallknecht, 2007).

### Avian influenza in other avian orders

Most of the remaining avian orders from which AIV has been isolated are associated from aquatic habitats, including Gaviiformes (loons), Podipedicepiformes (grebes), Procellariformes (shearwaters and petrels), Pelicaniformes (pelicans and cormorants), Ciconiiformes (herons and ibises), and Gruiformes (coots and moorhens) (Stallknecht and Shane, 1988b; Olsen et al., 2006). It is unclear if infection in any of these species is important to the maintenance of AIV in nature, or if these isolations represent accidental infections through shared environment with known reservoir species (e.g., waterfowl or gulls).

AIV have also been isolated or detected by RT-PCR in wild birds of some terrestrial orders, including Galliformes (pheasants), Columbiformes (doves and pigeons), Piciformes (woodpeckers), and Passeriformes (songbirds) (Stallknecht and Shane, 1988b; De Marco et al., 2005; Peterson et al., 2008). Again, the significance of birds in these orders to the maintenance of LPAI is unknown.

## AVIAN INFLUENZA IN THE ENVIRONMENT

AIV transmission in aquatic birds is thought to occur through an indirect fecal-oral route involving virus-laden water (Webster et al., 1978; Hinshaw et al., 1979). AIV have been isolated from natural, open, fresh water such as ponds (Hinshaw et al., 1979; Sivanandan et al., 1991; Ito et al., 1995) and, potentially, shared water sources have aided the introduction of AIV into domestic birds (Sivanandan et al., 1991; Terregino et al., 2007).

The maintenance of AIV in waterfowl populations may also depend on virus persistence in the environment. Experimental studies have shown that wild-type AIV can survive in water for extended periods (Webster et al., 1978; Stallknecht et al., 1990a; Stallknecht et al., 1990b; Brown et al., 2007; Brown et al., 2009). Although persistence in these studies varied between isolates tested under the same conditions, AIV survival was longest in cold fresh to brackish water (4-17°C, 0-20 ppt salinity) with neutral or slightly basic pH (7.4-8.2). Estimated persistence often exceeded 200 days under these conditions, and at least 10% of infective virus

remained after 25-30 d. Even under conditions of warm temperature (28°C) and high salinity (20-30 ppt), estimated virus persistence exceeded 20 d. Prolonged virus infectivity in water potentially enhances its transmissibility between birds.

If viruses are preserved over winter in the aquatic environment, these may serve as a source of infection as migratory species return the following spring (Webster et al., 1992b). However, to date, no studies have successfully documented infective AIV in frozen aquatic environments. Viral RNA has been detected in frozen pond sediments many months after waterfowl had departed and also in early spring as they returned (Lang et al., 2008), but the authors did not attempt to culture live viruses so infectivity is unknown. Avian influenza viral RNA was reportedly detected in Siberian lake ice (Zhang et al., 2006), however, Worobey (2008) identified that the RNA detected belonged to a positive control strain used in the study.

## AVIAN INFLUENZA AS A PUBLIC HEALTH ISSUE

Direct transmission of AIV from birds to humans has only been recognized in the last decade. Transmission of HPAI H5N1 from dead or ill poultry to humans, cats, dogs, and other mammals has challenged prior dogma that AIV must combine with a human influenza viruses in swine (the “mixing vessel”) before they are infective to humans. The high case-fatality rate for humans infected with HPAI H5N1 has led to the concern that if this virulent virus adapts to be successfully transmitted between humans then a deadly pandemic might result. Poultry-adapted AIV of other subtypes have also been involved in human morbidity and mortality, most notably enzootic LPAI H9N2 in Asia (Peiris et al., 1999; Butt et al., 2005), and epizootic HPAI H7N7 in the Netherlands (Fouchier et al., 2004).

Few studies have detailed the transmission risk of AIV, and particularly LPAI, among persons with regular contact with domestic birds. Iowa veterinarians exposed to domestic chickens, turkeys, ducks, geese, and/or quail had elevated antibody titers to AIV subtypes H5, H6, H7, and H9, and were 12-17 times more likely to be antibody positive than control subjects (Myers et al., 2007). Veterinarians that had examined birds with known AIV infection were more

likely to be seropositive than veterinarians without this exposure. These results indicate occupational exposure to AIV, but not necessarily increased risk of illness.

Contact with wild birds may rarely result in avian-to-human transmission of LPAIV. Persons with direct contact with infected waterfowl and their aquatic habitats, such as waterfowl hunters and waterfowl biologists, are considered at highest risk. Waterfowl banding activities occur primarily in the late summer and fall, concurrent with the period of peak AIV prevalence and shedding. Serological evidence of exposure to AIV subtype H11 was recorded in one of 39 (2.6%) duck hunters and two of 68 (2.9%) wildlife biologists in Iowa (Gill et al., 2006); all had 27-31 years of waterfowl exposure and handled >100 ducks annually. Seropositive individuals reported no associated illness, and the overall low seroprevalence among these groups suggests that successful transmission of LPAI between waterfowl and humans is rare. However, the risk of transfer of HPAI H5N1 from wild waterfowl sources to humans may be higher. In Azerbaijan, a family cluster of human H5N1 cases occurred after the affected patients had collected feathers from wild mute swans that had succumbed to HPAI H5N1 (Gilsdorf et al., 2006), and to date these cases are the only recorded influenza illnesses resulting from contact with wild birds.

## THE SHOREBIRD ANNUAL CYCLE, MIGRATION PHYSIOLOGY, AND THE DELAWARE BAY ECOSYSTEM

Delaware Bay is recognized as one of the most important and critical shorebird migration stopovers in the Western Hemisphere, and indeed, the world (Dunne et al., 1982; Stroud et al., 2006). Between 12-80% of the Atlantic flyway population of six beach-inhabiting shorebirds (Red Knot, Sanderling, Ruddy Turnstone, Semipalmated Sandpiper, Dunlin [*Calidris alpina*], and Short-billed Dowitcher [*Limnodromus griseus*]) stop at Delaware Bay during northward migration (U. S. Fish and Wildlife Service, 2003). Each of the above shorebird species undertake a series of long-distance, nonstop flights to travel between their wintering grounds in South and Central America and their breeding grounds in the Canadian and Alaskan Arctic.

Because shorebirds often cross vast areas of open water between stopovers, environmental and physiological conditions upon departure can have direct and immediate effects on survival. For example, Red Knots must gain a minimum estimated 47-65 g of body fat (41-57% of their mean arrival mass of 115 g) during the stopover at Delaware Bay to reach their breeding grounds in the Arctic (Baker et al., 2004).

Delaware Bay contains the largest population of breeding horseshoe crabs (*Limulus polyphemus*) along the Atlantic coast (U. S. Fish and Wildlife Service, 2003), which spawn *en masse*, especially at full and new moons, during late April-June. Shorebird migration is timed so the stopover at Delaware Bay coincides with the crab spawn; migrant shorebirds begin arriving at Delaware Bay in late April, reach peak numbers in mid-late May, and largely depart by the first week of June. Horseshoe crab eggs comprise 80-100% of a shorebird's diet while at Delaware Bay (Tsipoura and Burger, 1999), and individual birds may consume up to 2 eggs/second (Stillman et al., 2003).

Many long-distance migrant birds, and especially shorebirds, undergo atrophy of internal organs just before undertaking a leg of migration. Of these, the gastrointestinal tract undergoes the greatest decrease in mass (Piersma et al., 1999; Baker et al., 2004; Van Gils et al., 2005). Because horseshoe crab eggs are soft-shelled, it is thought that they are easily digested and shorebirds that feed on them do not need to increase gut mass during the stopover. Fat deposition is maximized and stopover length is minimized under these conditions.

At least 500,000 individual shorebirds stop over at Delaware Bay each spring (Clark et al., 1993), although some estimates are much higher (Dunne et al., 1982). While feeding together in mixed flocks on Delaware Bay beaches, shorebirds may reach densities of up to 210 birds/$m^2$ (Gillings et al., 2007). At no other aggregation site worldwide do shorebirds reach similar densities (C. Minton, personal communication).

## OBJECTIVES

### Chapter 3: Patterns of avian influenza virus infection and exposure among shorebirds and gulls at Delaware Bay

In this study, we concentrate on potential host related factors that may enhance or restrict AIV infection in shorebirds. Specific objectives were to: 1) characterize recent patterns of AIV prevalence, antibody prevalence, and subtype distribution and diversity across charadriiform species during the shorebird stopover season at Delaware Day, as well as across the years of the study, 2) identify population- and individual bird-related factors that are associated with AIV infection or antibody status in three key species (Ruddy Turnstones, Red Knots, and Sanderlings, and 3) provide possible explanations based on our current knowledge of shorebird behavior, migration physiology, stopover ecology, and immune responses for the patterns we observe.

### Chapter 4: Avian influenza virus infection dynamics in shorebird hosts

Our objectives were to describe temporal patterns of AIV prevalence and antibody prevalence within three shorebird species (Ruddy Turnstones, Red Knots, and Sanderlings) and one gull species (Laughing Gulls [*Leucophaeus atricilla*]) during the spring migratory stopover at Delaware Bay, and compare patterns between species. Such information provided insight into possible AIV sources, transmission, exposure, and maintenance patterns at this location, as well as potential exposures outside of the Delaware Bay stopover.

### Chapter 5: Spring migration stopover ecology of avian influenza virus shorebird hosts at Delaware Bay

Given the gaps in our knowledge of AIV dynamics in Ruddy Turnstones and other shorebirds that use Delaware Bay as a spring migratory stopover, and the potential consequences of transmission to nearby poultry, we conducted a radiotelemetry study of Ruddy Turnstones and Sanderlings during the 5-week stopover period. Our objectives were to answer the questions: 1) what habitats or locations are important for Ruddy Turnstones and Sanderlings

during their stopover at Delaware Bay, both during the day and at night? 2) Are habitats or locations used by shorebirds, particularly Ruddy Turnstones, overlapping with, or in close proximity to, areas used by other species important in the epidemiology of AIV (gulls or waterfowl) or with areas used for poultry production? 3) What movement patterns are exhibited by Ruddy Turnstones, which might spread AIV locally, during the stopover period? 4) Is long-distance movement of AIV likely by shorebirds migrating through Delaware Bay? Specifically, when do birds depart upon their next leg of migration and, in the context of infection dynamics, what proportion might be infected with AIV at the time of departure? The answers to the above questions would not only help describe AIV-shorebird-environment interactions in the context of local and global AIV epidemiology, but also inform conservation efforts for these declining shorebird populations.

**Chapter 6: Annual survival of Ruddy Turnstones is not affected by natural infection with low pathogenicity avian influenza viruses**

The aim of this study was to investigate potential effects of AIV infection on annual survival in Ruddy Turnstones. Our specific objectives were 1) to identify characteristics of individual birds or other potential factors such as year and location that are associated with resighting rate in future years and describe these associations; 2) to determine if there is any difference in resighting probabilities between AIV-infected and uninfected birds and evaluate each potential covariate's role as a confounder to this relationship; and 3) to evaluate if resighting rate is reduced in AIV-infected birds after all significant confounders are taken into account.

## LITERATURE CITED

ALEXANDER, D. J. 2007. An overview of the epidemiology of avian influenza. Vaccine 25: 5637-5644.

AUSTIN, F. J., and R. G. WEBSTER. 1993. Evidence of orthomyxoviruses and paramyxoviruses in fauna from Antarctica. Journal of Wildlife Diseases 29: 568-571.

BAKER, A. J., P. M. GONZÁLEZ, T. PIERSMA, L. J. NILES, I. D. L. S. D. NASCIMENTO, P. W. ATKINSON, N. A. CLARK, C. D. T. MINTON, M. K. PECK, and G. AARTS. 2004. Rapid population decline in red knots: fitness consequences of decreased refuelling rates and late arrival in Delaware Bay. Proceedings of the Royal Society B: Biological Sciences 271: 875-882.

BAO, Y. M., P. BOLOTOV, D. DERNOVOY, B. KIRYUTIN, L. ZASLAVSKY, T. TATUSOVA, J. OSTELL, and D. LIPMAN. 2008. The influenza virus resource at the National Center for Biotechnology Information. Journal of Virology 82: 596-601.

BECKER, W. B. 1966. The isolation and classification of tern virus: Influenza virus A/Tern/South Africa/1961. The Journal of Hygiene 64: 309-320.

BROWN, J. D., D. E. SWAYNE, R. J. COOPER, R. E. BURNS, and D. E. STALLKNECHT. 2007. Persistence of H5 and H7 avian influenza viruses in water. Avian Diseases 51: 285-289.

BROWN, J. D., G. GOEKJIAN, R. POULSON, S. VALEIKA, and D. E. STALLKNECHT. 2009. Avian influenza virus in water: infectivity is dependent on pH, salinity and temperature. Veterinary Microbiology 136: 20-26.

BUTT, K. M., G. J. D. SMITH, H. CHEN, L. J. ZHANG, Y. H. C. LEUNG, K. M. XU, W. LIM, R. G. WEBSTER, K. Y. YUEN, J. S. M. PEIRIS, and Y. GUAN. 2005. Human infection with an avian H9N2 influenza A virus in Hong Kong in 2003. Journal of Clinical Microbiology 43: 5760-5767.

CAMPITELLI, L., E. MOGAVERO, M. A. DE MARCO, M. DELOGU, S. PUZELLI, F. FREZZA, M. FACCHINI, C. CHIAPPONI, E. FONI, P. CORDIOLI, R. WEBBY, G. BARIGAZZI, R. G. WEBSTER, and I. DONATELLI. 2004. Interspecies transmission of an H7N3 influenza virus from wild birds to intensively reared domestic poultry in Italy. Virology 323: 24-36.

CAPUA, I., and D. J. ALEXANDER. 2007. Avian influenza infections in birds - a moving target. Influenza & Other Respiratory Viruses 1: 11-18.

CATTOLI, G., C. TERREGINO, V. GUBERTI, R. DE NARDI, A. DRAGO, A. SALVIATO, S. FASSINA, and I. CAPUA. 2007. Influenza virus surveillance in wild birds in Italy: Results of laboratory investigations in 2003-2005. Avian Diseases 51: 414-416.

CHEN, R., and E. C. HOLMES. 2009. Frequent inter-species transmission and geographic subdivision in avian influenza viruses from wild birds. Virology 383: 156-161.

CHEN, W. S., P. A. CALVO, D. MALIDE, J. GIBBS, U. SCHUBERT, I. BACIK, S. BASTA, R. O'NEILL, J. SCHICKLI, P. PALESE, P. HENKLEIN, J. R. BENNINK, and J. W. YEWDELL. 2001. A novel

influenza A virus mitochondrial protein that induces cell death. Nature Medicine 7: 1306-1312.

CLARK, K. E., L. J. NILES, and J. BURGER. 1993. Abundance and distribution of migrant shorebirds in Delaware Bay. Condor 95: 694-705.

D'AMICO, V. L., M. BERTELLOTTI, A. J. BAKER, and L. A. DIAZ. 2007. Exposure of Red Knots (*Calidris canutus rufa*) to select avian pathogens; Patagonia, Argentina. Journal of Wildlife Diseases 43: 794-797.

DE MARCO, M. A., G. E. FONI, L. CAMPITELLI, E. RAFFINI, L. DI TRANI, M. DELOGU, V. GUBERTI, G. BARIGAZZI, and I. DONATELLI. 2003. Circulation of influenza viruses in wild waterfowl wintering in Italy during the 1993-99 period: Evidence of virus shedding and seroconvcrsion in wild ducks. Avian Diseases 47: 861-866.

DE MARCO, M. A., L. CAMPITELLI, M. DELOGU, E. RAFFINI, E. FONI, L. DI TRANI, M. SCAFFIDI, and I. DONATELLI. 2005. Serological evidences showing the involvement of free-living pheasants in the influenza ecology. Italian Journal of Animal Science 4: 287-291.

DEIBEL, R., D. E. EMORD, W. DUKELOW, V. S. HINSHAW, and J. M. WOOD. 1985. Influenza viruses and paramyxoviruses in ducks in the Atlantic Flyway, 1977-1983, including an H5N2 isolate related to the virulent chicken virus. Avian Diseases 29: 970-985.

DUNNE, P., D. SIBLEY, C. SUTTON, and W. WANDER. 1982. Aerial surveys in Delaware Bay: confirming an enormous spring staging area for shorebirds. International Wader Study Group Bulletin 35: 32-33.

ESCUDERO, G., V. J. MUNSTER, M. BERTELLOTTI, and P. EDELAAR. 2008. Perpetuation of avian influenza in the Americas: Examining the role of shorebirds in Patagonia. Auk 125: 494-495.

FITCHNER, G. J. 1987. The Pennsylvania/Virginia experience in eradication of avian influenza H5N2. . *In* Proceedings of the Second International Symposium on Avian Influenza. U.S. Animal Health Association, Madison, WI, pp. 33-38.

FOUCHIER, R. A. M., B. OLSEN, T. M. BESTEBROER, S. HERFST, L. VAN DER KEMP, G. E. RIMMELZWAAN, and A. D. M. E. OSTERHAUS. 2003. Influenza A virus surveillance in wild birds in Northern Europe in 1999 and 2000. Avian Diseases 47: 857-860.

FOUCHIER, R. A. M., P. M. SCHNEEBERGER, F. W. ROZENDAAL, J. M. BROEKMAN, S. A. G. KEMINK, V. MUNSTER, T. KUIKEN, G. F. RIMMELZWAAN, M. SCHUTTEN, G. J. J. VAN DOORNUM, G. KOCH, A. BOSMAN, M. KOOPMANS, and A. D. M. E. OSTERHAUS. 2004. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. Proceedings of the National Academy of Sciences of the United States of America 101: 1356-1361.

FOUCHIER, R. A. M., V. MUNSTER, A. WALLENSTEN, T. M. BESTEBROER, S. HERFST, D. SMITH, G. F. RIMMELZWAAN, B. OLSEN, and A. D. M. E. OSTERHAUS. 2005. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from Black-headed Gulls. Journal of Virology 79: 2814-2822.

GAIDET, N., T. DODMAN, A. CARON, G. BALANCA, S. DESVAUX, F. GOUTARD, G. CATTOLI, F. LAMARQUE, W. HAGEMEIJER, and F. MONICAT. 2007. Avian influenza viruses in water birds, Africa. Emerging Infectious Diseases 13: 626-629.

GARAMSZEGI, L. Z., and A. P. MOLLER. 2007. Prevalence of avian influenza and host ecology. Proceedings Biological Sciences 274: 2003-2012.

GARCIA, M., D. L. SUAREZ, J. M. CRAWFORD, J. W. LATIMER, R. D. SLEMONS, D. E. SWAYNE, and M. L. PERDUE. 1997. Evolution of H5 subtype avian influenza A viruses in North America. Virus Research 51: 115-124.

GHERSI, B. M., D. L. BLAZES, E. ICOCHEA, R. I. GONZALEZ, T. KOCHEL, Y. TINOCO, M. M. SOVERO, S. LINDSTROM, B. SHU, A. KLIMOV, A. E. GONZALEZ, and J. M. MONTGOMERY. 2009. Avian influenza in wild birds, central coast of Peru. Emerging Infectious Diseases 15: 935-938.

GILL, J. S., R. WEBBY, M. J. R. GILCHRIST, and G. C. GRAY. 2006. Avian influenza among waterfowl hunters and wildlife professionals. Emerging Infectious Diseases 12: 1284-1286.

GILLINGS, S., P. W. ATKINSON, S. L. BARDSLEY, N. A. CLARK, S. E. LOVE, R. A. ROBINSON, R. A. STILLMAN, and R. G. WEBER. 2007. Shorebird predation of horseshoe crab eggs in Delaware Bay: species contrasts and availability constraints. Journal of Animal Ecology 76: 503-514.

GILSDORF, A., N. BOXALL, V. GASIMOV, I. AGAYEV, F. MAMMADZADE, P. URSU, E. GASIMOV, C. BROWN, S. MARDEL, D. JANKOVIC, G. PIMENTEL, I. AMIR AYOUB, E. MAHER LABIB ELASSAL, C. SALVI, D. LEGROS, C. PESSOA DA SILVA, R. HAY A. ANDRAGHETTI, G. RODIER, and B. GANTER. 2006. Two clusters of human infection with influenza A/H5N1 virus in the Republic of Azerbaijan, February–March 2006. Eurosurveillance 11: pii=620.

GORMAN, O. T., R. O. DONIS, Y. KAWAOKA, and R. G. WEBSTER. 1990. Evolution of influenza A virus PB2 genes: implications for evolution of the ribonucleoprotein complex and origin of human influenza A virus. Journal of Virology 64: 4893-4902.

GRAVES, I. L. 1992. Influenza viruses in birds of the Atlantic Flyway. Avian Diseases 36: 1-10.

HANSON, B. A., D. E. SWAYNE, D. A. SENNE, D. S. LOBPRIES, J. HURST, and D. E. STALLKNECHT. 2005. Avian influenza viruses and paramyxoviruses in wintering and resident ducks in Texas. Journal of Wildlife Diseases 41: 624-628.

HANSON, B. A., M. P. LUTTRELL, V. H. GOEKJIAN, L. NILES, D. E. SWAYNE, D. A. SENNE, and D. E. STALLKNECHT. 2008. Is the occurrence of avian influenza virus in Charadriiformes species and location dependent? Journal of Wildlife Diseases 44: 351-361.

HATCHETTE, T. F., D. WALKER, C. JOHNSON, A. BAKER, S. P. PRYOR, and R. G. WEBSTER. 2004. Influenza A viruses in feral Canadian ducks: extensive reassortment in nature. Journal of General Virology 85: 2327-2337.

HINSHAW, V. S., R. G. WEBSTER, and B. TURNER. 1979. Water-borne transmission of influenza A viruses. Intervirology 11: 66-68.

HINSHAW, V. S., W. J. BEAN, R. G. WEBSTER, and G. SRIRAM. 1980. Genetic reassortment of influenza A viruses in the intestinal tract of ducks. Virology 102: 412-419.

HINSHAW, V. S., J. M. WOOD, R. G. WEBSTER, R. DEIBEL, and B. TURNER. 1985. Circulation of influenza viruses and paramyxoviruses in waterfowl originating from 2 different areas of North America. Bulletin of the World Health Organization 63: 711-719.

HLINAK, A., R. U. MUHLE, O. WERNER, A. GLOBIG, E. STARICK, H. SCHIRRMEIER, B. HOFFMANN, A. ENGELHARDT, D. HUBNER, F. J. CONRATHS, D. WALLSCHLAGER, H. KRUCKENBERG, and T. MULLER. 2006. A virological survey in migrating waders and other waterfowl in one of the most important resting sites of Germany. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 53: 105-110.

IP, H. S., P. L. FLINT, J. C. FRANSON, R. J. DUSEK, D. V. DERKSEN, R. E. GILL, JR., C. R. ELY, J. M. PEARCE, R. B. LANCTOT, S. M. MATSUOKA, D. B. IRONS, J. B. FISCHER, R. M. OATES, M. R. PETERSEN, T. F. FONDELL, D. A. ROCQUE, J. C. PEDERSEN, and T. C. ROTHE. 2008. Prevalence of Influenza A viruses in wild migratory birds in Alaska: patterns of variation in detection at a crossroads of intercontinental flyways. Virology Journal 5: 71.

ITO, T., K. OKAZAKI, Y. KAWAOKA, A. TAKADA, R. G. WEBSTER, and H. KIDA. 1995. Perpetuation of influenza A viruses in Alaskan waterfowl reservoirs. Archives of Virology 140: 1163-1172.

ITO, T., H. GOTO, E. YAMAMOTO, H. TANAKA, M. TAKEUCHI, M. KUWAYAMA, Y. KAWAOKA, and K. OTSUKI. 2001. Generation of a highly pathogenic avian influenza A virus from an avirulent field isolate by passaging in chickens. The Journal of Virology 75: 4439-4443.

JACKWOOD, M. W., and D. E. STALLKNECHT. 2007. Molecular epidemiologic studies on North American H9 avian influenza virus isolates from waterfowl and shorebirds. Avian Diseases 51: 448-450.

JAHANGIR, A., Y. WATANABE, O. CHINEN, S. YAMAZAKI, K. SAKAI, M. OKAMURA, M. NAKAMURA, and K. TAKEHARA. 2008. Surveillance of avian influenza viruses in Northern pintails (Anas acuta) in Tohoku District, Japan. Avian Diseases 52: 49-53.

KISHIDA, N., Y. SAKODA, M. SHIROMOTO, G. R. BAI, N. ISODA, A. TAKADA, G. LAVER, and H. KIDA. 2008. H2N5 influenza virus isolates from terns in Australia: genetic reassortants between those of the Eurasian and American lineages. Virus Genes 37: 16-21.

KOEHLER, A. V., J. M. PEARCE, P. L. FLINT, J. C. FRANSON, and H. S. IP. 2008. Genetic evidence of intercontinental movement of avian influenza in a migratory bird: the northern pintail (*Anas acuta*). Molecular Ecology 17: 4754-4762.

KRAUSS, S., C. A. OBERT, J. FRANKS, D. WALKER, K. JONES, P. SEILER, L. NILES, S. P. PRYOR, J. C. OBENAUER, C. W. NAEVE, L. WIDJAJA, R. J. WEBBY, and R. G. WEBSTER. 2007. Influenza in migratory birds and evidence of limited intercontinental virus exchange. Plos Pathogens 3: 1684-1693.

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.

LANGSTAFF, I. G., and J. S. MCKENZIE. 2006. Surveillance for highly pathogenic avian influenza in migratory shorebirds at the terminus of the East Asian-Australasian Flyway. Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics, Cairns, Australia: ISVEE 11: 835.

LEBARBENCHON, C., C. M. CHANG, S. VAN DER WERF, J. T. AUBIN, Y. KAYSER, M. BALLESTEROS, F. RENAUD, F. THOMAS, and M. GAUTHIER-CLERC. 2007. Influenza A virus in birds during spring migration in the Camargue, France. Journal of Wildlife Diseases 43: 789-793.

LUDWIG, S., A. HAUSTEIN, E. F. KALETA, and C. SCHOLTISSEK. 1994. Recent influenza A (H1N1) infections of pigs and turkeys in Northern Europe. Virology 202: 281-286.

LUPIANI, B., and S. M. REDDY. 2009. The history of avian influenza. Comparative Immunology, Microbiology and Infectious Diseases 32: 311-323.

LVOV, D. K., S. S. YAMNIKOVA, A. S. GAMBARYAN, I. T. FEDYAKINA, and M. N. MATROSOVICH. 2001. Isolation of influenza viruses from wild birds in the Volga River basin and in the North Caspian Region. International Congress Series 1219: 251-258.

MAKAROVA, N. A., N. V. KAVERIN, S. KRAUSS, D. SENNE, and R. G. WEBSTER. 1999. Transmission of Eurasian avian H2 influenza virus to shorebirds in North America. Journal of General Virology 80: 3167-3171.

MILLER, G. D., J. M. WATTS, and G. R. SHELLAM. 2008. Viral antibodies in south polar skuas around Davis Station, Antarctica. Antarctic Science 20: 455-461.

MORGAN, I. R., and H. A. WESTBURY. 1981. Virological studies of Adelie penguins (*Pygoscelis adeliae*) in Antarctica. Avian Diseases 25: 1019-1026.

MYERS, J. P., M. SALLABERRY, E. ORTIZ, G. CASTRO, L. M. GORDON, J. L. MARON, C. T. SCHICK, E. TABILO, P. ANTAS, and T. BELOW. 1990. Migration routes of New World Sanderlings (*Calidris alba*). Auk 107: 172-180.

MYERS, K. P., S. F. SETTERQUIST, A. W. CAPUANO, and G. C. GRAY. 2007. Infection due to 3 avian influenza subtypes in United States veterinarians. Clinical Infectious Diseases 45: 4-9.

OBENAUER, J. C., J. DENSON, P. K. MEHTA, X. P. SU, S. MUKATIRA, D. B. FINKELSTEIN, X. Q. XU, J. H. WANG, J. MA, Y. P. FAN, K. M. RAKESTRAW, R. G. WEBSTER, E. HOFFMANN, S. KRAUSS, J. ZHENG, Z. W. ZHANG, and C. W. NAEVE. 2006. Large-scale sequence analysis of avian influenza isolates. Science 311: 1576-1580.

OIE. 2008. Article 10.4: Avian Influenza. *In* The Terrestrial Animal Health Code. The World Organisation for Animal Health (OIE), Paris, France.

OLSEN, B., V. J. MUNSTER, A. WALLENSTEN, J. WALDENSTROM, A. OSTERHAUS, and R. A. M. FOUCHIER. 2006. Global patterns of influenza A virus in wild birds. Science 312: 384-388.

PARMLEY, E. J., N. BASTIEN, T. F. BOOTH, V. BOWES, P. A. BUCK, A. BREAULT, D. CASWELL, P. Y. DAOUST, J. C. DAVIES, S. M. ELAHI, M. FORTIN, F. KIBENGE, R. KING, Y. LI, N. NORTH, D.

OJKIC, J. PASICK, S. P. PRYOR, J. ROBINSON, J. RODRIGUE, H. WHITNEY, P. ZIMMER, and F. A. LEIGHTON. 2008. Wild bird influenza survey, Canada, 2005. Emerging Infectious Diseases 14: 84-87.

PEIRIS, M., K. Y. YUEN, C. W. LEUNG, K. H. CHAN, P. L. S. IP, R. W. M. LAI, W. K. ORR, and K. F. SHORTRIDGE. 1999. Human infection with influenza H9N2. Lancet 354: 916-917.

PEREDA, A. J., M. UHART, A. A. PEREZ, M. E. ZACCAGNINI, L. LA SALA, J. DECARRE, A. GOIJMAN, L. SOLARI, R. SUAREZ, M. I. CRAIG, A. VAGNOZZI, A. RIMONDI, G. KONIG, M. V. TERRERA, A. KALOGHLIAN, H. C. SONG, E. M. SORRELL, and D. R. PEREZ. 2008. Avian influenza virus isolated in wild waterfowl in Argentina: Evidence of a potentially unique phylogenetic lineage in South America. Virology 378: 363-370.

PERKINS, D. E., P. A. SMITH, and H. G. GILCHRIST. 2007. The breeding ecology of Ruddy Turnstones (*Arenaria interpres*) in the eastern Canadian Arctic. Polar Record 43: 135-142.

PETERSON, A., S. BUSH, E. SPACKMAN, D. SWAYNE, and H. IP. 2008. Influenza A virus infections in land birds, People's Republic of China. Emerging Infectious Diseases 14: 1644-1646.

PIERSMA, T., G. A. GUDMUNDSSON, and K. LILLIENDAHL. 1999. Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. Physiological and Biochemical Zoology 72: 405-415.

PIERSMA, T. 2007. Using the power of comparison to explain habitat use and migration strategies of shorebirds worldwide. Journal of Ornithology 148: S45-S59.

SECRETARIA DE VIGILÂNCIA EM SAÚDE. 2004a. Boletim Eletrônico Epidemiológico, Ano 04, No. 5. Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasilia.

SECRETARIA DE VIGILÂNCIA EM SAÚDE. 2004b. Boletim Eletrônico Epidemiológico, Ano 04, No. 2. Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasilia.

SHARP, G. B., Y. KAWAOKA, S. M. WRIGHT, B. TURNER, V. HINSHAW, and R. G. WEBSTER. 1993. Wild ducks are the reservoir for only a limited number of influenza A subtypes. Epidemiology and Infection 110: 161-176.

SIVANANDAN, V., D. A. HALVORSON, E. LAUDERT, D. A. SENNE, and M. C. KUMAR. 1991. Isolation of H13N2 influenza A virus from turkeys and surface water. Avian Diseases 35: 974-977.

SPACKMAN, E., D. A. SENNE, S. DAVISON, and D. L. SUAREZ. 2003. Sequence analysis of recent H7 avian influenza viruses associated with three different outbreaks in commercial poultry in the United States. Journal of Virology 77: 13399-13402.

SPACKMAN, E., D. E. STALLKNECHT, R. D. SLEMONS, K. WINKER, D. L. SUAREZ, M. SCOTT, and D. E. SWAYNE. 2005. Phylogenetic analyses of type A influenza genes in natural reservoir species in North America reveals genetic variation. Virus Research 114: 89-100.

SPACKMAN, E., K. G. MCCRACKEN, K. WINKER, and D. E. SWAYNE. 2006. H7N3 avian influenza virus found in a South American wild duck is related to the Chilean 2002 poultry

outbreak, contains genes from equine and North American wild bird lineages, and is adapted to domestic turkeys. Journal of Virology 80: 7760-7764.

SPACKMAN, E., K. G. MCCRACKEN, K. WINKER, and D. E. SWAYNE. 2007. An avian influenza virus from waterfowl in South America contains genes from North American avian and equine lineages. Avian Diseases 51: 273-274.

STALLKNECHT, D. E., and S. M. SHANE. 1988a. Host range of avian influenza virus in free-living birds. Veterinary Research Communications 12: 125-141.

STALLKNECHT, D. E., and S. M. SHANE. 1988b. Host range of avian influenza virus in free living birds. Veterinary Research Communications 12: 125-141.

STALLKNECHT, D. E., M. T. KEARNEY, S. M. SHANE, and P. J. ZWANK. 1990a. Effects of pH, temperature, and salinity on persistence of avian influenza viruses in water. Avian Diseases 34: 412-418.

STALLKNECHT, D. E., S. M. SHANE, M. T. KEARNEY, and P. J. ZWANK. 1990b. Persistence of avian influenza viruses in water. Avian Diseases 34: 406-411.

STILLMAN, R. A., P. W. ATKINSON, N. A. CLARK, S. GILLINGS, I. G. HENDERSON, S. E. LOVE, R. A. ROBINSON, R. G. WEBER, and S. L. BARDSLEY. 2003. Functional responses of shorebirds feeding on horseshoe crab eggs. *In* Red Knots *Calidris canutus* in Delaware Bay 2002. Survival, Foraging and Marking Strategy. BTO Research Report No. 308. British Trust for Ornithology, pp. 19-28.

STROUD, D. A., A. BAKER, D. E. BLANCO, N. C. DAVIDSON, S. DELANY, B. GANTER, R. GILL, P. GONZALEZ, L. HAANSTRA, R. I. G. MORRISON, T. PIERSMA, D. A. SCOTT, O. THORUP, R. WEST, J. WILSON, and C. ZOKLER. 2006. The conservation and population status of the world's waders at the turn of the millenium. *In* Waterbirds Around the World, G. C. Boere, C. A. Galbraith, and D. A. Stroud, (eds.). TSO Scotland Ltd., Edinburgh, UK, pp. 643-648.

SUAREZ, D. L. 2000. Evolution of avian influenza viruses. Veterinary Microbiology 74: 15-27.

SUAREZ, D. L., D. A. SENNE, J. BANKS, I. H. BROWN, S. C. ESSEN, C. W. LEE, R. J. MANVELL, C. MATHIEU-BENSON, V. MORENO, J. C. PEDERSEN, B. PANIGRAHY, H. ROJAS, E. SPACKMAN, and D. J. ALEXANDER. 2004. Recombination resulting in virulence shift in avian influenza outbreak, Chile. Emerging Infectious Diseases 10: 693-699.

SÜSS, J., J. SCHÄFER, H. SINNECKER, and R. G. WEBSTER. 1994. Influenza virus subtypes in aquatic birds of eastern Germany. Archives of Virology 135: 101-114.

SWAYNE, D. E., and D. A. HALVORSON. 2003. Influenza. *In* Diseases of Poultry, Y. M. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. Mcdougald, and D. E. Swayne, (eds.). Blackwell Publishing, pp. 135-160.

TERREGINO, C., R. DE NARDI, V. GUBERTI, M. SCREMIN, E. RAFFINI, A. M. MARTIN, G. CATTOLI, L. BONFANTI, and I. CAPUA. 2007. Active surveillance for avian influenza viruses in wild birds and backyard flocks in Northern Italy during 2004 to 2006. Avian Pathology 36: 337-344.

TSIPOURA, N., and J. BURGER. 1999. Shorebird diet during spring migration stopover on Delaware Bay. Condor 101: 635-644.

U. S. FISH AND WILDLIFE SERVICE. 2003. Delaware Bay Shorebird-Horseshoe Crab Assessment Report and Peer Review. U. S. Fish and Wildlife Service Migratory Bird Publication R9-03/02, Arlington, VA. 99 pp.

U.S. ANIMAL HEALTH ASSOCIATION. 1994. Report of the Committee on Transmissible Diseases of Poultry and Other Avian Diseases. Criteria for determining that an AI virus isolation causing an outbreak must be considered for eradication. *In* Proceedings of the 98th Annual Meeting U.S. Animal Health Assiciation. U.S. Animal Health Association, Grand Rapids, MI, pp. 522.

VAN GILS, J. A., A. DEKINGA, B. SPAANS, W. K. VAHL, and T. PIERSMA. 2005. Digestive bottleneck affects foraging decisions in red knots Calidris canutus. II. Patch choice and length of working day. Journal of Animal Ecology 74: 120-130.

VAN GILS, J. A., V. J. MUNSTER, R. RADERSMA, D. LIEFHEBBER, R. A. FOUCHIER, and M. KLAASSEN. 2007. Hampered foraging and migratory performance in swans infected with low-pathogenic avian influenza A virus. PLoS ONE 2: e184.

WALLENSTEN, A., V. J. MUNSTER, J. ELMBERG, A. OSTERHAUS, R. A. M. FOUCHIER, and B. OLSEN. 2005. Multiple gene segment reassortment between Eurasian and American lineages of influenza A virus (H6N2) in guillemot (*Uria aalge*). Archives of Virology 150: 1685-1692.

WALLENSTEN, A., V. J. MUNSTER, M. KARLSSON, A. LUNDKVIST, M. BRYTTING, M. STERVANDER, A. OSTERHAUS, R. A. M. FOUCHIER, and B. OLSEN. 2006a. High prevalence of influenza A virus in ducks caught during spring migration through Sweden. Vaccine 24: 6734-6735.

WALLENSTEN, A., V. J. MUNSTER, A. OSTERHAUS, J. WALDENSTROM, J. BONNEDAHL, T. BROMAN, R. A. M. FOUCHIER, and B. OLSEN. 2006b. Mounting evidence for the presence of influenza A virus in the avifauna of the Antarctic region. Antarctic Science 18: 353-356.

WEBSTER, R. G., M. YAKHNO, V. S. HINSHAW, W. J. BEAN, and K. COPAL MURTI. 1978. Intestinal influenza: Replication and characterization of influenza viruses in ducks. Virology 84: 268-278.

WEBSTER, R. G., W. J. BEAN, O. T. GORMAN, T. M. CHAMBERS, and Y. KAWAOKA. 1992a. Evolution and ecology of influenza A viruses. Microbiological Reviews 56: 152-179.

WEBSTER, R. G., W. J. BEAN, O. T. GORMAN, T. M. CHAMBERS, and Y. KAWAOKA. 1992b. Evolution and ecology of influenza-A viruses. Microbiological Reviews 56: 152-179.

WEBSTER, R. G., S. KRAUSS, D. HULSE-POST, and K. STURM-RAMIREZ. 2007. Evolution of influenza A viruses in wild birds. Journal of Wildlife Diseases 43: S1-S6.

WIDJAJA, L., S. L. KRAUSS, R. J. WEBBY, T. XIE, and R. G. WEBSTER. 2004. Matrix gene of influenza A viruses isolated from wild aquatic birds: Ecology and emergence of influenza A viruses. Journal of Virology 78: 8771-8779.

WINKER, K., K. G. MCCRACKEN, D. D. GIBSON, C. L. PRUETT, R. MEIER, F. HUETTMANN, M. WEGE, I. V. KULIKOVA, Y. N. ZHURAVLEV, M. L. PERDUE, E. SPACKMAN, D. L. SUAREZ, and D. E. SWAYNE. 2007. Movements of birds and avian influenza from Asia into Alaska. Emerging Infectious Diseases 13: 547-552.

WINKER, K., E. SPACKMAN, and D. E. SWAYNE. 2008. Rarity of influenza A virus in spring shorebirds, southern Alaska. Emerging Infectious Diseases 14: 1314-1316.

WOROBEY, M. 2008. Phylogenetic evidence against evolutionary stasis and natural abiotic reservoirs of influenza A virus. Journal of Virology 82: 3769-3774.

YAMNIKOVA, S. S., A. S. GAMBARYAN, A. B. TUZIKOV, N. V. BOVIN, M. N. MATROSOVICH, I. T. FEDYAKINA, A. A. GRINEV, V. M. BLINOV, D. K. LVOV, D. L. SUAREZ, and D. E. SWAYNE. 2003. Differences between HA receptor-binding sites of avian influenza viruses isolated from Laridae and Anatidae. Avian Diseases 47: 1164-1168.

ZAMARIN, D., M. B. ORTIGOZA, and P. PALESE. 2006. Influenza A virus PB1-F2 protein contributes to viral pathogenesis in mice. Journal of Virology 80: 7976-7983.

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.

# CHAPTER 3

# PATTERNS OF AVIAN INFLUENZA VIRUS INFECTION AND EXPOSURE AMONG SHOREBIRDS AND GULLS AT DELAWARE BAY[1]

[1] Maxted, A. M., M. P. Luttrell, V. H. Goekjian, J. D. Brown, L. J. Niles, A. D. Dey, K. S. Kalasz, D. S. Mizrahi, D. E. Swayne, and D. E. Stallknecht. To be submitted to *PLoS ONE.*

## ABSTRACT

During 2006-2008, shorebirds and gulls were sampled at Delaware Bay during spring migration stopover and tested for avian influenza viruses (AIV) infection and antibodies by virus isolation in eggs and a commercial blocking ELISA, respectively. AIV were isolated from five of 13 species, and antibodies were detected in eight of nine species. At least 12 HA subtypes, all nine NA subtypes, and 30 subtype combinations were identified; these varied by year. Subtype diversity, as measured by the Shannon Index, was lowest in 2006 and highest in 2008. Ninety percent of 190 AIV isolations were from Ruddy Turnstones; prevalence varied by year and was lowest in 2007. Ruddy Turnstones also had the highest antibody prevalence among shorebird species. Red Knots had a low infection prevalence but a high antibody prevalence, suggesting previous infection prior to arrival at Delaware Bay. Sanderlings had both low infection and antibody prevalence suggesting rare exposure or resistance to AIV infection. Gulls had significantly higher antibody prevalence than shorebirds but few isolates were recovered, suggesting either endemicity or a previous infection prior to shorebird arrival. Controlling for year and date effects, odds of infection were higher in Ruddy Turnstones with heavier-than-expected mass, small body size, and in females. Although more study is needed, we speculate that AIV transmission among Ruddy Turnstones is linked to higher feeding and mass gain rates or to longer times-in-residence at Delaware Bay. Between-year differences in prevalence and subtype patterns might reflect partial population immunity to previously encountered AIV.

*Key Words:* AIV, avian influenza virus, Ruddy Turnstone, disease ecology, feeding rates, Delaware Bay, shorebirds, gulls

## INTRODUCTION

Some bird species associated with aquatic habitats, particularly in the Orders Anseriformes and Charadriiformes, are natural hosts for avian influenza viruses (AIV). Although waterfowl represent the primary reservoir of all AIV subtypes, some subtypes (i.e., H13 and H16) are more adapted to Charadriiformes species, particularly gulls (Hinshaw et al., 1983; Fouchier et al., 2005). Within Charadriiformes, there also is evidence, primarily from Delaware Bay, USA, that some shorebird (family Scolopacidae) species such as Ruddy Turnstones (*Arenaria interpres morinella*) also might act as reservoirs for these viruses (Kawaoka et al., 1988).

Each spring, >1 million shorebirds congregate at Delaware Bay during May-early June, *en route* to breeding grounds in the Arctic. This migration stopover is timed to horseshoe crab (*Limulus polyphemus*) spawning; birds feed intensively on the easily digested crab eggs and can double their mass within 3-4 wk (Robinson et al., 2003; Atkinson et al., 2007). Shorebird density can reach 210 birds/$m^2$ on Delaware Bay beaches (Gillings et al., 2007). Delaware Bay is apparently unique in global AIV epidemiology among shorebirds, possibly due to the large number and density of birds, flock species composition, stopover ecology of susceptible hosts, and/or environmental factors that facilitate viral persistence and transmission (Munster and Fouchier, 2009). Many widespread and localized surveys for AIV in healthy wild shorebirds have shown no more than limited infection outside of Delaware Bay (Fouchier et al., 2003; Chen et al., 2006; Hlinak et al., 2006; Hurt et al., 2006; D'Amico et al., 2007; Douglas et al., 2007; Gaidet et al., 2007; Munster et al., 2007; Terregino et al., 2007; Winker et al., 2007; Escudero et al., 2008; Hanson et al., 2008; Ip et al., 2008; Iverson et al., 2008; Pereda et al., 2008; Wahlgren et al., 2008; Winker et al., 2008; Dusek et al., 2009; Ghersi et al., 2009; Langstaff et al., 2009).

Two recent multi-year studies detailed AIV prevalence and annual subtype variation among shorebirds at Delaware Bay (Krauss et al., 2004; Hanson et al., 2008). During 1985-2000, AIV were isolated from 14% of pooled fecal and/or cloacal swab samples representing

4,266 individual shorebirds and gulls (Krauss et al., 2004), and during 2000-2005, overall prevalence in shorebirds and gulls was 4.6% (Hanson et al., 2008). Hanson et al. (2008) further described AIV prevalence by species and by year, with Ruddy Turnstones having the highest prevalence (11% overall; range=8-15% by year); prevalences in other shorebird species (0.8% overall; range=0-2.5% by species and year) and gulls (0.9%, one year only) were significantly lower. Both studies revealed a high richness of subtype diversity among shorebird isolates. Over 16 yr, Krauss et al. (2004) recorded 13 hemagglutinin (HA) subtypes (H1-H13) and all nine neuraminidase subtypes in 71 combinations. Hanson et al. (2008), over six yr, recorded 11 HA subtypes (H1-H7, H9-H12) and all nine NA subtypes in 39 combinations.

These differences in AIV prevalence and infecting subtypes across species and years currently cannot be explained, but defining these relationships will greatly increase our understanding of the epidemiology of AIV in this understudied reservoir at both a local and global scale. In this study, we concentrate on potential host related factors that could enhance or restrict AIV infection in shorebirds. Specific objectives were to: 1) characterize recent patterns of AIV prevalence, antibody prevalence, and subtype distribution and diversity across charadriiform species during the shorebird stopover season at Delaware Day, as well as across the years of the study, 2) identify population- and individual bird-related factors that are associated with AIV infection or antibody status in three key species (Ruddy Turnstones, Red Knots [*Calidris canutus rufa*], and Sanderlings [*Calidris alba*]), and 3) provide possible explanations based on our current knowledge of shorebird behavior, migration physiology, stopover ecology, and immune responses for the patterns we observe.

## MATERIALS AND METHODS

### Field and laboratory methods

Field work was conducted during 17-24 May 2006, 10 May-3 June 2007, and 7 May-4 June 2008. Shorebirds were captured with either cannon nets or mist nets by experienced personnel as part of long-term population studies. Following banding and measurement, swab

sampled were collected as previously described (Hanson et al., 2008). Briefly, the cloaca of each bird was swabbed by gently inserting a sterile cotton-tipped applicator. Fresh feces were swabbed in limited cases when the bird species identity was known with reasonable certainty, e.g., from within the borders of a monospecific gull breeding colony. Additionally, oropharyngeal swabs were collected from gull species in 2008 and analyzed separately from cloacal swabs. All swabs were placed in individual polypropylene tubes containing 2 mL of brain-heart infusion broth supplemented with antibiotics and antimycotics as described in Hanson et al. (2008). Tubes were kept on ice for usually <4 hr, then frozen in liquid nitrogen for transport to the lab. Samples were stored at -80°C until testing.

Swab samples were brought to room temperature, vortexed, centrifuged at 1,500x*g* for 15 min. and 0.25 mL of supernatant was injected into in each of four 9-d-old embryonated specific-pathogen-free chicken eggs. Upon death of the embryo, or after 96 hr, allantoic fluid was tested for hemagglutination as previously described (Swayne et al., 1998). Hemagglutinating viruses were subtyped by the National Veterinary Services Laboratory (NVSL; U.S. Department of Agriculture, Ames, IA) using HA- and NA-inhibition assays (2006-2007) or the Minnesota Center of Excellence for Influenza Research and Surveillance (University of Minnesota, St. Paul, MN) using gene sequencing (2008).

Blood samples, usually totaling <0.5% but not exceeding 1% of body mass in g, were collected by jugular venipuncture from a random subset of swabbed birds. Blood samples were kept on ice in the field; sera were stored at -20°C until testing. The presence of antibodies against AIV nucleoprotein was tested with the IDEXX FlockChek Avian Influenza MultiS-Screen Antibody blocking-format ELISA kit (IDEXX Laboratories, Westbrook, ME) according to kit instructions. A sample yielding a signal-to-noise (S/N) ratio of <0.5 was considered positive, per the manufacturer's recommendations.

The identity of birds was recorded whenever possible. Morphometric and demographic data for individual birds was obtained from the Shorebird Resighting Database (http://www.bandedbirds.org). Research was conducted under appropriate scientific collection permits and Institutional Animal Care and Use Committee review

**Statistical analyses**

Computations and statistical analyses were performed in program JMP version 8 (SAS Institute Inc., Cary, NC), unless otherwise noted. For birds that were sampled twice in the same year, one observation was randomly excluded. Because the bELISA imperfectly detects recent LPAI infection in wild bird species (test sensitivity=0.754, specificity=1; Brown et al., 2009), antibody prevalence calculations were adjusted according to Rogan and Gladen (1978):

$$P = \frac{P^T + Sp - 1}{Se + Sp - 1},$$

where P=true antibody prevalence, $P^T$=test antibody prevalence, Sp=test specificity, and Se=test sensitivity. Raw antibody status (i.e., antibody positive or negative) was used for individual bird-level analyses, acknowledging that some samples are falsely negative.

Binomial logistic regression was used to investigate patterns of AIV infection and antibody status across 13 charadriiform species tested and the three years of the study. Polytomous logistic regression was used to investigate differences in AIV HA and NA subtypes across species and years. Shannon diversity (H) and equitability ($E_H$) indices (Shannon, 1948) were calculated using program PAST (Hammer et al., 2001; freely available from http://folk.uio.no/ohammer/past/). Shannon's H index, commonly used to measure biodiversity, takes into account the number of species (S; or in this case, subtypes) as well as the number of individuals (isolates) counted (*n*), and can range from zero for viral communities with a single subtype to high values for communities with many subtypes, each with few isolates. Equitability is H/ln(S), ranges from zero to one, and is a measure of subtype distribution or how evenly isolates are spread across the subtypes present. Viral communities dominated by few subtypes

are characterized by equitability near zero. Diversities between years were tested with a *t*-test provided in PAST (Poole, 1974).

Generalized linear models (GLM) utilizing the binomial response distribution (i.e., logit link) were used to evaluate the associations between population- and individual- level variables and AIV infection status or antibody status in three species: Ruddy Turnstone, Red Knot, and Sanderling. The adjustment to the maximum likelihood estimator described by Firth (1993) was used when prompted by JMP. Variables measured at the level of the population included: year, day in May, and location of the capture event. Day in May is comparable to Julian date, except that is measured as days since 30 April in a given year, i.e., 1 May=1 and 1 June=32. Birds of the three above species were sampled at the following locations: in Delaware: Mispillion Harbor; in New Jersey: Sunray Beach, Stone Harbor Point, North Kimbles Beach/South Cooks Beach, North Cooks Beach/South Reeds Beach, North Reeds Beach, Moores Beach, Fortescue Beach, Gandys Beach, and an unnamed tidal creek mouth south of Gandys Beach. For all species, individual-level variables included indices of body size and condition. A Body Size Index (BSI) was computed using principal components analysis to reduce multicollinearity of three morphology measurements (culmen length, combined head and bill length, and flattened wing chord); BSI is the first principal component of these measurements, accounting for 61% of total variation in Ruddy Turnstone measurements, 68% in Red Knots, and 69% in Sanderlings. The unitless BSI is normally distributed around a population mean of zero, with negative BSI indicating smaller body size and positive BSI indicating larger body size, ranging from -3.88 to 4.77 in Ruddy Turnstones, -4.15 to 4.49 in Red Knots, and -4.89 to 3.70 in Sanderlings. Because birds are expected to gain mass over the course of the stopover, a bird's absolute mass is unlikely to reflect individual variation that relates to risk of AIV infection since mass is heavily dependent on day in May (Robinson et al., 2003; Atkinson et al., 2007) and patterns of mass gain can vary by year (Atkinson et al., 2007). Therefore, a Mass Index (MI) was calculated to obtain an individual's mass relative to the expected mass of the population on a given day

and year (Morrison et al., 2007). The MI is the unstandardized residual of a regression of mass on day, by individual years, using either a 4-node (Red Knots) or 5-node (Ruddy Turnstones and Sanderlings) knotted spline effect of day in standard least-squares regression to obtain maximum fit for population mass gain (Supplemental Figure 3.S1). Sex was included in models for Ruddy Turnstones (male or female based on plumage characteristics; birds of undetermined sex were excluded). Finally, for all three species, AIV infection status was considered in models for antibody status (but not vice versa because serological testing was conducted in only two years).

Population-level variables (year, day in May, and location) and their 2-way interactions were screened by backward stepwise regression for effect significance in association with AIV infection status or antibody status. Any significant variables and their interactions were included as covariates in further models. Each individual-level variable then was added to the model separately to determine its relationship to AIV infection or antibody status. Additionally, all possible models including individual-level main effects and 2-way interactions were evaluated; the model producing the smallest Akaike's Information Criterion corrected for small sample sizes ($AIC_c$) was selected as the best predictive model (Burnham and Anderson, 2002). In cases where two or more models were equivalent ($\Delta AIC_c \leq 2$), only the model with all variable effects significant at $\alpha=0.1$ is reported. Contingency table analyses and parametric (Student's *t*-test) and nonparametric (Mann-Whitney) univariate tests were used on variables deemed significant in the regression models, to further characterize their relationships to AIV infection or antibody status.

Unless otherwise noted, likelihood ratio $\chi^2$ tests are reported. Confidence intervals of proportions were computed using the score method (Agresti and Coull, 1998).

# RESULTS

## Patterns of AIV infection and prior exposure

During 2006-2008, 190 AIV were isolated from samples representing 3,233 individual birds of 13 Charadriiformes species (Table 3.1). AIV were detected in five species (Ruddy Turnstone, Red Knot, Sanderling, Laughing Gull [*Leucophaeus atricilla*], and Herring Gull [*Larus argentatus*]). Isolates were not distributed evenly among the positive species ($\chi^2$=180.7, df=4, *P*<0.0001); 90% of isolates were from Ruddy Turnstones. AIV prevalence in Ruddy Turnstones was higher than in the four other species in which AI viruses were detected, both combined (species contrast: $\chi^2$=130.2, df=1, *P*<0.0001) and individually (Table 3.2, Figure 3.1). Differences in prevalence were not detected for any species pair that did not include Ruddy Turnstones. No AIV were detected from 189 Semipalmated Sandpipers (*Calidris pusilla*), 112 Dunlins (*Calidris alpina hudsonia*), 60 Short-billed Dowitchers (*Limnodromus griseus*), 29 Least Sandpipers (*Calidris minutilla*), six Semipalmated Plovers (*Charadrius semipalmatus*), six Black Skimmers (*Rynchops niger*), two Great Black-backed Gulls (*Larus marinus*), or one Ring-billed Gull (*Larus delawarensis*).

Prevalence was different across years in Ruddy Turnstones and Sanderlings (Figure 3.1). Among Ruddy Turnstones, AIV prevalences in 2006 and 2008 were significantly higher than in 2007, but not significantly different from each other. During 2006-2008 mean prevalence in Ruddy Turnstones was 13%, which was higher than the 2000-2005 mean prevalence of 11% reported by Hanson et al. (2008) ($\chi^2$=4.0, df=1, *P*=0.046). Prevalences in 2006 and 2008 were significantly higher and prevalence in 2007 was significantly lower than the previous six-year mean ($\chi^2$=12.4, df=1, *P*=0.0004; $\chi^2$=13.9, df=1, *P*=0.0002; and $\chi^2$=19.3, df=1, *P*<0.0001, respectively). Prevalence in Sanderlings was higher in 2006 than in 2007-2008 (Figure 3.3). Mean prevalence from 2006-2008 did not differ from the 2000-2005 mean (1.1%; $\chi^2$=0.5, df=1, *P*=0.480), but prevalence in 2006 alone was higher ($\chi^2$=10.2, df=1, *P*=0.001). Prevalences in

Red Knots, Laughing Gulls, and Herring Gulls were not different across years and did not differ from 2000-2005 means (0.8%; $\chi^2$=0.02, df=1, *P*=0.899; 1.7%; $\chi^2$=0.02, df=1, *P*=0.886; and 0%; $\chi^2$=0.1, df=1, *P*=0.715, respectively). Yearly prevalence in Sanderlings from 2000-2008 was positively associated with prevalence in Ruddy Turnstones (binomial GLM: $\chi^2$=12.8, df=1, *P*=0.0003; model fit was improved when 2008 data was excluded: $\chi^2$=19.1, df=1, *P*<0.0001; Figure 3.2).

Serum samples from 861 birds of nine Charadriformes species were tested during 2007-2008. Anti-AIV antibodies were detected in eight species (Table 3.3, Figure 3.3). Adjusted antibody prevalence differed across species ($\chi^2$=401.3, df=8, *P*<0.0001), and as a group, gulls exhibited significantly higher antibody prevalence than shorebirds (81% vs. 65%; $\chi^2$=21.3, df=1, *P*<0.0001). Antibody prevalence did not differ between gull species ($\chi^2$=2.3, df=3, *P*=0.518), but differed significantly between shorebird species ($\chi^2$=390.5, df=4, *P*<0.0001). All between-species comparisons of adjusted antibody prevalence are listed in Table 3.4. Adjusted antibody prevalence did not differ between years for any species except Laughing Gulls, for which it was higher in 2007 than in 2008 (Figure 3.3).

Seventeen individual Ruddy Turnstones, five Red Knots, and four Sanderlings were sampled in >1 year. Virus isolation and serology results from these individuals are given in Table 3.5.

**Predictors of AIV infection and antibody status**

*Population-level variables*– Infection status in Ruddy Turnstones varied significantly by year and day of sampling and the interaction between them (whole model: $\chi^2$=65.7, df=6, *P*<0.0001; year effect: $\chi^2$=38.5, df=2, *P*<0.0001; day effect: $\chi^2$=15.4, df=2, *P*=0.0005; interaction effect: $\chi^2$=6.6, df=2, *P*=0.037), but antibody status varied by day only ($\chi^2$=63.9, df=1, *P*<0.0001). Although AIV infection status was associated with the sampling location in univariate analysis (simple logistic regression; Firth-adjusted $\chi^2$=30.5, df=9, *P*=0.0004), location did not influence

infection status after controlling for year and day effects (location effect: $\chi^2$=4.3, df=9, *P*=0.892). In Red Knots, no population variables were significantly associated with AIV infection status (all *P*>0.05), but antibody status was negatively associated with day ($\chi^2$=15.0, df=1, *P*=0.001). Only year was associated with AIV infection in Sanderlings ($\chi^2$=17.1, df=2, *P*=0.0002); no population variables were associated with antibody status.

*Individual-level variables*– Tables 3.6 and 3.7 show results from single individual-level variable models and the population and individual-level variables included in the best predictive model for AIV infection or antibody status, respectively (all controlling for significant confounding variables listed above).

Odds of infection were higher in Ruddy Turnstones with heavier-than-expected mass, small body size, and in females. On average, MI was 4.4 g (95% CI: 2.1-6.8 g) greater in virus-positive than in virus-negative birds (*t*=4.02, df=238, *P*<0.0001). Mass index in virus-positive birds was significantly greater than mean population MI (i.e., zero; *t*=4.30, df=167, *P*<0.0001). Body size index was not significantly different from zero in virus-positive birds but was significantly larger in negative birds (*t*=3.1, df=1,116, *P*=0.002). Neither the proportion of birds that were female among virus-positive (50%) nor negative (44%) birds was significantly different than that of all birds that we tested (45%; $\chi^2$=1.8, df=1, *P*=0.177 and $\chi^2$=0.3, df=1, *P*=0.587, respectively).

Because BSI was significantly larger in females than in males (0.67 vs. -0.33; *t*=13.5, df=1224, *P*<0.0001) (Nettleship, 2000), it seemed counterintuitive that odds of infection would be increased in both females and in birds of smaller body size. Therefore, we conducted further analyses separately for each sex to better characterize this relationship. Infection status was not associated with day in May among females like it was in males (Table 3.6), so day was dropped from female-specific models. In single individual-level variable analyses, MI was positively associated with infection status in both sexes but BSI was not associated with infection in either

(Table 3.6). However, in the multiple regression model, BSI was negatively associated with infection in females (i.e., odds of infection decreased as body size increased). For both sexes the odds of infection increased by a similar amount for each gram increase in MI.

In 2×2 contingency analysis, the relative risk (RR) for being antibody positive was 1.3 (95% CI: 1.1-1.5) times higher for AIV-positive than for AIV-negative birds and the RR for shedding AIV was 1.9 (95% CI: 1.1-3.4) times higher for antibody positive birds than negative birds ($\chi^2$=5.30, df=1, *P*=0.021, odds ratio [OR]=2.1 [95% CI: 1.1-4.1]). The proportion female, MI, and AIV prevalence did not differ from population means in either the antibody positive or negative groups (all *P*>0.05). The odds of being antibody positive increased with day and were higher in males, but the effects of MI, BSI, and infection status on antibody status varied between the sexes (Table 3.7). Day in May was the only variable associated with antibody prevalence in males; the situation was much more complex for females. The odds of being antibody-positive increased with day in May and MI, but the slope of the relationship between MI and antibody status was steeper among infection positive birds. The odds of being antibody positive increased with BSI among females that were actively shedding virus, but not among those that were virus-negative.

For Red Knots, odds of infection were higher in individuals with larger body size and with lower-than-expected mass. In univariate analysis, mean MI of infected birds was 11.8 g less than in uninfected birds, although not significantly so (*t*=1.3, df=702, *P*=0.199). Body Size Index was higher by 1.3 units (approximately one population standard deviation) in infected birds than in uninfected birds (*t*=2.1, df=710, *P*=0.038). The odds of being antibody positive decreased over the stopover season. Only one virus-positive Red Knot was tested serologically and it was positive.

In Sanderlings, odds of AIV infection were significantly higher in 2006 than in 2007 or 2008 (Table 3.7). Of eight AIV positive Sanderlings, seven were sampled in 2006 and one in 2008; none were tested serologically. No individual-level variables were associated with AIV

infection (Table 3.6), and the best predictive model contained only the variable year (Table 3.7). Antibody status was not associated with year, day, location, or BSI, and was only weakly associated with MI ($P$=0.084, Table 3.7). However, mean MI was 6.8 g (95% CI: 3.4-10.1 g) greater in antibody positive than in negative birds ($t$=5.1, df=5.3, $P$=0.003).

**Virus subtype distribution and diversity**

During 2006-2008, 12 HA subtypes, all nine NA subtypes, and 30 HA-NA subtype combinations were identified across all species of Charadriiformes (Tables 3.8-3.9). Ten percent of isolates ($n$=19) contained a HA, a NA, or both, that could not be identified by conventional inhibition assays (or gene sequencing in 2008). Hemagglutinin and NA subtypes were significantly associated ($\chi^2$=550.2, df=108, $P$<0.0001), i.e., certain HA and NA subtypes tended to occur together. No mixed-subtype infections were detected in any bird.

The individual HA and NA subtypes isolated varied significantly by year ($\chi^2$=273.4, df=24, p<0.0001 and $\chi^2$=248.5, df=18, p<0.0001, respectively; Table 3.8), as did HA-NA combinations (e.g., H7N3; $\chi^2$=289.5, df=60, $P$<0.0001; Table 3.9). Only 1-2 HA, NA, or HA-NA combinations comprised ≥20% of isolates in a given year. Diversity of HA and NA subtypes and their combinations was significantly or marginally different between all year-pairs 2006-2008 (Table 3.10); diversity was lowest in 2006 and highest in 2008.

The subtypes of all isolates from Sanderlings and Red Knots matched those of isolates from Ruddy Turnstones in the same year. Only two subtypes were not found in Ruddy Turnstones; H2N9 was isolated only from a Herring Gull, and H13N9 was isolated only from Laughing Gulls. Excluding these two, the probability of finding a given subtype combination in a species other than Ruddy Turnstones was related to its frequency in Ruddy Turnstones, both for combined data ($\chi^2$=23.0, df=1, $P$<0.0001) and in each year alone (2006: $\chi^2$=4.7, df=1, $P$=0.031; 2007: $\chi^2$=4.2, df=1, $P$=0.041; and 2008: $\chi^2$=11.8, df=1, $P$=0.0006). By inverse prediction, a

subtype had a 50% chance of being detected in another species in a given year if it was isolated from at least 16-18 Ruddy Turnstones (data not shown).

## DISCUSSION

### Differences in AIV infection and exposure patterns across species and years

We did not identify any new AIV hosts in this study. Eight species were negative for AIV; in some cases small sample sizes might have precluded detection (e.g., Great Black-backed Gull), but sample sizes in other species were adequate to detect AIV even at low prevalences (e.g., Semipalmated Sandpiper; Table 3.1). Historically, prevalence is very low (<1%) in Semipalmated Sandpipers, Dunlins, and Short-billed Dowitchers at Delaware Bay, if detected at all (Kawaoka et al., 1988; Hanson et al., 2008). AIV have not been previously detected at Delaware Bay in Black Skimmers, Great Black-backed Gulls, or Ring-billed Gulls (Kawaoka et al., 1988; Hanson et al., 2008), and Semipalmated Plovers have not been sampled previously at this location. However, sample sizes were too small in these latter species to suggest that they were uninfected.

Within a species, the significant variation in prevalence between years observed in this study is consistent with patterns described by Hanson et al. (2008). During 2000-2005, annual AIV prevalence in Ruddy Turnstones ranged from 8-15%. Our prevalence estimates for 2006 (19%) and 2008 (17%) are higher than any previously recorded prevalence estimates; in contrast, AIV prevalence during in 2007 (4.6%) was the lowest reported from this site to date. Similar cycles in yearly AIV prevalence at a given location, with low prevalence years following high prevalence years or vice versa, have also been observed in Mallards and other dabbling ducks (Hinshaw et al., 1985; Sharp et al., 1993; Munster et al., 2007). The extreme prevalence values in 2006, 2007, and 2008 might be partly the result of cyclical variation in prior population exposure and immunity, based on the proportion of birds infected in the previous year as suggested by Krauss et al (2004). Thus, the low observed prevalence in 2007 could be the result of high incidence in 2006, and vice versa for 2008. Addition of naïve birds to the

population through recruitment might also contribute, although the majority of birds migrating through Delaware Bay are adults that have utilized this site previously (shorebird resighting database; unpublished data), and it is unknown whether juvenile birds encounter AI viruses elsewhere before arriving at Delaware Bay. Interestingly, antibody prevalence among Ruddy Turnstones did not differ between 2007 and 2008, suggesting that there was an equal level of exposure to AIV in those years even though virus prevalences were markedly different. More study is needed to determine what effect prior exposure has on AIV prevalence in shorebirds.

Prevalence in Sanderlings significantly mirrored the prevalence in Ruddy Turnstones in a given year during 2000-2008, but was on average 10 times lower. Although we cannot say that Sanderlings acquire infections from Ruddy Turnstones, we can infer that the conditions that increase prevalence in Turnstones in a given year also increase prevalence in Sanderlings; this pattern suggests that Sanderlings are a “spillover” host for AIV at Delaware Bay (Fenton and Pedersen, 2005). This idea is supported by the fact that all of the subtypes isolated from Sanderlings were the dominant subtypes isolated from Ruddy Turnstones in that year. Red Knots were sporadically infected each year; no discernable pattern was seen between years or by prevalence in other species.

A substantial proportion of cloacal swab samples collected from Red Knots (26%) and Sanderlings (33%) were not tested in 2008 due to constraints on laboratory space and personnel. Unfortunately, untested samples were not distributed evenly over the stopover season; 80% of the tested samples were collected prior to 20 May (i.e., prior to the peak of the epizootic in Ruddy Turnstones). Traditionally, AIV are prevalent in these two species after 20 May (unpublished data; see Chapter 4, this thesis), thus, true prevalence in Red Knots and Sanderlings for 2008 are probably higher than are reported here. This pattern explains the increased strength of association between yearly prevalence in Sanderlings and Turnstones when 2008 data was excluded. Underestimates of prevalence in 2008 might also affect the comparisons of prevalence between species (Table 3.2).

Antibody prevalence varied widely among the species tested and were not uniformly consistent with AIV prevalence. As expected, Ruddy Turnstones had a relatively high antibody prevalence given that odds of infection in Turnstones are 9-20 times higher than in sympatric species at this time and location (Table 3.2). However, antibody prevalences in Red Knots and each of the four gull species, in which springtime prevalence at Delaware Bay is historically ≤3% (Hanson et al., 2008 and Table 3.1), were equal to that in Ruddy Turnstones. In individual birds, the presence of AIV-specific antibodies in the absence of infection during this time indicates AIV infection prior to the spring shorebird stopover period; on a population level this pattern is consistent with 1) long-lasting antibodies from prior exposures at Delaware Bay, 2) a recent AIV epizootic (prior to arrival at Delaware Bay) resulting in population seroconversion, or 3) regular encounters with AI viruses throughout the annual cycle, such as would be expected in an endemic reservoir host. Among gull species, AIV epidemics are thought to mostly occur in summer and fall when adults and nestlings are in dense concentrations on breeding colonies, and after juveniles have fledged (Olsen et al., 2006). However, at least among gulls in the Northern Hemisphere, prevalences during the nonbreeding season often exceed those found during and just after breeding (e.g., Cattoli et al., 2007; Lebarbenchon et al., 2007; Terregino et al., 2007; Hanson et al., 2008; Pannwitz et al., 2009) suggesting that AIV might circulate at low levels throughout the annual cycle. Little is known about AIV infection in these populations during other times of the year. Unfortunately, the length of time following infection that anti-AIV antibodies are detectable, the degree to which they protect against future infections, and the interplay of these two factors are all currently unknown.

The pattern of high antibody prevalence and low infection prevalence is not easily explained in Red Knots, since AIV infection has been documented only once outside of Delaware Bay (a virus isolated in Georgia, USA in September 2001; Hanson et al., 2008). No AIV has been detected in the *rufa* subspecies on the wintering grounds or at other spring migration stopovers (D'Amico et al., 2007; Escudero et al., 2008; Hanson et al., 2008), however,

many known wintering and stopover locations have not been sampled and detection might be difficult when infection is transient.

Sanderlings, Dunlins, and Short-billed Dowitchers exhibited relatively low AIV antibody prevalence, yet rarely were infected with AIV. Differences in the local foraging ecology of these species might help explain variations in prevalence. The typical foraging habitats of Dunlins and Dowitchers include marshes and mudflats while at Delaware Bay, in contrast to the sandy beaches preferred by other shorebird species (Burger et al., 1997). Such differences in spatial distribution might place them in contact with virus shed from Ruddy Turnstones or other species only infrequently. We detected anti-AIV NP antibodies in two of 14 Dunlins, but it is impossible to know if this reflects exposure to AIV at other locations or while at Delaware Bay. Sanderlings, however, readily feed alongside Ruddy Turnstones on the Bay beaches and presumably are exposed to feces that contain AIV.

**Predictors of AIV infection and antibody prevalence in individual shorebirds**

Hanson et al. (2008) found that prevalence in Ruddy Turnstones differed across mass classes, with low prevalence among birds at or near arrival mass and higher prevalence among birds that weighed more. This pattern suggests that the majority of birds become infected some time after arrival at Delaware Bay as they are gaining weight for the final leg of their migration to the Arctic. In this study, we examined the effect of relative rather than absolute mass as an indicator of condition in individual birds on AIV infection since the entire population gains weight over the stopover period. In both univariate and multivariate analyses, AIV infection was strongly associated with MI with higher prevalence among birds that weighed more than expected. Disregarding differences in body size, birds that are relatively heavier on a given day during the stopover either might have arrived earlier or had faster rates of mass gain than average (Robinson et al., 2003; Gillings et al., 2009). Thus we expect that odds of AIV infection are somehow linked to the “extra” time a bird has spent in residence at Delaware Bay prior to sampling or to conditions that would allow it to gain mass faster than conspecifics with similar

arrival dates. If risk of infection is linked to feeding as might be expected for a pathogen transmitted by the fecal-oral route, then birds with higher feeding rates would simultaneously gain mass more rapidly and have more exposure to AIV (i.e., birds that feed more intensively are more likely to become infected; Hall et al., 2007; Blanchet et al., 2009; Beldomenico and Begon, 2010). Our data do not allow us to distinguish between these two possible modes or others.

Higher relative mass among infected Ruddy Turnstones was somewhat unexpected since LPAI is primarily an intestinal disease (Webster et al., 1978) and could affect feeding behavior or food assimilation during the migratory stopover. Little is known about effect of LPAI virus infection on digestive function or mass gain in wild birds, but evidence to date suggests a negative relationship. Infected Mallards staging for fall migration were on average 1.7% lighter than uninfected birds, a result significant after controlling for body size, age, and other covariates (Latorre-Margalef et al., 2009b). Two AIV-infected wintering Bewick's Swans (*Cygnus columbianus bewickii*), showed reduced feeding performance, lower rates of mass gain, and delayed migration compared to uninfected birds (van Gils et al., 2007). Among wintering Greater White-fronted Geese (*Anser albifrons albifrons*) infected birds had lower body mass upon capture in one of four years; no difference was detected in the others (Kleijn et al., 2010). If AIV infection does interfere with mass gain during staging, or alternatively, if birds of low mass are more likely to be infected (Flint and Franson, 2009; Latorre-Margalef et al., 2009a), it is unclear why this relationship is not seen in shorebirds (although a negative association between MI and infection status approached statistical significance in Red Knots).

In Red Knots, birds of larger body size were more likely to be infected than those that were smaller. There is no size difference between sexes in Red Knots of subspecies *rufa* (Harrington, 2001), so higher odds of infection in larger birds of this subspecies are probably not due to a sex effect. However, a small minority of Knots migrating through Delaware Bay are thought to be *C. canutus roselaari,* which are somewhat larger than *rufa* (at least among

females; Harrington, 2001). Different wintering and breeding populations could have different exposure histories and susceptibility which might influence infection at Delaware Bay. Unfortunately, we can't confirm if there is a relationship between population and infection, and only one virus-positive bird was tested for antibodies (it was positive). Larger body size has been related to social dominance in other shorebird species (e.g., Burton and Evans, 2001), however, we do not know if this pattern holds for in Red Knots, if social dominance is acting on foraging Knots at Delaware Bay, or how this might affect AIV exposure or susceptibility.

Sex, MI, BSI and infection status all influenced antibody status in Ruddy Turnstones. Birds that were antibody positive were more likely to be infected than negative birds and vice versa, but it is unknown if we are detecting seroconversion in the face of infection or if the presence of antibodies is not protective against reinfection. We know that at least some birds that were infected in one year become reinfected in a later year (Table 3.5 and Hanson et al., 2008), but we do not know how long anti-AIV antibodies persist in this species or other shorebirds, or to what degree they protect against reinfection, especially with a different subtype. Also, the serological test that we used measures only antibodies to the viral NP, which are much less protective than antibodies against HA or NA proteins (Stitz et al., 1990; Suarez and Schultz-Cherry, 2000). The fact that antibody positive birds were more likely to be male and infected birds were more likely to be female suggests either that female birds become infected because they lack protective antibodies, or that migration chronology affects the pattern. On average males arrive at Delaware Bay slightly earlier than females, and it appears that the timing of the epizootic is shifted later for females (unpublished data; Chapter 4, this thesis). On a given day, then, we could have sampled females that were actively infected and males that had already recovered from infection and seroconverted. Associations between antibody status and MI and body size in females (after controlling for other covariates) probably reflect those birds that are more likely to be infected, i.e., the pattern reflects seroconversion in the wake of an epizootic. Annual resighting rates, and presumably survival, are not different between the

sexes (unpublished data; Chapter 6, this thesis). Thus, we do not expect that a difference in age structure between the sexes affects AIV prevalence through age-specific differences in susceptibility.

No individual-level characteristics were associated with antibody status in Red Knots, suggesting that factors that would cause exposure and seroconversion in Red Knots act equally on the population as a whole. Antibody status might have been weakly associated with MI in Sanderlings; however, so few individuals were positive that the power ability to detect an association was low.

**Subtype patterns and diversity**

Use of diversity and equitability indices to describe the number and distribution of subtypes detected during an AIV epizootic is a novel approach, one primarily used to measure biodiversity. Shannon's H diversity index is a measure not only of the number of subtypes we detected, but how evenly all isolates were distributed over the subtypes found; equitability is a measure of the observed diversity in relation to the maximum diversity possible given the number of isolates and different subtypes. Therefore, the dominance of one or few subtypes would result in low equitability. We found that both diversity and equitability were low in 2006 because of the extreme dominance of the H7N3 subtype in that year. Even though prevalence in 2007 was very low, diversity and equitability were higher than in 2006 because of the detection of an additional subtype and because of the distribution among a few subtypes other than the dominant subtype (H12N5). Diversity and equitability were the highest in 2008 because a large number of subtypes were detected and two subtypes made up ≥20% of isolates in that year.

Ruddy Turnstones are the main shorebirds species infected with AIV over the short stopover period and infections in other shorebird species might be linked to the annual prevalence, and subtypes circulating, in Ruddy Turnstones each spring. However, detection of additional subtypes in sympatric gull species not found in Ruddy Turnstones indicates that a

separate cycle of circulation might be occurring among breeding gulls. More detailed investigations into AIV ecology in gull species at Delaware Bay, especially longitudinal virus isolation and serology studies that include time periods before and after shorebird migration, are needed to characterize the potential epidemiologic link between the species groups.

## ACKNOWLEDGMENTS

This research was funded through Specific Cooperative Agreement 58-6612-2-0220 between the Southeast Poultry Research Laboratory, Agricultural Research Service, Department of Agriculture (USDA-ARS) and the Southeastern Cooperative Wildlife Disease Study and by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Department of Health and Human Services, under contract no. HHSN266200700007C. The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. Banding data and morphometric measurements are the property of the Natural Heritage & Endangered Species Program, Division of Fish and Wildlife, Delaware Department of Natural Resources and Environmental Control (DNREC), and the Nongame and Endangered Species Program, Division of Fish and Wildlife, New Jersey Department of Environmental Protection (NJDEP), and we thank these agencies for granting access to this data. We thank D. Senne of the National Veterinary Services Laboratory (USDA) for providing isolate subtyping support in 2006. We are grateful to the many people who provided field and laboratory support, particularly J. Smith, R. Paulson, B. Wilcox, S. Gibbs, G. Martin, J. Cumbee, L. Coffee, J. Murdock, W. Hamrick, S. McGraw, S. Keeler, C. McKinnon, M. Cole, D. Downs, and E. Casey.

## LITERATURE CITED

AGRESTI, A., and B. A. COULL. 1998. Approximate is better than "exact" for interval estimation of binomial proportions. American Statistician 52: 119-126.

ATKINSON, P. W., A. J. BAKER, K. A. BENNETT, N. A. CLARK, J. A. CLARK, K. B. COLE, A. DEKINGA, A. DEY, S. GILLINGS, P. M. GONZALEZ, K. KALASZ, C. D. T. MINTON, J. NEWTON, L. J. NILES, T. PIERSMA, R. A. ROBINSON, and H. P. SITTERS. 2007. Rates of mass gain and energy deposition in red knot on their final spring staging site is both time- and condition-dependent. Journal of Applied Ecology 44: 885-895.

BELDOMENICO, P. M., and M. BEGON. 2010. Response to comment by Loot et al. Trends in Ecology & Evolution 25: 132-132.

BLANCHET, S., F. THOMAS, and G. LOOT. 2009. Reciprocal effects between host phenotype and pathogens: new insights from an old problem. Trends in Parasitology 25: 364-369.

BROWN, J. D., D. E. STALLKNECHT, R. D. BERGHAUS, M. P. LUTTRELL, K. VELEK, W. KISTLER, T. COSTA, M. J. YABSLEY, and D. SWAYNE. 2009. Evaluation of a commercial blocking enzyme-linked immunosorbent assay to detect avian influenza virus antibodies in multiple experimentally infected avian species. Clinical and Vaccine Immunology 16: 824-829.

BURGER, J., L. NILES, and K. E. CLARK. 1997. Importance of beach, mudflat and marsh habitats to migrant shorebirds on Delaware Bay. Biological Conservation 79: 283-292.

BURNHAM, K. P., and D. R. ANDERSON. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. 2nd edition. Springer, New York. 488 pp.

BURTON, N. H. K., and P. R. EVANS. 2001. Aggressive behaviours and correlates of dominance in Purple Sandpipers *Calidris maritima* at a communal winter roost. Ibis 143: 248-254.

CATTOLI, G., C. TERREGINO, V. GUBERTI, R. DE NARDI, A. DRAGO, A. SALVIATO, S. FASSINA, and I. CAPUA. 2007. Influenza virus surveillance in wild birds in Italy: Results of laboratory investigations in 2003-2005. Avian Diseases 51: 414-416.

CHEN, H. X., H. G. SHEN, X. L. LI, J. Y. ZHOU, Y. Q. HOU, J. Q. GUO, and J. Q. HU. 2006. Seroprevalance and identification of influenza A virus infection from migratory wild waterfowl in China (2004-2005). Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 53: 166-170.

D'AMICO, V. L., M. BERTELLOTTI, A. J. BAKER, and L. A. DIAZ. 2007. Exposure of Red Knots (*Calidris canutus rufa*) to select avian pathogens; Patagonia, Argentina. Journal of Wildlife Diseases 43: 794-797.

DOUGLAS, K. O., M. C. LAVOIE, L. M. KIM, C. L. AFONSO, and D. L. SUAREZ. 2007. Isolation and genetic characterization of avian influenza viruses and a Newcastle disease virus from wild birds in Barbados: 2003-2004. Avian Diseases 51: 781-787.

DUSEK, R. J., J. B. BORTNER, T. J. DELIBERTO, J. HOSKINS, J. C. FRANSON, B. D. BALES, D. YPARRAGUIRRE, S. R. SWAFFORD, and H. S. IP. 2009. Surveillance for high pathogenicity

avian influenza virus in wild birds in the Pacific flyway of the United States, 2006-2007. Avian Diseases 53: 222-230.

ESCUDERO, G., V. J. MUNSTER, M. BERTELLOTTI, and P. EDELAAR. 2008. Perpetuation of avian influenza in the Americas: Examining the role of shorebirds in Patagonia. Auk 125: 494-495.

FENTON, A., and A. B. PEDERSEN. 2005. Community epidemiology framework for classifying disease threats. Emerging Infectious Diseases 11: 1815-1821.

FIRTH, D. 1993. Bias reduction of maximum likelihood estimates. Biometrika 80: 27-38.

FLINT, P. L., and J. C. FRANSON. 2009. Does influenza A affect body condition of wild mallard ducks, or vice versa? Proceedings of the Royal Society B: Biological Sciences 276: 2345-2346.

FOUCHIER, R. A. M., B. OLSEN, T. M. BESTEBROER, S. HERFST, L. VAN DER KEMP, G. E. RIMMELZWAAN, and A. D. M. E. OSTERHAUS. 2003. Influenza A virus surveillance in wild birds in Northern Europe in 1999 and 2000. Avian Diseases 47: 857-860.

FOUCHIER, R. A. M., V. MUNSTER, A. WALLENSTEN, T. M. BESTEBROER, S. HERFST, D. SMITH, G. F. RIMMELZWAAN, B. OLSEN, and A. D. M. E. OSTERHAUS. 2005. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from Black-headed Gulls. Journal of Virology 79: 2814-2822.

GAIDET, N., T. DODMAN, A. CARON, G. BALANCA, S. DESVAUX, F. GOUTARD, G. CATTOLI, F. LAMARQUE, W. HAGEMEIJER, and F. MONICAT. 2007. Avian influenza viruses in water birds, Africa. Emerging Infectious Diseases 13: 626-629.

GHERSI, B. M., D. L. BLAZES, E. ICOCHEA, R. I. GONZALEZ, T. KOCHEL, Y. TINOCO, M. M. SOVERO, S. LINDSTROM, B. SHU, A. KLIMOV, A. E. GONZALEZ, and J. M. MONTGOMERY. 2009. Avian influenza in wild birds, central coast of Peru. Emerging Infectious Diseases 15: 935-938.

GILLINGS, S., P. W. ATKINSON, S. L. BARDSLEY, N. A. CLARK, S. E. LOVE, R. A. ROBINSON, R. A. STILLMAN, and R. G. WEBER. 2007. Shorebird predation of horseshoe crab eggs in Delaware Bay: species contrasts and availability constraints. Journal of Animal Ecology 76: 503-514.

GILLINGS, S., P. W. ATKINSON, A. J. BAKER, K. A. BENNETT, N. A. CLARK, K. B. COLE, P. M. GONZALEZ, K. S. KALASZ, C. D. T. MINTON, L. J. NILES, R. C. PORTER, I. DE LIMA SERRANO, H. P. SITTERS, J. L. WOODS, and T. L. SHAFFER. 2009. Staging behavior in Red Knot (*Calidris canutus*) in Delaware Bay: implications for monitoring mass and population size. Auk 126: 54-63.

HALL, S., L. SIVARS-BECKER, C. BECKER, M. DUFFY, A. TESSIER, and C. CÁCERES. 2007. Eating yourself sick: transmission of disease as a function of foraging ecology. Ecology Letters 10: 207-218.

HAMMER, Ø., D. A. T. HARPER, and P. D. RYAN. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 1-9.

HANSON, B. A., M. P. LUTTRELL, V. H. GOEKJIAN, L. NILES, D. E. SWAYNE, D. A. SENNE, and D. E. STALLKNECHT. 2008. Is the occurrence of avian influenza virus in Charadriiformes species and location dependent? Journal of Wildlife Diseases 44: 351-361.

HARRINGTON, B. A. 2001. Red Knot (*Calidris canutus*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 563, http://bna.birds.cornell.edu/bna/species/563. Accessed January 2011.

HINSHAW, V. S., G. M. AIR, G. C. SCHILD, and R. W. NEWMAN. 1983. Characterization of a novel hemagglutinin subtype (H13) of influenza A viruses from gulls. Bulletin of the World Health Organization 61: 677-679.

HINSHAW, V. S., J. M. WOOD, R. G. WEBSTER, R. DEIBEL, and B. TURNER. 1985. Circulation of influenza viruses and paramyxoviruses in waterfowl originating from 2 different areas of North America. Bulletin of the World Health Organization 63: 711-719.

HLINAK, A., R. U. MUHLE, O. WERNER, A. GLOBIG, E. STARICK, H. SCHIRRMEIER, B. HOFFMANN, A. ENGELHARDT, D. HUBNER, F. J. CONRATHS, D. WALLSCHLAGER, H. KRUCKENBERG, and T. MULLER. 2006. A virological survey in migrating waders and other waterfowl in one of the most important resting sites of Germany. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 53: 105-110.

HURT, A. C., P. M. HANSBRO, P. SELLECK, B. OLSEN, C. MINTON, A. W. HAMPSON, and I. G. BARR. 2006. Isolation of avian influenza viruses from two different transhemispheric migratory shorebird species in Australia. Archives of Virology 151: 2301-2309.

IP, H. S., P. L. FLINT, J. C. FRANSON, R. J. DUSEK, D. V. DERKSEN, R. E. GILL, JR., C. R. ELY, J. M. PEARCE, R. B. LANCTOT, S. M. MATSUOKA, D. B. IRONS, J. B. FISCHER, R. M. OATES, M. R. PETERSEN, T. F. FONDELL, D. A. ROCQUE, J. C. PEDERSEN, and T. C. ROTHE. 2008. Prevalence of Influenza A viruses in wild migratory birds in Alaska: patterns of variation in detection at a crossroads of intercontinental flyways. Virology Journal 5: 71.

IVERSON, S. A., J. Y. TAKEKAWA, S. SCHWARZBACH, C. J. CARDONA, N. WARNOCK, M. A. BISHOP, G. A. SCHIRATO, S. PAROULEK, J. T. ACKERMAN, H. IP, and W. M. BOYCE. 2008. Low prevalence of avian influenza virus in shorebirds on the Pacific coast of North America. Waterbirds 31: 602-610.

KAWAOKA, Y., T. M. CHAMBERS, W. L. SLADEN, and R. G. WEBSTER. 1988. Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? Virology 163: 247-250.

KLEIJN, D., V. J. MUNSTER, B. S. EBBINGE, D. A. JONKERS, G. J. D. M. MÜSKENS, Y. VAN RANDEN, and R. A. M. FOUCHIER. 2010. Dynamics and ecological consequences of avian influenza virus infection in Greater White-fronted Geese in their winter staging areas. Proceedings of the Royal Society B: Biological Sciences 277: 2041-2048.

KRAUSS, S., D. WALKER, S. P. PRYOR, L. NILES, C. H. LI, V. S. HINSHAW, and R. G. WEBSTER. 2004. Influenza A viruses of migrating wild aquatic birds in North America. Vector-Borne and Zoonotic Diseases 4: 177-189.

LANGSTAFF, I. G., J. S. MCKENZIE, W. L. STANISLAWEK, C. E. M. REED, R. POLAND, and S. C. CORK. 2009. Surveillance for highly pathogenic avian influenza in migratory shorebirds at the terminus of the East Asian-Australasian Flyway. New Zealand Veterinary Journal 57: 160-165.

LATORRE-MARGALEF, N., G. GUNNARSSON, V. J. MUNSTER, R. A. M. FOUCHIER, A. D. M. E. OSTERHAUS, J. ELMBERG, B. OLSEN, A. WALLENSTEN, T. FRANSSON, L. BRUDIN, and J. WALDENSTROM. 2009a. Does influenza A affect body condition of wild mallard ducks, or vice versa? A reply to Flint and Franson. Proceedings of the Royal Society B: Biological Sciences 276: 2347-2349.

LATORRE-MARGALEF, N., G. GUNNARSSON, V. J. MUNSTER, R. A. M. FOUCHIER, A. D. M. E. OSTERHAUS, J. ELMBERG, B. OLSEN, A. WALLENSTEN, P. D. HAEMIG, T. FRANSSON, L. BRUDIN, and J. WALDENSTRÖM. 2009b. Effects of influenza A virus infection on migrating Mallard ducks. Proceedings of the Royal Society B: Biological Sciences 276: 1029-1036.

LEBARBENCHON, C., C. M. CHANG, S. VAN DER WERF, J. T. AUBIN, Y. KAYSER, M. BALLESTEROS, F. RENAUD, F. THOMAS, and M. GAUTHIER-CLERC. 2007. Influenza A virus in birds during spring migration in the Camargue, France. Journal of Wildlife Diseases 43: 789-793.

MORRISON, R. I. G., N. C. DAVIDSON, and J. R. WILSON. 2007. Survival of the fattest: body stores on migration and survival in Red Knots *Calidris canutus islandica*. Journal of Avian Biology 38: 479-487.

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.

MUNSTER, V. J., and R. A. M. FOUCHIER. 2009. Avian influenza virus: of virus and bird ecology. Vaccine 27: 6340-6344.

NETTLESHIP, D. N. 2000. Ruddy Turnstone (*Arenaria interpres*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 537, http://bna.birds.cornell.edu/bna/species/537. Accessed January 2011.

OLSEN, B., V. J. MUNSTER, A. WALLENSTEN, J. WALDENSTROM, A. OSTERHAUS, and R. A. M. FOUCHIER. 2006. Global patterns of influenza A virus in wild birds. Science 312: 384-388.

PANNWITZ, G., C. WOLF, and T. HARDER. 2009. Active surveillance for avian influenza virus infection in wild birds by analysis of avian fecal samples from the environment. Journal of Wildlife Diseases 45: 512-518.

PEREDA, A. J., M. UHART, A. A. PEREZ, M. E. ZACCAGNINI, L. LA SALA, J. DECARRE, A. GOIJMAN, L. SOLARI, R. SUAREZ, M. I. CRAIG, A. VAGNOZZI, A. RIMONDI, G. KONIG, M. V. TERRERA, A. KALOGHLIAN, H. C. SONG, E. M. SORRELL, and D. R. PEREZ. 2008. Avian influenza virus isolated in wild waterfowl in Argentina: Evidence of a potentially unique phylogenetic lineage in South America. Virology 378: 363-370.

POOLE, R. W. 1974. An Introduction to Quantitative Ecology. McGraw-Hill, New York, NY.

ROBINSON, R. A., P. W. ATKINSON, and N. A. CLARK. 2003. Arrival and weight gain of Red Knot *Calidris canutus,* Ruddy Turnstone *Arenaria interpres* and Sanderling *Calidris alba* staging in Delaware Bay in spring. British Trust for Ornithology Research Report 307: 51.

ROGAN, W. J., and B. GLADEN. 1978. Estimating prevalence from the results of a screening test. American Journal of Epidemiology 107: 71-76.

SHANNON, C. E. 1948. A mathematical theory of communication. Bell System Technical Journal 27: 379-423 and 623-656.

SHARP, G. B., Y. KAWAOKA, S. M. WRIGHT, B. TURNER, V. HINSHAW, and R. G. WEBSTER. 1993. Wild ducks are the reservoir for only a limited number of influenza A subtypes. Epidemiology and Infection 110: 161-176.

STITZ, L., C. SCHMITZ, D. BINDER, R. ZINKERNAGEL, E. PAOLETTI, and H. BECHT. 1990. Characterization and immunological properties of influenza A virus nucleoprotein (NP): Cell-associated NP isolated from infected cells or viral NP expresses by vaccinia recombinant virus do not confer protection. Journal of General Virology 71: 1169-1179.

SUAREZ, D. L., and S. SCHULTZ-CHERRY. 2000. Immunology of avian influenza virus: a review. Developmental and Comparative Immunology 24: 269-283.

SWAYNE, D. E., D. A. SENNE, and C. W. BEARD. 1998. Avian influenza. *In* A Laboratory Manual for the Isolation and Identification of Avian Pathogens, D. E. Swayne, (ed.). American Association of Avian Pathologists, Kennett Square, Pennsylvania, pp. 150-155.

TERREGINO, C., R. DE NARDI, V. GUBERTI, M. SCREMIN, E. RAFFINI, A. M. MARTIN, G. CATTOLI, L. BONFANTI, and I. CAPUA. 2007. Active surveillance for avian influenza viruses in wild birds and backyard flocks in Northern Italy during 2004 to 2006. Avian Pathology 36: 337-344.

VAN GILS, J. A., V. J. MUNSTER, R. RADERSMA, D. LIEFHEBBER, R. A. FOUCHIER, and M. KLAASSEN. 2007. Hampered foraging and migratory performance in swans infected with low-pathogenic avian influenza A virus. PLoS ONE 2: e184.

WAHLGREN, J., J. WALDENSTROM, S. SAHLIN, P. D. HAEMIG, R. A. M. FOUCHIER, A. D. M. E. OSTERHAUS, J. PINHASSI, J. BONNEDAHL, M. PISAREVA, M. GRUDININ, O. KISELEV, J. HERNANDEZ, K. I. FALK, Ã. LUNDKVIST, and B. OLSEN. 2008. Gene segment reassortment between American and Asian lineages of avian influenza virus from waterfowl in the Beringia area. Vector-Borne and Zoonotic Diseases 8: 783-790.

WEBSTER, R. G., M. YAKHNO, V. S. HINSHAW, W. J. BEAN, and K. COPAL MURTI. 1978. Intestinal influenza: replication and characterization of influenza viruses in ducks. Virology 84: 268-278.

WINKER, K., K. G. MCCRACKEN, D. D. GIBSON, C. L. PRUETT, R. MEIER, F. HUETTMANN, M. WEGE, I. V. KULIKOVA, Y. N. ZHURAVLEV, M. L. PERDUE, E. SPACKMAN, D. L. SUAREZ, and D. E. SWAYNE. 2007. Movements of birds and avian influenza from Asia into Alaska. Emerging Infectious Diseases 13: 547-552.

WINKER, K., E. SPACKMAN, and D. E. SWAYNE. 2008. Rarity of influenza A virus in spring shorebirds, southern Alaska. Emerging Infectious Diseases 14: 1314-1316.

Table 3.1. Avian influenza virus prevalence among 13 species of Charadriiformes sampled at Delaware Bay, springs 2006-2008.

| | Year | | | | | | | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2006 | | | 2007 | | | 2008 | | | | | |
| Family/Species | N[a] | Pos | Prevalence (%) (95% CI) | N[a] | Pos | Prevalence (%) (95% CI) | N[a] | Pos | Prevalence (%) (95% CI) | N[a] | Pos | Mean Prevalence (%) (95% CI) |
| Scolopacidae | | | | | | | | | | | | |
| Ruddy Turnstone *Arenaria interpres morinella* | 291 | 54 | 19 (15-23) | 413 | 19 | 4.6 (3.0-7.1) | 572 | 97 | 17 (14-20) | 1276 | 170 | 13 (12-15) |
| Red Knot *Calidris canutus rufa* | 193 | 2 | 1.0 (0.3-3.7) | 242 | 1 | 0.4 (0.1-2.3) | 274 | 2 | 0.7 (0.2-2.6) | 709 | 5 | 0.7 (0.3-1.6) |
| Sanderling *Calidris alba* | 113 | 7 | 6.2 (3.0-12) | 190 | 0 | 0 (0-2.0) | 221 | 1 | 0.5 (0.1-2.5) | 524 | 8 | 1.5 (0.8-3.0) |
| Semipalmated Sandpiper *Calidris pusilla* | 60 | 0 | 0 (0-6.0) | 28 | 0 | 0 (0-12) | 101 | 0 | 0 (0-3.7) | 189 | 0 | 0 (0-2.0) |
| Dunlin *Calidris alpina hudsonia* | 10 | 0 | 0 (0-28) | 31 | 0 | 0 (0-11) | 71 | 0 | 0 (0-5.1) | 112 | 0 | 0 (0-3.3) |
| Short-billed Dowitcher *Limnodromus griseus* | 17 | 0 | 0 (0-18) | 3 | 0 | 0 (0-56) | 40 | 0 | 0 (0-8.8) | 60 | 0 | 0 (0-6.0) |
| Least Sandpiper *Calidris minutilla* | 12 | 0 | 0 (0-24) | 2 | 0 | 0 (0-66) | 15 | 0 | 0 (0-20) | 29 | 0 | 0 (0-12) |
| Charadriidae | | | | | | | | | | | | |
| Semipalmated Plover *Charadrius semipalmatus* | 0 | n/a | n/a | 5 | 0 | 0 (0-43) | 1 | 0 | 0 (0-79) | 6 | 0 | 0 (0-39) |
| Laridae | | | | | | | | | | | | |
| Laughing Gull *Leucophaeus atricilla* | 60 | 1 | 1.7 (0.3-8.9) | 47 | 0 | 0 (0-7.6) | 142 | 4[b] | 2.8 (1.1-7.0) | 249 | 5[b] | 2.0 (0.9-4.6) |
| Herring Gull *Larus argentatus* | 0 | n/a | n/a | 29 | 1 | 3.4 (0.6-17) | 43 | 0 | 0 (0-8.2) | 72 | 1 | 1.4 (0.2-7.5) |
| Black Skimmer *Rynchops niger* | 6 | 0 | 0 (0-39) | 0 | n/a | n/a | 0 | n/a | n/a | 6 | 0 | 0 (0-39) |
| Great Black-backed Gull *Larus marinus* | 0 | n/a | n/a | 0 | n/a | n/a | 2 | 0 | 0 (0-66) | 2 | 0 | 0 (0-66) |
| Ring-billed Gull *Larus delawarensis* | 0 | n/a | n/a | 0 | n/a | n/a | 1 | 0 | 0 (0-79) | 1 | 0 | 0 (0-79) |

[a] Number of individuals, excludes multiple samples from the same bird within the same year
[b] A bird was considered positive if either the cloacal or oropharyngeal sample was positive (or both).

Table 3.2. Contrasts in AIV prevalence between five shorebird and gull species at Delaware Bay, springs 2006-2008, using binomial distribution GLM controlled for year differences. *P*-values are bolded where prevalence is significantly different between species.

| Species 1 | Species 2 | Contrast Value | $\chi^2$ | df | *P*-value | Adjusted OR (95% CI) |
|---|---|---|---|---|---|---|
| Ruddy Turnstone | Sanderling | 2.23 | 73.2 | 1 | **<0.0001** | 10.0 (5.2-22.3) |
| Ruddy Turnstone | Red Knot | 2.84 | 117.0 | 1 | **<0.0001** | 20.1 (9.7-51.5) |
| Ruddy Turnstone | Laughing Gull | 2.05 | 40.0 | 1 | **<0.0001** | 8.7 (3.9-24.6) |
| Ruddy Turnstone | Herring Gull | 1.85 | 9.1 | 1 | **0.003** | 9.8 (2.1-174.0) |
| Sanderling | Red Knot | 0.62 | 1.4 | 1 | 0.236 | 2.4 (0.8-7.4) |
| Sanderling | Laughing Gull | -0.18 | 0.1 | 1 | 0.748 | 0.9 (0.2-3.0) |
| Sanderling | Herring Gull | -0.38 | 0.2 | 1 | 0.688 | 0.2 (0.01-4.3) |
| Red Knot | Laughing Gull | -0.80 | 1.8 | 1 | 0.182 | 0.4 (0.1-1.5) |
| Red Knot | Herring Gull | -0.99 | 1.0 | 1 | 0.328 | 0.4 (0.1-8.2) |
| Laughing Gull | Herring Gull | -0.19 | 0.04 | 1 | 0.836 | 0.7 (0.04-4.9) |

Table 3.3. Calculated and adjusted anti-AIV antibody prevalences (95% CI) in nine Charadriiform bird species at Delaware Bay, springs 2007-2008.

| | Year | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2007 | | | | 2008 | | | | Total | | | |
| Family/Species | N[a] | Pos[b] | Calculated Ab-Prevalence[c] (%) | Adjusted Ab-Prevalence[d] (%) (95%CI) | N[a] | Pos[b] | Calculated Ab-Prevalence[c] (%) | Adjusted Ab-Prevalence[d] (%) (95%CI) | N[a] | Pos[b] | Calculated Ab-Prevalence[c] (%) | Adjusted Ab-Prevalence[d] (%) (95%CI) |
| Scolopacidae | | | | | | | | | | | | |
| Ruddy Turnstone | 127 | 79 | 62 | 83 (75-88) | 253 | 164 | 65 | 86 (81-90) | 380 | 243 | 64 | 85 (81-88) |
| Red Knot | 60 | 32 | 53 | 71 (58-81) | 151 | 93 | 62 | 82 (75-87) | 211 | 125 | 59 | 79 (73-84) |
| Sanderling | 36 | 1 | 2.8 | 3.7 (0.8-16) | 117 | 3 | 2.6 | 3.4 (1.3-2.6) | 153 | 4 | 2.6 | 3.5 (1.5-7.7) |
| Dunlin | 5 | 1 | 20 | 27 (5.9-68) | 9 | 1 | 11 | 15 (3.2-47) | 14 | 2 | 14 | 19 (6.3-45) |
| Short-billed Dowitcher | 0 | n/a | n/a | n/a | 8 | 0 | 0 | 0 (0-32) | 8 | 0 | 0 | 0 (0-32) |
| Laridae | | | | | | | | | | | | |
| Laughing Gull | 31 | 23 | 74 | 98 (86-100) | 52 | 25 | 48 | 64 (50-76) | 83 | 48 | 58 | 77 (67-85) |
| Herring Gull | 0 | n/a | n/a | n/a | 7 | 5 | 71 | 95 (58-100) | 7 | 5 | 71 | 95 (58-100) |
| Ring-billed Gull | 0 | n/a | n/a | n/a | 3 | 3 | 1 | 100 (44-100) | 3 | 3 | 1 | 100 (44-100) |
| Great Black-backed Gull | 0 | n/a | n/a | n/a | 2 | 2 | 1 | 100 (34-100) | 2 | 2 | 1 | 100 (34-100) |

[a] Number of samples tested
[b] Number of samples with S/N ratios ≤0.5 (test positive)
[c] Number of samples test positive divided by the number of samples tested
[d] Calculated antibody prevalence divided by 0.754; see text for details

Table 3.4. Contrasts in adjusted anti-AIV antibody prevalence between eight shorebird and gull species at Delaware Bay, springs 2007-2008, using binomial distribution GLM with Firth-adjusted maximum likelihood estimation. *P*-values are bolded where antibody prevalence is significantly different between species.

| Species 1 | Species 2 | Contrast Value | $\chi^2$ | df | *P*-value | OR (95% CI) |
|---|---|---|---|---|---|---|
| Ruddy Turnstone | Red Knot | 0.42 | 3.64 | 1 | 0.056 | 1.5 (1.0-2.3) |
| Ruddy Turnstone | Sanderling | 4.95 | 338.3 | 1 | **<0.0001** | 141.5 (64.1-380.1) |
| Ruddy Turnstone | Dunlin | 3.04 | 28.4 | 1 | **<0.0001** | 20.8 (6.5-90.1) |
| Ruddy Turnstone | Laughing Gull | 0.26 | 1.11 | 1 | 0.292 | 1.7 (0.9-3.0) |
| Ruddy Turnstone | Herring Gull | -0.39 | 0.12 | 1 | 0.719 | 0.7 (0.03-4.2) |
| Ruddy Turnstone | Ring-billed Gull | -0.23 | 0.02 | 1 | 0.874 | 0.8 (0.01-8.3) |
| Ruddy Turnstone | Great Black-backed Gull | 0.10 | <0.01 | 1 | 0.948 | 1.1 (0.01-13.9) |
| Red Knot | Sanderling | 4.53 | 235.6 | 1 | **<0.0001** | 92.9 (41.2-252.7) |
| Red Knot | Dunlin | 2.62 | 20.6 | 1 | **<0.0001** | 13.7 (4.2-59.6) |
| Red Knot | Laughing Gull | 0.11 | 0.14 | 1 | 0.703 | 1.1 (0.6-2.0) |
| Red Knot | Herring Gull | -0.81 | 0.61 | 1 | 0.434 | 0.4 (0.02-2.8) |
| Red Knot | Ring-billed Gull | -0.65 | 0.22 | 1 | 0.642 | 0.5 (0.004-5.5) |
| Red Knot | Great Black-backed Gull | -0.32 | 0.04 | 1 | 0.833 | 0.7 (0.01-9.1) |
| Sanderling | Dunlin | -1.92 | 5.18 | 1 | **0.023** | 0.5 (0.03-0.7) |
| Sanderling | Laughing Gull | -4.41 | 147.0 | 1 | **<0.0001** | 0.01 (0.004-0.03) |
| Sanderling | Herring Gull | -5.35 | 35.3 | 1 | **<0.0001** | 0.01 (0.0002-0.04) |
| Sanderling | Ring-billed Gull | -5.19 | 18.3 | 1 | **<0.0001** | 0.01 (<0.0001-0.07) |
| Sanderling | Great Black-backed Gull | -4.85 | 12.8 | 1 | **0.0003** | 0.01 (<0.0001-0.1) |
| Dunlin | Laughing Gull | -2.50 | 16.9 | 1 | **<0.0001** | 0.08 (0.02-0.3) |
| Dunlin | Herring Gull | -3.43 | 10.7 | 1 | **0.001** | 0.03 (0.001-0.3) |
| Dunlin | Ring-billed Gull | -3.27 | 6.17 | 1 | **0.013** | 0.04 (0.0003-0.5) |
| Dunlin | Great Black-backed Gull | -2.93 | 4.27 | 1 | **0.038** | 0.05 (0.0003-0.9) |
| Laughing Gull | Herring Gull | -0.93 | 0.79 | 1 | 0.374 | 0.4 (0.02-2.6) |
| Laughing Gull | Ring-billed Gull | -0.77 | 0.30 | 1 | 0.582 | 0.5 (0.003-5.1) |
| Laughing Gull | Great Black-backed Gull | -0.44 | 0.08 | 1 | 0.772 | 0.64 (0.005-8.4) |
| Herring Gull | Ring-billed Gull | -0.16 | 0.01 | 1 | 0.933 | 0.9 (0.02-151.4) |
| Herring Gull | Great Black-backed Gull | 0.50 | 0.06 | 1 | 0.800 | 1.6 (0.01-87.0) |
| Ring-billed Gull | Great Black-backed Gull | 0.34 | 0.02 | 1 | 0.877 | 1.4 (0.01-307.0) |

Table 3.5. Results of each virus isolation and bELISA serology test for individual shorebirds captured in two different years, Delaware Bay, 2006-2008. Positive and negative test results are denoted Pos and Neg, respectively. Serology was performed in 2007-2008 only.

| | Virus Isolation (Subtype) | | | bELISA | |
|---|---|---|---|---|---|
| | Year | | | Year | |
| Species/Individual | 2006 | 2007 | 2008 | 2007 | 2008 |
| Ruddy Turnstone | | | | | |
| AH4 | -[a] | Neg | Neg | nt[b] | Pos |
| AU3 | - | Neg | Neg | nt | Pos |
| HC4 | - | Neg | Neg | nt | Neg |
| JY5 | - | Neg | Neg | Pos | Pos |
| NU3 | - | Neg | Neg | nt | nt |
| NY3 | - | Neg | Pos (H4N6) | Neg | nt |
| UY4 | - | Neg | Neg | nt | nt |
| XNX | Neg | Neg | - | nt | - |
| XVT | Neg | - | Neg | - | Neg |
| XXE | Neg | - | Neg | - | nt |
| YAJ | Pos (H6N7) | Pos (H12N5) | - | nt | - |
| YAM | Pos (H9N2) | - | Neg | - | Pos |
| YAT | Pos (H7N3) | Neg | - | Neg | - |
| YAV | Neg | - | Pos (H11N9) | - | Pos |
| YEH | Pos (H7N3) | - | Pos (H12N5) | - | nt |
| YJT | Neg | Neg | - | Neg | - |
| YU3 | - | Neg | Neg | Pos | Pos |
| Red Knot | | | | | |
| LK5 | - | Neg | Neg | nt | nt |
| MK5 | - | Neg | Neg | nt | nt |
| PA8 | - | Neg | Neg | nt | nt |
| XJY | Neg | Neg | - | nt | - |
| XNP | Neg | Neg | - | Neg | - |
| Sanderling | | | | | |
| HE1 | - | Neg | Neg | nt | nt |
| MXV | Neg | - | Neg | - | nt |
| TK1 | - | Neg | Neg | nt | nt |
| VE1 | - | Neg | Neg | nt | Neg |

[a] denotes that the bird was not captured in this year
[b] nt=the bird was not tested at this capture event

Table 3.6. Single-variable and best predictive logistic regression models of individual bird-level attributes on AIV infection status, after controlling for significant population-level variables, in Ruddy Turnstones, Red Knots, and Sanderlings sampled during 2006-2008 at Delaware Bay. *P*-values of significant effects are bolded.

| Variable | $\chi^2$ | df | *P*-value | Level | OR (95% CI) |
|---|---|---|---|---|---|
| | | | *Single-Variable Models* | | |
| Ruddy Turnstone (controlled for year, day[a], and year×day) | | | | | |
| Body Size Index | 0.46 | 1 | 0.499 | continuous | 0.96 (0.85-1.09) |
| Mass index | 16.42 | 1 | **<0.0001** | continuous | 1.02 (1.01-1.04) |
| Sex | 1.66 | 1 | 0.197 | female vs. male | 1.3 (0.9-1.8) |
| Males only (controlled for year, day[a], and year×day) | | | | | |
| Body Size Index | 0.01 | 1 | 0.944 | continuous | 0.99 (0.82-1.20) |
| Mass index | 5.53 | 1 | **0.019** | continuous | 1.02 (1.00-1.04) |
| Females only (controlled for year) | | | | | |
| Body Size Index | 1.88 | 1 | 0.171 | continuous | 0.87 (0.72-1.06) |
| Mass index | 8.84 | 1 | **0.003** | continuous | 1.03 (1.01-1.04) |
| Red Knot | | | | | |
| Body Size Index | 4.16 | 1 | **0.041** | continuous | 1.91 (1.03-3.64) |
| Mass index | 1.60 | 1 | 0.206 | continuous | 0.97 (0.93-1.02) |
| Sanderling (controlled for year) | | | | | |
| Body Size Index | 2.17 | 1 | 0.141 | continuous | 0.36 (0.08-1.39)[b] |
| Mass index | 0.32 | 1 | 0.571 | continuous | 1.05 (0.90-1.23)[b] |
| | | | *Best Predictive Models* | | |
| Ruddy Turnstone | | | | | Adjusted OR (95% CI) |
| Whole model | 82.79 | 9 | **<0.0001** | | |
| Year | 36.16 | 2 | **<0.0001** | n/a[b] | n/a[b] |
| Day in May[a] | 19.35 | 2 | **<0.0001** | n/a[b] | n/a[b] |
| Year×Day in May | 10.22 | 2 | **0.006** | n/a[b] | n/a[b] |
| Mass index | 17.68 | 1 | **<0.0001** | continuous | 1.03 (1.01-1.04) |
| Sex | 4.50 | 1 | **0.034** | Female vs. male | 1.5 (1.0-2.2) |
| Body Size Index | 3.02 | 1 | 0.082 | continuous | 0.89 (0.77-1.02) |
| Males only | | | | | |
| Whole model | 47.99 | 7 | **<0.0001** | | |
| Year | 15.51 | 2 | **0.0004** | n/a[b] | n/a[b] |
| Day in May[a] | 24.84 | 2 | **<0.0001** | n/a[b] | n/a[b] |
| Year×Day in May | 7.25 | 2 | **0.027** | n/a[b] | n/a[b] |
| Mass index | 5.53 | 1 | **0.019** | continuous | 1.02 (1.00-1.04) |
| Females only | | | | | |
| Whole model | 39.02 | 4 | **<0.0001** | | |
| Year | 26.90 | 2 | **<0.0001** | 2006 vs. 2007 | 6.1 (2.7-13.8) |
| | | | | 2006 vs. 2008 | 1.2 (0.7-2.1) |
| | | | | 2008 vs. 2007 | 4.9 (2.1-10.8) |
| Mass index | 10.75 | 1 | **0.001** | continuous | 1.03 (1.01-1.05) |
| Body Size Index | 3.82 | 1 | 0.051 | continuous | 0.82 (0.67-1.00) |
| Red Knot | | | | | |
| Whole model | 7.45 | 2 | **0.024** | | |
| Body Size Index | 5.85 | 1 | **0.016** | continuous | 2.24 (1.17-4.42) |
| Mass index | 3.33 | 1 | 0.068 | continuous | 0.96 (0.92-1.00) |
| Sanderling | | | | | |
| Whole model | 17.14[c] | 2 | **0.0002** | | |
| Year | 17.14[c] | 2 | **0.0002** | 2006 vs. 2007 | 25.8 (3.1-3355.3)[c] |
| | | | | 2006 vs. 2008 | 10.1 (1.7-59.2)[c] |
| | | | | 2008 vs. 2007 | 2.6 (0.1-374.3)[c] |

[a] Modeled as a 3-node knotted spline effect
[b] Firth-adjusted estimates

Table 3.7. Single-variable logistic regression analyses of individual bird-level attributes on anti-AIV antibody status, after controlling for significant population-level variables, in Ruddy Turnstones, Red Knots, and Sanderlings sampled between 2006-2008 at Delaware Bay. *P*-values of significant effects are bolded.

| Variable | $\chi^2$ | df | *P*-value | Level | OR (95% CI) |
|---|---|---|---|---|---|
| | | | *Single-Variable Models* | | |
| Ruddy Turnstone (controlled for day) | | | | | |
| Body Size Index | 2.15 | 1 | 0.142 | continuous | 0.89 (0.75-1.04) |
| Mass index | 4.33 | 1 | **0.038** | continuous | 1.02 (1.00-1.04) |
| Sex | 2.65 | 1 | 0.104 | male vs. female | 1.5 (0.9-2.5) |
| Infection Status | 4.76 | 1 | **0.029** | positive vs. negative | 2.1 (1.1-4.3) |
| Males only (controlled for day) | | | | | |
| Body Size Index | 0.33 | 1 | 0.563 | continuous | 0.94 (0.75-1.17) |
| Mass index | 2.70 | 1 | 0.100 | continuous | 1.02 (1.00-1.04) |
| Infection status | 1.18 | 1 | 0.277 | positive vs. negative | 1.6 (0.7-4.2) |
| Females only (controlled for day) | | | | | |
| Body Size Index | 0.04 | 1 | 0.847 | continuous | 0.97 (0.71-1.32) |
| Mass index | 1.90 | 1 | 0.169 | continuous | 1.02 (0.99-1.05) |
| Infection status | 3.20 | 1 | 0.074 | positive vs. negative | 2.7 (0.9-9.2) |
| Red Knot (controlled for day) | | | | | |
| Body Size Index | 0.27 | 1 | 0.602 | continuous | 1.06 (0.86-1.31) |
| Mass index | 1.18 | 1 | 0.277 | continuous | 1.01 (0.99-1.02) |
| Infection Status | 0.08 | 1 | 0.773 | positive vs. negative | 1.6 (0.1-232.8)[a] |
| Sanderling | | | | | |
| Body Size Index | 1.10 | 1 | 0.294 | continuous | 2.03 (0.55-9.04)[a] |
| Mass index | 3.00 | 1 | 0.084 | continuous | 1.13 (0.99-1.31) |
| Infection Status | n/a[b] | n/a[b] | n/a[b] | n/a[b] | n/a[b] |
| | | | *Best Predictive Models* | | |
| All Ruddy Turnstones | | | | | Adjusted OR (95% CI) |
| Whole model | 69.14 | 4 | **<0.0001** | | |
| Day in May | 63.44 | 1 | **<0.0001** | continuous | 1.13 (1.10-1.17) |
| Sex | 3.99 | 1 | **0.046** | male vs. female | 1.7 (1.0-2.8) |
| Mass Index | 3.66 | 1 | 0.056 | continuous | 1.02 (1.00-1.04) |
| Infection status | 2.83 | 1 | 0.092 | positive vs. negative | 1.8 (0.9-3.8) |
| Males only | | | | | |
| Whole model | 36.23 | 1 | **<0.0001** | | |
| Day in May | 36.23 | 1 | **<0.0001** | continuous | 1.13 (1.08-1.19) |
| Females only | | | | | |
| Whole model | 41.18 | 6 | **<0.0001** | | |
| Day in May | 31.29 | 1 | **<0.0001** | continuous | 1.15 (1.09-1.22) |
| Infection status | 6.54 | 1 | **0.011** | n/a[c] | n/a[c] |
| Mass Index | 5.84 | 1 | **0.016** | n/a[c] | n/a[c] |
| Body Size Index | 4.55 | 1 | **0.033** | n/a[c] | n/a[c] |
| BSI × Infection status | 5.09 | 1 | **0.024** | n/a[c] | n/a[c] |
| MI × Infection status | 3.26 | 1 | 0.071 | n/a[c] | n/a[c] |
| Red Knot | | | | | |
| Whole model | 7.60 | 1 | **0.006** | | |
| Day in May | 7.60 | 1 | **0.006** | continuous | 0.95 (0.92-0.99) |
| Sanderling | | | | | |
| Whole model | 3.00 | 1 | 0.084 | | |
| Mass Index | 3.00 | 1 | 0.084 | continuous | 1.13 (0.99-1.31) |

[a] Firth-adjusted estimates
[b] No antibody positive Sanderlings were virus isolation-positive, thus, the regression is undefined
[c] ORs not meaningful due to interaction effects of the variable

Table 3.8. Distribution and diversity of AIV HA and NA subtypes isolated from all charadriiform birds at Delaware Bay, springs 2006-2008. Number and percent of all isolates in that year are given. Subtype diversity (H) and equitability ($E_H$) are according to Shannon (1948).

| | Year | | | |
|---|---|---|---|---|
| | 2006 | 2007 | 2008 | Total |
| HA subtype | | | | |
| H1 | - | - | 5 (4.8) | 5 (2.6) |
| H2 | - | 1 (4.8) | - | 1 (0.5) |
| H3 | - | - | 12 (12) | 12 (6.3) |
| H4 | - | - | 31 (30) | 31 (16) |
| H5 | - | 2 (9.5) | 1 (1.0) | 3 (1.6) |
| H6 | 1 (1.5) | 1 (4.8) | 3 (2.9) | 5 (2.6) |
| H7 | 50 (77) | 1 (4.8) | - | 51 (27) |
| H9 | 1 (1.5) | 2 (9.5) | - | 3 (1.6) |
| H10 | - | - | 26 (25) | 26 (14) |
| H11 | - | - | 4 (3.8) | 4 (2.1) |
| H12 | - | 14 (67) | 15 (14) | 29 (15) |
| H13 | - | - | 4 (3.8) | 4 (2.1) |
| Undetermined | 13 (20) | - | 3 (2.9) | 16 (8.4) |
| Total | 65 (100) | 21 (100) | 104 (100) | 190 (100) |
| | | | | |
| *n* subtypes | 3 | 6 | 9 | 12 |
| Diversity (H) | 0.19 | 1.15 | 1.79 | 1.97 |
| Equitability ($E_H$) | 0.17 | 0.64 | 0.81 | 0.79 |
| | | | | |
| NA subtype | | | | |
| N1 | - | 6 (29) | - | 6 (3.2) |
| N2 | 2 (3.1) | - | 10 (9.6) | 12 (6.3) |
| N3 | 47 (72) | 1 (4.8) | - | 48 (25) |
| N4 | 1 (1.5) | - | 1 (1.0) | 2 (1.1) |
| N5 | - | 13 (62) | 20 (19) | 33 (17) |
| N6 | - | - | 33 (32) | 33 (17) |
| N7 | 4 (6.2) | - | 30 (29) | 34 (18) |
| N8 | - | - | 3 (2.9) | 3 (1.6) |
| N9 | - | 1 (4.8) | 5 (4.8) | 6 (3.2) |
| Undetermined | 11 (17) | - | 2 (1.9) | 13 (6.8) |
| Total | 65 (100) | 21 (100) | 104 (100) | 190 (100) |
| | | | | |
| *n* subtypes | 4 | 4 | 7 | 9 |
| Diversity (H) | 0.51 | 0.94 | 1.57 | 1.83 |
| Equitability ($E_H$) | 0.37 | 0.68 | 0.81 | 0.83 |

Table 3.9. Distribution and diversity of AIV subtypes among Charadriiformes species at Delaware Bay, springs 2006-2008. Number and percent of all isolates in that year are given. All isolates are from Ruddy Turnstones except where indicated. Subtype diversity (H) and equitability ($E_H$) are according to Shannon (1948).

| | Year | | | |
|---|---|---|---|---|
| Subtype | 2006 | 2007 | 2008 | Total |
| H1N5 | - | - | 3 (2.9) | 3 (1.6) |
| H1N6 | - | - | 1 (1.0) | 1 (0.5) |
| H1N7 | - | - | 1 (1.0) | 1 (0.5) |
| H2N9 | - | 1[a] (4.8) | - | 1[a] (0.5) |
| H3N2 | - | - | 8 (7.7) | 8 (4.2) |
| H3N7 | - | - | 3 (2.9) | 3 (1.6) |
| H3N8 | - | - | 1 (1.0) | 1 (0.5) |
| H4N6 | - | - | 30[b] (29) | 30[b] (16) |
| H4N7 | - | - | 1 (1.0) | 1 (0.5) |
| H5N1 | - | 2 (9.5) | - | 2 (1.1) |
| H6N1 | - | 1 (4.8) | - | 1 (0.5) |
| H6N6 | - | - | 1 (1.0) | 1 (0.5) |
| H6N7 | 1 (1.5) | - | - | 1 (0.5) |
| H6N8 | - | - | 2 (1.9) | 2 (1.1) |
| H7N2 | 1 (1.5) | - | - | 1 (0.5) |
| H7N3 | 43[c] (66) | 1 (4.8) | - | 44[c] (23) |
| H7N4 | 1 (1.5) | - | - | 1 (0.5) |
| H7N7 | 3 (4.6) | - | - | 3 (1.6) |
| H9N1 | - | 2 (9.5) | - | 2 (1.1) |
| H9N2 | 1 (1.5) | - | - | 1 (0.5) |
| H10N2 | - | - | 1 (1.0) | 1 (0.5) |
| H10N6 | - | - | 1 (1.0) | 1 (0.5) |
| H10N7 | - | - | 24[d] (23) | 24[d] (13) |
| H11N2 | - | - | 1 (1.0) | 1 (0.5) |
| H11N5 | - | - | 1 (1.0) | 1 (0.5) |
| H11N9 | - | - | 2 (1.9) | 2 (1.1) |
| H12N1 | - | 1 (4.8) | - | 1 (0.5) |
| H12N4 | - | - | 1 (1.0) | 1 (0.5) |
| H12N5 | - | 13[e] (62) | 15[e] (14) | 28[e] (15) |
| H13N9 | - | - | 3[f] (2.9) | 3[f] (1.6) |
| Undetermined | 15[g] (23) | - | 4[g] (3.8) | 19[g] (10.0) |
| Total | 65 (100) | 21 (100) | 104 (100) | 190 (100) |
| | | | | |
| *n* subtypes | 6 | 7 | 19 | 30 |
| Diversity (H) | 0.61 | 1.33 | 2.12 | 2.37 |
| Equitability ($E_H$) | 0.34 | 0.68 | 0.72 | 0.70 |

[a] Isolated from a Herring Gull
[b] Includes one isolate from a Red Knot
[c] Includes two isolates from Red Knots and four isolates from Sanderlings
[d] Includes one isolate from a Sanderling
[e] Includes two isolates from Red Knots, one each in 2007 and 2008
[f] Isolated from Laughing Gulls
[g] In 2006, includes 10 isolates from Ruddy Turnstones, three from Sanderlings and one each from a Laughing Gull and a Red Knot. In 2008, includes three isolates from Ruddy Turnstones and one from a Laughing Gull.

Table 3.10. Between-year comparisons of Shannon's H diversity indices of AIV subtypes isolated from charadriiform species at Delaware Bay, 2006-2008.

| Year comparison | *t* | df | *P*-value |
|---|---|---|---|
| HA subtype diversity | | | |
| 2006 vs. 2007 | -3.2 | 29 | **0.003** |
| 2007 vs. 2008 | -2.8 | 25 | **0.011** |
| 2006 vs. 2008 | -11.9 | 108 | **<0.0001** |
| NA subtype diversity | | | |
| 2006 vs. 2007 | -1.8 | 47 | 0.084 |
| 2007 vs. 2008 | -3.5 | 28 | **0.002** |
| 2006 vs. 2008 | -7.0 | 83 | **<0.0001** |
| HA-NA combination diversity | | | |
| 2006 vs. 2007 | -2.0 | 40 | **0.048** |
| 2007 vs. 2008 | -3.0 | 31 | **0.005** |
| 2006 vs. 2008 | -7.2 | 100 | **<0.0001** |

## FIGURE LEGENDS

Figure 3.1. Avian influenza virus prevalence (95% CI) by year in five species of Charadriformes at Delaware Bay, 2006-2008. For each species, yearly prevalences not connected by the same letter are statistically different. Herring Gulls were not sampled in 2006. *Estimated prevalences in Red Knots and Sanderlings in 2008 are possibly depressed due to timing of the samples (see text).

Figure 3.2. Yearly prevalence in Sanderlings as a function of prevalence in Ruddy Turnstones during 2000-2007. Estimated prevalence in Sanderlings in 2008 is possibly depressed due to timing of the samples (see text); this data point is shown as an open circle and was excluded from the model. Line of fit (95% CI) was obtained using binomial distribution GLM.

Figure 3.3. Adjusted AIV antibody prevalence (95% CI) by year across in all nine species of Charadriiformes tested at Delaware Bay, 2007-2008. For each species, estimates of antibody prevalence not connected by the same letter are significantly different. Short-billed Dowitchers, Herring Gulls, Ring-billed Gulls, and Great Black-backed Gulls were not sampled in 2007.

Figure 3.4. Mean AIV prevalence and adjusted antibody prevalence (95% CI) by species, 2006-2008. Note that Ruddy Turnstones uniquely exhibited both “high” AIV prevalence and “high” antibody prevalence.

Supplemental Figure 3.S1. Expected mass gain of Ruddy Turnstones, Red Knots, and Sanderlings over the course of the stopover each year 2006-2008 at Delaware Bay. The regression curves were fit using a 4-node (Red Knot) or 5-node (Ruddy Turnstone and Sanderling) knotted spline effect of day on individual bird mass in standard least-squares linear regression. All fits are significant at the $\alpha$=0.0001 level. Also shown are the mean masses of birds on each individual catch where $n \geq 5$.

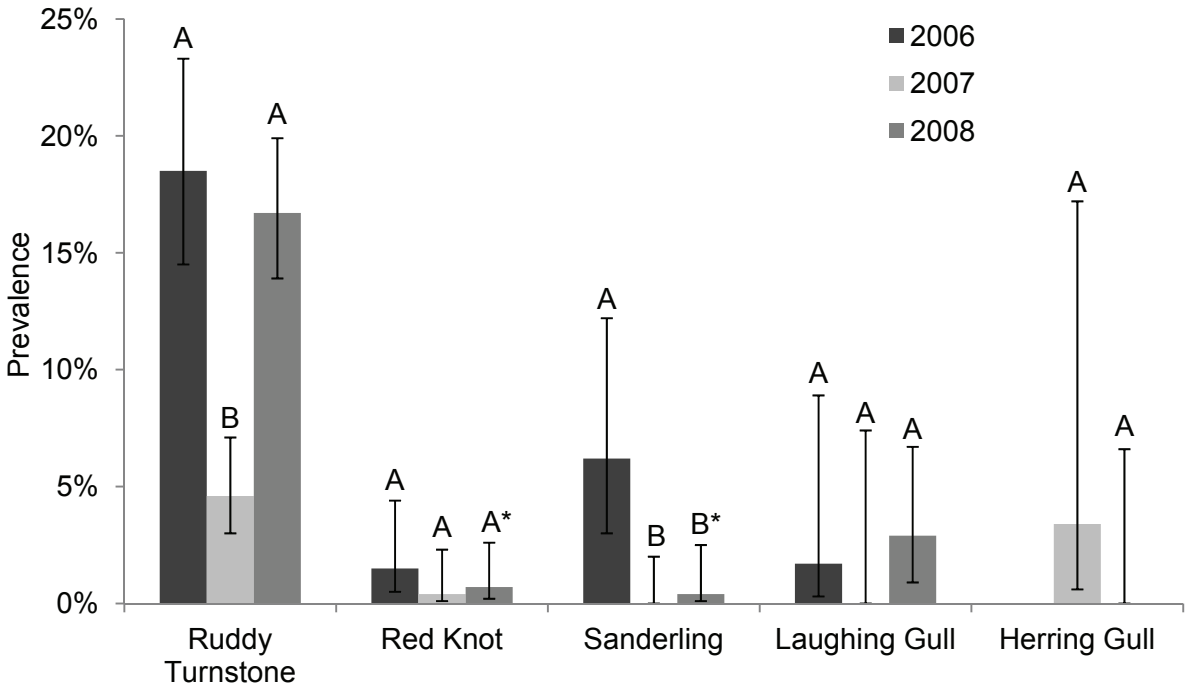

25%
20%
15%
10%
5%
0%
Prevalence
2006
2007
2008
A
B
A
A
A*
A
B
B*
A
A
A
A
A
Ruddy Turnstone
Red Knot
Sanderling
Laughing Gull
Herring Gull

Figure 3.1.

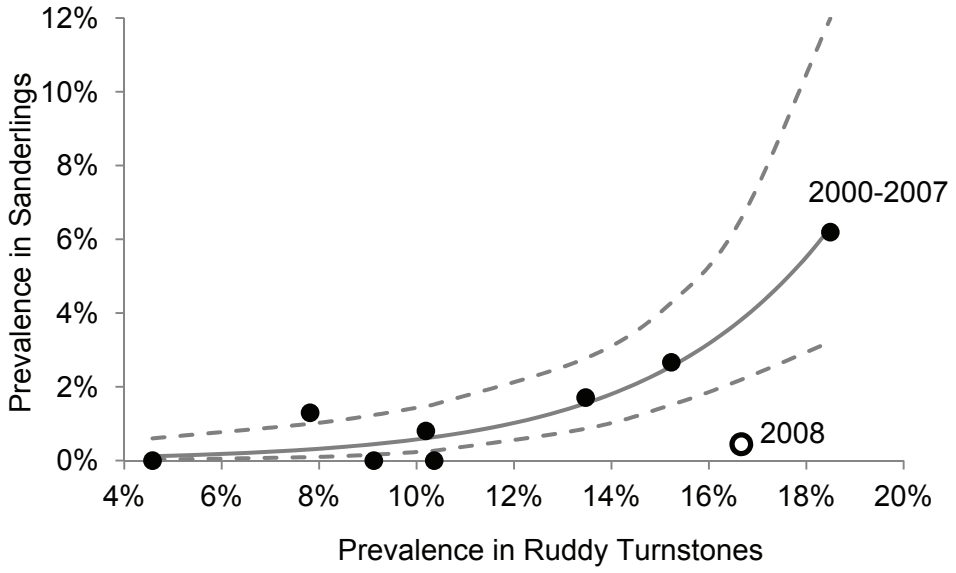

12%
10%
8%
6%
4%
2%
0%
Prevalence in Sanderlings
2000-2007
2008
4%
6%
8%
10%
12%
14%
16%
18%
20%
Prevalence in Ruddy Turnstones

Figure 3.2.

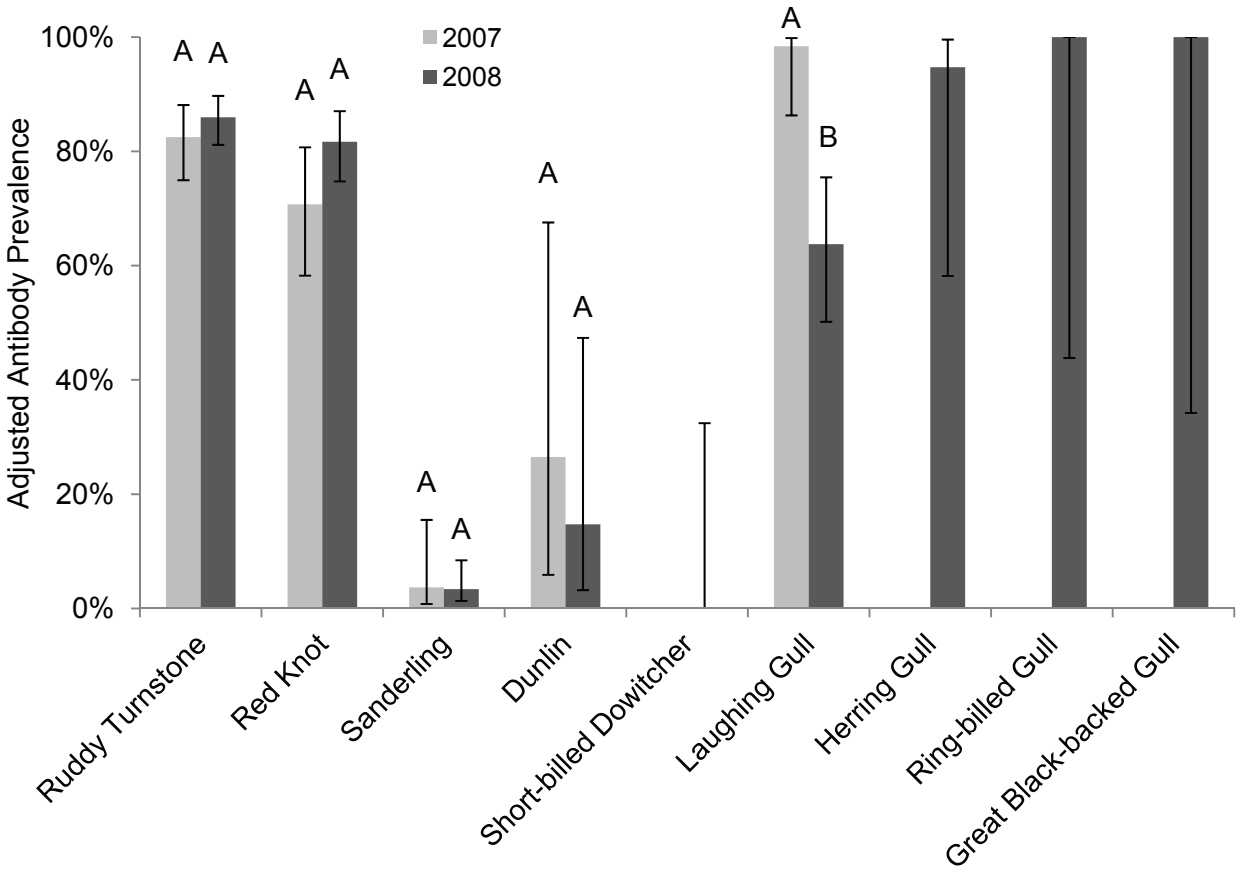

100%
80%
60%
40%
20%
0%
Adjusted Antibody Prevalence
2007
2008
A A
A A
A A
A
A
A
B
Ruddy Turnstone
Red Knot
Sanderling
Dunlin
Short-billed Dowitcher
Laughing Gull
Herring Gull
Ring-billed Gull
Great Black-backed Gull

Figure 3.3.

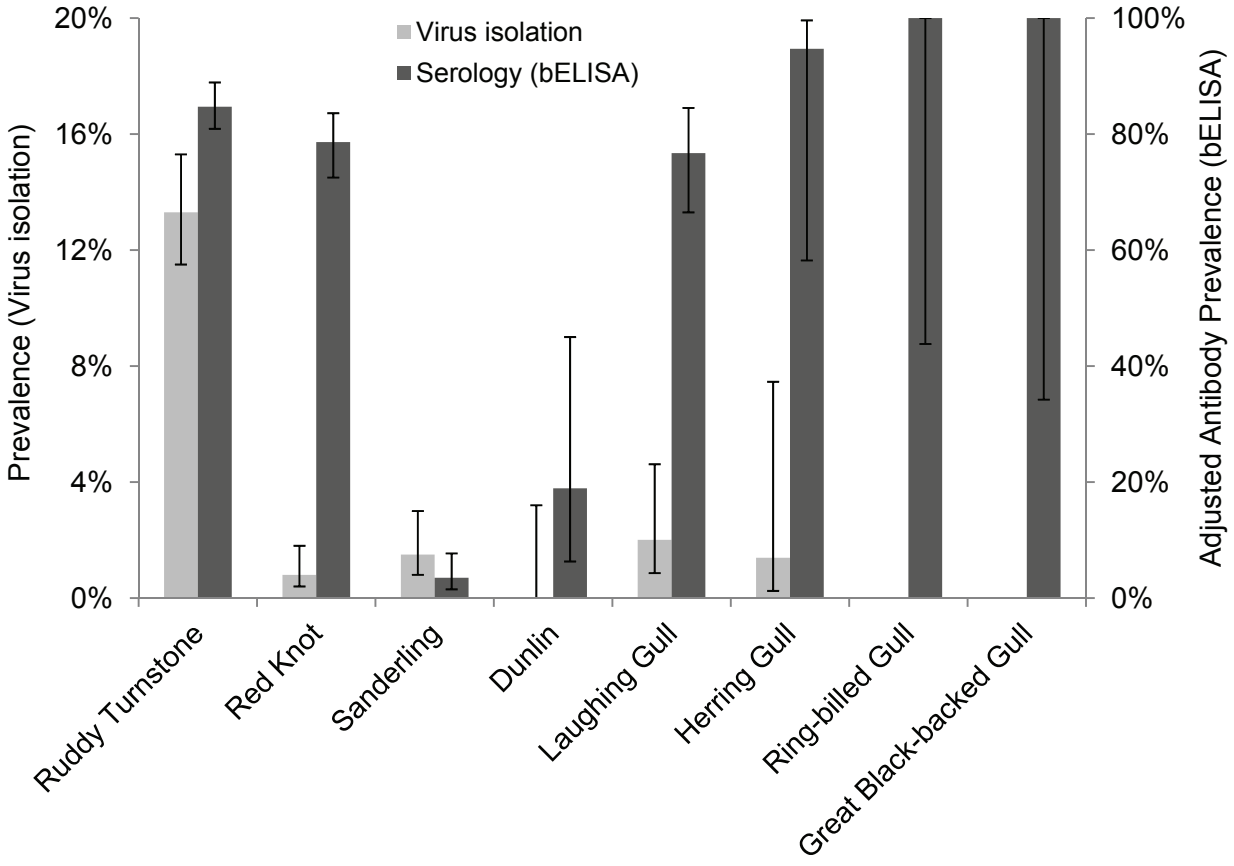
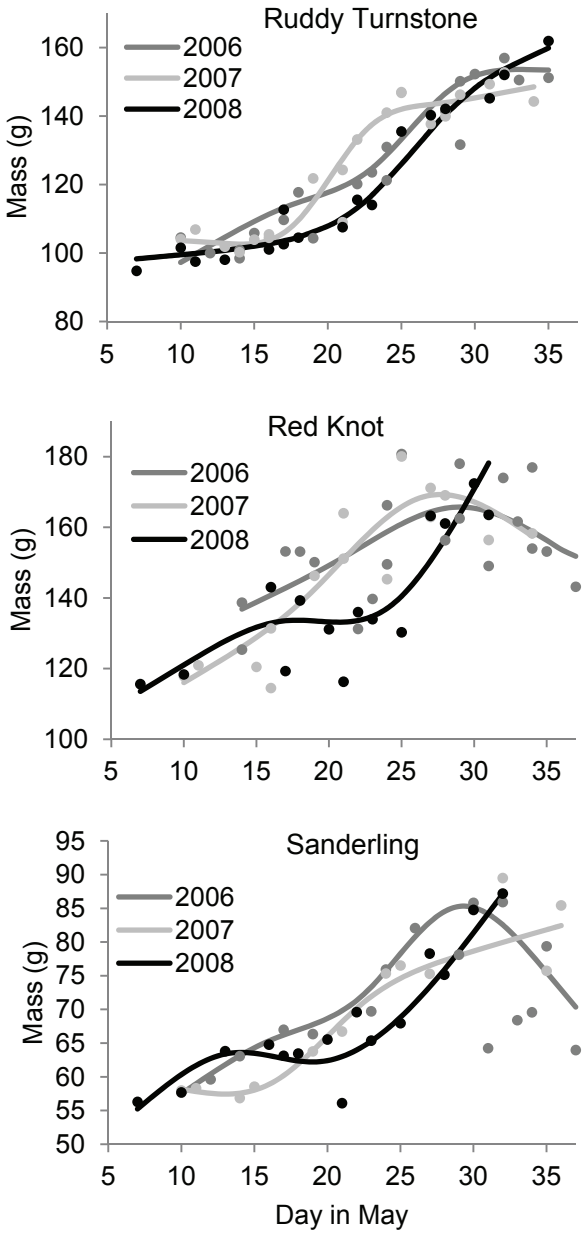

Virus isolation
Serology (bELISA)
Prevalence (Virus isolation)
Adjusted Antibody Prevalence (bELISA)
20%
16%
12%
8%
4%
0%
100%
80%
60%
40%
20%
0%
Ruddy Turnstone
Red Knot
Sanderling
Dunlin
Laughing Gull
Herring Gull
Ring-billed Gull
Great Black-backed Gull

Figure 3.4.




Supplemental Figure 3.S1.

# CHAPTER 4

# AVIAN INFLUENZA VIRUS INFECTION DYNAMICS IN SHOREBIRD HOSTS[1]

[1] Maxted, A. M., M. P. Luttrell, V. H. Goekjian, J. D. Brown, L. J. Niles, A. D. Dey, K. S. Kalasz, D. E. Swayne, and D. E. Stallknecht. Accepted by *Journal of Wildlife Diseases.* Reprinted here with permission of publisher.

## ABSTRACT

To gain insight into avian influenza virus (AIV) transmission, exposure, and maintenance patterns in shorebirds at Delaware Bay during spring migration, we examined temporal AIV prevalence trends in four Charadriiformes species using serial cross-sectional data from 2000-2008 and generalized linear and additive models. Prevalence of AIV in Ruddy Turnstones (*Arenaria interpres morinella*) increased after arrival, peaked in mid-late May, and decreased prior to departure. Antibody prevalence also increased over this period; together, these results suggested local infection and recovery prior to departure. Red Knots (*Calidris canutus rufa*), Sanderlings (*Calidris alba*), and Laughing Gulls (*Leucophaeus atricilla*) were rarely infected, but dynamical changes in antibody prevalence differed among species. In Red Knots, declining antibody prevalence over the stopover period suggested AIV exposure prior to arrival at Delaware Bay with limited infection at this site. Antibody prevalence was consistently high in Laughing Gulls and low in Sanderlings. Both prevalence and antibody prevalence in Sanderlings varied directly with those in Turnstones, suggesting virus spillover to Sanderlings. Results indicate that, although hundreds of thousands of birds concentrate at Delaware Bay during spring, dynamics of AIV infection differ among species, perhaps due to differences in susceptibility, potential for contact with AIV at this site, or prior exposure. Additionally, Ruddy Turnstones possibly act as a local AIV amplifying host rather than a reservoir.


*Key Words*: AIV, Avian influenza virus, Charadriiformes, Delaware Bay, disease ecology, infection dynamics, Ruddy Turnstone (*Arenaria interpres morinella*), shorebird

## INTRODUCTION

Although Anseriformes (swans, geese, and ducks) and Charadriiformes (gulls and shorebirds) are the natural reservoir of avian influenza viruses (AIV), our current understanding of AIV epidemiology in these reservoirs is limited by the patchy nature of surveillance across space, time, and species (Stallknecht and Brown, 2007). Among ducks, AIV prevalence typically peaks in late summer and autumn as birds, particularly naïve juveniles, aggregate on premigratory staging grounds (Olsen et al., 2006). Some species of Charadriiformes such as gulls (family Laridae) and shorebirds (families Charadriidae and Scolopacidae) also are important in global AIV epidemiology and certain subtypes are associated with and are particularly well adapted to gull species (Hinshaw et al., 1983; Yamnikova et al., 2003; Fouchier et al., 2005). While transmission among ducks occurs primarily through fecally contaminated water (Hinshaw et al., 1979), transmission and maintenance among Charadriiformes species is poorly understood.

Initial studies conducted at Delaware Bay on the Atlantic coast of North America reported that AIV prevalence was 2.4-20% among sampled shorebirds and gulls in spring compared to 3.5-8% in autumn and 0% in winter and summer (Kawaoka et al., 1988). Estimated mean springtime prevalence among shorebirds and gulls was 6.3% across 21 yr (Krauss et al., 2010). Both studies recognized one shorebird species, the Ruddy Turnstone (*Arenaria interpres morinella*), as disproportionately infected, and Hanson et al. (2008) reported that mean springtime prevalence was significantly higher among Ruddy Turnstones (11%) than among 10 other species (0.5%). Despite this consistent seasonal pattern of infection among shorebirds at Delaware Bay, AIV have not been detected more than occasionally in shorebirds at other times and locations, particularly in the Western Hemisphere (e.g., Escudero et al., 2008; Iverson et al., 2008; Winker et al., 2008; Ghersi et al., 2009).

An estimated >1 million shorebirds use Delaware Bay each spring (Clark et al., 1993), where, by feasting on eggs of horseshoe crabs (*Limulus polyphemus*) on spawning beaches,

they rapidly gain up to 70% of their arrival body masses to fuel long-distance flights to breeding grounds in the Arctic (Robinson et al., 2003). Each year, shorebirds begin arriving in early May following migrations from wintering and stopover areas in South America and the Caribbean (Myers et al., 1990; Morrison and Harrington, 1992) and depart for breeding grounds during late May-early June (Robinson et al., 2003). The stopover period approximates 5 wk, although individual birds can remain for shorter periods (Gillings et al., 2009). From 12-80% of the North American populations of six species use Delaware Bay in spring, including Red Knots (*Calidris canutus rufa*), Ruddy Turnstones, Sanderlings (*Calidris alba*), Semipalmated Sandpipers (*Calidris pusilla*), Dunlins (*Calidris alpina*) and Short-billed Dowitchers (*Limnodromus griseus*) (reviewed by US Fish and Wildlife Service, 2003). Additionally, adjacent salt marshes in New Jersey support breeding colonies of Laughing Gulls (*Leucophaeus atricilla*), Herring Gulls (*Larus argentatus*), and Great Black-backed Gulls (*Larus marinus*), and non-breeding Ring-billed Gulls (*Larus delawarensis*).that also feed on horseshoe crab eggs alongside shorebirds (Burger et al., 2007)

Delaware Bay is adjacent to the Delmarva Peninsula, an important poultry-producing region. Because transmission of AIV from wild birds to poultry occasionally occurs with appropriate contact (Spackman, 2009), understanding the scale, scope, and timing of AIV epidemics in nearby wild bird populations is important to address this risk.

Our objectives were to describe temporal patterns of AIV prevalence and antibody prevalence within three shorebird species (Ruddy Turnstones, Red Knots, and Sanderlings) and one gull species (Laughing Gulls) during the spring migratory stopover at Delaware Bay, and compare patterns between species. Such information provided insight into possible AIV sources, transmission, exposure, and maintenance patterns at this location, as well as potential exposures outside of the Delaware Bay stopover.

## MATERIALS AND METHODS

### Field and laboratory methods

Field work was conducted during 17-24 May 2006, 10 May-3 June 2007, and 7 May-4 June 2008 at Delaware Bay (39°N, 75°W). Shorebirds and gulls were captured with cannon nets as part of long-term population studies. Following banding and measurement, swab samples for virus isolation were collected, stored, processed, and tested in embryonated chicken eggs as previously described (Hanson et al., 2008). Cloacal swabs were collected from all birds; in addition, oropharyngeal swabs were collected from Laughing Gulls in 2008 and analyzed separately (none were positive). Fresh Laughing Gull feces were swabbed in limited cases and only from within the borders of monospecific breeding colonies. Presence of AIV was confirmed by hemagglutination (Swayne et al., 1998) and reverse transcriptase polymerase chain reaction (RT-PCR) for matrix gene (Spackman and Suarez, 2008) on allantoic fluid. All isolates were low pathogenic (LPAI) viruses (unpublished data).

During 2007-2008, blood samples were collected by jugular venipuncture from a random subset of swabbed birds. Collected volume ranged from <0.5% to 1% of a bird's body mass in g. Samples were kept on ice in the field and sera were stored at –20°C until testing. Antibodies against AIV nucleoprotein (NP) were tested with a commercial blocking enzyme-linked immunoassay (bELISA; FlockChek AI MultiS-Screen Antibody Test Kit, IDEXX Laboratories, Westbrook, Maine). Samples yielding S/N ratios of <0.5 were considered positive, per manufacturer's recommendations.

Morphometric data for individual sampled birds was obtained from the Shorebird Resighting Database (http://www.bandedbirds.org). Research was conducted under University of Georgia Animal Care and Use Committee approval and state and federal scientific collection permits.

**Statistical analyses**

Computations and statistical analyses were performed in JMP version 8 (SAS Institute Inc., Cary, North Carolina). Virus isolation data from four species (Ruddy Turnstone, Red Knot, Sanderling, and Laughing Gull) were evaluated for temporal trends within stopover seasons. Data from 2006-2008 were pooled with results from 2000-2005 originally reported in Hanson et al. (2008). For each species, data from all nine years (four years for Laughing Gulls; 2005-2008) were used to estimate the average trend, if any. Additionally, the sampling date range spanned the majority of the stopover season in 2002, 2007, and 2008 (25, 25, and 29 d, respectively) and allowed evaluation of each of these years separately. Within a logit-link generalized linear model (GLM) framework, changes in prevalence were modeled as a linear, quadratic, or 3-node knotted spline (Hastie and Tibshirani, 1990) function of date, to obtain maximum model fit. Because shorebirds are expected to gain mass over the stopover period, the above analyses were repeated using masses of sampled birds (available 2006-2008). Laughing Gulls were not weighed at the time of sampling and were excluded from these analyses. In some cases several models adequately described the prevalence trend, however we present the single model that minimized Akaike's Information Criterion, $AIC_c$ (Burnham and Anderson, 2002).

We also evaluated trends in antibody prevalence over time and over mass gain in each species using GLM. Because the bELISA imperfectly detects recent AIV infection in wild bird species (test sensitivity=0.754, specificity=1; Brown et al., 2009), antibody prevalence calculations were adjusted according to Rogan and Gladen (1978):

$$P = \frac{P^T + Sp - 1}{Se + Sp - 1},$$

where $P$=true antibody prevalence, $P^T$=test antibody prevalence, $Sp$=test specificity, and $Se$=test sensitivity. Thus, the adjusted antibody prevalence for each day or 10-g mass class (weighted by $n$) was used rather than raw antibody status of individual birds. Serology data were pooled by shorebird species because temporal patterns did not differ between years (year effect

with day as covariate, for Ruddy Turnstones: $\chi^2$=1.2, df=1, *P*=0.27; Red Knots: $\chi^2$=0.79, df=1, *P*=0.37; Sanderlings: $\chi^2$=0.02, df=1, *P*=0.88), but were examined for 2007-2008 separately in Laughing Gulls because the pattern varied between years ($\chi^2$=6.8, df=1, *P*=0.009). For Ruddy Turnstones, an estimate of the minimum proportion of birds that were exposed to AIV (i.e., seroconverted) during the stopover period was determined by subtracting the estimated antibody prevalence upon arrival at Delaware Bay from that upon departure.

Non-parametric Kendall's $\tau_b$ correlation was used to assess the relationship of AIV prevalence on a given year and day between species pairs. To reduce measurement bias, we included data only when $n \geq 5$ simultaneously for both species. We further used logistic regression to examine the relationship between isolating any AIV in a species (i.e., AIV presence/absence) and prevalence in Ruddy Turnstones on that date. Here, only data when $n \geq 30$ were used.

## RESULTS

Prevalence of AIV infection (Table 1) and AIV antibodies (Table 2) varied by species; both infection and antibody prevalence were highest in Ruddy Turnstones and lowest in Sanderlings. Red Knots and Laughing Gulls both exhibited high antibody prevalence but low infection prevalence. Annual AIV prevalence in Ruddy Turnstones was 10-20 times greater than in Red Knots and Sanderlings and 5-6 times greater than in Laughing Gulls.

### AIV dynamics in Ruddy Turnstones

Prevalence of AIV varied with time similarly for 2000-2008 pooled data (3-node knotted spline fit: $\chi^2$=20.0, df=2, *P*<0.0001) and in each individual year (2002: $\chi^2$=12.9, df=2, *P*=0.002; 2007: $\chi^2$=6.7, df=2, *P*=0.036; 2008: $\chi^2$=14.0, df=2, *P*=0.0009; Fig. 1). Although peak prevalence varied among years, peaks occurred within two calendar days of each other (Table 3). In all years, prevalences among birds sampled during periods of arrival and departure (<15 May or >May 30; Robinson et al., 2003) were lower than among birds sampled during 15-30 May

(2002: 4.7% vs. 12%, $\chi^2$=9.0, df=1, *P*=0.003; 2007: 1.2% vs. 6.9%, $\chi^2$=10.3, df=1, *P*=0.001; 2008: 11% vs. 20%, $\chi^2$=11.5, df=1, *P*=0.0007).

Estimated AIV prevalence also varied over mass, both for 2006-2008 pooled data (quadratic fit, $\chi^2$=28.4, df=2, *P*<0.0001) and in individual years (2007: quadratic fit, $\chi^2$=10.2, df=2, *P*=0.006; 2008: 3-node knotted spline fit, $\chi^2$=12.0, df=2, *P*=0.003; Fig. 2). In both 2007 and 2008, prevalence was lower among birds with masses suggesting recent arrival or impending departure (≤99 or ≥157g; Robinson et al. 2003) than among birds with mid-range masses (2007: 0.8% vs. 6.3%, $\chi^2$=8.3, df=1, *P*=0.004; 2008: 12% vs. 20%, $\chi^2$=3.8, df=1, *P*=0.050). Mass associated with peak prevalence varied by only 2g in different years (Table 3).

Adjusted antibody prevalence increased over the stopover season ($\chi^2$=116.0, df=1, P<0.0001; Fig. 3a) from <40% positive on 10 May to >95% positive by 25 May. Adjusted antibody prevalence was lower among birds sampled on or before 17 May, corresponding to the rapid prevalence increase (Fig. 1), than among birds sampled later (51% vs. 100%; $\chi^2$=143.3, df=1, P<0.0001). Adjusted antibody prevalence also increased with mass ($\chi^2$=100.7, df=1, P<0.0001). Antibody prevalence among birds weighing ≤99g was significantly lower than among heavier birds (41% vs. 91%; $\chi^2$=86.6, df=1, P<0.0001), and antibody prevalence among birds weighing ≥157g was higher than among lighter birds (100% vs. 82%; $\chi^2$=8.7, df=1, P=0.003).

*Evidence and estimates of seroconversion*– Individual Ruddy Turnstones that were tested both by VI and bELISA (n=363) were divided into four categories based on results of the two tests. Both the mean sample date and mean mass varied across categories (1-way ANOVA; date: F=22.8, df=3, P<0.0001; mass: F=21.0, df=3, P<0.0001) and increased in the order: VI negative and bELISA negative, VI positive and bELISA negative, VI positive and bELISA positive, VI negative and bELISA positive (Fig. 4).

Migrants typically arrive at Delaware Bay asynchronously during late April-mid May, but depart *en masse* over a few days in late May-early June (Clark et al., 1993; Gillings et al.,

2009). Assuming that arriving birds have similar masses regardless of arrival date (Gillings et al., 2009), we compared the expected antibody prevalence among Ruddy Turnstones at the mean arrival mass (96g; Niles et al. unpublished data, cited in US Fish and Wildlife Service, 2003) to the estimated antibody prevalence on the mean departure date (1 June; unpublished data), or 48% and 99%, respectively. Thus, approximately 51% of the Ruddy Turnstone population seroconverted during the stopover.

**AIV dynamics in Red Knots**

AIV prevalence in Red Knots remained low (<2%) over the stopover period during each year and was not associated with mass (2006-2008 pooled data; $\chi^2$=2.9, df=1, *P*=0.090; data not shown). In contrast to Ruddy Turnstones, adjusted antibody prevalence in Red Knots decreased significantly over time ($\chi^2$=15.0, df=1, *P*=0.001; Fig. 3b). Antibody prevalence varied with 10-g mass class in a quadratic manner ($\chi^2$=6.5, df=2, *P*=0.039), and was higher among mid-weight birds than among birds with either near-arrival (≤114g) or near-departure (≥176g) masses (82% vs. 70%; $\chi^2$=4.2, df=1, *P*=0.041).

**AIV dynamics in Sanderlings**

Viral prevalence increased with day during 2000-2008 ($\chi^2$=4.5, df=1, *P*=0.034) but not within any individual year (all *P*>0.05; data not shown). All Sanderling AIV isolations occurred during 17-30 May (prevalence=1.5%); this prevalence was significantly higher than the 0% prevalence during the arrival period ($\chi^2$=6.2, df=2, *P*=0.013) but not the departure period (*P*>0.05). Prevalence did not vary with mass, either for pooled 2006-2008 data or during 2008 alone (all *P*>0.05). However, a significant trend was observed when birds weighing >80g (representing <5% of tested birds) were excluded ($\chi^2$=4.8, df=1, *P*=0.028; Fig. 5a). Prevalences among arrival- (≤53g), intermediate- (54-90g), and departure-weight (≥91g) birds were 1.3%, 1.6% , and 0%, respectively, and were not statistically different ($\chi^2$=0.31, df=2, *P*=0.86).

Adjusted antibody prevalence did not vary over time ($\chi^2$=1.3, df=1, *P*=0.26; Fig. 3c) but increased with mass ($\chi^2$=4.0, df=1, *P*=0.045; Fig. 5b). Adjusted antibody prevalence did not differ among arrival-, mid-range, and departure-weight birds (0%, 3.2%, and 12% respectively; $\chi^2$=1.7, df=2, *P*=0.43). However, sample sizes were *n*=11 and *n*=13 in arrival- and departure-weight groups, respectively.

**AIV dynamics in Laughing Gulls**

Infection prevalence did not vary temporally within a stopover season during 2005-2008 or the individual years 2007 or 2008 (all *P*>0.05; data not shown). Adjusted daily antibody prevalences increased with time and were higher in 2007 than in 2008 (Fig. 3d); a significant year×day interaction was present (whole model: $\chi^2$=19.4, df=3, *P*=0.0002; day effect: $\chi^2$=12.1, df=1, *P*=0.0005; year effect: $\chi^2$=11.4, df=1, *P*=0.0008; year×day effect: $\chi^2$=4.6, df=1, *P*=0.032).

**AIV prevalence correlations between species**

Prevalence among Sanderlings was positively correlated with prevalence among Ruddy Turnstones captured on the same day (*n*=26 catches, Kendall's $\tau_b$=0.34, *P*=0.033). Daily prevalences among Red Knots and Laughing Gulls were not correlated with prevalence among Ruddy Turnstones (*n*=35, $\tau_b$=0.16, *P*=0.23; and *n*=10, $\tau_b$=-0.061, *P*=0.82, respectively), nor were prevalences correlated among Sanderlings, Red Knots, and Laughing Gulls (all *P*>0.05). Additionally, the probability of detecting AIV among a sample of ≥30 Sanderlings was positively associated with AIV prevalence among Ruddy Turnstones on that day (logistic regression: $\chi^2$=6.28, df=1, *P*=0.012) and was likely (≥50% probability) when Ruddy Turnstone prevalence exceeded 18.6%. There was no association between prevalence among Ruddy Turnstones and AIV presence among Red Knots ($\chi^2$<0.01, df=1, *P*=0.98) or Laughing Gulls ($\chi^2$=0.01, df=1, *P*=0.94) on a given date.

# DISCUSSION

## Ruddy Turnstones

There are few reports of AIV in Ruddy Turnstones at times and locations outside the Delaware Bay spring migratory stopover period. The consistent temporal pattern of infection and seroconversion (between and within years) at this site suggests an annual and localized epidemic; reported AIV isolations from Ruddy Turnstones at Delaware Bay each May since 1985 (Krauss et al., 2004; Hanson et al., 2008; Krauss et al., 2010) clearly demonstrate this predictable event.

All plots of AIV prevalence over time and mass gain had similar patterns of near zero prevalence during early May when Turnstones are at arrival weights, followed by abrupt increases in prevalence on approximately 16-17 May as the season progressed and birds gained weight. Antibody prevalence also increased during each stopover season; together these measures indicate that most exposures and infections occurred after arrival at Delaware Bay. The AIV infection duration is unknown in shorebirds, but the narrowness of the epidemic curve suggests short periods of shedding similar to the 2-8 d reported in wild Mallards (Latorre-Margalef et al., 2009). Infections decreased after 24 May, possibly due to an increase in population immunity. In 2007, the entire epidemic lasted less than 25 d before a complete fadeout, but in 2008 it lasted longer. We did not sample after 4 June because the small remaining number of birds were more dispersed and difficult to capture, but these data would have been useful to accurately determine the epidemic's span.

Similarly, patterns of antibody prevalence indicated population seroconversion in the wake of an epidemic. Generally, individual birds were virus- and antibody-negative at the beginning of the stopover, became infected, seroconverted within a few days of infection, and then recovered from infection but retained circulating antibodies. This pattern is expected with an acute infectious disease (Nunn and Altizer, 2006). We estimate that at least half of birds seroconverted during the stopover; this figure could include both previously unexposed birds

and birds that had been previously exposed (at Delaware Bay or elsewhere) but whose antibodies had fallen below detectable levels (i.e., they were re-exposed at Delaware Bay). Because virtually all Turnstones were antibody positive just prior to departure, the population proportion exposed at the stopover is likely higher than the above estimate.

Although the conditions that enhance AIV transmission in Ruddy Turnstones at Delaware Bay each spring are currently unknown, the high population density (up to 67 birds/$m^2$; Gillings et al. 2007) of this species at Delaware Bay is an obvious and unique situation; such densities are not encountered during other times in their annual cycle (Nettleship, 2000). The sudden aggregation of susceptible Ruddy Turnstones at Delaware Bay could provide the population threshold (Lloyd-Smith et al., 2005) needed to initiate and sustain annual AIV epidemics (Krauss et al., 2010). Up to 80% of the *morinella* subspecies migrates through Delaware Bay each year (Morrison et al., 2001), and aerial counts have exceeded 100,000 Turnstones on a single day (Clark et al., 1993). During the breeding, fall migration, and wintering periods Ruddy Turnstones are typically much more dispersed, often seen individually or in small flocks ($n$<50; Nettleship 2000). Thus, potential bird-to-bird transmission of AIV could be interrupted when they do not regularly encounter a large number of conspecifics.

A majority of Turnstones did not have detectable antibodies against AIV upon arrival at Delaware Bay. Though the duration for which anti-NP antibodies can be detected following initial exposure is unknown, it seems reasonable that the majority of Ruddy Turnstones had not been recently exposed to AIV, and indeed might never have been exposed (e.g., young adult birds migrating through Delaware Bay for the first time) or were last exposed during the preceding spring. Further, previous population exposure might have involved different AIV subtypes to which detected antibodies were not protective or only partially protective.

In addition to population density and immunity, increased potential virus contact at Delaware Bay due to the local density of other AIV reservoir species also should be considered. The largest Laughing Gull breeding colony on the Atlantic coast and smaller colonies of Herring

Gulls, Great Black-backed Gulls, egrets, herons, and other waterbirds are located in close proximity on the Cape May Peninsula in New Jersey, as are migrant and resident waterfowl. Laughing and Herring Gulls number tens of thousands of breeding pairs (Pierotti and Good, 1994; Burger, 1996) and often feed alongside shorebirds on the beaches (Burger et al., 2007). Gulls are recognized AIV reservoirs and transmission might be associated with breeding behavior (Velarde et al., 2010). The high prevalence of AIV antibodies detected in gulls in the present study supports their possible involvement. Infection data in gulls, waterfowl, and other resident waterbirds prior to shorebird arrival would help determine if epidemiologic links exist between species groups.

Two additional unique and poorly understood factors that might enhance AIV transmission or susceptibility at this site are environmental conditions that allow effective exposure to and transmission of AIV and increased susceptibility related to the physiologic changes associated with long-distance migration and rapid weight gain. Migration and refueling at stopovers are physiologically stressful activities, and stress hormones such as corticosterone that could be important to shorebird stopover physiology (Piersma et al., 2000; Mizrahi et al., 2001) might also be immunosuppressive.

Because the majority of Ruddy Turnstones are infected with and recover from AIV during the spring stopover, and from Delaware Bay disperse onto the breeding grounds and remain dispersed during fall migration and winter, it is possible that AIV infection does not persist year-round in this species. This is supported by the low antibody prevalence we observed on arrival. Rather than being an AIV reservoir, which implies endemicity, it's possible that Ruddy Turnstones are a local amplifying host under conditions of high density, low flock immunity, and increased exposure to AIV. Similar conditions might exist at other locations involving Ruddy Turnstones or another permissive species; in October 2007, AIV were detected in Ruddy Turnstones in Peru (Ghersi et al., 2009). While shorebirds have been implicated in rare transhemispheric movement of AIV gene segments (Krauss et al., 2007), a recent analysis

suggests that AIV are more likely to be carried long distances by gulls and that shorebirds, such as Ruddy Turnstones, are probably local secondary hosts (Pearce et al., 2010).

**Other species**

Of the three syntopic species studied, only Sanderlings exhibited dynamical changes in AIV prevalence over the stopover period. Prevalence increased with day (≤30 May), and with mass (≤80g), and was positively correlated with prevalence in Ruddy Turnstones. Sanderlings appear relatively resistant to AIV infection (perhaps due to decreased susceptibility or limited contact), given low prevalence and antibody prevalence at Delaware Bay, and zero prevalence at other times and locations (Hlinak et al., 2006; Munster et al., 2007; Winker et al., 2007; Escudero et al., 2008; Hanson et al., 2008; Iverson et al., 2008; Ghersi et al., 2009). Because infections in Sanderlings are sporadic and likely depend on the infected proportion of syntopic birds, they are probably due to spillover events from Ruddy Turnstones acting as amplifying hosts (Fenton and Pedersen, 2005).

The high antibody prevalence in Red Knots, which declined over the season, was an unexpected finding. Red Knots are not often found infected at Delaware Bay or the limited number of other locations where they have been sampled (D'Amico et al., 2007; Hanson et al., 2008). Although the duration of antibody detectability is unknown, this pattern suggests that Red Knots were exposed to AIV recently prior to arrival at Delaware Bay, perhaps on their wintering grounds or at another stopover during northward migration. Red Knots often winter in large flocks at Tierra del Fuego in Argentina and Chile and congregate at several stopovers prior to Delaware Bay (Harrington, 2001), therefore, opportunity could exist for AIV spread within localized populations throughout much of the year. Although AIV has not been detected on wintering grounds, available information is limited. More information regarding when and where Red Knots are exposed or infected would be helpful to understand what role, if any, LPAI infection has had on recent population declines (Baker et al., 2004; Niles et al., 2009).

High antibody prevalence could also result from long-lasting immunity following prior, or repeated, exposure(s) at Delaware Bay (Buehler et al., 2010). However, antibody prevalence declines rather than remaining high over the stopover duration, suggesting that Red Knots are not re-exposed to AIV while at Delaware Bay. This pattern is difficult to understand because Knots and Turnstones feed together on the Bay beaches. Perhaps subtle differences in host species ecology support transmission of virus among Turnstones but not to Knots. For example, Red Knots primarily consume horseshoe crab eggs available at the surface, but Ruddy Turnstones regularly dig several centimeters deep to reach buried egg masses (Tsipoura and Burger, 1999). If fecally excreted virus becomes rapidly unavailable on the beach surface (e.g., through UV irradiation or percolation into the sand due to wave action) but remains infectious when adsorbed to subsurface sand particles (Jin et al., 1997) or horseshoe crab eggs, then Red Knots might encounter viable AIV less often than Ruddy Turnstones. Sanderlings and gulls often raid Turnstone excavations whereas Red Knots rarely do (Burger et al., 2007; Vahl et al., 2007), perhaps helping to explain why Sanderlings and gulls are infected more often. Other differences in host ecology such as roost environments could also contribute to differential transmission. Additionally, we did not examine whether Red Knots shed AIV from the respiratory rather than the intestinal tract.

Laughing Gulls exhibited significant but variable increases in antibody prevalence over the stopover period; sample sizes were relatively small. Given that a majority of Ruddy Turnstones were exposed to AIV over the stopover season and that Laughing Gulls and Turnstones share multiple habitats in the Delaware Bay area (data not shown), it is not surprising that many Laughing Gulls also were exposed. To date, Laughing Gulls have not been extensively sampled at Delaware Bay or elsewhere (reviewed by Bogomolni et al., 2008) and infection data are limited in this study. More systematic sampling of Laughing Gulls including time periods before shorebird arrival and after their departure would help to further characterize AIV dynamics in this species.

**Conclusions**

This study is the first to detail AIV infection dynamics over the entire course of an epidemic within Charadriiformes populations. The observed dynamical changes in prevalence and antibody prevalence allowed insight into when and where these four species were infected with or exposed to AIV. Although Ruddy Turnstones are seemingly the most important species in AIV epidemics at Delaware Bay, the detailed dynamical changes reported here indicate that they largely become infected, seroconvert, and recover locally. The source of viruses for these annual epidemics has not been identified, but AIV could arrive with a few infected Ruddy Turnstones (or other shorebirds) or be acquired from local sources such as gulls or waterfowl. Because infection apparently is a local phenomenon, Ruddy Turnstones might primarily act as a local virus amplifier, potentially generating reassortant viruses derived from many distant sources (Pearce et al., 2010). Other species, such as Red Knots, might be exposed at other (unidentified) sites, but further research is needed to define their possible roles in AIV epidemiology.

## ACKNOWLEDGMENTS

This research was funded through Specific Cooperative Agreement 58-6612-2-0220 between the Southeast Poultry Research Laboratory, Agricultural Research Service, Department of Agriculture (USDA-ARS) and the Southeastern Cooperative Wildlife Disease Study, and by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Department of Health and Human Services, under contract no. HHSN266200700007C. The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. Banding data and morphometric measurements are the property of the Natural Heritage & Endangered Species Program, Division of Fish and Wildlife, Delaware Department of Natural Resources and Environmental Control, and the Nongame and Endangered Species Program, Division of Fish and Wildlife, New Jersey Department of Environmental Protection; we thank these agencies for granting

access to these data. We are grateful to many people who provided field and laboratory support, particularly E. Casey, L. Coffee, M. Cole, J. Cumbee, D. Downs, S. Gibbs, W. Hamrick, S. Keeler, G. Martin, S. McGraw, C. McKinnon, J. Murdock, R. Paulson, J. Smith, and B. Wilcox.

## LITERATURE CITED

BAKER, A. J., P. M. GONZALEZ, T. PIERSMA, L. J. NILES, I. D. S. DO NASCIMENTO, P. W. ATKINSON, N. A. CLARK, C. D. T. MINTON, M. K. PECK, and G. AARTS. 2004. Rapid population decline in Red Knots: fitness consequences of decreased refueling rates and late arrival in Delaware Bay. Proceedings of the Royal Society B: Biological Sciences 271: 875-882.

BOGOMOLNI, A. L., R. J. GAST, J. C. ELLIS, M. DENNETT, K. R. PUGLIARES, B. J. LENTELL, and M. J. MOORE. 2008. Victims or vectors: a survey of marine vertebrate zoonoses from coastal waters of the Northwest Atlantic. Diseases of Aquatic Organisms 81: 13-38.

BROWN, J. D., D. E. STALLKNECHT, R. D. BERGHAUS, M. P. LUTTRELL, K. VELEK, W. KISTLER, T. COSTA, M. J. YABSLEY, and D. SWAYNE. 2009. Evaluation of a commercial blocking enzyme-linked immunosorbent assay to detect avian influenza virus antibodies in multiple experimentally infected avian species. Clinical and Vaccine Immunology 16: 824-829.

BUEHLER, D. M., B. I. TIELEMAN, and T. PIERSMA. 2010. How do migratory species stay healthy over the annual cycle? A conceptual model for immune function and for resistance to disease. Integrative and Comparative Biology 50: 346-357.

BURGER, J. 1996. Laughing Gull (*Larus atricilla*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 225, http://bna.birds.cornell.edu/bna/species/225. Accessed January 2011.

BURGER, J., S. A. CARLUCCI, C. W. JEITNER, and L. NILES. 2007. Habitat choice, disturbance, and management of foraging shorebirds and gulls at a migratory stopover. Journal of Coastal Research 23: 1159-1166.

BURNHAM, K. P., and D. R. ANDERSON. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. 2nd edition. Springer, New York. 488 pp.

CLARK, K. E., L. J. NILES, and J. BURGER. 1993. Abundance and distribution of migrant shorebirds in Delaware Bay. Condor 95: 694-705.

D'AMICO, V. L., M. BERTELLOTTI, A. J. BAKER, and L. A. DIAZ. 2007. Exposure of Red Knots (*Calidris canutus rufa*) to select avian pathogens; Patagonia, Argentina. Journal of Wildlife Diseases 43: 794-797.

ESCUDERO, G., V. J. MUNSTER, M. BERTELLOTTI, and P. EDELAAR. 2008. Perpetuation of avian influenza in the Americas: Examining the role of shorebirds in Patagonia. Auk 125: 494-495.

FENTON, A., and A. B. PEDERSEN. 2005. Community epidemiology framework for classifying disease threats. Emerging Infectious Diseases 11: 1815-1821.

FOUCHIER, R. A. M., V. MUNSTER, A. WALLENSTEN, T. M. BESTEBROER, S. HERFST, D. SMITH, G. F. RIMMELZWAAN, B. OLSEN, and A. D. M. E. OSTERHAUS. 2005. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from Black-headed Gulls. Journal of Virology 79: 2814-2822.

GHERSI, B. M., D. L. BLAZES, E. ICOCHEA, R. I. GONZALEZ, T. KOCHEL, Y. TINOCO, M. M. SOVERO, S. LINDSTROM, B. SHU, A. KLIMOV, A. E. GONZALEZ, and J. M. MONTGOMERY. 2009. Avian influenza in wild birds, central coast of Peru. Emerging Infectious Diseases 15: 935-938.

GILLINGS, S., P. W. ATKINSON, A. J. BAKER, K. A. BENNETT, N. A. CLARK, K. B. COLE, P. M. GONZALEZ, K. S. KALASZ, C. D. T. MINTON, L. J. NILES, R. C. PORTER, I. DE LIMA SERRANO, H. P. SITTERS, J. L. WOODS, and T. L. SHAFFER. 2009. Staging behavior in Red Knot (*Calidris canutus*) in Delaware Bay: implications for monitoring mass and population size. Auk 126: 54-63.

HANSON, B. A., M. P. LUTTRELL, V. H. GOEKJIAN, L. NILES, D. E. SWAYNE, D. A. SENNE, and D. E. STALLKNECHT. 2008. Is the occurrence of avian influenza virus in Charadriiformes species and location dependent? Journal of Wildlife Diseases 44: 351-361.

HARRINGTON, B. A. 2001. Red Knot (*Calidris canutus*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 563, http://bna.birds.cornell.edu/bna/species/563. Accessed January 2011.

HASTIE, T. J., and R. J. TIBSHIRANI. 1990. Generalized Additive Models. Chapman and Hall, London, United Kingdom. 352 pp.

HINSHAW, V. S., R. G. WEBSTER, and B. TURNER. 1979. Water-borne transmission of influenza A viruses. Intervirology 11: 66-68.

HINSHAW, V. S., G. M. AIR, G. C. SCHILD, and R. W. NEWMAN. 1983. Characterization of a novel hemagglutinin subtype (H13) of influenza A viruses from gulls. Bulletin of the World Health Organization 61: 677-679.

HLINAK, A., R. U. MUHLE, O. WERNER, A. GLOBIG, E. STARICK, H. SCHIRRMEIER, B. HOFFMANN, A. ENGELHARDT, D. HUBNER, F. J. CONRATHS, D. WALLSCHLAGER, H. KRUCKENBERG, and T. MULLER. 2006. A virological survey in migrating waders and other waterfowl in one of the most important resting sites of Germany. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 53: 105-110.

IVERSON, S. A., J. Y. TAKEKAWA, S. SCHWARZBACH, C. J. CARDONA, N. WARNOCK, M. A. BISHOP, G. A. SCHIRATO, S. PAROULEK, J. T. ACKERMAN, H. IP, and W. M. BOYCE. 2008. Low prevalence of avian influenza virus in shorebirds on the Pacific coast of North America. Waterbirds 31: 602-610.

JIN, Y., M. V. YATES, S. S. THOMPSON, and W. A. JURY. 1997. Sorption of viruses during flow through saturated sand columns. Environmental Science & Technology 31: 548-555.

KAWAOKA, Y., T. M. CHAMBERS, W. L. SLADEN, and R. G. WEBSTER. 1988. Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? Virology 163: 247-250.

KRAUSS, S., D. WALKER, S. P. PRYOR, L. NILES, C. H. LI, V. S. HINSHAW, and R. G. WEBSTER. 2004. Influenza A viruses of migrating wild aquatic birds in North America. Vector-Borne and Zoonotic Diseases 4: 177-189.

KRAUSS, S., C. A. OBERT, J. FRANKS, D. WALKER, K. JONES, P. SEILER, L. NILES, S. P. PRYOR, J. C. OBENAUER, C. W. NAEVE, L. WIDJAJA, R. J. WEBBY, and R. G. WEBSTER. 2007. Influenza in migratory birds and evidence of limited intercontinental virus exchange. Plos Pathogens 3: 1684-1693.

KRAUSS, S., D. E. STALLKNECHT, N. J. NEGOVETICH, L. J. NILES, R. J. WEBBY, and R. G. WEBSTER. 2010. Coincident Ruddy Turnstone migration and horseshoe crab spawning creates an ecological 'hot spot' for influenza viruses. Proceedings of the Royal Society B: Biological Sciences 277: 3373-3379.

LATORRE-MARGALEF, N., G. GUNNARSSON, V. J. MUNSTER, R. A. M. FOUCHIER, A. D. M. E. OSTERHAUS, J. ELMBERG, B. OLSEN, A. WALLENSTEN, P. D. HAEMIG, T. FRANSSON, L. BRUDIN, and J. WALDENSTRÖM. 2009. Effects of influenza A virus infection on migrating Mallard ducks. Proceedings of the Royal Society B: Biological Sciences 276: 1029-1036.

LLOYD-SMITH, J. O., P. C. CROSS, C. J. BRIGGS, M. DAUGHERTY, W. M. GETZ, J. LATTO, M. S. SANCHEZ, A. B. SMITH, and A. SWEI. 2005. Should we expect population thresholds for wildlife disease? Trends in Ecology & Evolution 20: 511-519.

MIZRAHI, D. S., R. L. HOLBERTON, and S. A. GAUTHREAUX. 2001. Patterns of corticosterone secretion in migrating Semipalmated Sandpipers at a major spring stopover site. Auk 118: 79-91.

MORRISON, R. I. G., and B. A. HARRINGTON. 1992. The migration system of the Red Knot *Calidris canutus rufa* in the New World. Wader Study Group Bulletin 64 (Supplement): 71-84.

MORRISON, R. I. G., R. E. GILL, B. A. HARRINGTON, S. SKAGEN, G. W. PAGE, C. L. GRATTO-TREVOR, and S. M. HAIG. 2001. Estimates of Shorebird Populations in North America. Canadian Wildlife Service Occasional Paper 104: 64.

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.

MYERS, J. P., M. SALLABERRY, E. ORTIZ, G. CASTRO, L. M. GORDON, J. L. MARON, C. T. SCHICK, E. TABILO, P. ANTAS, and T. BELOW. 1990. Migration routes of New World Sanderlings (*Calidris alba*). Auk 107: 172-180.

NETTLESHIP, D. N. 2000. Ruddy Turnstone (*Arenaria interpres*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 537, http://bna.birds.cornell.edu/bna/species/537. Accessed January 2011.

NILES, L. J., J. BART, H. P. SITTERS, A. D. DEY, K. E. CLARK, P. W. ATKINSON, A. J. BAKER, K. A. BENNETT, K. S. KALASZ, N. A. CLARK, J. CLARK, S. GILLINGS, A. S. GATES, P. M. GONZALEZ, D. E. HERNANDEZ, C. D. T. MINTON, R. I. G. MORRISON, R. R. PORTER, R. KEN ROSS, and C. R. VEITCH. 2009. Effects of horseshoe crab harvest in Delaware Bay on Red Knots: are harvest restrictions working? Bioscience 59: 153-164.

NUNN, C. L., and S. ALTIZER. 2006. Host-parasite dynamics and epidemiological principles. *In* Infectious Diseases in Primates: Behavior, Ecology, and Evolution, C. L. Nunn, and S. Altizer, (eds.). Oxford University Press, New York, pp. 98-133.

OLSEN, B., V. J. MUNSTER, A. WALLENSTEN, J. WALDENSTROM, A. OSTERHAUS, and R. A. M. FOUCHIER. 2006. Global patterns of influenza A virus in wild birds. Science 312: 384-388.

PEARCE, J. M., A. M. RAMEY, H. S. IP, and R. E. GILL. 2010. Limited evidence of trans-hemispheric movement of avian influenza viruses among contemporary North American shorebird isolates. Virus Research 148: 44-50.

PIEROTTI, R. J., and T. P. GOOD. 1994. Herring Gull (*Larus argentatus*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 124, http://bna.birds.cornell.edu/bna/species/124. Accessed January 2011.

PIERSMA, T., J. RENEERKENS, and M. RAMENOFSKY. 2000. Baseline corticosterone peaks in shorebirds with maximal energy stores for migration: a general preparatory mechanism for rapid behavioral and metabolic transitions? General and Comparative Endocrinology 120: 118-126.

ROBINSON, R. A., P. W. ATKINSON, and N. A. CLARK. 2003. Arrival and weight gain of Red Knot *Calidris canutus,* Ruddy Turnstone *Arenaria interpres* and Sanderling *Calidris alba* staging in Delaware Bay in spring. British Trust for Ornithology Research Report 307: 51.

ROGAN, W. J., and B. GLADEN. 1978. Estimating prevalence from the results of a screening test. American Journal of Epidemiology 107: 71-76.

SPACKMAN, E., and D. SUAREZ. 2008. Type A influenza virus detection and quantitation by real-time RT-PCR. Methods in Molecular Biology 436: 19-26.

SPACKMAN, E. 2009. The ecology of avian influenza virus in wild birds: What does this mean for poultry? Poultry Science 88: 847-850.

STALLKNECHT, D. E., and J. D. BROWN. 2007. Wild birds and the epidemiology of avian influenza. Journal of Wildlife Diseases 43: S15-S20.

SWAYNE, D. E., D. A. SENNE, and C. W. BEARD. 1998. Avian influenza. *In* A Laboratory Manual for the Isolation and Identification of Avian Pathogens, D. E. Swayne, (ed.). American Association of Avian Pathologists, Kennett Square, Pennsylvania, pp. 150-155.

TSIPOURA, N., and J. BURGER. 1999. Shorebird diet during spring migration stopover on Delaware Bay. Condor 101: 635-644.

US FISH AND WILDLIFE SERVICE. 2003. Delaware Bay Shorebird-Horseshoe Crab Assessment Report and Peer Review. US Fish and Wildlife Service Migratory Bird Publication R9-03/02, Arlington, Virginia. 99 pp.

VAHL, W. K., J. VAN DER MEER, K. MEIJER, T. PIERSMA, and F. J. WEISSING. 2007. Interference competition, the spatial distribution of food and free-living foragers. Animal Behaviour 74: 1493-1503.

VELARDE, R., S. E. CALVIN, D. OJKIC, I. K. BARKER, and É. NAGY. 2010. Avian influenza virus H13 circulating in Ring-billed Gulls (*Larus delawarensis*) in southern Ontario, Canada. Avian Diseases 54: 411-419.

WINKER, K., K. G. MCCRACKEN, D. D. GIBSON, C. L. PRUETT, R. MEIER, F. HUETTMANN, M. WEGE, I. V. KULIKOVA, Y. N. ZHURAVLEV, M. L. PERDUE, E. SPACKMAN, D. L. SUAREZ, and D. E. SWAYNE. 2007. Movements of birds and avian influenza from Asia into Alaska. Emerging Infectious Diseases 13: 547-552.

WINKER, K., E. SPACKMAN, and D. E. SWAYNE. 2008. Rarity of influenza A virus in spring shorebirds, southern Alaska. Emerging Infectious Diseases 14: 1314-1316.

YAMNIKOVA, S. S., A. S. GAMBARYAN, A. B. TUZIKOV, N. V. BOVIN, M. N. MATROSOVICH, I. T. FEDYAKINA, A. A. GRINEV, V. M. BLINOV, D. K. LVOV, D. L. SUAREZ, and D. E. SWAYNE. 2003. Differences between HA receptor-binding sites of avian influenza viruses isolated from Laridae and Anatidae. Avian Diseases 47: 1164-1168.

Table 4.1. Springtime stopover site AIV prevalence among four species of Charadriiformes at Delaware Bay, 2000-2008.

| | Ruddy Turnstone | | Red Knot | | Sanderling | | Laughing Gull | |
|---|---|---|---|---|---|---|---|---|
| | *n* | Prevalence (%) | *n* | Prevalence (%) | *n* | Prevalence (%) | *n* | Prevalence (%) |
| Year(s) | (Positive) | (95% CI) | (Positive) | (95% CI) | (Positive) | (95% CI) | (Positive) | (95% CI) |
| 2002 | 736 (75) | 10 (8.2-13) | 364 (2) | 0.5 (0.2-2.0) | 248 (2) | 0.8 (0.2-2.9) | n/a[a] | n/a[a] |
| 2007 | 415 (19) | 4.6 (3.0-7.0) | 242 (1) | 0.4 (0.1-2.3) | 191 (0) | 0.0 (0.0-2.0) | 48 (0) | 0.0 (0.0-7.4) |
| 2008 | 582 (97) | 17 (14-20) | 277 (2) | 0.7 (0.2-3.0) | 225 (1) | 0.4 (0.1-2.5) | 149 (4)[b] | 2.7 (1.0-6.7) |
| 2000-2008 | 3,660 (431) | 12 (12-13) | 2,438 (21) | 0.9 (0.6-1.3) | 1,269 (15) | 1.2 (0.7-1.9) | 332 (6)[a] | 1.8 (0.8-3.9)[a] |

[a] Laughing Gulls were sampled during 2005-2008 only

[b] Two cloacal swabs and two fecal swabs positive

Table 4.2. Springtime stopover site AIV antibody prevalence among four species of Charadriiformes at Delaware Bay, 2007-2008.

| Species | Year(s) | *n* (Positive) | Calculated antibody prevalence (%) | Adjusted antibody prevalence (%)[a] (95% CI) |
|---|---|---|---|---|
| Ruddy Turnstone | 2007-2008 | 380 (243) | 64 | 85 (78-91) |
| Red Knot | 2007-2008 | 211 (125) | 59 | 79 (70-87) |
| Sanderling | 2007-2008 | 153 (4) | 2.6 | 3.4 (0.1-6.8) |
| Laughing Gull | 2007 | 31 (23) | 74 | 98 (78-100) |
| | 2008 | 52 (25) | 48 | 64 (46-82) |

[a] Adjusted for test sensitivity=0.754 (Brown et al., 2009), according to Rogan and Gladen (1978).

Table 4.3. Estimated peak AIV prevalence by day and body mass in Ruddy Turnstones, 2000-2008, Delaware Bay. See text for details of model fit.

| | By day | | By mass[a] | |
|---|---|---|---|---|
| Year | Peak prevalence (%) (95% CI) | Date of peak prevalence | Peak prevalence (%) (95% CI) | Mass at peak prevalence (g) |
| 2002 | 14 (11-19) | 22 May | - | - |
| 2007 | 8.2 (4.7-14) | 22 May | 9.3 (5.3-16) | 133 |
| 2008 | 23 (18-28) | 24 May | 24 (18-30) | 132 |
| 2000-2008 | 13 (11-14) | 24 May | - | - |
| 2006-2008 | - | - | 19 (16-22) | 134 |

[a] Mass data were not available prior to 2006.

## FIGURE LEGENDS

Figure 4.1. Avian influenza virus prevalence in Ruddy Turnstones over the course of the spring stopover at Delaware Bay, (A) for 2000-2008 pooled data, and for each year sufficient serial data were available: (B) 2002, (C) 2007, and (D) 2008. Lines of fit (95% CI) and prevalence (±SE) for each day (B, C, and D) or 5-d time span (A) when $n \geq 5$ are shown. All lines of fit are significant at $\alpha=0.05$.

Figure 4.2. Avian influenza virus prevalence during (A) 2006-2008, (B) 2007, and (C) 2008, and (D) antibody prevalence during 2007-2008 in Ruddy Turnstones by 10-g mass classes, Delaware Bay. Lines of fit and 95% CI are shown (all fits are significant at $\alpha=0.01$). Also shown are prevalences and adjusted antibody prevalences (±SE) positioned at the midpoint of each class (e.g., adjusted antibody prevalence among birds 90-99g is plotted at 95g).

Figure 4.3. Estimated AIV antibody prevalence (95% CI) over the stopover season at Delaware Bay in (A) Ruddy Turnstones, (B) Red Knots, (C) Sanderlings, and (D) Laughing Gulls. Also shown are adjusted antibody prevalences (±SE) on each sample date (data from both years combined for Ruddy Turnstones, Red Knots, and Sanderlings). All fits are significant at $\alpha=0.001$.

Figure 4.4. Mean (±SE) (A) sample date and (B) mass of Ruddy Turnstones testing positive (+) or negative (-) on virus isolation (VI) and serology (bELISA). Within each panel, the mean sample date or mass among birds belonging to categories not connected by the same letter are significantly different (Tukey-Kramer post-hoc tests; $\alpha=0.05$).

Figure 4.5. Avian influenza virus (A) prevalence (2006-2008) and (B) antibody prevalence by 10-g mass class (2007-2008) in Sanderlings. Lines of fit and 95% CI are shown (all fits are significant at $\alpha=0.05$); see text for details.

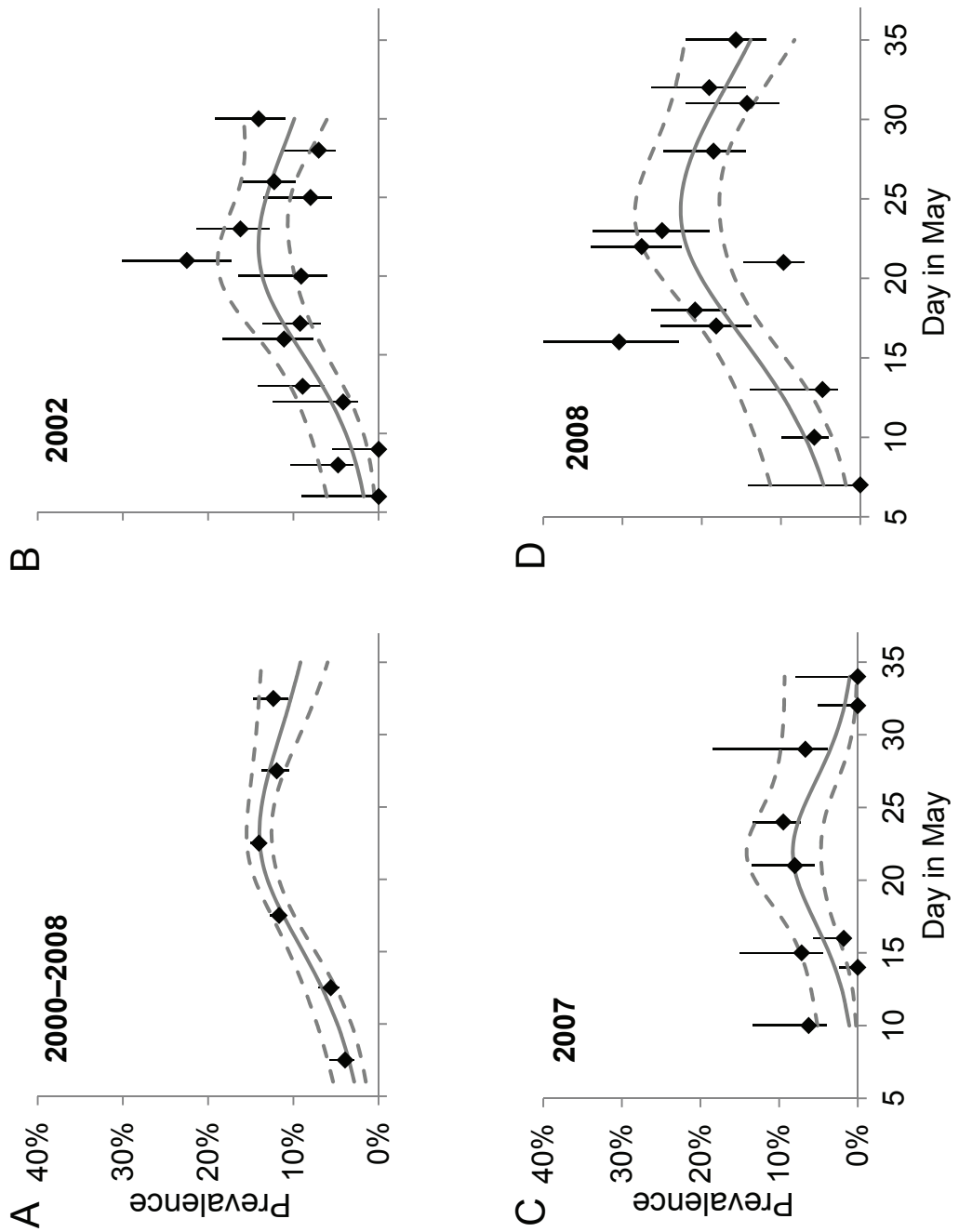

A
2000–2008
B
2002
C
2007
D
2008
Prevalence
40%
30%
20%
10%
0%
5
10
15
20
25
30
35
Day in May

Figure 4.1.

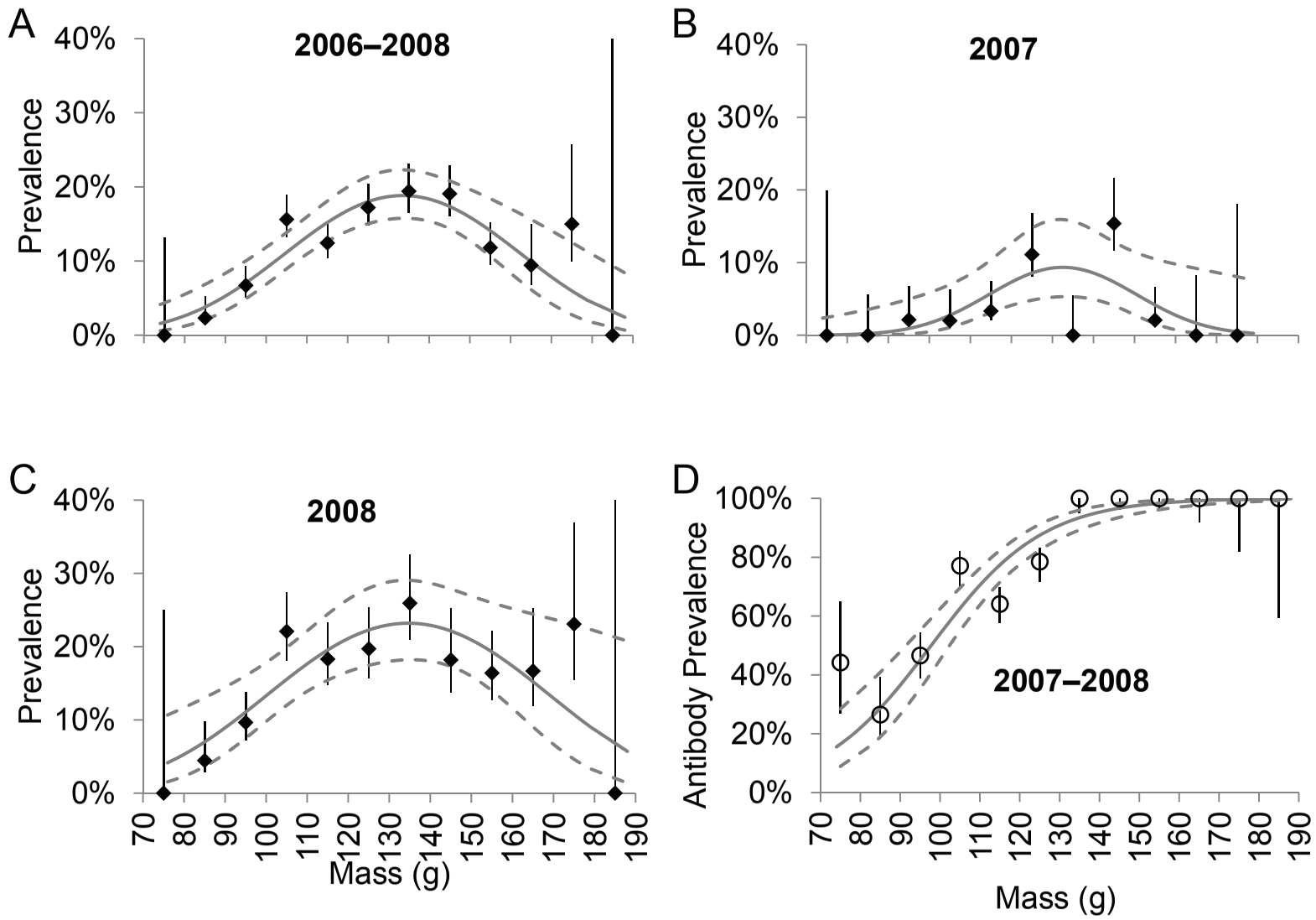

A
2006–2008
Prevalence
40%
30%
20%
10%
0%
B
2007
Prevalence
40%
30%
20%
10%
0%
C
2008
Prevalence
40%
30%
20%
10%
0%
70
80
90
100
110
120
130
140
150
160
170
180
190
Mass (g)
D
Antibody Prevalence
100%
80%
60%
40%
20%
0%
2007–2008
70
80
90
100
110
120
130
140
150
160
170
180
190
Mass (g)

Figure 4.2.

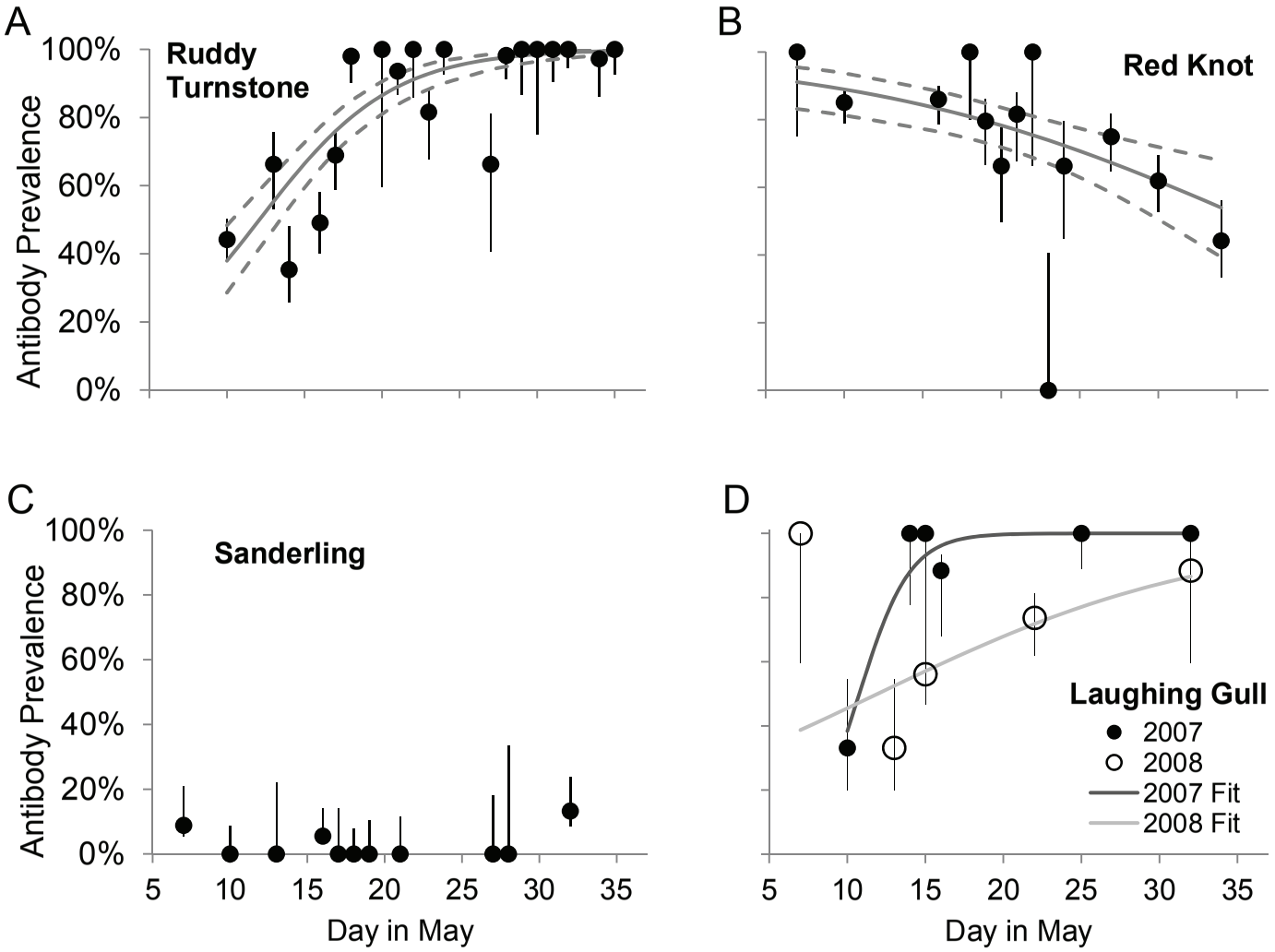

A
Ruddy Turnstone
B
Red Knot
C
Sanderling
D
Laughing Gull
2007
2008
2007 Fit
2008 Fit
Antibody Prevalence
100%
80%
60%
40%
20%
0%
5
10
15
20
25
30
35
Day in May

Figure 4.3.

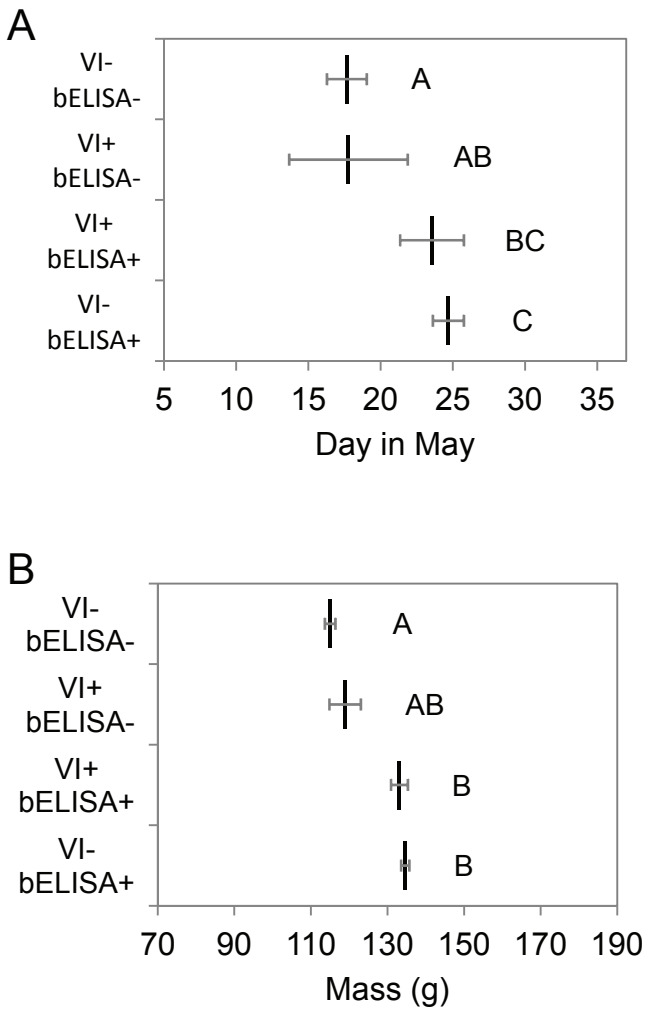

A
VI-
bELISA-
VI+
bELISA-
VI+
bELISA+
VI-
bELISA+
A
AB
BC
C
5
10
15
20
25
30
35
Day in May
B
VI-
bELISA-
VI+
bELISA-
VI+
bELISA+
VI-
bELISA+
A
AB
B
B
70
90
110
130
150
170
190
Mass (g)

Figure 4.4.

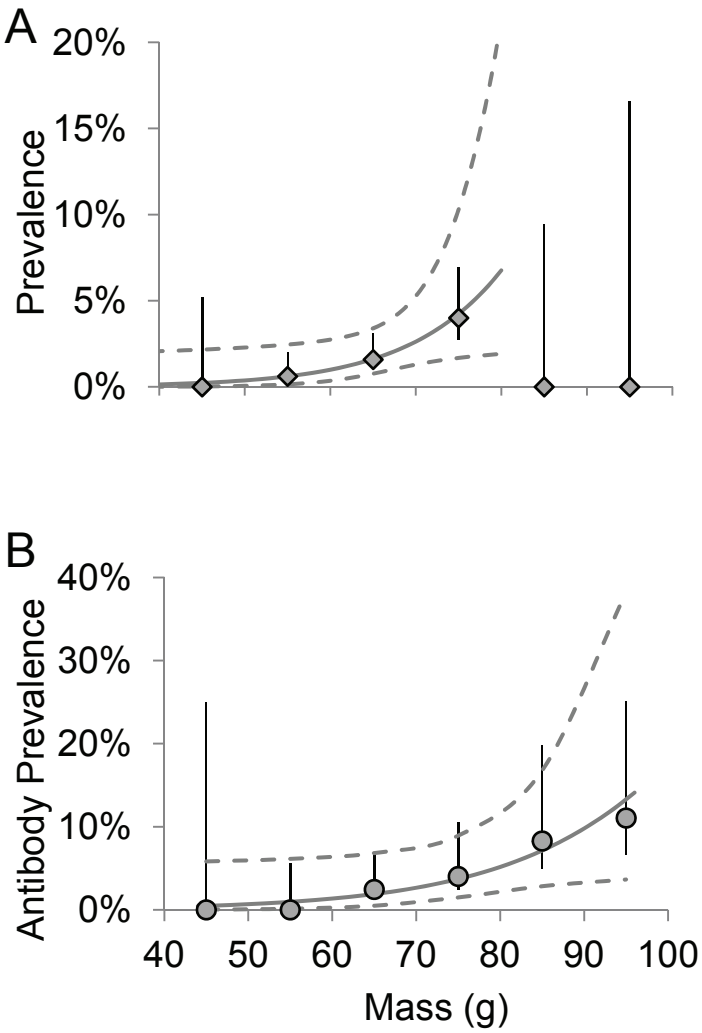

A
20%
15%
10%
5%
0%
Prevalence
B
40%
30%
20%
10%
0%
Antibody Prevalence
40
50
60
70
80
90
100
Mass (g)

Figure 4.5.

# CHAPTER 5

# SPRING MIGRATION STOPOVER ECOLOGY OF AVIAN INFLUENZA VIRUS SHOREBIRD HOSTS AT DELAWARE BAY[1]

[1] Maxted, A. M., H. P. Sitters, M. P. Luttrell, A. D. Dey, K. S. Kalasz, L. J. Niles, and D. E. Stallknecht. To be submitted to *Waterbirds*.

## ABSTRACT

Although shorebirds at Delaware Bay are infected with low pathogenicity avian influenza viruses (LPAIV) annually, little is known about affected species' habitat preferences or movement patterns that might influence virus transmission and spread. During the 5-wk spring migration stopover period in 2007-2008 we conducted a radiotelemetry study of often-infected Ruddy Turnstones (*Arenaria interpres morinella*; *n*=60) and rarely infected Sanderlings (*Calidris alba*; *n*=20) to identify locations and habitats important to these species (during day and night), determine the extent of overlap with other AIV reservoir species or poultry production areas, reveal possible movements of AIV around the Bay, and assess whether long-distance movement of AIV is likely after shorebird departure. Ruddy Turnstones and Sanderlings both fed on Bay beaches during the day. However, Sanderlings used remote sandy points and islands at night while Ruddy Turnstones primarily used salt marsh harboring waterfowl and gull breeding colonies suggesting that this environment supports AIV circulation. Shorebird locations were farther from agricultural land and poultry operations than random locations, suggesting selection away from poultry. Further, there was no areal overlap between shorebird home ranges and poultry production areas. Only 37% (22/60) of Ruddy Turnstones crossed into Delaware from banding sites in New Jersey suggesting partial site fidelity and AIV gene pool separation between the states. Ruddy Turnstones departed *en masse* around 1 June when AIV prevalence was low or declining, suggesting that a limited number of birds could disperse AIV onto the breeding grounds. This study provides needed insight into AIV and migratory host ecology and results can inform both AIV prevention and shorebird conservation efforts.


*Key Words: Arenaria interpres morinella*, avian influenza virus, *Calidris alba*, Delaware Bay, migration, radio telemetry, resource selection, Ruddy Turnstone, Sanderling, stopover ecology

## INTRODUCTION

Avian influenza viruses (AIV) are of interest to both veterinary and public health, but their transmission from natural reservoir species into domestic animals or humans have been documented rarely. Low pathogenicity AIVs (LPAIV) are maintained in nature by certain groups of wild aquatic birds, including waterfowl (particularly ducks) and gulls (Webster et al., 1992; Olsen et al., 2006). Although some studies have found AIV in shorebirds (order Chardriiformes, strictly families Scolopacidae and Charadriidae), infections in shorebirds have been localized and limited to few species (Krauss et al., 2004; Hurt et al., 2006; Hanson et al., 2008). Many recent surveillance studies worldwide have failed to find AIV more than occasionally in shorebirds (e.g., D'Amico et al., 2007; Munster et al., 2007; Escudero et al., 2008; Iverson et al., 2008; Winker et al., 2008; Dusek et al., 2009; Langstaff et al., 2009).

The most regular occurrence of AIV in shorebirds is at Delaware Bay on the Atlantic coast of North America during spring migration stopover during May-early June. Here, prevalence in Ruddy Turnstones (*Arenaria interpres morinella*) can exceed 15% in some years (Hanson et al., 2008); sympatric shorebird species are less often infected. It is not fully understood why AIV prevalence is high in shorebirds at this location, but very dense concentrations, up to 67 birds/$m^2$ (Gillings et al., 2007), of a species permissive to AIV (Ruddy Turnstones) is certainly a major factor (Krauss et al., 2010). Delaware Bay is a major spring migratory stopover site for several species of shorebirds, including Red Knots (*Calidris canutus rufa*), Ruddy Turnstones, Sanderlings (*Calidris alba*), Semipalmated Sandpipers (*Calidris pusilla*), Dunlins (*Calidris alpina*), and Short-billed Dowitchers (*Limnodromus griseus*) (Dunne et al., 1982; Clark et al., 1993). Each of these populations have experienced declines since 1998 (Niles et al., 2009), most likely due to reduced availability of their main diet at this site, the eggs of Horseshoe Crabs (*Limulus polyphemus*); this is the result of overharvest in preceding decades. Other, non-shorebird species utilize this site and might contiribute to AIV maintenance; these include migrating and breeding waterfowl, gulls, terns, and wading birds.

With the possible exception of Herring Gulls (*Larus argentatus smithsonianus*) and Laughing Gulls (*Leucophaeus atricilla*) (Kawaoka et al., 1988; Hanson et al., 2008), there has been limited study of AIV in these groups at this location.

Despite the close proximity of major poultry-producing (the Delmarva Peninsula) and seasonal tourist areas (Cape May and the Atlantic shore of New Jersey) to Delaware Bay, general stopover ecology and movement patterns of Ruddy Turnstones, which could be shedding AIV, at this stopover site are virtually unknown. The H5 and H7 subtypes, both documented in Ruddy Turnstones at Delaware Bay (Hanson et al., 2008), are of particular interest because of their ability to mutate into highly pathogenic forms. Investigations into the movement patterns of potential AIV reservoir species, both at global and local scales, have been recommended to better understand global ecology of AIV (FAO, 2007). Furthermore, other shorebird species are rarely infected at this site, despite feeding on Horseshoe Crab eggs alongside infected Ruddy Turnstones on Delaware Bay beaches. Subtle differences in habitats used or other aspects of their stopover ecologies might help explain why virus circulation is virtually limited to Ruddy Turnstones. Finally, it is unknown whether shorebirds such as Ruddy Turnstones are capable of transporting AIV long distances, possibly even between continents. Ruddy Turnstones use Delaware Bay *en route* to breeding grounds in the low Arctic (Nettleship, 1973; Perkins et al., 2007).

Given these gaps in our knowledge of AIV dynamics in Ruddy Turnstones and other shorebirds that use Delaware Bay as a spring migratory stopover, and the potential consequences of transmission to nearby poultry, we conducted a radiotelemetry study of Ruddy Turnstones and Sanderlings during the 5-wk stopover period. Sanderlings are rarely infected but share feeding habitat with Turnstones and were studied for comparison. Our objectives were to answer the questions: 1) What habitats or locations are important for Ruddy Turnstones and Sanderlings during their stopover at Delaware Bay, both during the day and at night? 2) Are habitats or locations used by shorebirds, particularly Ruddy Turnstones, overlapping with, or in

close proximity to, areas used by other species important in AIV epidemiology (gulls or waterfowl) or with areas used for poultry production? 3) What movement patterns are exhibited by Ruddy Turnstones, which could spread AIV locally, during the stopover period? 4) Is long-distance movement of AIV likely by shorebirds migrating through Delaware Bay? Specifically, when do birds depart upon their next leg of migration and, in the context of infection dynamics, what proportion might be infected with AIV at the time of departure? The answers to the above questions would not only help describe AIV-shorebird-environment interactions in the context of local and global AIV epidemiology, but also inform conservation efforts for these declining shorebird populations.

## MATERIALS AND METHODS

### Study site

Delaware Bay is located on the Atlantic coast of North America, between the U.S. states of Delaware and New Jersey (38° 47’ to 39° 20’ N, 74° 50’ to 75° 30’ W). Primary shorebird habitats include sandy beaches bordered by tidal *Spartina* spp. salt marshes and interspersed by small beachfront communities. Additionally, large tracts of tidal salt marsh are on the adjacent Cape May peninsula behind populated barrier islands along the Atlantic Ocean. Delaware Bay beaches support the greatest concentrations of migrating shorebirds including Red Knots, Ruddy Turnstones, Sanderlings, and Semipalmated Sandpipers, but tidal mudflats and marshes support higher numbers of certain species (Short-billed Dowitchers, Least Sandpipers [*Calidris minutilla*], Semipalmated Plovers [*Charadrius semipalmatus*], and Black-bellied Plovers [*Pluvialis squatarola*]) (Burger et al., 1997). The Atlantic coastal salt marshes also support large breeding colonies of Laughing Gulls and Herring Gulls, smaller numbers of Great Black-Backed Gulls (*Larus marinus*), and rookeries of mixed herons, egrets, and ibises.

**Field and laboratory methods**

Field work was conducted during May-early June, 2007-2008. Shorebirds were captured with cannon nets on Delaware Bay beaches by experienced personnel for inclusion in long-term population studies. Birds were fitted with a numbered metal U.S. Fish and Wildlife Service band, a lime green Darvic color band, and a lime green laser-inscribed plastic leg flag containing a unique 3-digit alphanumeric code (Clark et al., 2005). Following banding and measurement, cloacal swab samples were collected, stored, processed, and tested by virus isolation in embryonated chicken eggs as previously described (Hanson et al., 2008). The presence of AIV in allantoic fluid was confirmed by hemagglutination and reverse transcriptase polymerase chain reaction (RT-PCR) for matrix gene (Swayne et al., 1998; Spackman and Suarez, 2008).

**Radiotelemetry methods**

Sixty Ruddy Turnstones and 20 Sanderlings were fitted with back-mounted radiotransmitters (Model BD2, Holohil Systems Ltd., Ontario) that weighed, on average, 1.6% and 1.9% of body mass for Ruddy Turnstones and Sanderlings, respectively (transmitters weighed 1.6 g and 1.2 g for Ruddy Turnstones and Sanderlings, respectively). All individual transmitters weighed <2.3% of the bird's body weight. We attached radiotags by snipping a 1×2-cm patch of feathers close to the skin over the synsacrum and used cyanoacrylate glue gel to secure the package directly to the skin, allowing the antenna to lie freely over the tail. Six groups of 10 Ruddy Turnstones each and four groups of five Sanderlings each were radiotagged at adjacent Reed's and Cook's Beaches on the New Jersey shore of Delaware Bay (Fig. 5.1). Ruddy Turnstones were tagged on 10, 14, and 21 May 2007, and 7, 13, and 16 May 2008. Sanderlings were tagged on 10, 13, 17, and 18 May 2008.

We attempted to locate each radiotagged bird each day and night as weather permitted. We used handheld receivers (Model R4000, ATS Inc., Isanti, MN) and 3-element yagi antennae to search for and triangulate signals on the ground. Aerial telemetry was conducted every 2-3 d, and whenever possible, we paired a day flight with a night flight on the same date. A 2-element

H-antenna was attached to each wing of the Cessna 172 airplane, and two observers each surveyed a separate set of frequencies. We flew at 120-135 km/hr at 150-200 m altitude along the entire shoreline of the Delaware Bay and also flew transects over large expanses of marsh (e.g., along the New Jersey Atlantic coast). Locations were recorded as the position of the airplane when a given radio signal was loudest. Nine day and six night flights were conducted during 13 May-2 June 2007, and 10 day and six night flights were performed during 11 May-5 June 2008.

Additionally, teams of experienced observers scanned shorebird flocks using spotting scopes for marked individuals around Delaware Bay. Scanners concentrated their efforts on beaches where the largest numbers of birds congregated, enabling a large proportion of marked individuals to be seen (Gillings et al., 2009). Resighting data and morphometric and demographic data recorded at the time of capture were obtained from the Shorebird Resighting Database (www.bandedbirds.org). A body size index (BSI) was computed using principal components analysis to reduce multicollinearity of three morphology measurements (culmen length, combined head and bill length, and flattened wing chord); BSI is the first principal component of these measurements. The unitless BSI is normally distributed around a population mean of zero, with negative BSI indicating smaller body size and positive BSI indicating larger body size. A mass index (MI) was calculated to obtain an individual's mass relative to the expected mass of the population on a given day and year (Morrison et al., 2007). The MI is the unstandardized residual of a regression of mass on day, by individual years, using 5-node knotted spline effect of day in standard least-squares regression to obtain maximum fit for population mass gain (data not shown).

**Data analyses**

*GIS*– Each bird location was plotted in Google Earth (v. 5.2.1, Google Inc.) then transferred into ArcGIS 9.2 (ESRI, Redlands, CA). Base layer and topographic feature shapefiles were obtained from Delaware DataMIL (http://datamil.delaware.org/) and New Jersey

Department of Environmental Protection (NJDEP) Bureau of Geographic Information Systems (http://www.state.nj.us/dep/gis/). Only one location was retained per individual bird within the same daylight category (day or night, as determined by civil sunrise and sunset) on a given calendar date. The Moran's I statistic was used to test whether various point attributes (e.g., species, year, daylight category, or individual bird) clustered within the distribution of telemetry locations.

*Use areas*– Using only data obtained from aerial tracking to minimize bias relating to detection probability, 75% kernel density home range estimates were calculated using Hawth's Tools add-in to ArcGIS (available from http://www.spatialecology.com), for each combination of species, year, and daylight category. A smoothing parameter of 5,000 was used. Overlap between use areas was calculated using ArcGIS.

*Resource Selection*– Using ArcGIS, areal habitat was reclassified from 2007 land-use-land-cover shapefiles into 11 general habitat types: beach, barren/altered land, agriculture, shrubland, forest, wooded wetland, open water, freshwater wetland, urban, residential, and salt marsh. Additionally, because of our interest in shorebird locations in relation to poultry production locations, concentrated animal feeding operations (CFOs) were classified separately from other agriculture and included as a twelfth habitat class although they comprised a tiny proportion (<0.2%) of the landscape within 30 km of Delaware Bay. The vast majority of these CFOs are poultry operations, especially for broiler production (U.S. Department of Agriculture, 2009). Distance from each bird location to the nearest other features (stream, road, shoreline, and to each of the 12 habitat types) was measured. Measurements were repeated on 1,000 random points generated within the area covered by the flight transects (conservatively estimated to be the ground area within 3.2 km perpendicular to the aircraft's approximate flight line). Because point-to-feature distances were not normally distributed, relationships between used/available status and the distance to habitat and landscape feature variables were characterized by non-parametric univariate analyses using PROC NPAR1WAY in SAS version

9.2 (SAS Institute Inc., Cary, NC). The 2-sample Kolomogorov-Smirnov test was used to determine if the distribution of distances to each feature or habitat type were the same between bird locations and random locations, and the Wilcoxon test was used to compare central tendency, i.e., whether bird locations were located closer or further from each feature or habitat than random locations. These tests were also used to compare resource selection between species.

*Proximity to agriculture and poultry production areas*– In addition to the proximity tests above, minimum convex polygons (MCP) were calculated from all available shorebird location data and compared to CFO locations.

*Movements*– Four quadrants were defined as follows: Lower New Jersey, locations south and southeast of the Maurice River including all locations along and near the Atlantic coast; Upper New Jersey, points north and west of the Maurice River; Lower Delaware, locations south and east of Big Stone Beach; Upper Delaware, locations north and west of Big Stone Beach (Fig. 5.1). Whether birds moved between quadrants or crossed the bay and the first dates they were recorded in a new quadrant or in Delaware were examined. The MCP of each individual bird was compared to MCPs produced by 100 “random walks” with the number of locations obtained for that bird and across the range of distances between successive locations, using Hawth’s Tools. Movement was considered non-random if area of bird MCPs were smaller than the area of ≥95% of MCPs produced by random walks.

*Departure dates*– Departures were analyzed by product-limit (i.e., Kaplan-Meier; Kaplan and Meier, 1958) survival analysis, where departure from Delaware Bay represent “failures”. Departure dates were defined as the last date each bird was detected by resighting or radiotelemetry by ground or air. When a bird was last detected at night, it was given an additional half-day increment (e.g., 28.5 May). It was presumed that the radiotransmitter became dislodged or nonfunctional when individual radio signals were not detected after 20 May; the last dates these birds were detected were right-censored in the analyses. Likewise,

birds that were detected on the last day of tracking (3 June 2007 and 5 June 2008) were right-censored because they were still present at the conclusion of the study. Using log-rank analysis (Peto and Peto, 1972), distributions of departure dates were compared between years in Ruddy Turnstones. Because departure rates were not proportional over time between Ruddy Turnstones and Sanderlings, the assumption of proportional hazards was violated (Cox and Oakes, 1984) and the distribution of departure dates could not be compared statistically between species. Within each year-species combination, departure dates were further compared by date of capture, sex (male or female; birds of unknown sex were excluded), raw mass (in 10-g classes), body size index (BSI; in classes by the number of standard deviations above or below zero), mass index (MI; in 10-g classes above or below expected mass), and AIV infection status (positive or negative) recorded at the time of capture. The latter comparison included 2008 Ruddy Turnstone data only, since no Sanderlings or Ruddy Turnstones tagged in 2007 were AIV positive. Proportions of birds remaining at Delaware Bay over time were estimated for each group using the log-normal distribution.

Computations and statistical analyses not performed in ArcGIS were carried out in SAS version 9.2 or JMP version 8 (SAS Institute Inc., Cary, NC). Scientific collection permits were provided by U. S. Fish and Wildlife Service, Delaware Division of Fish and Wildlife, DNREC, and New Jersey Division of Fish and Wildlife, NJDEP.

## RESULTS

A total of 1,355 locations were obtained from ground and aerial telemetry, capture data, and resightings of radiotagged birds (*n*=1,089 Ruddy Turnstone locations, *n*=226 Sanderling locations). In 2007 and 2008, respectively, Ruddy Turnstones were tracked for medians of 17 (range: 5.5-22) and 20 (range: 2.5-29) d. Sanderlings were tracked for a median of 16 (range: 5-26) d in 2008. Individual Ruddy Turnstones in 2007 and 2008 and Sanderlings in 2008 were located on a median of 10, 12, and 10 days (range: 3-23), and six, seven, and three nights (range: 0-15), respectively. The total number of locations per bird ranged from 4-36.

Seven hundred thirty-seven locations were determined by aerial telemetry (*n*=563 Ruddy Turnstone locations and *n*=174 Sanderling locations). Individual birds were located a median of nine (range: 1-15) times by aerial telemetry, or on a median of six (range: 1-9) day flights and three (range: 0-6) night flights.

Morphometic and demographic charcteristics of radiotagged birds are summarized in Table 5.1. Among radiotagged Ruddy Turnstones, BSI was significantly larger in 2007 than in 2008 ($t$=2.1, df=58, $P$=0.038); all other measures were equivalent between years ($P$>0.05).

**Avian influenza virology**

All 30 tagged Ruddy Turnstones were AIV-negative in 2007 (Table 5.1). In 2008, five of 29 tagged Turnstones were AIV-positive (17%; one bird not tested). Prevalence increased with capture date (logistic regression; $\chi^2$=6.9, df=1, $P$=0.009) and was 0% (0/10), 11% (1/9) and 40% (4/10) among birds captured on 7, 13, and 16 May 2008, respectively. All subtypes were of low pathogenicity (H6N8 [*n*=1], H10N7 [*n*=1], and H12N5 [*n*=3]; unpublished data). All 14 Sanderlings tested were negative for AIV.

**Use areas and habitat use**

Fig. 5.2 illustrates the areas used by Ruddy Turnstones and Sanderlings during the day and at night. Both species primarily used Delaware Bay beaches during the day, particularly along the Cape May peninsula in New Jersey, from Fortescue to Baypoint in New Jersey, and a few discrete locations in Delaware (Port Mahon, Bower's Beach, and Mispillion Harbor). In 2008, strong westerly winds caused a large focus of horseshoe crab spawning at Moore's Beach and nearby locations (NJDEP, unpublished data); hence, this area was used more heavily in 2008 compared to 2007. Not surprisingly, due their common diet of horseshoe crab eggs, Sanderlings and Ruddy Turnstones used similar stretches of Delaware Bay beaches in 2008. The daytime use areas, defined by 75% kernel density contours, overlapped by 64% between species. However, daytime locations clustered by species (Moran's I=0.16, Z=2.37, $P$=0.018).

For both species, areas used at night were significantly different than those used during the day (Ruddy Turnstone: Moran's I=0.49, Z=4.08, *P*<0.0001; Sanderling: Moran's I=0.76, Z=9.63, *P*<0.0001). At night, Ruddy Turnstones primarily used areas of expansive salt marsh, particularly along the barrier islands of the Atlantic Ocean in the Cape May Peninsula (hereafter, Atlantic marshes), Egg Island and restored former salt hay farms (Balletto et al., 2005; Hinkle and Mitsch, 2005) just west of the Maurice River in Upper New Jersey, and Bombay Hook National Wildlife Refuge in Delaware. Two birds also roosted on stone breakwaters off Cape Henlopen, Delaware. In 2008, salt marshes surrounding West Creek were heavily used by Turnstones, while Bombay Hook was used relatively infrequently (Fig. 5.2). Turnstone night locations clustered by year (Moran's I=0.09, *Z*=3.44, *P*=0.0006), but day locations did not (Moran's I=-0.02, *Z*=-0.29, *P*=0.772). Sanderlings used a small number of locations at night including Stone Harbor Point, Egg Island Point, Dyer's Cove, Mispillion Harbor, and several sandy points and mudflats from West Creek, Moore's Beach, and Thompson's Beach. Although Ruddy Turnstones also roosted on sandy points such as Stone Harbor Point, they roosted more often in the nearby salt marshes (Fig. 5.3). Nighttime use areas overlapped by only 20% between species. Nighttime locations clustered strongly by species (Moran's I=1.06, *Z*=18.34, *P*<0.0001)

Table 5.2 lists the proportion of aerial telemetry locations within each habitat for each species and daylight class, as well as the proportion of randomly selected points in each habitat. During the day for both species, a majority of bird locations were on beaches. Salt marsh was also used by both species. For both species >25% of locations were in open water, probably because of both location inaccuracy and the species' use of tidal habitats, which by definition are often covered by water. Ninety-nine percent of points in open water were within 200 m of either salt marsh or beach habitat. Points located in residential areas invariably were in beachfront communities, likely due to bird use of beaches surrounding and under houses. The distribution of habitats in which daytime aerial telemetry points were located was similar

between species, and between years among Ruddy Turnstones. However, night locations revealed markedly different habitat use between species. While >80% of Ruddy Turnstone night locations were in salt marsh, only 26% of Sanderling locations were in salt marsh. Likewise, >70% of Sanderling night locations were in beach or open water, <20% of Ruddy Turnstone locations were in these habitats. The distribution of terrestrial habitats in which aerial telemetry points were located was markedly different than the underlying distribution of random points on the landscape for both species in both daylight classes (Table 5.2). Excluding telemetry locations in open water, the distribution of locations in habitats differed from the underlying (i.e., random) distribution for all species-year-daylight classes (Kolomogorov-Smirnov 2-sample test, all $P<0.0001$).

Likewise, the distribution of distances from each aerial telemetry point to the nearest block of habitat (e.g., agriculture, beach, forest), or landscape feature (e.g., road, stream, shoreline) varied from the expected distribution (i.e., the distribution of distances from random points to each habitat or feature) for every habitat-species-daylight class combination (Kolomogorov-Smirnov 2-sample tests, all $P<0.05$). During the day, Ruddy Turnstones locations were further from agriculture, CFOs, forest, freshwater wetlands, wooded wetlands, and urban areas, and nearer to beaches, recreational areas, streams, and shoreline than random points. At night, they were further from agriculture, barren/altered land, CFOs, forest, freshwater and wooded wetlands, residential and urban areas, and roads, and nearer to salt marsh and streams than random. During the day, Sanderlings were located nearer to beaches, recreational areas, residential areas, streams, and shoreline and further from agriculture, CFOs, forest, freshwater wetlands, urban areas, and wooded wetlands than random locations. At night, they were closer to beaches, streams, and shoreline and further from agriculture, barren/altered lands, CFOs forest, freshwater wetlands, recreational lands, residential and urban areas, wooded wetlands, and roads than random (Wilcoxon tests, all $P<0.05$).

Although median distances to habitats and features was similar between Ruddy Turnstones and Sanderlings during the day, these differed at night for every habitat and feature except for distance to urban and wooded wetland habitats and to streams (Table 5.3). During the day, Turnstones were closer to salt marsh and farther from beach and wooded wetland habitats than Sanderlings. At night, Ruddy Turnstones were significantly closer to barren/altered land, freshwater wetland, recreational and residential areas, salt marsh, and roads than Sanderlings, and significantly further from Agriculture, CFOs, beaches, forest, shrubland, and the shoreline than Sanderlings (Table 5.3).

Proximity to landscape and habitat features did not differ between Ruddy Turnstones that were AIV-positive or -negative at the time of capture (all $P>0.05$), except that AIV-positive birds were located further from barren/altered lands than AIV-negative birds (median 2.5 vs. 1.9 km for negative birds; Wilcoxon $Z=2.20$, $P=0.028$).

**Proximity of Ruddy Turnstones to areas used for poultry production**

Compared to random locations, Ruddy Turnstone locations were further from both agriculture in general (median 1.9 vs. 0.6 km; Wilcoxon Z=20.87, $P<0.0001$) and confined feeding operations in specific (median 14.7 vs. 9.6 km; Wilcoxon $Z=10.30$, $P<0.0001$). Nighttime Ruddy Turnstone locations were significantly further from both agriculture and CFOs than daytime locations (median 2.9 vs. 1.8 km, Wilcoxon Z=11.05, $P<0.0001$; median 19.7 vs. 13.2 km, Wilcoxon Z=9.10, $P<0.0001$, respectively). Ruddy Turnstones were located closer to CFOs when they were in Delaware compared to New Jersey (median 6.7 vs. 15.7 km; Wilcoxon Z=-11.6, $P<0.0001$). The minimum distance between any Ruddy Turnstone location and the nearest CFO was 3.2 km. No CFOs were located within the MCP created by all bird locations (Fig. 5.4). The shortest distance between a CFO and the edge of the MCP was 1.7 km.

**Ruddy Turnstone movements**

Birds moved non-randomly on the landscape (all MCP smaller than >95% of those produced by random walks), but apparently moved independently of each other. In each year,

locations of individual birds were randomly distributed within the distribution of all aerial telemetry locations (2007: Moran's I=0.08, Z=1.08, *P*=0.280; 2008: Moran's I=-0.12, Z=-0.97, *P*=0.332).

In 2007, 23 (77%) Ruddy Turnstones moved away from lower New Jersey and 14 (47%) crossed Delaware Bay at least once. In 2008, these numbers were 17 (57%) and eight (27%), respectively. The proportion of birds moving away from lower New Jersey and crossing Delaware Bay did not differ between years (Fisher's exact tests; *P*=0.170 and *P*=0.180, respectively). In 2007, 36% (5/14) of birds that crossed the bay crossed back into New Jersey. In 2008, 63% (5/8) crossed back; this difference between years is not significant (Fisher's exact test, *P*=0.443).

Turnstones that moved away from lower New Jersey were first detected elsewhere a median of nine (2007) and seven (2008) d from date of capture (range: 0-20 d). They were first detected in Delaware a median of 13 and 9.5 d after capture in 2007 and 2008, respectively (range: 3-24 d). The median dates a bird was first detected elsewhere was 23 May 2007 (range: 13-31 May) and 19 May 2008 (range 11 May-5 June), and the median dates on which a bird was first detected in Delaware was 27 May 2007 (range: 24-31 May) and 20 May 2008 (range: 11 May-1 June; Fig. 5.5).

Using data from individual birds only when day and night flights were paired (*n*=113), median distance moved from day to night locations was 11.2 km (range: 0.2-58.1 km). Ninety percent of night locations were ≤22.7 km from the corresponding day location. Distance moved did not vary between years (Wilcoxon Z=-0.39, *P*=0.694) or by date (Kruskal-Wallis $\chi^2$=7.63, df=2, *P*=0.471). Rarely did birds cross the bay; in only five (4%) instances did Ruddy Turnstones cross the Bay between day and night locations; three from upper New Jersey beaches to Bombay Hook National Wildlife Refuge and two from beaches in Delaware to West Creek and the Atlantic marshes, respectively.

Among the five Ruddy Turnstones that were AIV-positive at the time of capture, three (60%) moved away from lower New Jersey and two (40%) crossed the Bay. These two birds were first detected in Delaware on 22 May and 1 June 2008, or six and 19 d after capture, respectively. Median distance moved between day and night locations was 18.0 km (*n*=11, range: 3.3-27.7 km); this was not different from AIV-negative birds (Wilcoxon Z=1.5, *P*=0.129). Movement patterns of AIV-infected birds are displayed in Fig. 5.6; these were similar to movement patterns of uninfected birds.

**Departure dates**

Table 5.4 lists, for each species-year combination, the median departure date and estimated dates that 25%, 50%, and 75% of birds had departed. In Ruddy Turnstones, departure dates were distributed significantly later in 2008 than 2007 (Log-Rank $\chi^2$=13.76, df=1, *P*=0.0002; Fig. 5.7A). Estimated mean departure was nearly two days later in 2008, however, the span of time over which the middle 50% of Ruddy Turnstones departed remained approximately the same between years (2.5 d in 2007 and 3.0 d in 2008; Table 5.4). Departure dates differed with capture date in 2007 but not in 2008 (Table 5.5); birds from the earliest capture in 2007 (10 May) departed earlier than birds captured later (Fig. 5.8A). In 2008, but not 2007, departure dates were different across 10-g mass classes and 10-g MI classes (Table 5.5); birds that were heaviest at the time of capture, either absolutely or relatively, departed earlier than lighter birds (Fig. 5.8B and C). Departure dates in each year did not differ by sex or BSI (Table 5.5). There was no difference in departure dates between birds that were AIV positive at the time of capture and those that were negative (2008 data only, Table 5.5).

In Sanderlings, heavier birds (both absolutely and relatively) departed earlier than lighter birds (Table 5.5). Sanderlings (but not Ruddy Turnstones) with larger relative body size (BSI) departed earlier than smaller birds (Table 5.5).

Fig. 5.7B shows estimated departures of Sanderlings and Ruddy Turnstones in 2008. The median departure date of Sanderlings was 28 May, compared to 2 June for Ruddy

Turnstones (Table 5.4). Comparatively, Sanderlings departed on a wider range of dates. The middle 50% of Sanderlings departed during 27 May-3 June (seven days), while the same proportion of Ruddy Turnstones departed during 31 May-3 June (three days).

## DISCUSSION

### Use areas, habitat use, and proximity to other AIV reservoir species

Overlap of daytime use areas was substantial between species, and between years in Ruddy Turnstones. Shorebirds using Delaware Bay during spring migration, including Ruddy Turnstones and Sanderlings, rely heavily on Horseshoe Crab eggs for rapid weight gain (Atkinson et al., 2007). It is not surprising, then, that locations used by both species during the day historically have supported large numbers of spawning crabs (Smith et al., 2002) and high egg densities (Botton et al., 1994). Spatial distribution of Red Knots, a sympatric species, was also linked to the available number of Horseshoe Crab eggs (Karpanty et al., 2006). A period of sustained westerly winds in May 2008 resulted in lower spawning crabs along the relatively north-south aspect of the beaches along the Cape May Penninsula, and higher numbers along the east-west aspect of lower Delaware Bay from East Point to Dennis Creek in New Jersey.

A key finding regarding habitat use of Ruddy Turnstones is that their primary night roost locations are in expansive salt marsh habitat. These wetland areas are frequented by known AIV reservoir species including waterfowl (e.g., Mallards [*Anas platyrhynchos*]) and gulls (Laughing Gulls, Herring Gulls). In particular, the nighttime area used in the Atlantic marshes (Fig. 5.3) is home to the largest number of breeding Laughing Gull pairs on the eastern seaboard (Burger, 1996). The AIV subtypes isolated from shorebirds at Delaware Bay (Krauss et al., 2007; Hanson et al., 2008) have included subtypes normally associated with duck species (e.g, H3, H4, and H6) as well as those adapted to gulls (e.g, H13, H16) (Olsen et al., 2006). Predominant AIV subtypes among Ruddy Turnstones during 2007-2008 were H4N6, H10N7, and H12N5 (26%, 20%, and 23% of 116 isolates, respectively; unpublished data). Because AIV infection is waterborne, it is possible that tidal marshes are an important source of AIV to which

Ruddy Turnstones are exposed upon arrival at Delaware Bay. Although Ruddy Turnstones feed primarily on Horseshoe Crab eggs while at this stopover (Tsipoura and Burger, 1999), they are known to take a variety of food items (Gill, 1986) including gull feces and eggs (King, 1982; Brearey and Hilden, 1985). Thus, gulls or their nesting habitat could be a source of AIV for Turnstones.

Alternatively, this habitat could provide conditions appropriate for AIV transmission among Ruddy Turnstones. Because Ruddy Turnstones, Red Knots, and Sanderlings share common feeding habitat during the day (Delaware Bay beaches) (Clark et al., 1993; Burger et al., 1997; Burger et al., 2007), and Red Knots and Sanderlings have very low AIV prevalence (Hanson et al., 2008), perhaps differences in nighttime habitat use allow for variable transmission to and within each species. Sanderlings (and Red Knots; unpublished data) do not extensively share Ruddy Turnstone nighttime roost locations, instead preferring remote sandy points and islands. Beach sand might not be an effective medium for AIV transmission because frequent wave action possibly causes percolation of virus through the sand and ultraviolet (UV) radiation and drying at the surface likely cause rapid inactivation of virus. Because AIV is efficiently transmitted through contaminated water (Hinshaw et al., 1979), the tidal marshes and their standing water are perhaps a better medium for transmission, particularly at night when viruses aren't inactivated by UV light. Although the length of time that AIV survives in water decreases with increasing salinity (Stallknecht et al., 1990; Brown et al., 2009), survival of even a few days in saltwater could be sufficient for sustained transmission, especially when birds concentrate in large numbers on just a few identified night roost areas. More study is necessary to discover the exact infection sources and transmission mechanisms of AIV within and among shorebirds, gulls, waterfowl, and other waterbirds at Delaware Bay.

**Proximity to poultry production areas**

Transmission of AIV from wild birds to poultry species is a high concern for this agricultural industry, particularly the H5 and H7 subtypes capable of mutating into highly

pathogenic forms. Subtypes H5 or H7 were detected in Delaware Bay shorebirds in five of six years during 2000-2005 (Hanson et al., 2008), highlighting the need for excellent biosecurity in this context.

All Ruddy Turnstone locations were at least several km from known CFOs. However, the locations of some small poultry holders (e.g., free range) that do not "look" like CFOs on aerial photography are unknown. Although shorebirds were not detected in areas of poultry production, we did not systematically search these areas. Assuming that shorebirds took the most direct route between locations, or at least flew along the shoreline, no CFOs were under the flight line of shorebirds that could be shedding AIV. However, small backyard flocks or free range poultry were seen on the Cape May Peninsula during ground tracking; these were under the flight path of Turnstones from their daytime feeding locations along the lower New Jersey beaches and their night roost locations in the Atlantic marshes. While no transmissions of AIV from infected shorebirds to nearby domestic birds have been documented, small poultry holders should be educated about the risk of AIV transmission and all nearby domestic birds should be kept indoors during the shorebird stopover period. Further, potentially exposed domestic birds should not be transferred or sold at live bird markets until it's certain they are free of infection.

**Movement patterns**

A minority of Ruddy Turnstones crossed the Bay from New Jersey into Delaware; this finding is consistent with capture-resighting studies (unpublished data). Ruddy Turnstones were relatively faithful to a particular side of Delaware Bay, just as they are faithful to wintering sites (Metcalfe and Furness, 1985; Nettleship, 2000). Predicted population AIV prevalence on the median day of bay crossing was 5% (95% CI: 2-11%) in 2007, and 20% (95% CI: 16-25%) in 2008 (unpublished data). Peak prevalence occurs on about 22-24 May. Thus, potential exists for local movement of AIV by Ruddy Turnstones within the Delaware Bay ecosystem. However, cross-bay circulation of all strains present in a given year might be limited. For example, during May-June 2008, only four (22%) of 18 AIV subtypes isolated from Ruddy Turnstones were

detected in both states; four (22%) were detected only in Delaware and 10 (56%) were detected only in New Jersey (unpublished data).

**Departure dates and AIV prevalence**

Ruddy turnstones use Delaware Bay *en route* to breeding grounds in the low Arctic (Nettleship, 1973; Perkins et al., 2007). Predicted AIV prevalence on Ruddy Turnstones' median departure dates in 2007 and 2008 were 2% (95% CI: 0.5-9%) and 16% (95% CI: 11-23%), respectively (unpublished data). Thus, a small number of Ruddy Turnstones potentially could transport AIV from Delaware Bay toward the breeding grounds. However, it is possible that birds that became infected late in the stopover season, and thus were shedding virus during the period of peak departure, remained at Delaware Bay longer than uninfected birds. Indeed, median departure date was about two days later in 2008, when AIV prevalence was high, than in 2007, when prevalence was much lower. Migration delays have been observed in AIV-infected Mallards (Latorre-Margalef et al., 2009) and Bewick's Swans (*Cygnus columbianus bewickii*) (van Gils et al., 2007) when compared to uninfected conspecifics. Alternatively, the observed migration delay could have been due to weather- or weight gain-related factors or a combination of factors. In 2008, birds that were AIV-positive at the time of capture (13-16 May) presumably recovered from infection before departure approximately 17-20 d later. No difference was observed in the departure dates between birds that were initially infected and uninfected, but birds could have become infected after they were radiotagged. Thus, it is unclear from our data if AIV infection influences departure from Delaware Bay, or if Turnstones transport virus northward following departure.

Although some AIV isolates recovered from shorebirds show evidence of intercontinental exchange of entire virus or gene segments (Krauss et al., 2007), most shorebird infections are thought to occur locally as spillover events (Pearce et al., 2010). Because Ruddy Turnstones typically disperse onto breeding grounds after departing Delaware Bay (Nettleship, 2000;

Perkins et al., 2007), transmission is thought not to be sustained throughout the annual cycle. Indeed, AIV is detected rarely in Ruddy Turnstones outside of Delaware Bay.

**Conservation aspects**

This study identified additional sites and habitats that are potentially critical to shorebirds during their stopover at Delaware Bay. For example, it was previously unknown that Ruddy Turnstones used salt marshes extensively at night and Sanderlings used mudflats and sandy points. That relatively few nighttime congregation sites were used highlights the need for continued protection of these areas. Because these shorebirds were captured in one location and generally stayed in New Jersey, future study should include birds from other sites around Delaware Bay, especially Delaware, to help identify other critical nighttime roosting locations that these site-faithful birds did not visit.

## ACKNOWLEDGMENTS

This research was funded through Specific Cooperative Agreement 58-6612-2-0220 between the Southeast Poultry Research Laboratory, Agricultural Research Service, Department of Agriculture (USDA-ARS) and the Southeastern Cooperative Wildlife Disease Study, and by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Department of Health and Human Services, under contract no. HHSN266200700007C. The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. Banding data and morphometric measurements are the property of the Natural Heritage & Endangered Species Program, Division of Fish and Wildlife, Delaware Department of Natural Resources and Environmental Control, and the Nongame and Endangered Species Program, Division of Fish and Wildlife, New Jersey Department of Environmental Protection (NJDEP); we thank these agencies for granting access to these data. We are grateful to C. Minton, R. Veitch, and several others whose knowledge of shorebird ecology helped guide this study. We thank S. Schweitzer (University of Georgia) and NJDEP for lending their radiotelemetry equipment and technical

support to this study; we particularly thank W. Pitts (NJDEP). We are grateful to many people who provided field support, particularly E. Casey, L. Coffee, M. Cole, J. Cumbee, D. Downs, W. Hamrick, S. Keeler, G. Martin, S. McGraw, C. McKinnon, J. Murdock, and B. Wilcox. Jim Strong Aviation provided expert aviation service.

## LITERATURE CITED

ATKINSON, P. W., A. J. BAKER, K. A. BENNETT, N. A. CLARK, J. A. CLARK, K. B. COLE, A. DEKINGA, A. DEY, S. GILLINGS, P. M. GONZALEZ, K. KALASZ, C. D. T. MINTON, J. NEWTON, L. J. NILES, T. PIERSMA, R. A. ROBINSON, and H. P. SITTERS. 2007. Rates of mass gain and energy deposition in red knot on their final spring staging site is both time- and condition-dependent. Journal of Applied Ecology 44: 885-895.

BALLETTO, J. H., M. V. HEIMBUCH, and H. J. MAHONEY. 2005. Delaware Bay salt marsh restoration: mitigation for a power plant cooling water system in New Jersey, USA. Ecological Engineering 25: 204-213.

BOTTON, M. L., R. E. LOVELAND, and T. R. JACOBSEN. 1994. Site selection by migratory shorebirds in Delaware Bay, and its relationship to beach characteristics and abundance of horseshoe crab (*Limulus polyphemus*) eggs. Auk 111: 605-616.

BREAREY, D., and O. HILDEN. 1985. Nesting and egg predation by Turnstones *Arenaria interpres* in Larid colonies. Ornis Scandinavica 16: 283-292.

BROWN, J. D., G. GOEKJIAN, R. POULSON, S. VALEIKA, and D. E. STALLKNECHT. 2009. Avian influenza virus in water: Infectivity is dependent on pH, salinity and temperature. Veterinary Microbiology 136: 20-26.

BURGER, J. 1996. Laughing Gull (*Larus atricilla*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 225, http://bna.birds.cornell.edu/bna/species/225. Accessed January 2011.

BURGER, J., L. NILES, and K. E. CLARK. 1997. Importance of beach, mudflat and marsh habitats to migrant shorebirds on Delaware Bay. Biological Conservation 79: 283-292.

BURGER, J., S. A. CARLUCCI, C. W. JEITNER, and L. NILES. 2007. Habitat choice, disturbance, and management of foraging shorebirds and gulls at a migratory stopover. Journal of Coastal Research 23: 1159-1166.

CLARK, K. E., L. J. NILES, and J. BURGER. 1993. Abundance and distribution of migrant shorebirds in Delaware Bay. Condor 95: 694-705.

CLARK, N. A., S. GILLINGS, A. J. BAKER, P. M. GONZALEZ, and R. PORTER. 2005. The production and use of permanently inscribed leg flags for waders. Wader Study Group Bulletin 108: 38-41.

COX, D. R., and D. OAKES. 1984. Analysis of Survival Data. Chapman and Hall, London, United Kingdom.

D'AMICO, V. L., M. BERTELLOTTI, A. J. BAKER, and L. A. DIAZ. 2007. Exposure of Red Knots (*Calidris canutus rufa*) to select avian pathogens; Patagonia, Argentina. Journal of Wildlife Diseases 43: 794-797.

DUNNE, P., D. SIBLEY, C. SUTTON, and W. WANDER. 1982. Aerial surveys in Delaware Bay: confirming an enormous spring staging area for shorebirds. International Wader Study Group Bulletin 35: 32-33.

DUSEK, R. J., J. B. BORTNER, T. J. DELIBERTO, J. HOSKINS, J. C. FRANSON, B. D. BALES, D. YPARRAGUIRRE, S. R. SWAFFORD, and H. S. IP. 2009. Surveillance for high pathogenicity avian influenza virus in wild birds in the Pacific flyway of the United States, 2006-2007. Avian Diseases 53: 222-230.

ESCUDERO, G., V. J. MUNSTER, M. BERTELLOTTI, and P. EDELAAR. 2008. Perpetuation of avian influenza in the Americas: Examining the role of shorebirds in Patagonia. Auk 125: 494-495.

FAO. 2007. Wild Birds and Avian Influenza: an Introduction to Applied Field Research and Disease Sampling Techniques. *In* D. Whitworth, S. Newman, T. Mundkur, and P. Harris, (eds.) FAO Animal Production and Health Manual FAO, Rome, 120 pp.

GILL, R. E. 1986. What won't turnstones eat? British Birds 79: 402-403.

GILLINGS, S., P. W. ATKINSON, S. L. BARDSLEY, N. A. CLARK, S. E. LOVE, R. A. ROBINSON, R. A. STILLMAN, and R. G. WEBER. 2007. Shorebird predation of horseshoe crab eggs in Delaware Bay: species contrasts and availability constraints. Journal of Animal Ecology 76: 503-514.

GILLINGS, S., P. W. ATKINSON, A. J. BAKER, K. A. BENNETT, N. A. CLARK, K. B. COLE, P. M. GONZALEZ, K. S. KALASZ, C. D. T. MINTON, L. J. NILES, R. C. PORTER, I. DE LIMA SERRANO, H. P. SITTERS, J. L. WOODS, and T. L. SHAFFER. 2009. Staging behavior in Red Knot (*Calidris canutus*) in Delaware Bay: implications for monitoring mass and population size. Auk 126: 54-63.

HANSON, B. A., M. P. LUTTRELL, V. H. GOEKJIAN, L. NILES, D. E. SWAYNE, D. A. SENNE, and D. E. STALLKNECHT. 2008. Is the occurrence of avian influenza virus in Charadriiformes species and location dependent? Journal of Wildlife Diseases 44: 351-361.

HINKLE, R. L., and W. J. MITSCH. 2005. Salt marsh vegetation recovery at salt hay farm wetland restoration sites on Delaware Bay. Ecological Engineering 25: 240-251.

HINSHAW, V. S., R. G. WEBSTER, and B. TURNER. 1979. Water-borne transmission of influenza A viruses. Intervirology 11: 66-68.

HURT, A. C., P. M. HANSBRO, P. SELLECK, B. OLSEN, C. MINTON, A. W. HAMPSON, and I. G. BARR. 2006. Isolation of avian influenza viruses from two different transhemispheric migratory shorebird species in Australia. Archives of Virology 151: 2301-2309.

IVERSON, S. A., J. Y. TAKEKAWA, S. SCHWARZBACH, C. J. CARDONA, N. WARNOCK, M. A. BISHOP, G. A. SCHIRATO, S. PAROULEK, J. T. ACKERMAN, H. IP, and W. M. BOYCE. 2008. Low prevalence of avian influenza virus in shorebirds on the Pacific coast of North America. Waterbirds 31: 602-610.

KAPLAN, E. L., and P. MEIER. 1958. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 53: 457-481.

KARPANTY, S. M., J. D. FRASER, J. BERKSON, L. J. NILES, A. DEY, and E. P. SMITH. 2006. Horseshoe crab eggs determine red knot distribution in Delaware Bay. Journal of Wildlife Management 70: 1704-1710.

KAWAOKA, Y., T. M. CHAMBERS, W. L. SLADEN, and R. G. WEBSTER. 1988. Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? Virology 163: 247-250.

KING, B. 1982. Turnstone feeding on gull excrement British Birds 75: 88-88.

KRAUSS, S., D. WALKER, S. P. PRYOR, L. NILES, C. H. LI, V. S. HINSHAW, and R. G. WEBSTER. 2004. Influenza A viruses of migrating wild aquatic birds in North America. Vector-Borne and Zoonotic Diseases 4: 177-189.

KRAUSS, S., C. A. OBERT, J. FRANKS, D. WALKER, K. JONES, P. SEILER, L. NILES, S. P. PRYOR, J. C. OBENAUER, C. W. NAEVE, L. WIDJAJA, R. J. WEBBY, and R. G. WEBSTER. 2007. Influenza in migratory birds and evidence of limited intercontinental virus exchange. Plos Pathogens 3: 1684-1693.

KRAUSS, S., D. E. STALLKNECHT, N. J. NEGOVETICH, L. J. NILES, R. J. WEBBY, and R. G. WEBSTER. 2010. Coincident Ruddy Turnstone migration and horseshoe crab spawning creates an ecological 'hot spot' for influenza viruses. Proceedings of the Royal Society B: Biological Sciences 277: 3373-3379.

LANGSTAFF, I. G., J. S. MCKENZIE, W. L. STANISLAWEK, C. E. M. REED, R. POLAND, and S. C. CORK. 2009. Surveillance for highly pathogenic avian influenza in migratory shorebirds at the terminus of the East Asian-Australasian Flyway. New Zealand Veterinary Journal 57: 160-165.

LATORRE-MARGALEF, N., G. GUNNARSSON, V. J. MUNSTER, R. A. M. FOUCHIER, A. D. M. E. OSTERHAUS, J. ELMBERG, B. OLSEN, A. WALLENSTEN, P. D. HAEMIG, T. FRANSSON, L. BRUDIN, and J. WALDENSTRÖM. 2009. Effects of influenza A virus infection on migrating Mallard ducks. Proceedings of the Royal Society B: Biological Sciences 276: 1029-1036.

METCALFE, N. B., and R. W. FURNESS. 1985. Survival, winter population stability and site fidelity in the Turnstone *Arenaria interpres*. Bird Study 32: 207-214.

MORRISON, R. I. G., N. C. DAVIDSON, and J. R. WILSON. 2007. Survival of the fattest: body stores on migration and survival in Red Knots *Calidris canutus islandica*. Journal of Avian Biology 38: 479-487.

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.

NETTLESHIP, D. N. 1973. Breeding ecology of Turnstones *Arenaria interpres* at Hazen Camp, Ellesmere Island, N.W.T. Ibis 115: 202-217.

NETTLESHIP, D. N. 2000. Ruddy Turnstone (*Arenaria interpres*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 537, http://bna.birds.cornell.edu/bna/species/537. Accessed January 2011.

NILES, L. J., J. BART, H. P. SITTERS, A. D. DEY, K. E. CLARK, P. W. ATKINSON, A. J. BAKER, K. A. BENNETT, K. S. KALASZ, N. A. CLARK, J. CLARK, S. GILLINGS, A. S. GATES, P. M.

GONZALEZ, D. E. HERNANDEZ, C. D. T. MINTON, R. I. G. MORRISON, R. R. PORTER, R. KEN ROSS, and C. R. VEITCH. 2009. Effects of horseshoe crab harvest in Delaware Bay on Red Knots: are harvest restrictions working? Bioscience 59: 153-164.

OLSEN, B., V. J. MUNSTER, A. WALLENSTEN, J. WALDENSTROM, A. OSTERHAUS, and R. A. M. FOUCHIER. 2006. Global patterns of influenza A virus in wild birds. Science 312: 384-388.

PEARCE, J. M., A. M. RAMEY, H. S. IP, and R. E. GILL. 2010. Limited evidence of trans-hemispheric movement of avian influenza viruses among contemporary North American shorebird isolates. Virus Research 148: 44-50.

PERKINS, D. E., P. A. SMITH, and H. G. GILCHRIST. 2007. The breeding ecology of Ruddy Turnstones (*Arenaria interpres*) in the eastern Canadian Arctic. Polar Record 43: 135-142.

PETO, R., and J. PETO. 1972. Asymptotically efficient rank invariant procedures. Journal of the Royal Statistical Society Series B (Methodological) 135: 185-207.

SMITH, D. R., P. S. POOLER, B. L. SWAN, S. F. MICHELS, W. R. HALL, P. J. HIMCHAK, and M. J. MILLARD. 2002. Spatial and temporal distribution of horseshoe crab (Limulus polyphemus) spawning in Delaware Bay: Implications for monitoring. Estuaries 25: 115-125.

SPACKMAN, E., and D. SUAREZ. 2008. Type A influenza virus detection and quantitation by real-time RT-PCR. Methods in Molecular Biology 436: 19-26.

STALLKNECHT, D. E., M. T. KEARNEY, S. M. SHANE, and P. J. ZWANK. 1990. Effects of pH, temperature, and salinity on persistence of avian influenza viruses in water. Avian Diseases 34: 412-418.

SWAYNE, D. E., D. A. SENNE, and C. W. BEARD. 1998. Avian influenza. *In* A Laboratory Manual for the Isolation and Identification of Avian Pathogens, D. E. Swayne, (ed.). American Association of Avian Pathologists, Kennett Square, Pennsylvania, pp. 150-155.

TSIPOURA, N., and J. BURGER. 1999. Shorebird diet during spring migration stopover on Delaware Bay. Condor 101: 635-644.

U.S. DEPARTMENT OF AGRICULTURE. 2009. U. S. Summary and State Data. *In* 2007 Census of Agriculture. http://www.agcensus.usda.gov/Publications/2007/index.asp. Accessed.

VAN GILS, J. A., V. J. MUNSTER, R. RADERSMA, D. LIEFHEBBER, R. A. FOUCHIER, and M. KLAASSEN. 2007. Hampered foraging and migratory performance in swans infected with low-pathogenic avian influenza A virus. PLoS ONE 2: e184.

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.

WINKER, K., E. SPACKMAN, and D. E. SWAYNE. 2008. Rarity of influenza A virus in spring shorebirds, southern Alaska. Emerging Infectious Diseases 14: 1314-1316.

Table 5.1. Descriptive statistics of radiotagged Ruddy Turnstones and Sanderlings at Delaware Bay, 2007-2008, by species and year.

| | | | Percent (*n*/total) | | | Mean (range) | | |
|---|---|---|---|---|---|---|---|---|
| Species | Year | *n* tagged | AIV positive | Recaptured birds | Female | Weight (g) | Body size index (BSI) | Mass index (MI) (g) |
| Ruddy Turnstone | 2007 | 30 | 0 (0/30) | 3.3 (1/30) | 33 (10/30) | 106.6 (79-125) | 0.60 (-1.31-3.28) | -4.66 (-48.40-16.34) |
| Ruddy Turnstone | 2008 | 30 | 17 (5/29)[a] | 13 (4/30) | 43 (12/28)[b] | 99.5 (81-121) | -0.12 (-2.79-2.68) | -1.08 (-17.29-18.33) |
| Sanderling | 2008 | 20 | 0 (0/14)[a] | 16 (3/19)[c] | n/a | 63.7 (52-84) | -0.53 (-2.75-1.49) | 1.44 (-10.38-20.62) |

[a] One Ruddy Turnstone and six Sanderlings were not tested for AIV in 2008

[b] Excludes two birds of unknown sex

[c] Morphometric and demographic data not recorded for one Sanderling

Table 5.2. Distribution of habitats in which aerial telemetry points (Ruddy Turnstones and Sanderlings) and GIS-generated random points were located.

| | *n* (%) | | | |
|---|---|---|---|---|
| | 2007 | 2008 | | |
| Habitat | Ruddy Turnstone | Ruddy Turnstone | Sanderling | Random Points |
| Day locations | | | | |
| Salt Marsh | 42 (25)[a] | 34 (17) | 16 (14) | 477 (48) |
| Agriculture | 0 (0) | 0 (0) | 0 (0) | 147 (15) |
| Wooded Wetland | 0 (0) | 0 (0) | 2 (1.7) | 103 (10) |
| Forest | 0 (0) | 0 (0) | 0 (0) | 67 (6.7) |
| Residential | 3 (1.8) | 3 (1.5) | 0 (0) | 66 (6.6) |
| Urban | 0 (0) | 0 (0) | 0 (0) | 53 (5.3) |
| Freshwater Wetland | 0 (0) | 0 (0) | 0 (0) | 31 (3.1) |
| Beach | 78 (46) | 70 (36) | 60 (52) | 18 (1.8) |
| Shrubland | 3 (1.8) | 12 (6.1) | 5 (4.3) | 16 (1.6) |
| Barren/Altered Land | 1 (0.6) | 0 (0) | 0 (0) | 11 (1.1) |
| Recreation | 0 (0) | 0 (0) | 0 (0) | 11 (1.1) |
| Open Water | 42 (25) | 77 (39) | 33 (28) | 0 (0)[a] |
| Night locations | | | | |
| Salt Marsh | 69 (82) | 92 (81) | 15 (26) | 477 (48) |
| Agriculture | 0 (0) | 0 (0) | 0 (0) | 147 (15) |
| Wooded Wetland | 0 (0) | 0 (0) | 1 (1.7) | 103 (10) |
| Forest | 0 (0) | 0 (0) | 0 (0) | 67 (6.7) |
| Residential | 0 (0) | 0 (0) | 0 (0) | 66 (6.6) |
| Urban | 0 (0) | 0 (0) | 0 (0) | 53 (5.3) |
| Freshwater Wetland | 0 (0) | 0 (0) | 0 (0) | 31 (3.1) |
| Beach | 1 (1.2) | 3 (2.6) | 10 (17) | 18 (1.8) |
| Shrubland | 1 (1.2) | 0 (0) | 1 (1.7) | 16 (1.6) |
| Barren/Altered Land | 3 (3.6) | 0 (0) | 0 (0) | 11 (1.1) |
| Recreation | 0 (0) | 0 (0) | 0 (0) | 11 (1.1) |
| Open Water | 10 (12) | 19 (17) | 31 (53) | 0 (0)[a] |

[a] Random points were excluded from location in open water

Table 5.3. Between-species comparison of distances from aerial telemetry locations to various habitat classes and features, by daylight class. Asymptotic (2-sample) Kolomogorov-Smirnoff statistics (KSa) test whether the distributions are the same, whereas Wilcoxon Z tests compare central tendency. Positive Z values indicate that Sanderling locations tended to be further from the habitat or feature than Ruddy Turnstone locations. *P*-values of significant tests are bolded.

| | Day | | | | Night | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Wilcoxon | | | | Wilcoxon | |
| Habitat/Feature | $KS_a$ | *P*-value | Z | *P*-value | $KS_a$ | *P*-value | Z | *P*-value |
| Agriculture | 1.27 | 0.079 | -1.66[c] | 0.097 | 2.96 | **<0.0001** | -3.38[c] | **0.0007** |
| Barren/Altered Land | 0.84 | 0.481 | -0.39 | 0.698 | 3.46 | **<0.0001** | 5.71 | **<0.0001** |
| Beach | 1.09 | **0.0003** | -3.95 | **<0.0001** | 3.87 | **<0.0001** | -6.52 | **<0.0001** |
| CFO[a] | 0.95 | 0.329 | -0.39 | 0.699 | 3.83 | **<0.0001** | -5.63 | **<0.0001** |
| Forest | 0.75 | 0.626 | -1.45 | 0.145 | 1.94 | **0.001** | -3.72 | **0.0002** |
| Freshwater Wetland | 1.04 | 0.233 | 0.11 | 0.913 | 2.90 | **<0.0001** | 2.40 | **0.016** |
| Recreation | 1.21 | 0.108 | 1.61 | 0.108 | 3.56 | **<0.0001** | 6.02 | **<0.0001** |
| Residential | 1.16 | 0.137 | -1.60 | 0.109 | 2.12 | **0.0002** | 3.06 | **0.002** |
| Salt Marsh | 1.27 | 0.080 | 2.12 | **0.034** | 3.13 | **<0.0001** | 6.79 | **<0.0001** |
| Shrubland | 0.84 | 0.486 | -0.40 | 0.692 | 3.24 | **<0.0001** | -7.30 | **<0.0001** |
| Urban | 0.98 | 0.290 | 0.97 | 0.332 | 1.21 | 0.105 | -0.02 | 0.402 |
| Wooded Wetland | 1.64 | **0.009** | -2.41 | **0.016** | 0.72 | 0.684 | 0.72 | 0.469 |
| Roads | 0.95 | 0.323 | -0.54 | 0.589 | 1.96 | **0.0009** | 3.37 | **0.0007** |
| Streams | 1.08 | 0.190 | 1.51 | 0.130 | 0.52 | 0.947 | -0.55 | 0.582 |
| Shoreline | 1.02 | 0.246 | -1.23 | 0.218 | 4.31 | **<0.0001** | -8.47 | **<0.0001** |

[a] Confined Feeding Operation

Table 5.4. Timing of departure of radiotagged Ruddy Turnstones and Sanderlings from Delaware Bay, 2007-2008. Days are measured as the number of days after 30 April.

| | | | | | Log-normal fit estimates (95% CI)[a] | | |
|---|---|---|---|---|---|---|---|
| Species | Year | *n* tagged | *n* right-censored | Observed median departure day | 25% departure day | 50% departure day | 75% departure day |
| Ruddy Turnstone | 2007 | 30 | 2 | 31.25 | 29.7 (28.7-30.2) | 30.9 (30.3-31.6) | 32.2 (31.4-33.0) |
| Ruddy Turnstone | 2008 | 30 | 5 | 32.5 | 31.3 (30.4-32.2) | 32.8 (31.9-33.6) | 34.3 (33.5-35.3) |
| Sanderling | 2008 | 20 | 3 | 28.25 | 26.8 (24.6-29.2) | 30.2 (27.9-32.7) | 34.1 (31.1-37.4) |

[a] Kaplan-Meier survival regression

Table 5.5. Effects of date of capture, weight, body size index, mass index, and AIV infection status on springtime departure date of Ruddy Turnstones and Sanderlings from Delaware Bay, by species and year, using the proportional hazards model.

| Species and year | *n* | Log-rank $\chi^2$ | df | *P*-value |
|---|---|---|---|---|
| Ruddy Turnstone 2007 | | | | |
| Capture date | 30 | 8.4 | 2 | **0.015** |
| Sex | 30 | 1.7 | 1 | 0.194 |
| Weight[a] | 30 | 5.4 | 5 | 0.375 |
| BSI[b] | 30 | 7.8 | 5 | 0.170 |
| MI[a] | 30 | 3.6 | 4 | 0.467 |
| AIV status | n/a[c] | n/a | n/a | n/a |
| Ruddy Turnstone 2008 | | | | |
| Capture date | 30 | 0.2 | 2 | 0.917 |
| Sex | 28 | 0.1 | 1 | 0.755 |
| Weight[a] | 30 | 12.1 | 4 | **0.016** |
| BSI[b] | 30 | 6.4 | 5 | 0.269 |
| MI[a] | 30 | 13.6 | 3 | **0.004** |
| AIV status | 29 | 0.9 | 1 | 0.351 |
| Sanderling 2008 | | | | |
| Capture date | 20 | 3.5 | 3 | 0.316 |
| Weight[a] | 19 | 12.5 | 3 | **0.006** |
| BSI[b] | 19 | 16.2 | 4 | **0.003** |
| MI[a] | 19 | 13.8 | 4 | **0.008** |
| AIV status | n/a[c] | n/a | n/a | n/a |

[a] By 10-g class

[b] By SD class

[c] No AIV were isolated from radiotagged Ruddy Turnstones in 2007 or Sanderlings in 2008

## FIGURE LEGENDS

Figure 5.1. Map of Delaware Bay with locations mentioned in the text labeled. State outlines are in gray. Dashed lines indicate the division between upper and lower areas of each state. The location of capture is indicated by a star.

Figure 5.2. Day (solid lines) and night (dashed lines) 75% kernel density contours for (A) Ruddy Turnstones in 2007, (B) Ruddy Turnstones in 2008, and (C) Sanderlings in 2008 during spring migration stopover at Delaware Bay. State outlines and tidal salt marsh habitat are shaded gray.

Figure 5.3. Closeup of night use areas of Ruddy Turnstones and Sanderlings on Cape May Peninsula, New Jersey. Open water is shaded light gray, salt marsh habitat is medium gray, and beaches are dark gray. Other habitats are unshaded.

Figure 5.4. Location of confined animal feeding operations (black dots) in relation to the area used by shorebirds at Delaware Bay. The heavy black line represents the minimum convex polygon (MCP) created by all radio telemetry locations of Sanderlings and Ruddy Turnstones in 2007 and 2008. Note that no CFOs fell within the MCP. State outlines are shown in gray.

Figure 5.5. Distribution of dates on which Ruddy Turnstones were first detected (A) outside of lower New Jersey, and (B) in Delaware.

Figure 5.6. Movements of five Ruddy Tunstones that were AIV-positive at the time of capture. Day locations are circles, night locations are crosses.

Figure 5.7. (A) The proportion of radiotagged Ruddy Turnstones remaining in Delaware Bay by day in May and year. (B) The proportion of Ruddy Turnstones and Sanderlings remaining in Delaware Bay by day in May 2008. Shown are Kaplan-Meier survival curves line of fit (95% CI) by lognormal regression.

Figure 5.8. Estimated mean departure date (95% CI) of radiotagged Ruddy Turnstones by (A) capture date in 2007, (B) mass class in 2008, and (C) MI class in 2008.

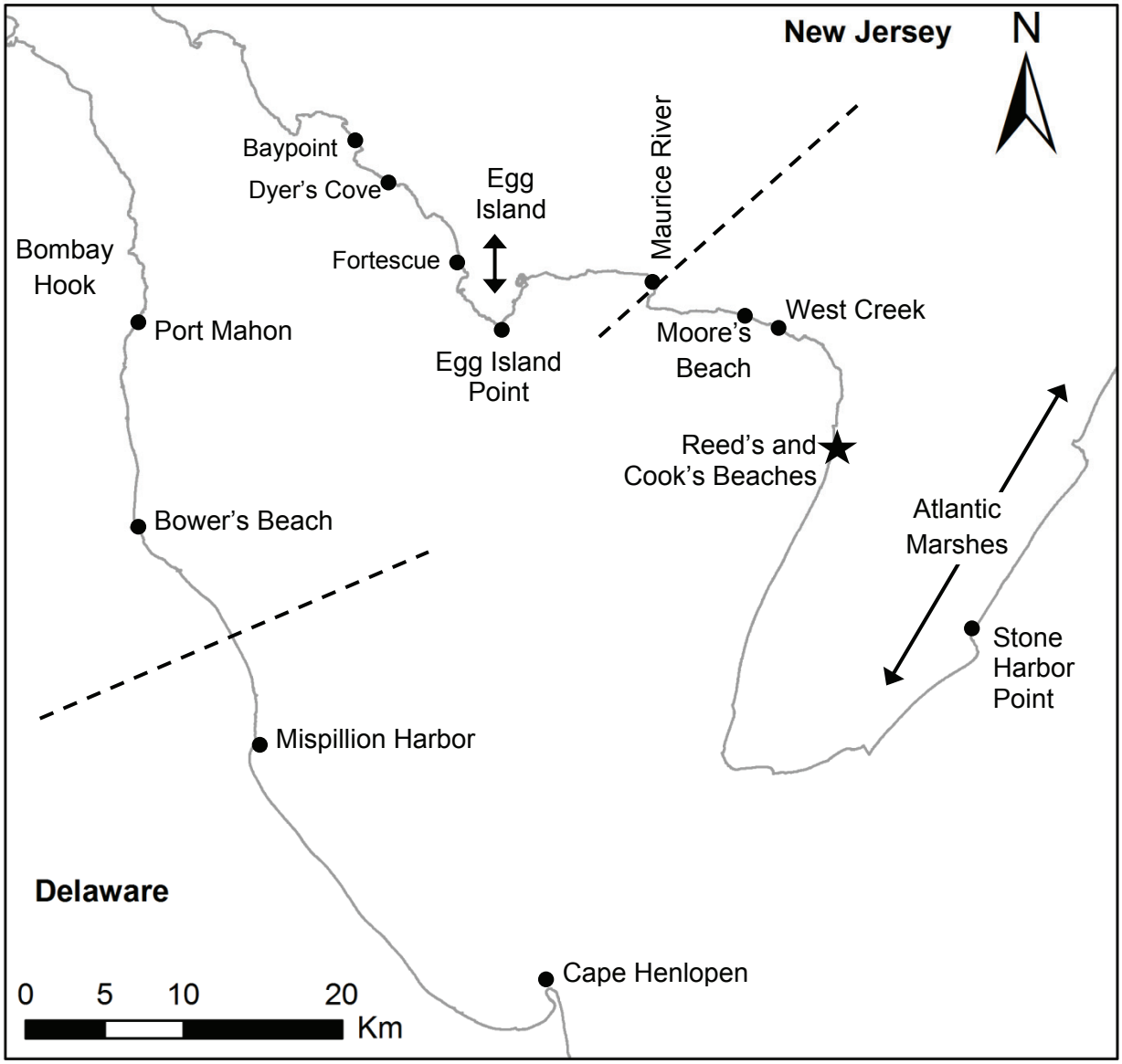

New Jersey
N
Baypoint
Dyer's Cove
Egg
Island
Maurice River
Bombay
Hook
Fortescue
Port Mahon
Egg Island
Point
Moore's
Beach
West Creek
Reed's and
Cook's Beaches
Atlantic
Marshes
Bower's Beach
Stone
Harbor
Point
Mispillion Harbor
Delaware
Cape Henlopen
0 5 10 20
Km

Figure 5.1.

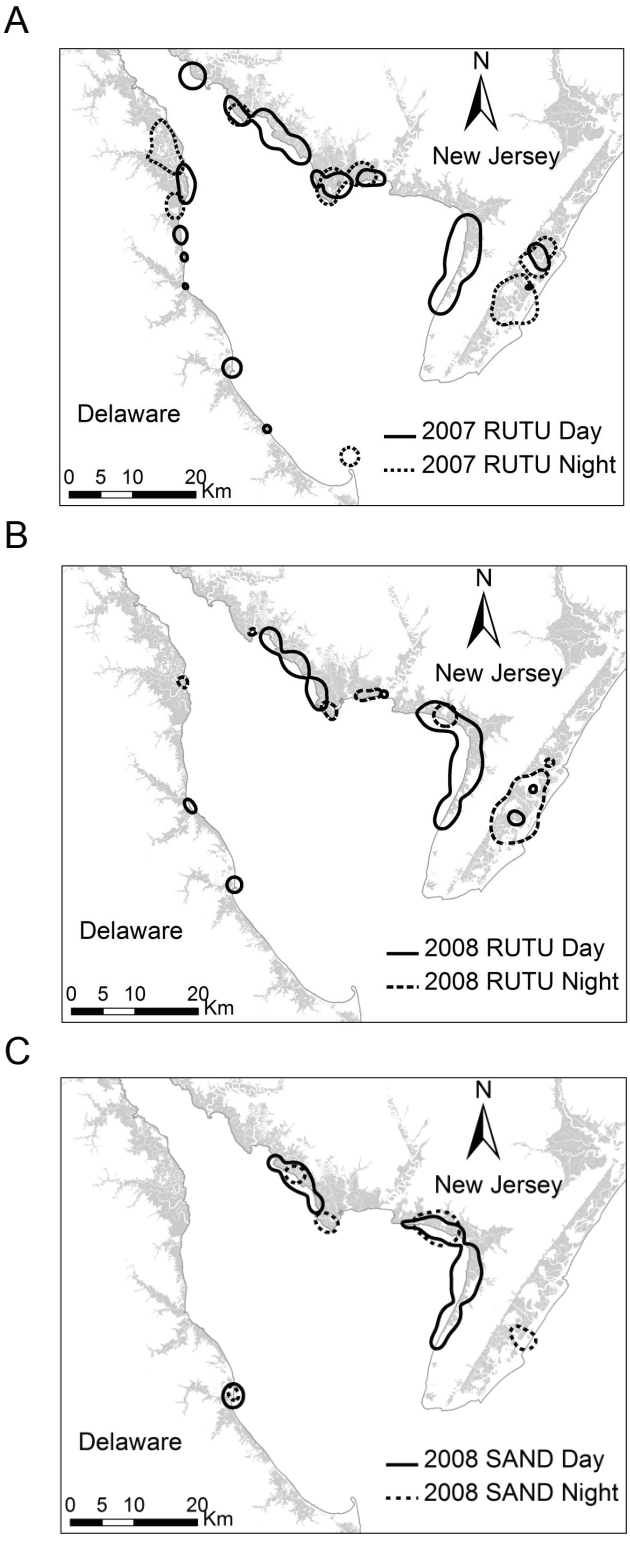

A
N
New Jersey
Delaware
2007 RUTU Day
2007 RUTU Night
0 5 10 20 Km
B
N
New Jersey
Delaware
2008 RUTU Day
2008 RUTU Night
0 5 10 20 Km
C
N
New Jersey
Delaware
2008 SAND Day
2008 SAND Night
0 5 10 20 Km

Figure 5.2.

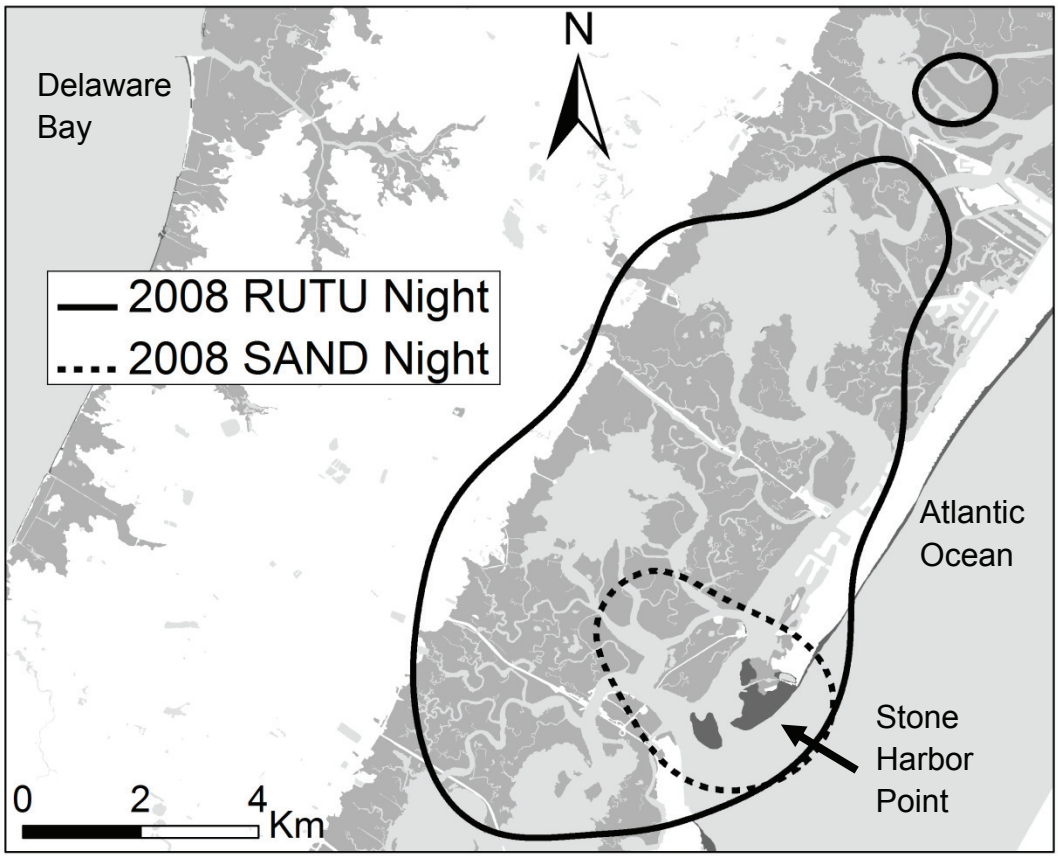

N
Delaware
Bay
2008 RUTU Night
2008 SAND Night
Atlantic
Ocean
Stone
Harbor
Point
0
2
4
Km

Figure 5.3.

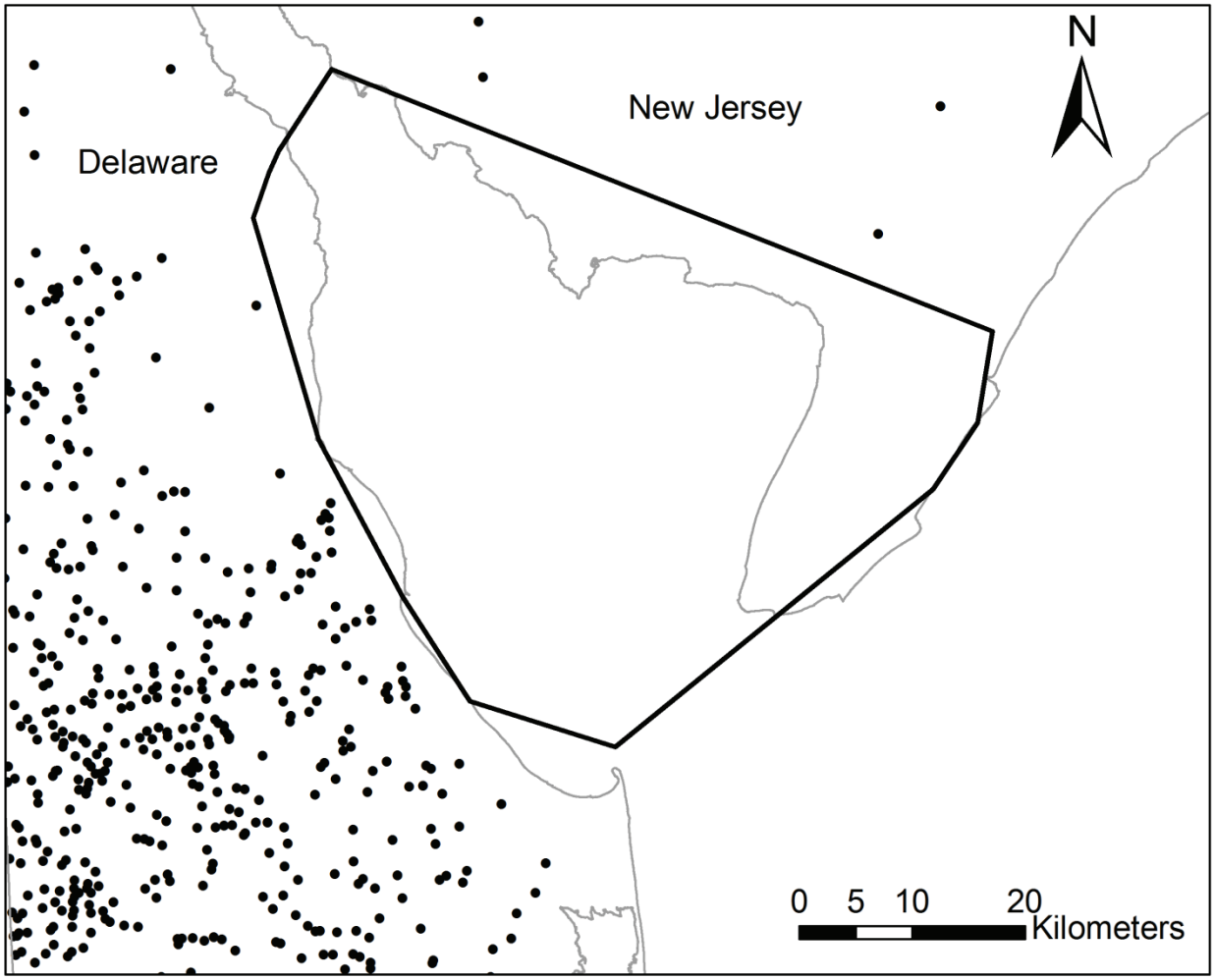



Figure 5.4.

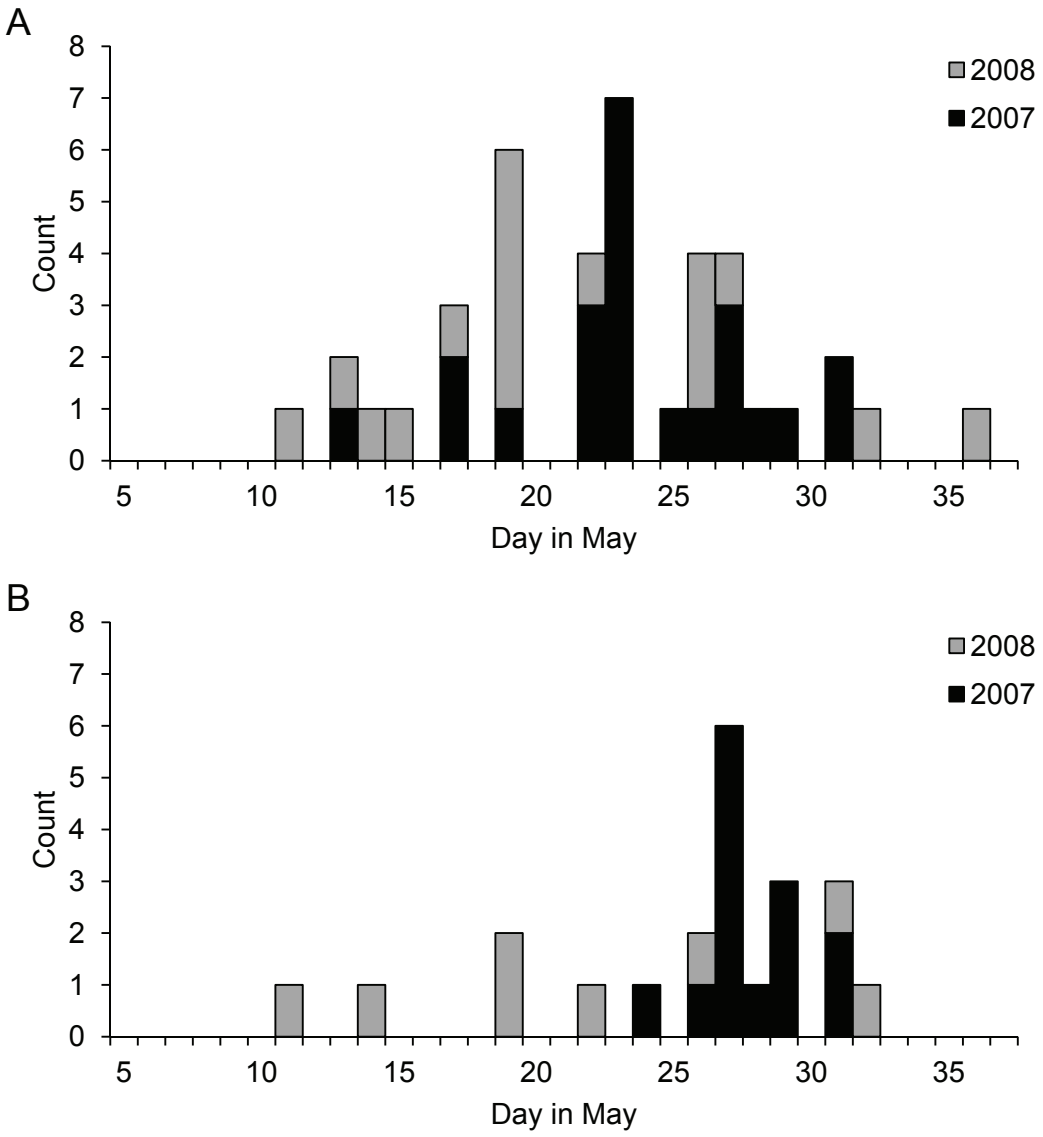



Figure 5.5.

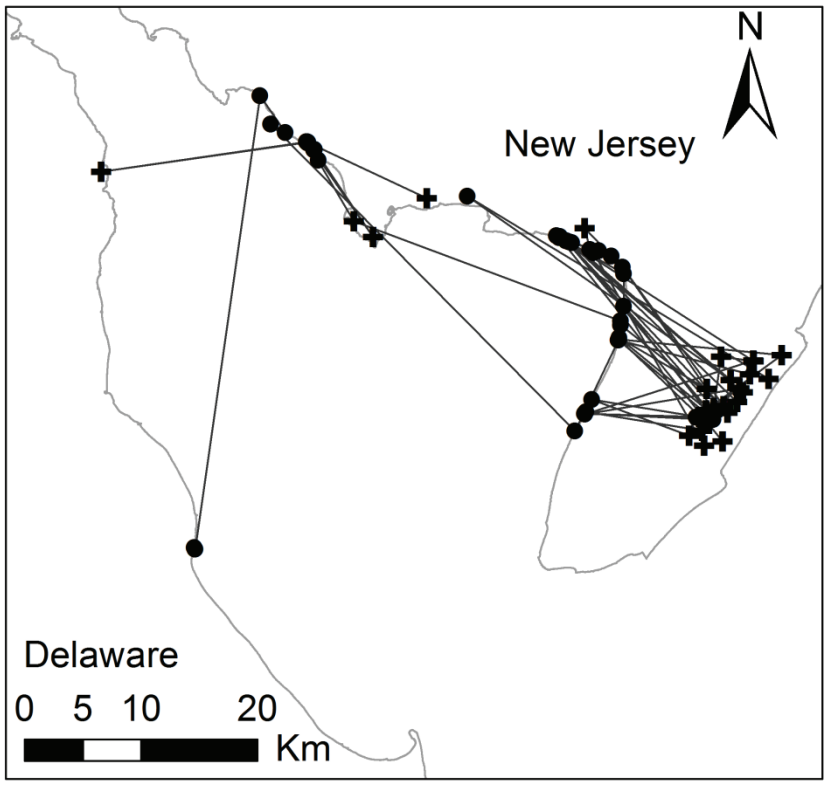



Figure 5.6.

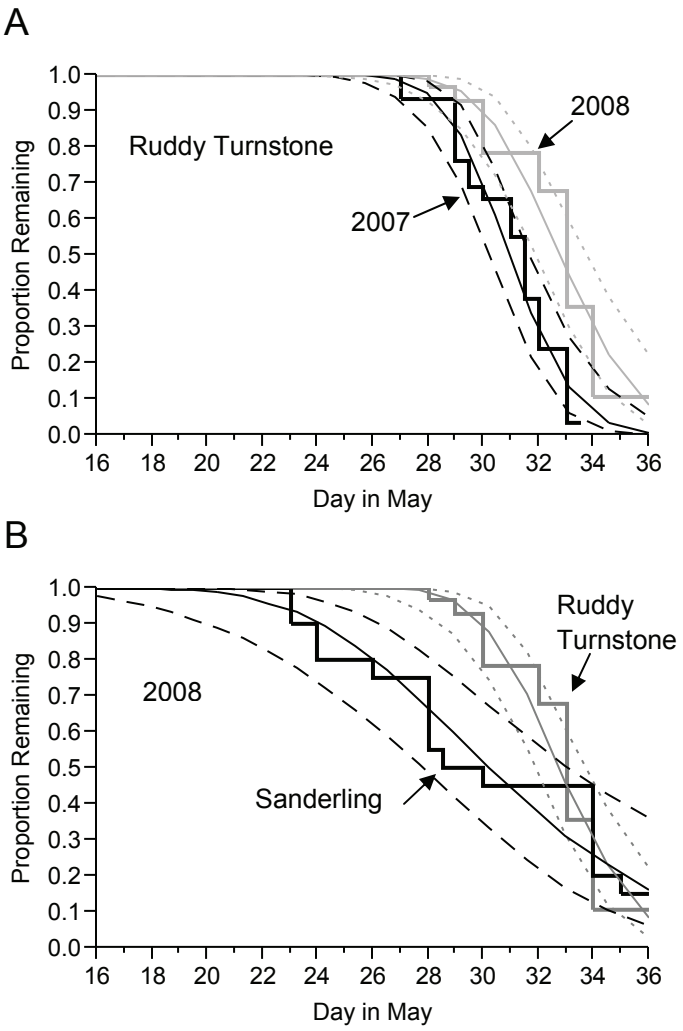

A
Ruddy Turnstone
2008
2007
Proportion Remaining
Day in May
B
2008
Ruddy
Turnstone
Sanderling
Proportion Remaining
Day in May

Figure 5.7.

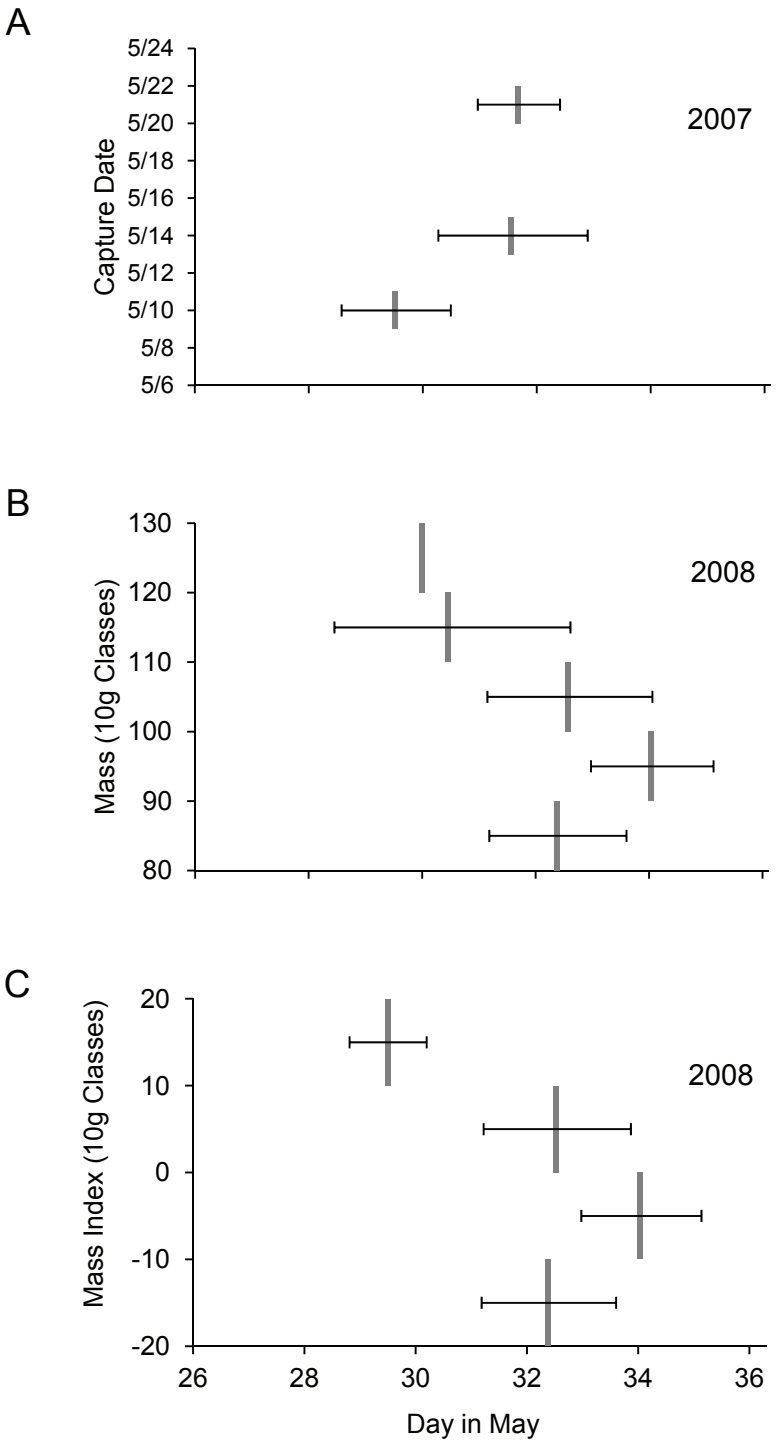

A
5/24
5/22
5/20
5/18
5/16
5/14
5/12
5/10
5/8
5/6
Capture Date
2007
B
130
120
110
100
90
80
Mass (10g Classes)
2008
C
20
10
0
-10
-20
Mass Index (10g Classes)
2008
26
28
30
32
34
36
Day in May

Figure 5.8.

# CHAPTER 6

# ANNUAL SURVIVAL OF RUDDY TURNSTONES IS NOT AFFECTED BY NATURAL INFECTION WITH LOW PATHOGENICITY AVIAN INFLUENZA VIRUSES[1]

[1] Maxted, A. M., R. R. Porter, M. P. Luttrell, V. H. Goekjian, A. D. Dey, K. S. Kalasz, L. J. Niles, and D. E. Stallknecht. Submitted to *The Auk* on 9/18/2011.

## ABSTRACT

The population of Ruddy Turnstones (*Arenaria interpres morinella*) that migrates through Delaware Bay has undergone severe declines in recent years, attributable to reduced availability of horseshoe crab (*Limulus polyphemus)* eggs at this critical spring migration stopover site. Concurrently, this population has experienced annual occurrences of low pathogenicity avian influenza virus (AIV) infections at this same site. Using a prospective cohort study design with birds individually flagged during May-June 2006-2008, we evaluated resighting rates (a proxy for annual survival) between AIV-infected and uninfected birds one yr after capture, testing, and measurement. Overall resighting rate was 46%, which varied by year and increased with relative mass of the bird when captured. Also, birds from which blood samples were collected were more likely to be resighted than those that were unsampled; however, blood-sampled birds were relatively heavier than unsampled birds. Resighting rates were not different between AIV-infected and uninfected birds in any period. In multivariate analyses, infection status was also unrelated to resighting rate after controlling for year, day, state, sex, body size, mass index, or whether the bird was blood-sampled. Thus, apparent annual survival in Ruddy Turnstones was not reduced by AIV infection at this migratory stopover. However, it is unknown whether intestinal AIV infection might cause subtle reductions in weight gain which could negatively influence reproduction.

*Key Words:* Ruddy Turnstone, *Arenaria interpres*, avian influenza virus, annual survival, resighting rate, Delaware Bay, shorebird

## INTRODUCTION

The population of Ruddy Turnstones (*Arenaria interpres morinella*) that migrates through Delaware Bay each spring en route to breeding grounds in the Arctic has undergone significant population declines over the last two decades (Niles et al., 2009). These declines are thought primarily to be the consequence of reduced availability of their main food source and foraging habitats at this location (Burger et al., 2004; Niles et al., 2009). Delaware Bay is the last stopover site before reaching their breeding grounds, and weight gained here must be sufficient to sustain birds for the last leg of migration and through periods of potentially harsh weather upon arrival in the Arctic (Moore et al., 2005). An estimated 50-80% of the *morinella* subspecies uses this site each spring (reviewed by U. S. Fish and Wildlife Service, 2003) making it a geographic and energetic bottleneck in their annual cycle (Buehler and Piersma, 2008).

Severe drops in spawning horseshoe crabs (*Limulus polyphemus*) numbers in Delaware Bay since the early 1990's, due to harvesting as bait for an emerging conch fishery, have led to measureable declines in the number of their eggs available to time-constrained migratory shorebirds during their stopover period (reviewed by Niles et al., 2009). At least six shorebird species migrating through Delaware Bay have undergone population declines in the last two decades; peak Ruddy Turnstone numbers, as determined by aerial counts, declined by 77% during 1998-2007 (Niles et al., 2009). Much evidence has linked shorebird population declines to the reduced availability of horseshoe crab eggs at this critical stopover (Baker et al., 2004; Niles et al., 2009). An increasing proportion of Red Knots (*Calidris canutus rufa*) migrating through Delaware Bay are poorly conditioned late in the stopover season, just prior to the major period of departure (Baker et al., 2004). If the same is true for Ruddy Turnstones, then fewer body stores could be immediately available upon arrival at their breeding grounds. Because breeding activities commence within a few days of arrival, females arriving with greater reserves could accelerate the onset of egg production over those whose energy for breeding is acquired solely on location (Morrison and Hobson, 2004), which could confer a reproductive advantage

(Moore et al., 2005). Although breeding onset dates remain relatively constant (Perkins et al., 2007), weather conditions such as snow cover and available food resources upon arrival can be highly variable and energy expenditure during incubation under harsh conditions can be high (Piersma and Morrison, 1994). Excess body stores acquired while at Delaware Bay, then, could confer both survival and reproductive advantages. To reach the Arctic (without excess energy reserves), Ruddy Turnstones must weigh an estimated 150 g upon departure (Gudmundsson et al., 1991), or gain more than 50% of their lean body mass while at Delaware Bay (reviewed by U. S. Fish and Wildlife Service, 2003).

Although the primary population concerns for subspecies *morinella* Ruddy Turnstones are reduced availability of food and foraging habitats at Delaware Bay (Burger et al., 2004; Niles et al., 2009), this population also is infected annually with low pathogenicity avian influenza viruses (AIV) at this location. Springtime prevalence ranges from 4.6-18.6% (Krauss et al., 2004; Hanson et al., 2008; A. Maxted, unpublished data). Although no measurable mortality has been associated with these infections, whether AIV infection has had any role in recent population declines remains to be investigated. Avian influenza viruses replicate primarily within the intestine of their natural waterbird hosts, such as ducks (Webster et al., 1978). We hypothesize that the additional stress of an intestinal disease on digestion during this period of limited food availability could reduce weight gain, perhaps leading to population effects such as reduced annual survival or reproductive performance (Tompkins et al., 2002; McWilliams and Karasov, 2005; Moore et al., 2005). Our aim was to investigate potential effects of AIV infection on annual survival in Ruddy Turnstones. Specific objectives were 1) to identify characteristics of individual birds or other potential factors such as year and location that are associated with resighting rate in future years and describe these associations; 2) to determine if any difference in resighting probabilities between AIV-infected and uninfected birds existed and evaluate each potential covariate's role as a confounder to this relationship; and 3) evaluate if resighting rate is reduced in AIV-infected birds after significant confounders are taken into account.

## MATERIALS AND METHODS

### Study design

This is a prospective cohort study utilizing mark-resight data. Birds were sampled for AIV at the time of capture and later classified as infected (positive) or not infected (negative) based on laboratory results of virus isolation on cloacal swabs. All sampled birds that carried an alphanumeric inscribed flag were followed through subsequent spring stopover seasons (i.e., years) and whether each individual bird was resighted at Delaware Bay in the next year was the outcome (independent) variable. Resighting rates were compared between groups of birds testing either AIV-positive or -negative.

### Field and laboratory methods

Field work was conducted during May-early June, 2006-2009 at Delaware Bay (39°N, 75°W). Shorebirds and gulls were captured with cannon nets as part of long-term population studies. Following banding and measurement, cloacal swab samples for virus isolation were collected, stored, processed, and tested in embryonated chicken eggs as previously described (Hanson et al., 2008). Presence of AIV was confirmed by hemagglutination (Swayne et al., 1998) and reverse transcriptase polymerase chain reaction (RT-PCR) for matrix gene (Spackman and Suarez, 2008) on allantoic fluid. All viruses were low pathogenicity (LPAI) subtypes (unpublished data). Blood samples totaling ≤0.8 mL (0.4-1.0% of body mass in g) were collected by jugular venipuncture from a nonsystematic subset of swabbed birds. Detailed virus isolation and serology results will be reported elsewhere.

Throughout each spring stopover period, teams of experienced observers scanned shorebird flocks around Delaware Bay for marked individuals. Scanners concentrated their efforts on beaches where the largest numbers of birds congregated, enabling a large proportion of marked individuals to be seen (Gillings et al., 2009). Although not every marked bird was detected in this manner and a small proportion of birds flagged at Delaware Bay likely used

alternate stopover sites during spring migration (e.g., Placyk and Harrington, 2004), we assume resighting rates are proportional to annual survival and are used as a proxy measure to compare groups of individual animals within the same population. Morphometric and demographic data at the time of capture and resighting data were obtained from the Shorebird Resighting Database (www.bandedbirds.org). Research was conducted under University of Georgia Animal Care and Use Committee approval and state and federal scientific collection permits.

**Statistical analyses**

Only individually identifiable AIV-tested birds were included in analyses. Data included information recorded at the time of capture during 2006-2008 (measurements of culmen, head+bill, and wing lengths; mass; sex; day; location; whether it was bled; and virus isolation results) and resightings during 2007-2009. Individuals were recorded as either “seen” or “not seen” in the next year. When birds were captured twice in the same year ($n$=11), only data from one randomly-selected date was included in analyses. For birds captured in more than one year ($n$=17), each capture was considered independent and was included. Individual birds can become reinfected with AIV in subsequent years (unpublished data), suggesting that infection probability is independent between years. For consistency, we did not include recaptures as resightings.

Variables evaluated as confounders included capture year, day, state (Delaware or New Jersey), sex (male or female based on plumage characteristics), and physical attributes such as relative mass and body size. A Body Size Index (BSI) was computed as the first principal component from principal components analysis (PCA) on wing, culmen, and combined head and bill lengths. A Mass Index (MI) was calculated as the number of grams above or below the expected mass on that capture date; expected mass was determined by 4- or 5-node knotted spline least-squares regression all captured bird masses on date, for each year. Simple contingency analyses (for categorical variables) or logistic regression (for continuous variables)

were performed to analyze potential relationships between AIV infection, potential confounders, and whether a bird was resighted. Additionally, multivariate logistic regression was used to evaluate resighting probability as a function of AIV infection, both when controlling for each single covariate, and in combination with multiple covariates. For the latter analysis, all potential confounders and their two-way interactions were evaluated in mixed stepwise regression where significance probabilities for a term to enter or leave the model were set at 0.20, and lower order terms were included when interaction terms were significant. Interaction terms involving the state of capture were excluded because no birds were sampled in Delaware in 2007. Candidate models from the last several steps were evaluated manually for fit and parameter significance, and the model with the lowest Akaike's Information Criterion (corrected for small sample size; $AIC_c$) was selected as the "best" model (Burnham and Anderson, 2002). Finally, significance of adding AIV infection status to this model (i.e., the significance of AIV status to resighting status, controlling for all other significant variables) was ascertained.

Computations and statistical analyses were carried out in program JMP version 8 (SAS Institute Inc., Cary, NC). Likelihood ratio $\chi^2$ tests are reported. Confidence intervals of proportions were computed using the score method (Agresti and Coull, 1998).

## RESULTS

### General resighting rates

During 2006-2008, 2,928 individual Ruddy Turnstones were captured, measured, and flagged (if not already flagged). Of these, 1,233 (42%) were tested for AIV by virus isolation; overall prevalence was 13% (95% CI: 11-15%). Among tested birds, 46% (567 of 1,233) were seen one yr following capture.

Among birds captured on the same day and location, resighting rates were not different between 1,233 AIV-tested and 314 untested birds (46% vs. 44%; $\chi^2$=0.36, df=1, *P*=0.55). Thus,

we do not believe that the additional brief handling for AIV sampling affected resighting rate in the following year.

Birds were more likely to be seen in the state in which they were captured. Birds resighted in Delaware were 4.1 (3.3-5.1) times more likely to have been captured in Delaware than New Jersey and birds seen in New Jersey were 20.4 (6.6-62.7) times more likely to have been captured in New Jersey than Delaware. Among resighted birds, 77% (436 of 567) were seen only in the state of capture, 18% (101 of 567) were seen only in the opposite state, and 5.3% (30 of 567) were seen in both states.

**Potential covariate screening**

Table 6.1 contains the results of contingency analyses and simple logistic regression between possible confounding variables and whether or not a bird was seen one yr later (resighting rate). Resighting rates varied by year of capture, MI, and whether or not a bird was blood-sampled (Fig. 6.1). Resighting rates were higher for birds captured in 2006 than in 2007-2008 (55% vs. 44%; $\chi^2$=10.9, df=1, *P*=0.001; relative risk=1.3 [1.1-1.4]). Resighting rate increased with MI; birds that were heavier than expected on their capture date (i.e., with MI>0) were 1.3 (1.1-1.5) times more likely to be seen in the next year than birds that were lighter than expected (MI<0). Interestingly, birds that were blood-sampled for serology studies in addition to cloacal swabbing were 1.2 (1.0-1.3) times more likely to be seen in the next year compared to birds that were only swabbed (Fig. 6.1). However, on average, MI of blood-sampled birds was 6.4 g greater than that of unsampled birds (+3.1 g vs. -3.3 g; *t*=7.7, df=1,228, *P*<0.0001) and after controlling for MI, resighting probability was not related to whether or not a bird was blood-sampled (whole model: $\chi^2$=16.1, df=2, *P*=0.0003; MI effect: $\chi^2$=10.3, df=1, *P*=0.001; bled status effect: $\chi^2$=2.8, df=1, *P*=0.096; Fig. 6.1). Thus, the difference in resighting rate is probably attributable to the difference in MI between blood-sampled and unsampled groups. One of two

birds that were inadvertently blood-sampled twice within the same stopover season, each seven days apart, was seen in the following year.

**Effect of AIV infection status on resighting rate**

Seventy-three of 163 (45%) AIV-positive Turnstones were seen in the year following capture, compared to 494 of 1,070 (46%) AIV-negative Turnstones; this difference is not significant (Table 6.3). There were no significant differences in resighting probability at one yr between AIV-positive and AIV-negative birds, for any capture year cohort (Table 6.2; Fig. 6.2). Infection status was also not associated with resighting probability at one yr post-capture after controlling for year, day, state, sex, BSI, MI, or whether or not the bird had been bled (Table 6.4; Figs. 6.2-6.3). Models including interaction terms between AIV infection status and each of the above variables were also evaluated but no interactions were significantly associated with resighting rate (all $P>0.05$; data not shown). In multivariate models, after controlling for all significant confounders, AIV infection status was not associated with resighting probability at one yr post-capture (Table 6.3).

## DISCUSSION

Only a few recent studies have examined the effects of LPAI infection on wild bird populations. A small number of LPAIV-infected Bewick's swans (*Cygnus columbianus bewickii*) had lower feeding rates and rates of apparent mass change, exhibited delayed migration, and migrated a shorter distance to the next stopover site than did uninfected birds (van Gils et al., 2007). Resighting rates in the following year were apparently equal between antibody positive and negative groups (3 of 4 and 5 of 8 seen again, respectively), but the amount of time that had passed between exposure to AIV and the time of blood sampling was unknown. Fall-migrating Mallards (*Anas platyrhynchos*) exhibited no differences in stopover duration, speed of migration, the time or distance between banding and band recovery, or the geographical distribution of band recoveries between AIV-infected and uninfected birds even though infected birds weighed significantly (but slightly) less than uninfected birds at the time of capture

(Latorre-Margalef et al., 2009). No recaptures between years were reported. Similarly, there were no difference in short-term (12 d) dispersal between AIV-infected and uninfected Greater White-fronted Geese (*Anser albifrons albifrons*), but infected birds weighed less than uninfected birds at the time of capture in one of four years (Kleijn et al., 2010). Again, it is unknown whether any tested birds were resighted in a subsequent winter. To the best of our knowledge, this is the first study to compare annual resighting or recapture rates of large numbers of individually marked wild birds naturally infected with AIV to those of uninfected birds. Further, the presence of both AIV infected and uninfected birds in nearly every group of birds we sampled (data not shown) meant that year, day, state, location and certain demographic features of the population (e.g., on average males arrive at Delaware Bay earlier than females, unpublished data) were represented in both groups.

In addition to not surviving to the following spring, there are a number of reasons why a flagged bird may not be seen: 1) it may use an alternate stopover site during northward migration and not be present at Delaware Bay that spring; 2) it is missed by scanning efforts, e.g., by using a remote location within the Bay that is not frequented by the scanning team; or 3) its flag code was recorded incorrectly, was ascribed to the wrong species, or was excluded by some other clerical error. We estimate that >25% of birds that are alive are missed by scanning efforts each year, thus our resighting rates represent only the minimum proportion of birds alive in that year. For our resighting rate comparisons between AIV infected and uninfected birds to be valid, AIV infection itself must not alter the probability that a bird uses Delaware Bay (vs. an alternative stopover site) during a later migration or its detection probability while visiting Delaware Bay in a later year, given that the bird survived. While we do not have information to directly support or refute these assumptions, we have little reason to believe that acute AIV infection substantially alters the stopover behavior of Turnstones in subsequent years. If AIV infection in a previous year does indeed cause a bird to seek alternative stopover sites or areas within Delaware Bay, then apparent survival in the infected class would be reduced. We did not

detect differences in apparent survival between classes, so we can safely assume that AIV infection does not cause Ruddy Turnstones to alter their spring migration patterns.

Resighting rate of flagged Ruddy Turnstones in the first year post-capture were high (46%); similar to resighting rate of flagged, sympatric Red Knots (41%; Gillings et al., 2009). However, two studies of wintering Turnstones in the United Kingdom reported annual resighting rates of 95% and 86%, respectively (Metcalfe and Furness, 1985; Burton and Evans, 1997); our resighting rate seems low by comparison. These studies were conducted over areas much smaller than Delaware Bay, over longer time spans, with smaller total numbers of Turnstones. It is important to remember, however, that the goal of this study was to compare resighting rates between two groups of birds rather than precisely estimate annual survival (e.g., for use in population dynamical models).

Ruddy Turnstones are relatively faithful to their feeding areas both while at the Bay, and from year to year. Thus, a bird banded in Delaware was more likely to be seen in Delaware than in New Jersey (and vice versa) in following years. Since AIV positive and negative birds were found at all locations, however, our comparisons between these groups should not be affected.

It is not clear why resighting rates are lower in the 2007 and 2008 capture cohorts than in birds captured in 2006, Resighting rate depends not only on annual survival, but also on use of Delaware Bay rather than alternate locations, and detection probability while at the Bay. Likely, between-year variations in bird use of areas accessible to resighters, weather conditions affecting detection probability, and resighting effort (e.g., person-hours spent) account for most of the difference. Our analysis was limited to birds that were tested for AIV, and while we tested birds throughout the stopover season in 2007 and 2008 (over 25 and 29 d, respectively) sampling was limited to an 8 d span in 2006. However, sampling day in May was not associated with resighting rate, either alone (Table 6.1) or when considered by year (data not shown, $P$=0.618). A larger proportion of birds were sampled in Delaware in 2006 than in 2008 (27% vs. 10%; $\chi^2$=44.5, df=1, $P$<0.0001), so these difference in resighting rates may simply be due to the

state in which birds were sampled. Still, resighting rates for birds captured in 2006 were higher than those captured in 2008 for both states (Fig. 6.2), suggesting that differences in detection probability and resighting effort play the larger role in yearly resighting rates. Variable climatic conditions or food resources at other parts of the birds' annual cycle may have caused fluctuations in survival between years, but so far, no specific factors have been identified.

It has been well-documented in migratory birds that better condition (i.e., higher mass in relation to body size, presumably in the form of fat stores) is associated with higher annual survival rates (reviewed by Newton, 2006). This may be especially true of long-distance migrant shorebirds that often cross large bodies of water during legs of their migration; birds that do not carry sufficient fuel in the form of fat to reach the next stopover site may perish en route (Pfister et al., 1998). At Delaware Bay, Red Knots known to survive to a year later were significantly heavier than birds that were never seen again (Baker et al., 2004). In subspecies *islandica* Red Knots, known survivors had significantly greater condition index (equivalent to MI in this study) than the general population (Morrison et al., 2007). Resighting rates of southward-bound Semipalmated Sandpipers (*Calidris pusilla*) increased with estimated percent body fat at departure (Pfister et al., 1998). Ruddy Turnstones in this study followed the same pattern; birds with higher relative mass were more likely to be seen in the following year.

Interestingly, AIV infection probability also increased with MI; birds in the heaviest quartile were 2.7 (1.7-4.4) times more likely to be infected than those in the lightest quartile (data not shown). However, since AIV infection status was not related to resighting probability even after controlling for the effects of MI, it is likely that MI acts independently on both resighting rate and AIV infection probability. This result provides further support that survival to the next year is not affected by AIV infection status. However, a large proportion of this Ruddy Turnstone population likely becomes infected each spring at Delaware Bay; >50% of the population seroconverts during the stopover period (unpublished data). Our measure of the relationship between MI and AIV infection status is only a reflection of the risk of infection at the

time of sampling (e.g., having been present at the Bay longer than the average bird, therefore having increased exposure and likelihood of infection) rather than the risk of infection at any time during the stopover. Likewise, we may not be able to detect an association between AIV infection and resighting in a later year because some birds that were AIV negative at the time of sampling actually were infected either before or after that time point (within the same season), thus leading to misclassification biases.

Initially, it appeared that birds that had been bled were more likely to be seen in the following year than those that were only swabbed, a result that would be counter to the commonly held belief that bleeding has either neutral or negative effects on survival (Sheldon et al., 2008; Brown and Brown, 2009). Closer inspection revealed that resighting rate in bled birds was skewed by the fact that bled birds tended to be heavier than average, for which resighting rates truly were higher. Although no systematic attempts were made to bleed only birds that had achieved a certain weight, or those that were among the heaviest captured on a given day, apparently our selection of birds to bleed was subject to investigator bias. This unexpected finding underscores the need to control for potential confounding variables in statistical analyses, especially those that may consciously or unconsciously affect inclusion or exclusion of a study subject. Only one other study has evaluated potential effects of blood sampling on shorebirds, and like ours, found that annual resighting rates were not different between bled and unbled groups, but that the timing of sampling during the breeding season could cause nest desertion in some species (Colwell et al., 1988). A recent evaluation of blood sampling in Cliff Swallows (*Petrochelidon pyrrhonota*) estimated that 1-year survival rates were reduced by a greater amount in bled birds from nesting colonies that had not been fumigated against ectoparasites compared to those that were parasite-free (Brown and Brown, 2009), suggesting that birds already stressed by ectoparasitism were more likely to be negatively affected by blood sampling. Although this population of Ruddy Turnstones may already be stressed by inadequate

food resources and AIV infection while at Delaware Bay, no negative effects of blood sampling were identified.

Although we found no effect of AIV infection on annual survival, it is still possible that AIV infection may be associated with other population effects such as reduced reproductive success or fecundity. Turnstones rely on fat stores acquired at Delaware Bay not only for travel to breeding grounds in the Arctic, but to sustain them through uncertain weather and environmental conditions upon arrival and to initiate breeding (Morrison and Hobson, 2004; Moore et al., 2005; Drent et al., 2006). If breeding fails, birds begin southward migration immediately and do not attempt to renest (Nettleship, 1973), hence, failed breeders invest more in their own survival than reproductive activities. Slight reductions in digestion efficiency (and mass gain) owing to intestinal AIV infection during the stopover at Delaware Bay may be sufficient to reduce reproductive ability, even if there is no apparent effect on annual survival (Moore et al., 2005). However, reproductive success of Ruddy Turnstones in relation to AIV infection status at Delaware Bay is unknown and this possible association remains to be tested.

## ACKNOWLEDGEMENTS

This research was funded through Specific Cooperative Agreement 58-6612-2-0220 between the Southeast Poultry Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture (USDA-ARS) and the Southeastern Cooperative Wildlife Disease Study (SCWDS). Additionally, this work was funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Department of Health and Human Services, under contract no. HHSN266200700007C. The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. Banding data and morphometric measurements are the property of the Natural Heritage & Endangered Species Program, Division of Fish and Wildlife, Delaware Department of Natural Resources and Environmental Control (DNREC), and the Nongame and Endangered Species Program, Division of Fish and Wildlife, New Jersey Department of Environmental Protection

(NJDEP), and we thank these agencies for granting access to these data. We are grateful to the many people, too numerous to list here, who provided field and laboratory support.

## LITERATURE CITED

AGRESTI, A., and B. A. COULL. 1998. Approximate is better than "exact" for interval estimation of binomial proportions. American Statistician 52: 119-126.

BAKER, A. J., P. M. GONZALEZ, T. PIERSMA, L. J. NILES, I. D. S. DO NASCIMENTO, P. W. ATKINSON, N. A. CLARK, C. D. T. MINTON, M. K. PECK, and G. AARTS. 2004. Rapid population decline in Red Knots: fitness consequences of decreased refueling rates and late arrival in Delaware Bay. Proceedings of the Royal Society B: Biological Sciences 271: 875-882.

BROWN, M. B., and C. R. BROWN. 2009. Blood sampling reduces annual survival in Cliff Swallows (*Petrochelidon pyrrhonota*). Auk 126: 853-861.

BUEHLER, D. M., and T. PIERSMA. 2008. Travelling on a budget: predictions and ecological evidence for bottlenecks in the annual cycle of long-distance migrants. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 247-266.

BURGER, J., C. JEITNER, K. CLARK, and L. J. NILES. 2004. The effect of human activities on migrant shorebirds: successful adaptive management. Environmental Conservation 31: 283-288.

BURNHAM, K. P., and D. R. ANDERSON. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. 2nd edition. Springer, New York. 488 pp.

BURTON, N. H. K., and P. R. EVANS. 1997. Survival and winter site-fidelity of Turnstones *Arenaria interpres* and Purple Sandpipers *Calidris maritima* in northeast England. Bird Study 44: 35-44.

COLWELL, M. A., C. L. GRATTO, L. W. ORING, and A. J. FIVIZZANI. 1988. Effects of blood sampling on shorebirds: injuries, return rates, and clutch desertions. The Condor 90: 942-945.

DRENT, R., A. FOX, and J. STAHL. 2006. Travelling to breed. Journal of Ornithology 147: 122-134.

GILLINGS, S., P. W. ATKINSON, A. J. BAKER, K. A. BENNETT, N. A. CLARK, K. B. COLE, P. M. GONZALEZ, K. S. KALASZ, C. D. T. MINTON, L. J. NILES, R. C. PORTER, I. DE LIMA SERRANO, H. P. SITTERS, J. L. WOODS, and T. L. SHAFFER. 2009. Staging behavior in Red Knot (*Calidris canutus*) in Delaware Bay: implications for monitoring mass and population size. Auk 126: 54-63.

GUDMUNDSSON, G. A., A. LINDSTROM, and T. ALERSTAM. 1991. Optimal fat loads and long-distance flights by migrating Knots *Calidris canutus,* Sanderlings *C. alba*, and Turnstones *Arenaria interpres*. Ibis 133: 140-152.

HANSON, B. A., M. P. LUTTRELL, V. H. GOEKJIAN, L. NILES, D. E. SWAYNE, D. A. SENNE, and D. E. STALLKNECHT. 2008. Is the occurrence of avian influenza virus in Charadriiformes species and location dependent? Journal of Wildlife Diseases 44: 351-361.

KLEIJN, D., V. J. MUNSTER, B. S. EBBINGE, D. A. JONKERS, G. J. D. M. MÜSKENS, Y. VAN RANDEN, and R. A. M. FOUCHIER. 2010. Dynamics and ecological consequences of avian influenza

virus infection in Greater White-fronted Geese in their winter staging areas. Proceedings of the Royal Society B: Biological Sciences 277: 2041-2048.

KRAUSS, S., D. WALKER, S. P. PRYOR, L. NILES, C. H. LI, V. S. HINSHAW, and R. G. WEBSTER. 2004. Influenza A viruses of migrating wild aquatic birds in North America. Vector-Borne and Zoonotic Diseases 4: 177-189.

LATORRE-MARGALEF, N., G. GUNNARSSON, V. J. MUNSTER, R. A. M. FOUCHIER, A. D. M. E. OSTERHAUS, J. ELMBERG, B. OLSEN, A. WALLENSTEN, P. D. HAEMIG, T. FRANSSON, L. BRUDIN, and J. WALDENSTRÖM. 2009. Effects of influenza A virus infection on migrating Mallard ducks. Proceedings of the Royal Society B: Biological Sciences 276: 1029-1036.

MCWILLIAMS, S. R., and W. H. KARASOV. 2005. Migration takes guts: digestive physiology of migratory birds and its ecological significance. *In* Birds of Two Worlds: the Ecology and Evolution of Migration, R. Greenberg, and P. P. Marra, (eds.). Johns Hopkins University Press, Baltimore, pp. 67-78.

METCALFE, N. B., and R. W. FURNESS. 1985. Survival, winter population stability and site fidelity in the Turnstone *Arenaria interpres*. Bird Study 32: 207-214.

MOORE, F. R., R. J. SMITH, and R. SANDBERG. 2005. Stopover ecology of intercontinental migrants: en route problems and consequences for reproductive performance. *In* Birds of Two Worlds: the Ecology and Evolution of Migration, R. Greenberg, and P. P. Marra, (eds.). Johns Hopkins University Press, Baltimore, pp. 251-261.

MORRISON, R. I. G., and K. A. HOBSON. 2004. Use of body stores in shorebirds after arrival on High-Arctic breeding grounds. Auk 121: 333-344.

MORRISON, R. I. G., N. C. DAVIDSON, and J. R. WILSON. 2007. Survival of the fattest: body stores on migration and survival in Red Knots *Calidris canutus islandica*. Journal of Avian Biology 38: 479-487.

NETTLESHIP, D. N. 1973. Breeding ecology of Turnstones *Arenaria interpres* at Hazen Camp, Ellesmere Island, N.W.T. Ibis 115: 202-217.

NEWTON, I. 2006. Can conditions experienced during migration limit the population levels of birds? Journal of Ornithology 147: 146-166.

NILES, L. J., J. BART, H. P. SITTERS, A. D. DEY, K. E. CLARK, P. W. ATKINSON, A. J. BAKER, K. A. BENNETT, K. S. KALASZ, N. A. CLARK, J. CLARK, S. GILLINGS, A. S. GATES, P. M. GONZALEZ, D. E. HERNANDEZ, C. D. T. MINTON, R. I. G. MORRISON, R. R. PORTER, R. KEN ROSS, and C. R. VEITCH. 2009. Effects of horseshoe crab harvest in Delaware Bay on Red Knots: are harvest restrictions working? Bioscience 59: 153-164.

PERKINS, D. E., P. A. SMITH, and H. G. GILCHRIST. 2007. The breeding ecology of Ruddy Turnstones (*Arenaria interpres*) in the eastern Canadian Arctic. Polar Record 43: 135-142.

PFISTER, C., M. J. KASPRZYK, and B. A. HARRINGTON. 1998. Body-fat levels and annual return in migrating Semipalmated Sandpipers. Auk 115: 904-915.

PIERSMA, T., and R. I. G. MORRISON. 1994. Energy expenditure and water turnover of incubating Ruddy Turnstones: high costs under high Arctic climatic conditions. Auk 111: 366-376.

PLACYK, J. S., and B. A. HARRINGTON. 2004. Prey abundance and habitat use by migratory shorebirds at coastal stopover sites in Connecticut. Journal of Field Ornithology 75: 223-231.

SHELDON, L. D., E. H. CHIN, S. A. GILL, G. SCHMALTZ, A. E. M. NEWMAN, and K. K. SOMA. 2008. Effects of blood collection on wild birds: an update. Journal of Avian Biology 39: 369-378.

SPACKMAN, E., and D. SUAREZ. 2008. Type A influenza virus detection and quantitation by real-time RT-PCR. Methods in Molecular Biology 436: 19-26.

SWAYNE, D. E., D. A. SENNE, and C. W. BEARD. 1998. Avian influenza. *In* A Laboratory Manual for the Isolation and Identification of Avian Pathogens, D. E. Swayne, (ed.). American Association of Avian Pathologists, Kennett Square, Pennsylvania, pp. 150-155.

TOMPKINS, D. M., A. P. DOBSON, P. ARNEBERG, M. E. BEGON, I. M. CATTADORI, J. V. GREENMAN, J. A. P. HEESTERBEEK, P. J. HUDSON, D. NEWBORN, A. PUGLIESE, A. P. RIZZOLI, R. ROSA, F. ROSSO, and K. WILSON. 2002. Parasites and host population dynamics. *In* The Ecology of Wildlife Diseases, P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson, (eds.). Oxford University Press, New York, pp. 45-62.

U. S. FISH AND WILDLIFE SERVICE. 2003. Delaware Bay Shorebird-Horseshoe Crab Assessment Report and Peer Review. U. S. Fish and Wildlife Service Migratory Bird Publication R9-03/02, Arlington, VA. 99 pp.

VAN GILS, J. A., V. J. MUNSTER, R. RADERSMA, D. LIEFHEBBER, R. A. FOUCHIER, and M. KLAASSEN. 2007. Hampered foraging and migratory performance in swans infected with low-pathogenic avian influenza A virus. PLoS ONE 2: e184.

WEBSTER, R. G., M. YAKHNO, V. S. HINSHAW, W. J. BEAN, and K. COPAL MURTI. 1978. Intestinal influenza: replication and characterization of influenza viruses in ducks. Virology 84: 268-278.

Table 6.1. Univariate associations between the listed variables and whether an individual bird was seen one year following capture. P-values and relative risks/odds ratios of significant associations are bolded.

| Parameter | $\chi^2$ | df | P | Level | Relative risk (categorical variables) or odds ratio (continuous variables) (95% CI) |
|---|---|---|---|---|---|
| Year | 12.00 | 2 | **0.003** | **2006 vs. 2007** | **1.32 (1.13-1.54)** |
| | | | | **2006 vs. 2008** | **1.22 (1.06-1.40)** |
| | | | | 2007 vs. 2008 | 1.08 (0.93-1.26) |
| Day in May | 0.377 | 1 | 0.540 | continuous | 0.995 (0.978-1.011)[a] |
| State | 1.86 | 1 | 0.173 | DE vs. NJ | 1.12 (0.96-1.32) |
| Sex[b] | 0.17 | 1 | 0.683 | Female vs. male | 1.02 (0.92-1.14) |
| Body Size Index | 0.02 | 1 | 0.894 | continuous | 1.005 (0.929-1.088)[a] |
| Mass Index | 13.32 | 1 | **0.0003** | **continuous** | **1.014 (1.007-1.022)[a]** |
| Blood-sampled status | 6.20 | 1 | **0.013** | **Bled vs. not bled** | **1.14 (1.03-1.33)** |

[a] Per 1-unit increase in variable

[b] n=1,198; Birds of unknown sex excluded

Table 6.2. The results of contingency analyses of the effect of AIV infection status on resighting probability of Ruddy Turnstones one yr following sampling, by capture year. Note that there was no effect of AIV infection status on resighting probability for any capture year-resighting period combination.

| Capture year | AIV status | *n* | % Seen | $\chi^2$ | df | *P* | Relative risk (95% CI)[a] |
|---|---|---|---|---|---|---|---|
| 2006 | Positive | 49 | 49.0 | 0.78 | 1 | 0.378 | 0.88 (0.64-1.19) |
| | Negative | 229 | 55.9 | | | | |
| 2007 | Positive | 19 | 31.6 | 0.83 | 1 | 0.362 | 0.75 (0.38-1.47) |
| | Negative | 386 | 42.0 | | | | |
| 2008 | Positive | 95 | 45.3 | 0.01 | 1 | 0.939 | 1.01 (0.79-1.29) |
| | Negative | 455 | 44.8 | | | | |
| 2006-2008 | Positive | 163 | 44.8 | 0.11 | 1 | 0.741 | 0.97 (0.81-1.16) |
| | Negative | 1,070 | 46.2 | | | | |

[a] Resighting probability if AIV positive / resighting probability if AIV negative

Table 6.3. Multivariate logistic regression models to describe resighting probability of flagged Ruddy Turnstones one yr post-capture, both excluding and including AIV infection status as a predictor variable of interest (shown in italics). *P*-values and adjusted odds ratios of significant effects are bolded. Note that AIV infection status did not influence resighting probability.

| Parameter | $\chi^2$ | df | *P* | Level | Adjusted odds ratio (95% CI)[a] |
|---|---|---|---|---|---|
| Whole Model | 25.37 | 3 | **<0.0001** | n/a | n/a |
| Year | 12.05 | 2 | **0.002** | **2006 vs. 2007** | **1.71 (1.26-2.34)** |
| | | | | **2006 vs. 2008** | **1.47 (1.10-1.97)** |
| | | | | 2008 vs. 2007 | 1.16 (0.89-1.51) |
| Mass Index | 13.61 | 1 | **0.0002** | **continuous** | **1.015 (1.007-1.023)[b]** |
| Whole Model (Including AIV status) | 26.77 | 4 | **<0.0001** | n/a | n/a |
| Year | 12.90 | 2 | **0.002** | **2006 vs. 2007** | **1.76 (1.29-2.41)** |
| | | | | **2006 vs. 2008** | **1.48 (1.10-1.98)** |
| | | | | 2008 vs. 2007 | 1.19 (0.92-1.56) |
| Mass Index | 14.41 | 1 | **0.0001** | **continuous** | **1.015 (1.007-1.023)[b]** |
| *AIV Infection Status* | *1.40* | *1* | *0.237* | *Neg. vs. Pos.* | *1.23 (0.87-1.73)* |

[a] Adjusted for all included covariates

[b] For each 1-g increase in MI

Table 6.4. Bivariate logistic regression analysis of AIV infection status on resighting probability, with single parameters included as covariates. *P*-values of significant effects are bolded. Note that AIV infection status was not significantly associated with resighting probability in any case.

| | Whole model | | | Parameter effect | | | AIV status effect | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameter | $\chi^2$ | df | *P* | $\chi^2$ | df | *P* | $\chi^2$ | df | *P* |
| Year | 12.56 | 3 | **0.006** | 12.45 | 2 | **0.002** | 0.56 | 1 | 0.456 |
| Day in May | 0.47 | 2 | 0.793 | 0.36 | 1 | 0.551 | 0.09 | 1 | 0.766 |
| State | 2.02 | 2 | 0.364 | 1.91 | 1 | 0.167 | 0.16 | 1 | 0.686 |
| Sex | 0.30 | 2 | 0.862 | 0.17 | 1 | 0.675 | 0.13 | 1 | 0.719 |
| Body Size Index | 0.13 | 2 | 0.939 | 0.02 | 1 | 0.898 | 0.11 | 1 | 0.743 |
| Mass Index | 13.87 | 2 | **0.001** | 13.74 | 1 | **0.0002** | 0.55 | 1 | 0.459 |
| Bled status | 6.36 | 2 | **0.042** | 6.25 | 1 | **0.012** | 0.16 | 1 | 0.685 |

## FIGURE LEGENDS

Fig.6.1. Relationship between resighting probability one year following capture/AIV testing and (A) the year of capture, (B) the type(s) of samples collected, (C) mass index, and (D) the interaction between mass index and the types of samples collected. In (C), the proportions resighted (±SE) are shown for each 5-g MI class where $n \geq 5$. In (D), sample type(s) were not significantly different after controlling for the effect of MI. In (A), (B), and (D), resighting rates in classes not connected by the same letter are significantly different ($\alpha$=0.05).

Fig. 6.2. Proportion (95% CI) of Ruddy Turnstones resighted one year after capture by AIV infection status and year of capture. There were no significant differences of resighting probability between infected and uninfected groups in any capture year-resighting period combination.

Fig. 6.3. Resighting probability (95% CI; dashed lines) of Ruddy Turnstones one year post-capture by MI and AIV infection status. Also shown are the proportion (±SE) of birds seen by 5-g MI class and infection status where $n \geq 5$. Although the effect of MI on resighting probability appears steeper for AIV-positive birds, this interaction is not significant ($\chi^2$=2.7, df=1, $P$=0.10).

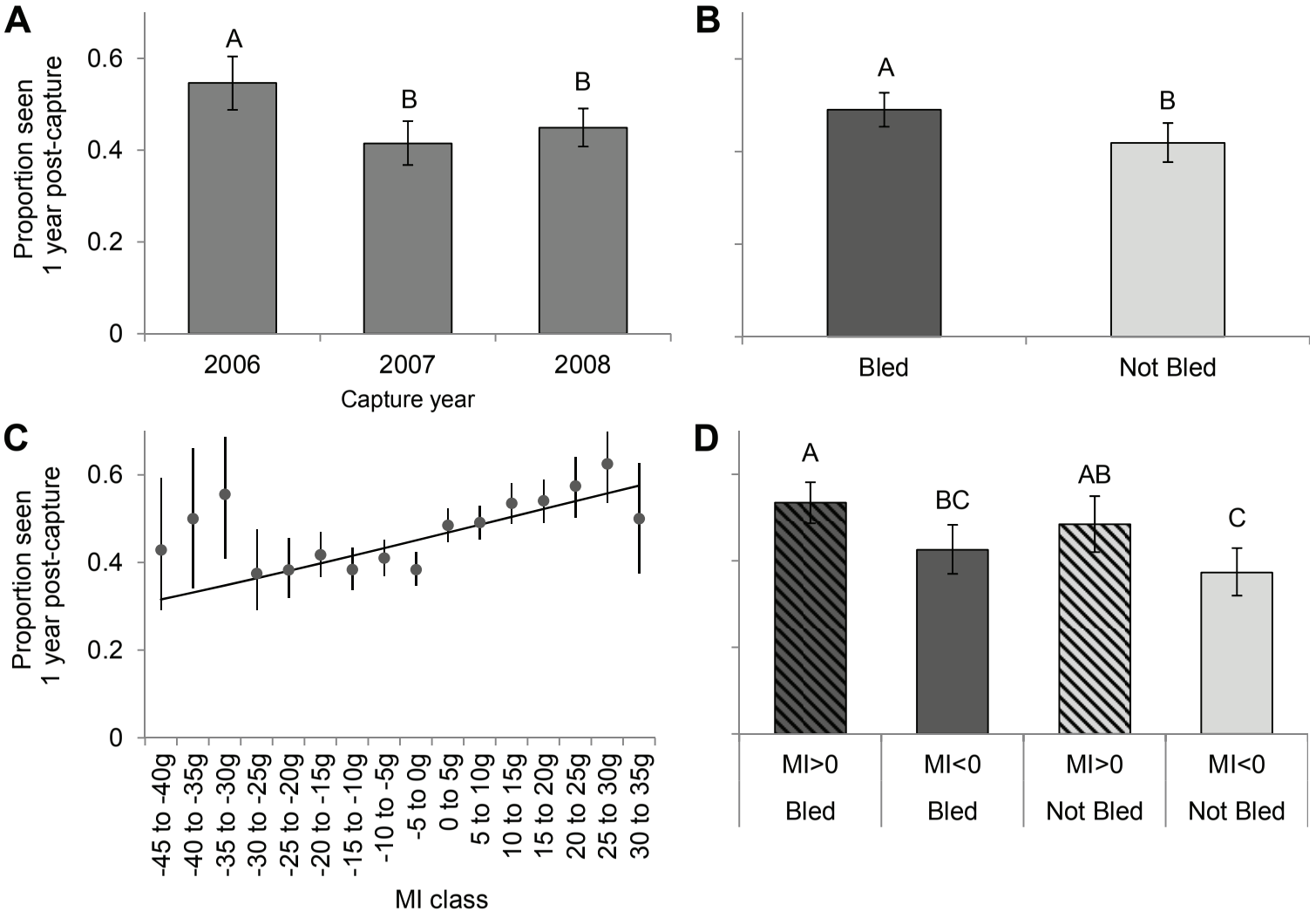

A
Proportion seen
1 year post-capture
0.6
0.4
0.2
0
A
B
B
2006
2007
2008
Capture year
B
A
B
Bled
Not Bled
C
Proportion seen
1 year post-capture
0.6
0.4
0.2
0
-45 to -40g
-40 to -35g
-35 to -30g
-30 to -25g
-25 to -20g
-20 to -15g
-15 to -10g
-10 to -5g
-5 to 0g
0 to 5g
5 to 10g
10 to 15g
15 to 20g
20 to 25g
25 to 30g
30 to 35g
MI class
D
A
BC
AB
C
MI>0
Bled
MI<0
Bled
MI>0
Not Bled
MI<0
Not Bled

Fig. 6.1.

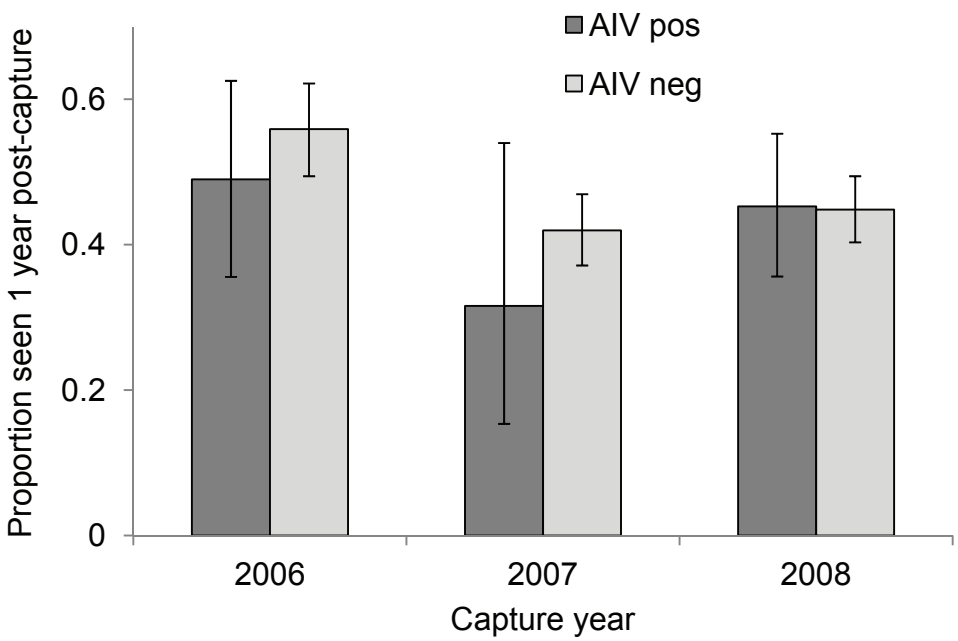

AIV pos
AIV neg
Proportion seen 1 year post-capture
0.6
0.4
0.2
0
2006
2007
2008
Capture year

Fig. 6.2.

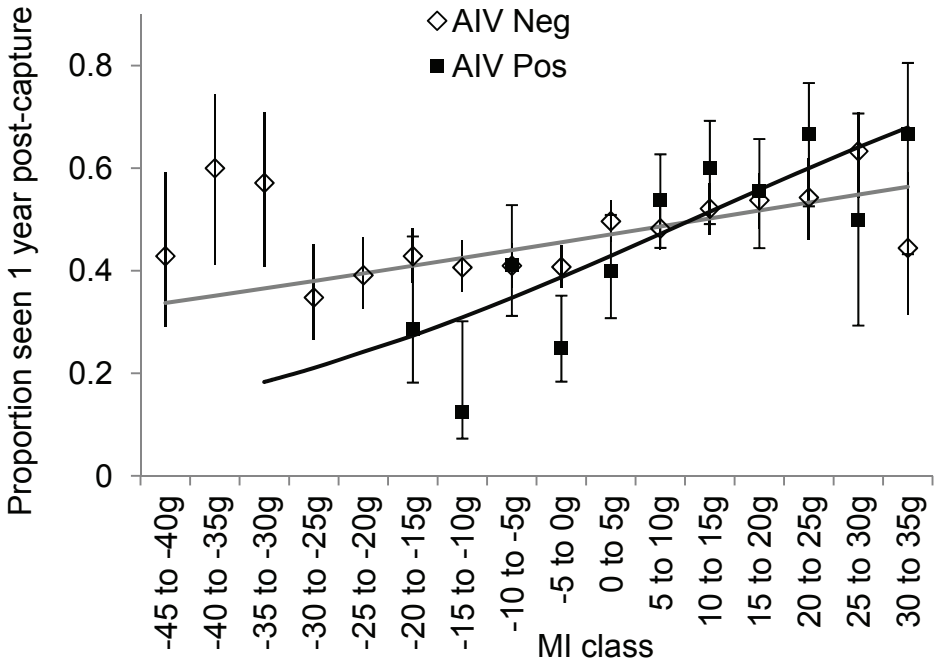

AIV Neg
AIV Pos
Proportion seen 1 year post-capture
0
0.2
0.4
0.6
0.8
-45 to -40g
-40 to -35g
-35 to -30g
-30 to -25g
-25 to -20g
-20 to -15g
-15 to -10g
-10 to -5g
-5 to 0g
0 to 5g
5 to 10g
10 to 15g
15 to 20g
20 to 25g
25 to 30g
30 to 35g
MI class

Fig. 6.3.

# CHAPTER 7

# CONCLUSIONS

## MAIN CONCLUSIONS

### Ruddy Turnstones are the major player in local AIV epidemiology

Among shorebird and gull species sampled at Delaware Bay during spring, Ruddy Turnstones were disproportionately infected with AIV. This finding corroborates several previous studies (Kawaoka et al., 1988; Krauss et al., 2004; Hanson et al., 2008). In this study, though, we found evidence that Ruddy Turnstones might not be an AIV "reservoir host" species, but rather are likely a local "amplifying host" population 1) that is susceptible to infection upon arrival, 2) in which a large proportion of individuals become infected (from each other or a common environmental source) and shed virus in a short time period, and 3) that largely recovers prior to northward migration.

### Largely, Ruddy Turnstones become infected locally

Serial, cross-sectional serosurveys revealed that most Ruddy Turnstones arrived at Delaware Bay without detectable anti-AIV nucleoprotein antibodies, but nearly all birds were antibody-positive at the end of the stopover period. Likewise, very few Turnstones were shedding AIV at the beginning of the stopover period; peak prevalence was near the middle of the stopover period. Although we cannot rule out that some Turnstones arrive at Delaware Bay already infected, most become infected locally.

### Infection in Ruddy Turnstones is probably linked to feeding behavior or nighttime roost location

Although other studies have speculated that e.g., local population density, foraging and migratory behavior, bird susceptibility, or other factors influence high AIV infection prevalence

among Turnstones at Delaware Bay, this is the first study to analyze these possible influences in depth. As illustrated in Chapter 3, a major predictor of infection within Ruddy Turnstones was a positive mass index (MI), i.e., weighing more than the expected population mean mass on the date of capture even after controlling for body size. Because fluctuations in shorebird mass at a stopover location are driven by food consumption (Atkinson et al., 2007), we concluded that birds that weighed more relative to the population either arrived earlier than average and had thus been feeding longer than the average bird, or had fed at a faster rate than the average bird. In either scenario, the intake of more food, which possibly exposed these birds to more AIV because of fecal-oral transmission, would lead to the combined conditions of higher relative weight and AIV infection at the time of capture (Hall et al., 2007).

As shown in Chapter 5, Ruddy Turnstones used substantially different habitat at night (salt marsh) when compared with sympatric species that are less often infected. Because AIV can be transmitted indirectly through water (Hinshaw et al., 1979; VanDalen et al., 2010), salt marsh habitat might promote AIV transmission between Turnstones (but not often to other species) through contact with AIV-contaminated pools of water. Even if not exposed during foraging on beaches, Turnstones that have been at Delaware Bay the longest presumably spent more nights in salt marsh, and therefore more contact with AIV-contaminated water, than recently arrived conspecifics.

**Viruses detected in other shorebird species are likely due to virus spillover**

In Chapters 3-4, we demonstrated that both annual and daily AIV prevalence in Sanderlings varied directly with prevalence in Ruddy Turnstones, suggesting that locally-amplified AIV from Ruddy Turnstones infects less-susceptible Sanderlings (the “spillover host”; Fenton and Pedersen, 2005). Thus, infections in Sanderlings depend on infections in sympatric Turnstones, and sustained Sanderling-to-Sanderling likely does not occur. No previous study has identified such a relationship between AIV infections between two bird species. The

subtypes of all AIVs isolated from other shorebirds were also isolated from Ruddy Turnstones, additionally suggesting virus spillover between species.

**Red Knots arrive with population immunity to AIV; they might be exposed to AIV at a wintering location or a prior migratory stopover site**

Red Knots uniquely had a high proportion of individuals with anti-AIV NP antibodies (79%) but a low proportion of AIV infections (0.7%; see Chapter 3). Further analysis of serial, cross sectional data (Chapter 4) revealed that antibody prevalence was high upon bird arrival at Delaware Bay and even decreased over the stopover period. While detection of antibodies indicates prior exposure, it's difficult to interpret when Red Knots were previously exposed. The pattern of decreasing population antibody prevalence is suggestive of recent infection with little re-exposure while at Delaware Bay. The populations of Red Knots that migrate through Delaware Bay rarely have been tested for AIV at other times and locations (e.g., on wintering grounds or previous migratory stopovers, but see D'Amico et al., 2007). Further study is needed to determine when and where these Red Knots are exposed.

**The variety of AIV subtypes detected indicates multiple introduction sources**

While different subtypes dominated each year (H7N3 in 2006; H12N5 in 2007; H4N6 and H10N7 in 2008), during each spring of this study, at least 7-20 AIV subtype combinations (4-10 HA subtypes; 4-7 NA subtypes) were detected among shorebirds and gulls. The domination of 1-2 subtypes each year represents clonal virus expansion, but the extensive subtype variety, even in low-prevalence years, can only result from multiple AIV sources.

**Virus pools of gulls are likely somewhat separate from those of shorebirds**

Ruddy Turnstones were most often infected and AIV isolated from this species were highly diverse. All AIV subtypes isolated from other shorebird species were also isolated from Ruddy Turnstones; however, not all subtypes found in gull species were isolated from Turnstones. In 2007, H2N9 was isolated only from a Herring Gull, and in 2008, H13N9 was isolated only from three Laughing Gulls. Thus, not all AIV circulating in gulls at Delaware Bay in

spring pass on to Ruddy Turnstones and other shorebirds, indicating partial separation of AIV gene pools between species groups.

**Neither AIV infection nor blood-sampling affects Ruddy Turnstone annual survival**

Chapter 6 presents an analysis of shorebird resighting data (a proxy measure for annual survival) in relation to AIV infection status and other individual bird characteristics. There was no difference in resighting rates, one year after capture, between Ruddy Turnstones that were infected at the time of capture and those that were uninfected. Thus, we concluded that infection did not affect annual survival. However, it was likely that some birds that were uninfected at the time of capture were indeed infected at some other point during the stopover; nearly all sampled Turnstones are antibody-positive by the end of the stopover season (Chapter 4). Certainly, there is some degree of misclassification bias toward the null. However, Ruddy Turnstone population numbers are declining no faster than other shorebirds that migrate though Delaware Bay (Niles et al., 2009), suggesting that AIV infection at Delaware Bay has minimal effect on this specie's population dynamics.

Concern was raised by study collaborators that blood sampling might have a negative impact on shorebird annual survival in these already declining, nutritionally stressed shorebird populations. Results presented in Chapter 6 suggest that blood-sampled Ruddy Turnstones were *more* likely to be seen in the following spring than unsampled birds; however, this was an artifact of individual bird selection for blood sampling (investigator bias). Bled birds tended to be heavier than average, and heavier birds were more likely to be resighted than lighter birds. Once controlled for weight, there was no difference in resighting rates between blood-sampled and unsampled birds. Similarly in Red Knots and Sanderlings, there was no difference in resighting rates between blood-sampled and unsampled birds (data not shown).

**Ruddy Turnstones selected areas away from agriculture and poultry production**

A major concern regarding AIV circulation in wild birds is the likelihood of introduction into domestic bird flocks. Once introduced, even low pathogenicity AI (LPAI) can cause

economic losses; development of highly pathogenic AI (HPAI) can be economically catastrophic (Clark and Hall, 2006). In Chapter 5, analysis of actual shorebird location data, as determined by radiotelemetry, demonstrated that shorebirds selected areas further from both agricultural lands in general and confined animal feeding operations (largely poultry production facilities) in specific. Thus, introduction of AIV into commercial poultry flocks from shorebirds shedding AIV at this specific time and location is probably minimal. However, proper biosecurity measures (e.g., housing birds indoors) should be followed, particularly among small backyard flocks, to reduce AIV introduction risk.

## CONTRIBUTIONS OF THIS RESEARCH TO KNOWLEDGE OF AIV IN SHOREBIRDS

The spring shorebird migration stopover event at the Delaware Bay ecosystem is indeed a unique situation in global AIV ecology. Munster and Fouchier (2009) outlined several possible ecological factors contributing to this unique situation:

> *"It is…plausible that the Delaware Bay area, where many initial shorebird studies were performed, combines a unique set of ecological factors that meet the requirements for efficient influenza A virus circulation and transmission. These ecological factors are probably a combination between host species and virus specific ecological factors such as foraging behavior, migratory behavior, habitat preference, susceptibility, local bird density and species composition, aggregation of host species, environmental persistence of the virus, receptor specificity, tissue tropism and replication and transmission kinetics. Locations comparable to Delaware Bay with respect to virus-host ecology have yet to be identified elsewhere in the world."*

The research contained in this dissertation addresses most of these ecological factors either through direct hypothesis testing of data, or through discussion of their possible

contribution to observed results. Namely, foraging behavior, migratory behavior, habitat preference, susceptibility, local bird density and species composition, and aggregation of host species were addressed. Environmental persistence of the virus, receptor specificity, tissue tropism, and replication and transmission kinetics were not specifically addressed by this research.

Specific findings of this research that further our knowledge of AIV in shorebird hosts and in the Delaware Bay ecosystem, and hypotheses to explain these results, are listed in Table 7.1.

## LITERATURE CITED

ATKINSON, P. W., A. J. BAKER, K. A. BENNETT, N. A. CLARK, J. A. CLARK, K. B. COLE, A. DEKINGA, A. DEY, S. GILLINGS, P. M. GONZALEZ, K. KALASZ, C. D. T. MINTON, J. NEWTON, L. J. NILES, T. PIERSMA, R. A. ROBINSON, and H. P. SITTERS. 2007. Rates of mass gain and energy deposition in red knot on their final spring staging site is both time- and condition-dependent. Journal of Applied Ecology 44: 885-895.

CLARK, L., and J. HALL. 2006. Avian influenza in wild birds: Status as reservoirs, and risks to humans and agriculture. Ornithological Monographs 60: 3-29.

D'AMICO, V. L., M. BERTELLOTTI, A. J. BAKER, and L. A. DIAZ. 2007. Exposure of Red Knots (*Calidris canutus rufa*) to select avian pathogens; Patagonia, Argentina. Journal of Wildlife Diseases 43: 794-797.

FENTON, A., and A. B. PEDERSEN. 2005. Community epidemiology framework for classifying disease threats. Emerging Infectious Diseases 11: 1815-1821.

HALL, S., L. SIVARS-BECKER, C. BECKER, M. DUFFY, A. TESSIER, and C. CÁCERES. 2007. Eating yourself sick: transmission of disease as a function of foraging ecology. Ecology Letters 10: 207-218.

HANSON, B. A., M. P. LUTTRELL, V. H. GOEKJIAN, L. NILES, D. E. SWAYNE, D. A. SENNE, and D. E. STALLKNECHT. 2008. Is the occurrence of avian influenza virus in Charadriiformes species and location dependent? Journal of Wildlife Diseases 44: 351-361.

HINSHAW, V. S., R. G. WEBSTER, and B. TURNER. 1979. Water-borne transmission of influenza A viruses. Intervirology 11: 66-68.

KAWAOKA, Y., T. M. CHAMBERS, W. L. SLADEN, and R. G. WEBSTER. 1988. Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? Virology 163: 247-250.

KRAUSS, S., D. WALKER, S. P. PRYOR, L. NILES, C. H. LI, V. S. HINSHAW, and R. G. WEBSTER. 2004. Influenza A viruses of migrating wild aquatic birds in North America. Vector-Borne and Zoonotic Diseases 4: 177-189.

MUNSTER, V. J., and R. A. M. FOUCHIER. 2009. Avian influenza virus: of virus and bird ecology. Vaccine 27: 6340-6344.

NILES, L. J., J. BART, H. P. SITTERS, A. D. DEY, K. E. CLARK, P. W. ATKINSON, A. J. BAKER, K. A. BENNETT, K. S. KALASZ, N. A. CLARK, J. CLARK, S. GILLINGS, A. S. GATES, P. M. GONZALEZ, D. E. HERNANDEZ, C. D. T. MINTON, R. I. G. MORRISON, R. R. PORTER, R. KEN ROSS, and C. R. VEITCH. 2009. Effects of horseshoe crab harvest in Delaware Bay on Red Knots: are harvest restrictions working? Bioscience 59: 153-164.

VANDALEN, K. K., A. B. FRANKLIN, N. L. MOOERS, H. J. SULLIVAN, and S. A. SHRINER. 2010. Shedding light on avian influenza H4N6 infection in Mallards: Modes of transmission and implications for surveillance. PLoS ONE 5: e12851.

Table 7.1. Summary of evidence presented in this dissertation regarding differences in springtime AIV prevalence patterns among select shorebird and gull species at Delaware Bay.

| Species | New evidence presented here | Hypotheses |
|---|---|---|
| Red Knot | Arrive at Delaware Bay in spring with population immunity | Infected somewhere else, or long duration of immunity |
| | Low prevalence | Not exposed, or protective immunity |
| | Same subtypes as Ruddy Turnstones | Virus spillover from Ruddy Turnstones |
| Sanderling | No population immunity upon arrival, low prevalence | Generally resistant species, or not exposed |
| | Prevalence correlated with Ruddy Turnstones, and same subtypes as Ruddy Turnstones | Virus spillover from Ruddy Turnstones |
| | Sandy beach areas selected day and night | Habitat preferences (surface sand) not ideal for AIV transmission |
| Laughing Gull | Different subtypes than shorebirds | Partially separate AIV transmission cycles/gene pools |
| | Constant seasonal population immunity | Prolonged virus circulation |
| Ruddy Turnstone | Low population immunity upon arrival | Seasonally susceptible, short duration of immunity |
| | Salt marsh use unlike other shorebird species (especially at night), also used by gulls, waterfowl | Habitat preferences might support transmission among conspecifics, be an initial source of infection for Ruddy Turnstones, and limit transmission to sympatric species with different habitat preferences |
| | Low prevalence upon arrival; epidemic curve | Local transmission of virus |
| | No effect of AIV infection on annual survival | Natural, permissive host for LPAIV. High annual incidence leading to misclassification bias |

# REFERENCES

AGRESTI, A., and B. A. COULL. 1998. Approximate is better than "exact" for interval estimation of binomial proportions. American Statistician 52: 119-126.

ALEXANDER, D. J. 2007. An overview of the epidemiology of avian influenza. Vaccine 25: 5637-5644.

ATKINSON, P. W., A. J. BAKER, K. A. BENNETT, N. A. CLARK, J. A. CLARK, K. B. COLE, A. DEKINGA, A. DEY, S. GILLINGS, P. M. GONZALEZ, K. KALASZ, C. D. T. MINTON, J. NEWTON, L. J. NILES, T. PIERSMA, R. A. ROBINSON, and H. P. SITTERS. 2007. Rates of mass gain and energy deposition in red knot on their final spring staging site is both time- and condition-dependent. Journal of Applied Ecology 44: 885-895.

AUSTIN, F. J., and R. G. WEBSTER. 1993. Evidence of orthomyxoviruses and paramyxoviruses in fauna from Antarctica. Journal of Wildlife Diseases 29: 568-571.

BAKER, A. J., P. M. GONZALEZ, T. PIERSMA, L. J. NILES, I. D. S. DO NASCIMENTO, P. W. ATKINSON, N. A. CLARK, C. D. T. MINTON, M. K. PECK, and G. AARTS. 2004. Rapid population decline in Red Knots: fitness consequences of decreased refueling rates and late arrival in Delaware Bay. Proceedings of the Royal Society B: Biological Sciences 271: 875-882.

BALLETTO, J. H., M. V. HEIMBUCH, and H. J. MAHONEY. 2005. Delaware Bay salt marsh restoration: mitigation for a power plant cooling water system in New Jersey, USA. Ecological Engineering 25: 204-213.

BAO, Y. M., P. BOLOTOV, D. DERNOVOY, B. KIRYUTIN, L. ZASLAVSKY, T. TATUSOVA, J. OSTELL, and D. LIPMAN. 2008. The influenza virus resource at the National Center for Biotechnology Information. Journal of Virology 82: 596-601.

BECKER, W. B. 1966. The isolation and classification of tern virus: Influenza virus A/Tern/South Africa/1961. Journal of Hygiene 64: 309-320.

BELDOMENICO, P. M., and M. BEGON. 2010. Response to comment by Loot et al. Trends in Ecology & Evolution 25: 132-132.

BIN MUZAFFAR, S., R. C. YDENBERG, and I. L. JONES. 2006. Avian influenza: an ecological and evolutionary perspective for waterbird scientists. Waterbirds 29: 243-257.

BLANCHET, S., F. THOMAS, and G. LOOT. 2009. Reciprocal effects between host phenotype and pathogens: new insights from an old problem. Trends in Parasitology 25: 364-369.

BOGOMOLNI, A. L., R. J. GAST, J. C. ELLIS, M. DENNETT, K. R. PUGLIARES, B. J. LENTELL, and M. J. MOORE. 2008. Victims or vectors: a survey of marine vertebrate zoonoses from coastal waters of the Northwest Atlantic. Diseases of Aquatic Organisms 81: 13-38.

BOTTON, M. L., R. E. LOVELAND, and T. R. JACOBSEN. 1994. Site selection by migratory shorebirds in Delaware Bay, and its relationship to beach characteristics and abundance of horseshoe crab (*Limulus polyphemus*) eggs. Auk 111: 605-616.

BREAREY, D., and O. HILDEN. 1985. Nesting and egg predation by Turnstones *Arenaria interpres* in Larid colonies. Ornis Scandinavica 16: 283-292.

BROWN, J. D., D. E. SWAYNE, R. J. COOPER, R. E. BURNS, and D. E. STALLKNECHT. 2007. Persistence of H5 and H7 avian influenza viruses in water. Avian Diseases 51: 285-289.

BROWN, J. D., G. GOEKJIAN, R. POULSON, S. VALEIKA, and D. E. STALLKNECHT. 2009a. Avian influenza virus in water: infectivity is dependent on pH, salinity and temperature. Veterinary Microbiology 136: 20-26.

BROWN, J. D., D. E. STALLKNECHT, R. D. BERGHAUS, M. P. LUTTRELL, K. VELEK, W. KISTLER, T. COSTA, M. J. YABSLEY, and D. SWAYNE. 2009b. Evaluation of a commercial blocking enzyme-linked immunosorbent assay to detect avian influenza virus antibodies in multiple experimentally infected avian species. Clinical and Vaccine Immunology 16: 824-829.

BROWN, M. B., and C. R. BROWN. 2009. Blood sampling reduces annual survival in Cliff Swallows (*Petrochelidon pyrrhonota*). Auk 126: 853-861.

BUEHLER, D. M., and T. PIERSMA. 2008. Travelling on a budget: predictions and ecological evidence for bottlenecks in the annual cycle of long-distance migrants. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 247-266.

BUEHLER, D. M., B. I. TIELEMAN, and T. PIERSMA. 2010. How do migratory species stay healthy over the annual cycle? A conceptual model for immune function and for resistance to disease. Integrative and Comparative Biology 50: 346-357.

BURGER, J. 1996. Laughing Gull (*Larus atricilla*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 225, http://bna.birds.cornell.edu/bna/species/225. Accessed January 2011.

BURGER, J., L. NILES, and K. E. CLARK. 1997. Importance of beach, mudflat and marsh habitats to migrant shorebirds on Delaware Bay. Biological Conservation 79: 283-292.

BURGER, J., C. JEITNER, K. CLARK, and L. J. NILES. 2004. The effect of human activities on migrant shorebirds: successful adaptive management. Environmental Conservation 31: 283-288.

BURGER, J., S. A. CARLUCCI, C. W. JEITNER, and L. NILES. 2007. Habitat choice, disturbance, and management of foraging shorebirds and gulls at a migratory stopover. Journal of Coastal Research 23: 1159-1166.

BURNHAM, K. P., and D. R. ANDERSON. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. 2nd edition. Springer, New York, New York. 488 pp.

BURTON, N. H. K., and P. R. EVANS. 1997. Survival and winter site-fidelity of Turnstones *Arenaria interpres* and Purple Sandpipers *Calidris maritima* in northeast England. Bird Study 44: 35-44.

BURTON, N. H. K., and P. R. EVANS. 2001. Aggressive behaviours and correlates of dominance in Purple Sandpipers *Calidris maritima* at a communal winter roost. Ibis 143: 248-254.

BUTT, K. M., G. J. D. SMITH, H. CHEN, L. J. ZHANG, Y. H. C. LEUNG, K. M. XU, W. LIM, R. G. WEBSTER, K. Y. YUEN, J. S. M. PEIRIS, and Y. GUAN. 2005. Human infection with an avian H9N2 influenza A virus in Hong Kong in 2003. Journal of Clinical Microbiology 43: 5760-5767.

CAMPITELLI, L., E. MOGAVERO, M. A. DE MARCO, M. DELOGU, S. PUZELLI, F. FREZZA, M. FACCHINI, C. CHIAPPONI, E. FONI, P. CORDIOLI, R. WEBBY, G. BARIGAZZI, R. G. WEBSTER, and I. DONATELLI. 2004. Interspecies transmission of an H7N3 influenza virus from wild birds to intensively reared domestic poultry in Italy. Virology 323: 24-36.

CAPUA, I., and D. J. ALEXANDER. 2007. Avian influenza infections in birds - a moving target. Influenza & Other Respiratory Viruses 1: 11-18.

CATTOLI, G., C. TERREGINO, V. GUBERTI, R. DE NARDI, A. DRAGO, A. SALVIATO, S. FASSINA, and I. CAPUA. 2007. Influenza virus surveillance in wild birds in Italy: Results of laboratory investigations in 2003-2005. Avian Diseases 51: 414-416.

CHEN, H. X., H. G. SHEN, X. L. LI, J. Y. ZHOU, Y. Q. HOU, J. Q. GUO, and J. Q. HU. 2006. Seroprevalance and identification of influenza A virus infection from migratory wild waterfowl in China (2004-2005). Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 53: 166-170.

CHEN, R., and E. C. HOLMES. 2009. Frequent inter-species transmission and geographic subdivision in avian influenza viruses from wild birds. Virology 383: 156-161.

CHEN, W. S., P. A. CALVO, D. MALIDE, J. GIBBS, U. SCHUBERT, I. BACIK, S. BASTA, R. O'NEILL, J. SCHICKLI, P. PALESE, P. HENKLEIN, J. R. BENNINK, and J. W. YEWDELL. 2001. A novel influenza A virus mitochondrial protein that induces cell death. Nature Medicine 7: 1306-1312.

CLARK, K. E., L. J. NILES, and J. BURGER. 1993. Abundance and distribution of migrant shorebirds in Delaware Bay. Condor 95: 694-705.

CLARK, L., and J. HALL. 2006. Avian influenza in wild birds: Status as reservoirs, and risks to humans and agriculture. Ornithological Monographs 60: 3-29.

CLARK, N. A., S. GILLINGS, A. J. BAKER, P. M. GONZALEZ, and R. PORTER. 2005. The production and use of permanently inscribed leg flags for waders. Wader Study Group Bulletin 108: 38-41.

COLWELL, M. A., C. L. GRATTO, L. W. ORING, and A. J. FIVIZZANI. 1988. Effects of blood sampling on shorebirds: injuries, return rates, and clutch desertions. Condor 90: 942-945.

COX, D. R., and D. OAKES. 1984. Analysis of Survival Data. Chapman and Hall, London, United Kingdom. 201 pp.

D'AMICO, V. L., M. BERTELLOTTI, A. J. BAKER, and L. A. DIAZ. 2007. Exposure of Red Knots (*Calidris canutus rufa*) to select avian pathogens; Patagonia, Argentina. Journal of Wildlife Diseases 43: 794-797.

DE MARCO, M. A., G. E. FONI, L. CAMPITELLI, E. RAFFINI, L. DI TRANI, M. DELOGU, V. GUBERTI, G. BARIGAZZI, and I. DONATELLI. 2003. Circulation of influenza viruses in wild waterfowl wintering in Italy during the 1993-99 period: Evidence of virus shedding and seroconvcrsion in wild ducks. Avian Diseases 47: 861-866.

DE MARCO, M. A., L. CAMPITELLI, M. DELOGU, E. RAFFINI, E. FONI, L. DI TRANI, M. SCAFFIDI, and I. DONATELLI. 2005. Serological evidences showing the involvement of free-living pheasants in the influenza ecology. Italian Journal of Animal Science 4: 287-291.

DEIBEL, R., D. E. EMORD, W. DUKELOW, V. S. HINSHAW, and J. M. WOOD. 1985. Influenza viruses and paramyxoviruses in ducks in the Atlantic Flyway, 1977-1983, including an H5N2 isolate related to the virulent chicken virus. Avian Diseases 29: 970-985.

DOUGLAS, K. O., M. C. LAVOIE, L. M. KIM, C. L. AFONSO, and D. L. SUAREZ. 2007. Isolation and genetic characterization of avian influenza viruses and a Newcastle disease virus from wild birds in Barbados: 2003-2004. Avian Diseases 51: 781-787.

DRENT, R., A. FOX, and J. STAHL. 2006. Travelling to breed. Journal of Ornithology 147: 122-134.

DUNNE, P., D. SIBLEY, C. SUTTON, and W. WANDER. 1982. Aerial surveys in Delaware Bay: confirming an enormous spring staging area for shorebirds. International Wader Study Group Bulletin 35: 32-33.

DUSEK, R. J., J. B. BORTNER, T. J. DELIBERTO, J. HOSKINS, J. C. FRANSON, B. D. BALES, D. YPARRAGUIRRE, S. R. SWAFFORD, and H. S. IP. 2009. Surveillance for high pathogenicity avian influenza virus in wild birds in the Pacific flyway of the United States, 2006-2007. Avian Diseases 53: 222-230.

ESCUDERO, G., V. J. MUNSTER, M. BERTELLOTTI, and P. EDELAAR. 2008. Perpetuation of avian influenza in the Americas: Examining the role of shorebirds in Patagonia. Auk 125: 494-495.

FAO. 2007. Wild Birds and Avian Influenza: an Introduction to Applied Field Research and Disease Sampling Techniques. *In* D. Whitworth, S. Newman, T. Mundkur, and P. Harris, (eds.) FAO Animal Production and Health Manual FAO, Rome, 120 pp.

FENTON, A., and A. B. PEDERSEN. 2005. Community epidemiology framework for classifying disease threats. Emerging Infectious Diseases 11: 1815-1821.

FIRTH, D. 1993. Bias reduction of maximum likelihood estimates. Biometrika 80: 27-38.

FITCHNER, G. J. 1987. The Pennsylvania/Virginia experience in eradication of avian influenza H5N2. . *In* Proceedings of the Second International Symposium on Avian Influenza. U.S. Animal Health Association, Madison, WI, pp. 33-38.

FLINT, P. L., and J. C. FRANSON. 2009. Does influenza A affect body condition of wild mallard ducks, or vice versa? Proceedings of the Royal Society B: Biological Sciences 276: 2345-2346.

FOUCHIER, R. A. M., B. OLSEN, T. M. BESTEBROER, S. HERFST, L. VAN DER KEMP, G. E. RIMMELZWAAN, and A. D. M. E. OSTERHAUS. 2003. Influenza A virus surveillance in wild birds in Northern Europe in 1999 and 2000. Avian Diseases 47: 857-860.

FOUCHIER, R. A. M., V. MUNSTER, A. WALLENSTEN, T. M. BESTEBROER, S. HERFST, D. SMITH, G. F. RIMMELZWAAN, B. OLSEN, and A. D. M. E. OSTERHAUS. 2005. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from Black-headed Gulls. Journal of Virology 79: 2814-2822.

GAIDET, N., T. DODMAN, A. CARON, G. BALANCA, S. DESVAUX, F. GOUTARD, G. CATTOLI, F. LAMARQUE, W. HAGEMEIJER, and F. MONICAT. 2007. Avian influenza viruses in water birds, Africa. Emerging Infectious Diseases 13: 626-629.

GARAMSZEGI, L. Z., and A. P. MOLLER. 2007. Prevalence of avian influenza and host ecology. Proceedings Biological Sciences 274: 2003-2012.

GARCIA, M., D. L. SUAREZ, J. M. CRAWFORD, J. W. LATIMER, R. D. SLEMONS, D. E. SWAYNE, and M. L. PERDUE. 1997. Evolution of H5 subtype avian influenza A viruses in North America. Virus Research 51: 115-124.

GHERSI, B. M., D. L. BLAZES, E. ICOCHEA, R. I. GONZALEZ, T. KOCHEL, Y. TINOCO, M. M. SOVERO, S. LINDSTROM, B. SHU, A. KLIMOV, A. E. GONZALEZ, and J. M. MONTGOMERY. 2009. Avian influenza in wild birds, central coast of Peru. Emerging Infectious Diseases 15: 935-938.

GILL, J. S., R. WEBBY, M. J. R. GILCHRIST, and G. C. GRAY. 2006. Avian influenza among waterfowl hunters and wildlife professionals. Emerging Infectious Diseases 12: 1284-1286.

GILL, R. E. 1986. What won't turnstones eat? British Birds 79: 402-403.

GILLINGS, S., P. W. ATKINSON, S. L. BARDSLEY, N. A. CLARK, S. E. LOVE, R. A. ROBINSON, R. A. STILLMAN, and R. G. WEBER. 2007. Shorebird predation of horseshoe crab eggs in Delaware Bay: species contrasts and availability constraints. Journal of Animal Ecology 76: 503-514.

GILLINGS, S., P. W. ATKINSON, A. J. BAKER, K. A. BENNETT, N. A. CLARK, K. B. COLE, P. M. GONZALEZ, K. S. KALASZ, C. D. T. MINTON, L. J. NILES, R. C. PORTER, I. DE LIMA SERRANO, H. P. SITTERS, J. L. WOODS, and T. L. SHAFFER. 2009. Staging behavior in Red Knot (*Calidris canutus*) in Delaware Bay: implications for monitoring mass and population size. Auk 126: 54-63.

GILSDORF, A., N. BOXALL, V. GASIMOV, I. AGAYEV, F. MAMMADZADE, P. URSU, E. GASIMOV, C. BROWN, S. MARDEL, D. JANKOVIC, G. PIMENTEL, I. AMIR AYOUB, E. MAHER LABIB ELASSAL,

C. SALVI, D. LEGROS, C. PESSOA DA SILVA, R. HAY A. ANDRAGHETTI, G. RODIER, and B. GANTER. 2006. Two clusters of human infection with influenza A/H5N1 virus in the Republic of Azerbaijan, February–March 2006. Eurosurveillance 11: pii=620.

GORMAN, O. T., R. O. DONIS, Y. KAWAOKA, and R. G. WEBSTER. 1990. Evolution of influenza A virus PB2 genes: implications for evolution of the ribonucleoprotein complex and origin of human influenza A virus. Journal of Virology 64: 4893-4902.

GRAVES, I. L. 1992. Influenza viruses in birds of the Atlantic Flyway. Avian Diseases 36: 1-10.

GUDMUNDSSON, G. A., A. LINDSTROM, and T. ALERSTAM. 1991. Optimal fat loads and long-distance flights by migrating Knots *Calidris canutus,* Sanderlings *C. alba*, and Turnstones *Arenaria interpres*. Ibis 133: 140-152.

HALL, S., L. SIVARS-BECKER, C. BECKER, M. DUFFY, A. TESSIER, and C. CÁCERES. 2007. Eating yourself sick: transmission of disease as a function of foraging ecology. Ecology Letters 10: 207-218.

HAMMER, Ø., D. A. T. HARPER, and P. D. RYAN. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 1-9.

HANSON, B. A., D. E. SWAYNE, D. A. SENNE, D. S. LOBPRIES, J. HURST, and D. E. STALLKNECHT. 2005. Avian influenza viruses and paramyxoviruses in wintering and resident ducks in Texas. Journal of Wildlife Diseases 41: 624-628.

HANSON, B. A., M. P. LUTTRELL, V. H. GOEKJIAN, L. NILES, D. E. SWAYNE, D. A. SENNE, and D. E. STALLKNECHT. 2008. Is the occurrence of avian influenza virus in Charadriiformes species and location dependent? Journal of Wildlife Diseases 44: 351-361.

HARRINGTON, B. A. 2001. Red Knot (*Calidris canutus*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 563, http://bna.birds.cornell.edu/bna/species/563. Accessed January 2011.

HASTIE, T. J., and R. J. TIBSHIRANI. 1990. Generalized Additive Models. Chapman and Hall, London, United Kingdom. 352 pp.

HATCHETTE, T. F., D. WALKER, C. JOHNSON, A. BAKER, S. P. PRYOR, and R. G. WEBSTER. 2004. Influenza A viruses in feral Canadian ducks: extensive reassortment in nature. Journal of General Virology 85: 2327-2337.

HINKLE, R. L., and W. J. MITSCH. 2005. Salt marsh vegetation recovery at salt hay farm wetland restoration sites on Delaware Bay. Ecological Engineering 25: 240-251.

HINSHAW, V. S., R. G. WEBSTER, and B. TURNER. 1979. Water-borne transmission of influenza A viruses. Intervirology 11: 66-68.

HINSHAW, V. S., W. J. BEAN, R. G. WEBSTER, and G. SRIRAM. 1980. Genetic reassortment of influenza A viruses in the intestinal tract of ducks. Virology 102: 412-419.

HINSHAW, V. S., G. M. AIR, G. C. SCHILD, and R. W. NEWMAN. 1983. Characterization of a novel hemagglutinin subtype (H13) of influenza A viruses from gulls. Bulletin of the World Health Organization 61: 677-679.

HINSHAW, V. S., J. M. WOOD, R. G. WEBSTER, R. DEIBEL, and B. TURNER. 1985. Circulation of influenza viruses and paramyxoviruses in waterfowl originating from 2 different areas of North America. Bulletin of the World Health Organization 63: 711-719.

HLINAK, A., R. U. MUHLE, O. WERNER, A. GLOBIG, E. STARICK, H. SCHIRRMEIER, B. HOFFMANN, A. ENGELHARDT, D. HUBNER, F. J. CONRATHS, D. WALLSCHLAGER, H. KRUCKENBERG, and T. MULLER. 2006. A virological survey in migrating waders and other waterfowl in one of the most important resting sites of Germany. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 53: 105-110.

HURT, A. C., P. M. HANSBRO, P. SELLECK, B. OLSEN, C. MINTON, A. W. HAMPSON, and I. G. BARR. 2006. Isolation of avian influenza viruses from two different transhemispheric migratory shorebird species in Australia. Archives of Virology 151: 2301-2309.

IP, H. S., P. L. FLINT, J. C. FRANSON, R. J. DUSEK, D. V. DERKSEN, R. E. GILL, JR., C. R. ELY, J. M. PEARCE, R. B. LANCTOT, S. M. MATSUOKA, D. B. IRONS, J. B. FISCHER, R. M. OATES, M. R. PETERSEN, T. F. FONDELL, D. A. ROCQUE, J. C. PEDERSEN, and T. C. ROTHE. 2008. Prevalence of Influenza A viruses in wild migratory birds in Alaska: patterns of variation in detection at a crossroads of intercontinental flyways. Virology Journal 5: 71.

ITO, T., K. OKAZAKI, Y. KAWAOKA, A. TAKADA, R. G. WEBSTER, and H. KIDA. 1995. Perpetuation of influenza A viruses in Alaskan waterfowl reservoirs. Archives of Virology 140: 1163-1172.

ITO, T., H. GOTO, E. YAMAMOTO, H. TANAKA, M. TAKEUCHI, M. KUWAYAMA, Y. KAWAOKA, and K. OTSUKI. 2001. Generation of a highly pathogenic avian influenza A virus from an avirulent field isolate by passaging in chickens. Journal of Virology 75: 4439-4443.

IVERSON, S. A., J. Y. TAKEKAWA, S. SCHWARZBACH, C. J. CARDONA, N. WARNOCK, M. A. BISHOP, G. A. SCHIRATO, S. PAROULEK, J. T. ACKERMAN, H. IP, and W. M. BOYCE. 2008. Low prevalence of avian influenza virus in shorebirds on the Pacific coast of North America. Waterbirds 31: 602-610.

JACKWOOD, M. W., and D. E. STALLKNECHT. 2007. Molecular epidemiologic studies on North American H9 avian influenza virus isolates from waterfowl and shorebirds. Avian Diseases 51: 448-450.

JAHANGIR, A., Y. WATANABE, O. CHINEN, S. YAMAZAKI, K. SAKAI, M. OKAMURA, M. NAKAMURA, and K. TAKEHARA. 2008. Surveillance of avian influenza viruses in Northern pintails (Anas acuta) in Tohoku District, Japan. Avian Diseases 52: 49-53.

JIN, Y., M. V. YATES, S. S. THOMPSON, and W. A. JURY. 1997. Sorption of viruses during flow through saturated sand columns. Environmental Science & Technology 31: 548-555.

KAPLAN, E. L., and P. MEIER. 1958. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 53: 457-481.

KARPANTY, S. M., J. D. FRASER, J. BERKSON, L. J. NILES, A. DEY, and E. P. SMITH. 2006. Horseshoe crab eggs determine red knot distribution in Delaware Bay. Journal of Wildlife Management 70: 1704-1710.

KAWAOKA, Y., T. M. CHAMBERS, W. L. SLADEN, and R. G. WEBSTER. 1988. Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? Virology 163: 247-250.

KING, B. 1982. Turnstone feeding on gull excrement British Birds 75: 88-88.

KISHIDA, N., Y. SAKODA, M. SHIROMOTO, G. R. BAI, N. ISODA, A. TAKADA, G. LAVER, and H. KIDA. 2008. H2N5 influenza virus isolates from terns in Australia: genetic reassortants between those of the Eurasian and American lineages. Virus Genes 37: 16-21.

KLEIJN, D., V. J. MUNSTER, B. S. EBBINGE, D. A. JONKERS, G. J. D. M. MÜSKENS, Y. VAN RANDEN, and R. A. M. FOUCHIER. 2010. Dynamics and ecological consequences of avian influenza virus infection in Greater White-fronted Geese in their winter staging areas. Proceedings of the Royal Society B: Biological Sciences 277: 2041-2048.

KOEHLER, A. V., J. M. PEARCE, P. L. FLINT, J. C. FRANSON, and H. S. IP. 2008. Genetic evidence of intercontinental movement of avian influenza in a migratory bird: the northern pintail (*Anas acuta*). Molecular Ecology 17: 4754-4762.

KRAUSS, S., D. WALKER, S. P. PRYOR, L. NILES, C. H. LI, V. S. HINSHAW, and R. G. WEBSTER. 2004. Influenza A viruses of migrating wild aquatic birds in North America. Vector-Borne and Zoonotic Diseases 4: 177-189.

KRAUSS, S., C. A. OBERT, J. FRANKS, D. WALKER, K. JONES, P. SEILER, L. NILES, S. P. PRYOR, J. C. OBENAUER, C. W. NAEVE, L. WIDJAJA, R. J. WEBBY, and R. G. WEBSTER. 2007. Influenza in migratory birds and evidence of limited intercontinental virus exchange. Plos Pathogens 3: 1684-1693.

KRAUSS, S., D. E. STALLKNECHT, N. J. NEGOVETICH, L. J. NILES, R. J. WEBBY, and R. G. WEBSTER. 2010. Coincident Ruddy Turnstone migration and horseshoe crab spawning creates an ecological 'hot spot' for influenza viruses. Proceedings of the Royal Society B: Biological Sciences 277: 3373-3379.

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.

LANGSTAFF, I. G., and J. S. MCKENZIE. 2006. Surveillance for highly pathogenic avian influenza in migratory shorebirds at the terminus of the East Asian-Australasian Flyway. Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics, Cairns, Australia: ISVEE 11: 835.

LANGSTAFF, I. G., J. S. MCKENZIE, W. L. STANISLAWEK, C. E. M. REED, R. POLAND, and S. C. CORK. 2009. Surveillance for highly pathogenic avian influenza in migratory shorebirds at the terminus of the East Asian-Australasian Flyway. New Zealand Veterinary Journal 57: 160-165.

LATORRE-MARGALEF, N., G. GUNNARSSON, V. J. MUNSTER, R. A. M. FOUCHIER, A. D. M. E. OSTERHAUS, J. ELMBERG, B. OLSEN, A. WALLENSTEN, T. FRANSSON, L. BRUDIN, and J. WALDENSTROM. 2009a. Does influenza A affect body condition of wild mallard ducks, or vice versa? A reply to Flint and Franson. Proceedings of the Royal Society B: Biological Sciences 276: 2347-2349.

LATORRE-MARGALEF, N., G. GUNNARSSON, V. J. MUNSTER, R. A. M. FOUCHIER, A. D. M. E. OSTERHAUS, J. ELMBERG, B. OLSEN, A. WALLENSTEN, P. D. HAEMIG, T. FRANSSON, L. BRUDIN, and J. WALDENSTRÖM. 2009b. Effects of influenza A virus infection on migrating Mallard ducks. Proceedings of the Royal Society B: Biological Sciences 276: 1029-1036.

LEBARBENCHON, C., C. M. CHANG, S. VAN DER WERF, J. T. AUBIN, Y. KAYSER, M. BALLESTEROS, F. RENAUD, F. THOMAS, and M. GAUTHIER-CLERC. 2007. Influenza A virus in birds during spring migration in the Camargue, France. Journal of Wildlife Diseases 43: 789-793.

LUDWIG, S., A. HAUSTEIN, E. F. KALETA, and C. SCHOLTISSEK. 1994. Recent influenza A (H1N1) infections of pigs and turkeys in Northern Europe. Virology 202: 281-286.

LUPIANI, B., and S. M. REDDY. 2009. The history of avian influenza. Comparative Immunology, Microbiology and Infectious Diseases 32: 311-323.

LVOV, D. K., S. S. YAMNIKOVA, A. S. GAMBARYAN, I. T. FEDYAKINA, and M. N. MATROSOVICH. 2001. Isolation of influenza viruses from wild birds in the Volga River basin and in the North Caspian Region. International Congress Series 1219: 251-258.

MAKAROVA, N. A., N. V. KAVERIN, S. KRAUSS, D. SENNE, and R. G. WEBSTER. 1999. Transmission of Eurasian avian H2 influenza virus to shorebirds in North America. Journal of General Virology 80: 3167-3171.

MCWILLIAMS, S. R., and W. H. KARASOV. 2005. Migration takes guts: digestive physiology of migratory birds and its ecological significance. *In* Birds of Two Worlds: the Ecology and Evolution of Migration, R. Greenberg, and P. P. Marra, (eds.). Johns Hopkins University Press, Baltimore, MD, pp. 67-78.

METCALFE, N. B., and R. W. FURNESS. 1985. Survival, winter population stability and site fidelity in the Turnstone *Arenaria interpres*. Bird Study 32: 207-214.

MILLER, G. D., J. M. WATTS, and G. R. SHELLAM. 2008. Viral antibodies in south polar skuas around Davis Station, Antarctica. Antarctic Science 20: 455-461.

MIZRAHI, D. S., R. L. HOLBERTON, and S. A. GAUTHREAUX. 2001. Patterns of corticosterone secretion in migrating Semipalmated Sandpipers at a major spring stopover site. Auk 118: 79-91.

MOORE, F. R., R. J. SMITH, and R. SANDBERG. 2005. Stopover ecology of intercontinental migrants: en route problems and consequences for reproductive performance. *In* Birds of Two Worlds: the Ecology and Evolution of Migration, R. Greenberg, and P. P. Marra, (eds.). Johns Hopkins University Press, Baltimore, MD, pp. 251-261.

MORGAN, I. R., and H. A. WESTBURY. 1981. Virological studies of Adelie penguins (*Pygoscelis adeliae*) in Antarctica. Avian Diseases 25: 1019-1026.

MORRISON, R. I. G., and B. A. HARRINGTON. 1992. The migration system of the Red Knot *Calidris canutus rufa* in the New World. Wader Study Group Bulletin 64 (Supplement): 71-84.

MORRISON, R. I. G., R. E. GILL, B. A. HARRINGTON, S. SKAGEN, G. W. PAGE, C. L. GRATTO-TREVOR, and S. M. HAIG. 2001. Estimates of Shorebird Populations in North America. Canadian Wildlife Service Occasional Paper 104: 64.

MORRISON, R. I. G., and K. A. HOBSON. 2004. Use of body stores in shorebirds after arrival on High-Arctic breeding grounds. Auk 121: 333-344.

MORRISON, R. I. G., N. C. DAVIDSON, and J. R. WILSON. 2007. Survival of the fattest: body stores on migration and survival in Red Knots *Calidris canutus islandica*. Journal of Avian Biology 38: 479-487.

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. D. M. E. 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.

MUNSTER, V. J., and R. A. M. FOUCHIER. 2009. Avian influenza virus: of virus and bird ecology. Vaccine 27: 6340-6344.

MYERS, J. P., M. SALLABERRY, E. ORTIZ, G. CASTRO, L. M. GORDON, J. L. MARON, C. T. SCHICK, E. TABILO, P. ANTAS, and T. BELOW. 1990. Migration routes of New World Sanderlings (*Calidris alba*). Auk 107: 172-180.

MYERS, K. P., S. F. SETTERQUIST, A. W. CAPUANO, and G. C. GRAY. 2007. Infection due to 3 avian influenza subtypes in United States veterinarians. Clinical Infectious Diseases 45: 4-9.

NETTLESHIP, D. N. 1973. Breeding ecology of Turnstones *Arenaria interpres* at Hazen Camp, Ellesmere Island, N.W.T. Ibis 115: 202-217.

NETTLESHIP, D. N. 2000. Ruddy Turnstone (*Arenaria interpres*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 537, http://bna.birds.cornell.edu/bna/species/537. Accessed January 2011.

NEWTON, I. 2006. Can conditions experienced during migration limit the population levels of birds? Journal of Ornithology 147: 146-166.

NILES, L. J., J. BART, H. P. SITTERS, A. D. DEY, K. E. CLARK, P. W. ATKINSON, A. J. BAKER, K. A. BENNETT, K. S. KALASZ, N. A. CLARK, J. CLARK, S. GILLINGS, A. S. GATES, P. M. GONZALEZ, D. E. HERNANDEZ, C. D. T. MINTON, R. I. G. MORRISON, R. R. PORTER, R. KEN ROSS, and C. R. VEITCH. 2009. Effects of horseshoe crab harvest in Delaware Bay on Red Knots: are harvest restrictions working? Bioscience 59: 153-164.

NUNN, C. L., and S. ALTIZER. 2006. Host-parasite dynamics and epidemiological principles. *In* Infectious Diseases in Primates: Behavior, Ecology, and Evolution, C. L. Nunn, and S. Altizer, (eds.). Oxford University Press, New York, pp. 98-133.

OBENAUER, J. C., J. DENSON, P. K. MEHTA, X. P. SU, S. MUKATIRA, D. B. FINKELSTEIN, X. Q. XU, J. H. WANG, J. MA, Y. P. FAN, K. M. RAKESTRAW, R. G. WEBSTER, E. HOFFMANN, S. KRAUSS, J. ZHENG, Z. W. ZHANG, and C. W. NAEVE. 2006. Large-scale sequence analysis of avian influenza isolates. Science 311: 1576-1580.

OIE. 2008. Article 10.4: Avian Influenza. *In* The Terrestrial Animal Health Code. The World Organisation for Animal Health (OIE), Paris, France.

OLSEN, B., V. J. MUNSTER, A. WALLENSTEN, J. WALDENSTROM, A. OSTERHAUS, and R. A. M. FOUCHIER. 2006. Global patterns of influenza A virus in wild birds. Science 312: 384-388.

PANNWITZ, G., C. WOLF, and T. HARDER. 2009. Active surveillance for avian influenza virus infection in wild birds by analysis of avian fecal samples from the environment. Journal of Wildlife Diseases 45: 512-518.

PARMLEY, E. J., N. BASTIEN, T. F. BOOTH, V. BOWES, P. A. BUCK, A. BREAULT, D. CASWELL, P. Y. DAOUST, J. C. DAVIES, S. M. ELAHI, M. FORTIN, F. KIBENGE, R. KING, Y. LI, N. NORTH, D. OJKIC, J. PASICK, S. P. PRYOR, J. ROBINSON, J. RODRIGUE, H. WHITNEY, P. ZIMMER, and F. A. LEIGHTON. 2008. Wild bird influenza survey, Canada, 2005. Emerging Infectious Diseases 14: 84-87.

PEARCE, J. M., A. M. RAMEY, H. S. IP, and R. E. GILL. 2010. Limited evidence of trans-hemispheric movement of avian influenza viruses among contemporary North American shorebird isolates. Virus Research 148: 44-50.

PEIRIS, M., K. Y. YUEN, C. W. LEUNG, K. H. CHAN, P. L. S. IP, R. W. M. LAI, W. K. ORR, and K. F. SHORTRIDGE. 1999. Human infection with influenza H9N2. Lancet 354: 916-917.

PEREDA, A. J., M. UHART, A. A. PEREZ, M. E. ZACCAGNINI, L. LA SALA, J. DECARRE, A. GOIJMAN, L. SOLARI, R. SUAREZ, M. I. CRAIG, A. VAGNOZZI, A. RIMONDI, G. KONIG, M. V. TERRERA, A. KALOGHLIAN, H. C. SONG, E. M. SORRELL, and D. R. PEREZ. 2008. Avian influenza virus isolated in wild waterfowl in Argentina: Evidence of a potentially unique phylogenetic lineage in South America. Virology 378: 363-370.

PERKINS, D. E., P. A. SMITH, and H. G. GILCHRIST. 2007. The breeding ecology of Ruddy Turnstones (*Arenaria interpres*) in the eastern Canadian Arctic. Polar Record 43: 135-142.

PETERSON, A., S. BUSH, E. SPACKMAN, D. SWAYNE, and H. IP. 2008. Influenza A virus infections in land birds, People's Republic of China. Emerging Infectious Diseases 14: 1644-1646.

PETO, R., and J. PETO. 1972. Asymptotically efficient rank invariant procedures. Journal of the Royal Statistical Society Series B (Methodological) 135: 185-207.

PFISTER, C., M. J. KASPRZYK, and B. A. HARRINGTON. 1998. Body-fat levels and annual return in migrating Semipalmated Sandpipers. Auk 115: 904-915.

PIEROTTI, R. J., and T. P. GOOD. 1994. Herring Gull (*Larus argentatus*). *In* The Birds of North America Online, A. Poole, (ed.). Cornell Lab of Ornithology, Ithaca, New York. Species Account no. 124, http://bna.birds.cornell.edu/bna/species/124. Accessed January 2011.

PIERSMA, T., and R. I. G. MORRISON. 1994. Energy expenditure and water turnover of incubating Ruddy Turnstones: high costs under high Arctic climatic conditions. . Auk 111: 366-376.

PIERSMA, T., G. A. GUDMUNDSSON, and K. LILLIENDAHL. 1999. Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. Physiological and Biochemical Zoology 72: 405-415.

PIERSMA, T., J. RENEERKENS, and M. RAMENOFSKY. 2000. Baseline corticosterone peaks in shorebirds with maximal energy stores for migration: a general preparatory mechanism for rapid behavioral and metabolic transitions? General and Comparative Endocrinology 120: 118-126.

PIERSMA, T. 2007. Using the power of comparison to explain habitat use and migration strategies of shorebirds worldwide. Journal of Ornithology 148: S45-S59.

PLACYK, J. S., and B. A. HARRINGTON. 2004. Prey abundance and habitat use by migratory shorebirds at coastal stopover sites in Connecticut. Journal of Field Ornithology 75: 223-231.

POOLE, R. W. 1974. An Introduction to Quantitative Ecology. McGraw-Hill, New York, NY. 532 pp.

ROBINSON, R. A., P. W. ATKINSON, and N. A. CLARK. 2003. Arrival and weight gain of Red Knot *Calidris canutus,* Ruddy Turnstone *Arenaria interpres* and Sanderling *Calidris alba* staging in Delaware Bay in spring. British Trust for Ornithology Research Report 307: 51.

ROGAN, W. J., and B. GLADEN. 1978. Estimating prevalence from the results of a screening test. American Journal of Epidemiology 107: 71-76.

SECRETARIA DE VIGILÂNCIA EM SAÚDE. 2004a. Boletim Eletrônico Epidemiológico, Ano 04, No. 5. Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasilia.

SECRETARIA DE VIGILÂNCIA EM SAÚDE. 2004b. Boletim Eletrônico Epidemiológico, Ano 04, No. 2. Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasilia.

SHANNON, C. E. 1948. A mathematical theory of communication. Bell System Technical Journal 27: 379-423 and 623-656.

SHARP, G. B., Y. KAWAOKA, S. M. WRIGHT, B. TURNER, V. HINSHAW, and R. G. WEBSTER. 1993. Wild ducks are the reservoir for only a limited number of influenza A subtypes. Epidemiology and Infection 110: 161-176.

SHELDON, L. D., E. H. CHIN, S. A. GILL, G. SCHMALTZ, A. E. M. NEWMAN, and K. K. SOMA. 2008. Effects of blood collection on wild birds: an update. Journal of Avian Biology 39: 369-378.

SIVANANDAN, V., D. A. HALVORSON, E. LAUDERT, D. A. SENNE, and M. C. KUMAR. 1991. Isolation of H13N2 influenza A virus from turkeys and surface water. Avian Diseases 35: 974-977.

SMITH, D. R., P. S. POOLER, B. L. SWAN, S. F. MICHELS, W. R. HALL, P. J. HIMCHAK, and M. J. MILLARD. 2002. Spatial and temporal distribution of horseshoe crab (*Limulus polyphemus*) spawning in Delaware Bay: Implications for monitoring. Estuaries 25: 115-125.

SPACKMAN, E., D. A. SENNE, S. DAVISON, and D. L. SUAREZ. 2003. Sequence analysis of recent H7 avian influenza viruses associated with three different outbreaks in commercial poultry in the United States. Journal of Virology 77: 13399-13402.

SPACKMAN, E., D. E. STALLKNECHT, R. D. SLEMONS, K. WINKER, D. L. SUAREZ, M. SCOTT, and D. E. SWAYNE. 2005. Phylogenetic analyses of type A influenza genes in natural reservoir species in North America reveals genetic variation. Virus Research 114: 89-100.

SPACKMAN, E., K. G. MCCRACKEN, K. WINKER, and D. E. SWAYNE. 2006. H7N3 avian influenza virus found in a South American wild duck is related to the Chilean 2002 poultry outbreak, contains genes from equine and North American wild bird lineages, and is adapted to domestic turkeys. Journal of Virology 80: 7760-7764.

SPACKMAN, E., K. G. MCCRACKEN, K. WINKER, and D. E. SWAYNE. 2007. An avian influenza virus from waterfowl in South America contains genes from North American avian and equine lineages. Avian Diseases 51: 273-274.

SPACKMAN, E., and D. SUAREZ. 2008. Type A influenza virus detection and quantitation by real-time RT-PCR. Methods in Molecular Biology 436: 19-26.

SPACKMAN, E. 2009. The ecology of avian influenza virus in wild birds: What does this mean for poultry? Poultry Science 88: 847-850.

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., M. T. KEARNEY, S. M. SHANE, and P. J. ZWANK. 1990a. Effects of pH, temperature, and salinity on persistence of avian influenza viruses in water. Avian Diseases 34: 412-418.

STALLKNECHT, D. E., S. M. SHANE, M. T. KEARNEY, and P. J. ZWANK. 1990b. Persistence of avian influenza viruses in water. Avian Diseases 34: 406-411.

STALLKNECHT, D. E., and J. D. BROWN. 2007. Wild birds and the epidemiology of avian influenza. Journal of Wildlife Diseases 43: S15-S20.

STITZ, L., C. SCHMITZ, D. BINDER, R. ZINKERNAGEL, E. PAOLETTI, and H. BECHT. 1990. Characterization and immunological properties of influenza A virus nucleoprotein (NP): Cell-associated NP isolated from infected cells or viral NP expresses by vaccinia recombinant virus do not confer protection. Journal of General Virology 71: 1169-1179.

STROUD, D. A., A. BAKER, D. E. BLANCO, N. C. DAVIDSON, S. DELANY, B. GANTER, R. GILL, P. GONZALEZ, L. HAANSTRA, R. I. G. MORRISON, T. PIERSMA, D. A. SCOTT, O. THORUP, R. WEST, J. WILSON, and C. ZOKLER. 2006. The conservation and population status of the world's waders at the turn of the millenium. *In* Waterbirds Around the World, G. C.

Boere, C. A. Galbraith, and D. A. Stroud, (eds.). TSO Scotland Ltd., Edinburgh, UK, pp. 643-648.

SUAREZ, D. L. 2000. Evolution of avian influenza viruses. Veterinary Microbiology 74: 15-27.

SUAREZ, D. L., and S. SCHULTZ-CHERRY. 2000. Immunology of avian influenza virus: a review. Developmental and Comparative Immunology 24: 269-283.

SUAREZ, D. L., D. A. SENNE, J. BANKS, I. H. BROWN, S. C. ESSEN, C. W. LEE, R. J. MANVELL, C. MATHIEU-BENSON, V. MORENO, J. C. PEDERSEN, B. PANIGRAHY, H. ROJAS, E. SPACKMAN, and D. J. ALEXANDER. 2004. Recombination resulting in virulence shift in avian influenza outbreak, Chile. Emerging Infectious Diseases 10: 693-699.

SÜSS, J., J. SCHÄFER, H. SINNECKER, and R. G. WEBSTER. 1994. Influenza virus subtypes in aquatic birds of eastern Germany. Archives of Virology 135: 101-114.

SWAYNE, D. E., D. A. SENNE, and C. W. BEARD. 1998. Avian influenza. *In* A Laboratory Manual for the Isolation and Identification of Avian Pathogens, D. E. Swayne, (ed.). American Association of Avian Pathologists, Kennett Square, Pennsylvania, pp. 150-155.

SWAYNE, D. E., and D. A. HALVORSON. 2003. Influenza. *In* Diseases of Poultry, Y. M. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. Mcdougald, and D. E. Swayne, (eds.). Blackwell Publishing, pp. 135-160.

TERREGINO, C., R. DE NARDI, V. GUBERTI, M. SCREMIN, E. RAFFINI, A. M. MARTIN, G. CATTOLI, L. BONFANTI, and I. CAPUA. 2007. Active surveillance for avian influenza viruses in wild birds and backyard flocks in Northern Italy during 2004 to 2006. Avian Pathology 36: 337-344.

THRUSFIELD, M. 2005. Veterinary Epidemiology. 3rd edition. Blackwell Publishing, Oxford, United Kingdom. 610 pp.

TOMPKINS, D. M., A. P. DOBSON, P. ARNEBERG, M. E. BEGON, I. M. CATTADORI, J. V. GREENMAN, J. A. P. HEESTERBEEK, P. J. HUDSON, D. NEWBORN, A. PUGLIESE, A. P. RIZZOLI, R. ROSA, F. ROSSO, and K. WILSON. 2002. Parasites and host population dynamics. *In* The Ecology of Wildlife Diseases, P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson, (eds.). Oxford University Press, New York, NY, pp. 45-62.

TRACEY, J. P., R. WOODS, D. ROSHIER, P. WEST, and G. R. SAUNDERS. 2004. The role of wild birds in the transmission of avian influenza for Australia: an ecological perspective. Emu 104: 109-124.

TSIPOURA, N., and J. BURGER. 1999. Shorebird diet during spring migration stopover on Delaware Bay. Condor 101: 635-644.

U. S. FISH AND WILDLIFE SERVICE. 2003. Delaware Bay Shorebird-Horseshoe Crab Assessment Report and Peer Review. U. S. Fish and Wildlife Service Migratory Bird Publication R9-03/02, Arlington, VA. 99 pp.

U.S. ANIMAL HEALTH ASSOCIATION. 1994. Report of the Committee on Transmissible Diseases of Poultry and Other Avian Diseases. Criteria for determining that an AI virus isolation

causing an outbreak must be considered for eradication. *In* Proceedings of the 98th Annual Meeting U.S. Animal Health Assiciation. U.S. Animal Health Association, Grand Rapids, MI, pp. 522.

U.S. DEPARTMENT OF AGRICULTURE. 2009. U. S. Summary and State Data. *In* 2007 Census of Agriculture. http://www.agcensus.usda.gov/Publications/2007/index.asp.

VAHL, W. K., J. VAN DER MEER, K. MEIJER, T. PIERSMA, and F. J. WEISSING. 2007. Interference competition, the spatial distribution of food and free-living foragers. Animal Behaviour 74: 1493-1503.

VAN GILS, J. A., A. DEKINGA, B. SPAANS, W. K. VAHL, and T. PIERSMA. 2005. Digestive bottleneck affects foraging decisions in red knots Calidris canutus. II. Patch choice and length of working day. Journal of Animal Ecology 74: 120-130.

VAN GILS, J. A., V. J. MUNSTER, R. RADERSMA, D. LIEFHEBBER, R. A. FOUCHIER, and M. KLAASSEN. 2007. Hampered foraging and migratory performance in swans infected with low-pathogenic avian influenza A virus. PLoS ONE 2: e184.

VANDALEN, K. K., A. B. FRANKLIN, N. L. MOOERS, H. J. SULLIVAN, and S. A. SHRINER. 2010. Shedding light on avian influenza H4N6 infection in Mallards: Modes of transmission and implications for surveillance. PLoS ONE 5: e12851.

VELARDE, R., S. E. CALVIN, D. OJKIC, I. K. BARKER, and É. NAGY. 2010. Avian influenza virus H13 circulating in Ring-billed Gulls (*Larus delawarensis*) in southern Ontario, Canada. Avian Diseases 54: 411-419.

WAHLGREN, J., J. WALDENSTROM, S. SAHLIN, P. D. HAEMIG, R. A. M. FOUCHIER, A. D. M. E. OSTERHAUS, J. PINHASSI, J. BONNEDAHL, M. PISAREVA, M. GRUDININ, O. KISELEV, J. HERNANDEZ, K. I. FALK, Ã. LUNDKVIST, and B. OLSEN. 2008. Gene segment reassortment between American and Asian lineages of avian influenza virus from waterfowl in the Beringia area. Vector-Borne and Zoonotic Diseases 8: 783-790.

WALLENSTEN, A., V. J. MUNSTER, J. ELMBERG, A. OSTERHAUS, R. A. M. FOUCHIER, and B. OLSEN. 2005. Multiple gene segment reassortment between Eurasian and American lineages of influenza A virus (H6N2) in guillemot (*Uria aalge*). Archives of Virology 150: 1685-1692.

WALLENSTEN, A., V. J. MUNSTER, M. KARLSSON, A. LUNDKVIST, M. BRYTTING, M. STERVANDER, A. OSTERHAUS, R. A. M. FOUCHIER, and B. OLSEN. 2006a. High prevalence of influenza A virus in ducks caught during spring migration through Sweden. Vaccine 24: 6734-6735.

WALLENSTEN, A., V. J. MUNSTER, A. OSTERHAUS, J. WALDENSTROM, J. BONNEDAHL, T. BROMAN, R. A. M. FOUCHIER, and B. OLSEN. 2006b. Mounting evidence for the presence of influenza A virus in the avifauna of the Antarctic region. Antarctic Science 18: 353-356.

WEBSTER, R. G., M. YAKHNO, V. S. HINSHAW, W. J. BEAN, and K. COPAL MURTI. 1978. Intestinal influenza: Replication and characterization of influenza viruses in ducks. Virology 84: 268-278.

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., S. KRAUSS, D. HULSE-POST, and K. STURM-RAMIREZ. 2007. Evolution of influenza A viruses in wild birds. Journal of Wildlife Diseases 43: S1-S6.

WIDJAJA, L., S. L. KRAUSS, R. J. WEBBY, T. XIE, and R. G. WEBSTER. 2004. Matrix gene of influenza A viruses isolated from wild aquatic birds: Ecology and emergence of influenza A viruses. Journal of Virology 78: 8771-8779.

WINKER, K., K. G. MCCRACKEN, D. D. GIBSON, C. L. PRUETT, R. MEIER, F. HUETTMANN, M. WEGE, I. V. KULIKOVA, Y. N. ZHURAVLEV, M. L. PERDUE, E. SPACKMAN, D. L. SUAREZ, and D. E. SWAYNE. 2007. Movements of birds and avian influenza from Asia into Alaska. Emerging Infectious Diseases 13: 547-552.

WINKER, K., E. SPACKMAN, and D. E. SWAYNE. 2008. Rarity of influenza A virus in spring shorebirds, southern Alaska. Emerging Infectious Diseases 14: 1314-1316.

WOROBEY, M. 2008. Phylogenetic evidence against evolutionary stasis and natural abiotic reservoirs of influenza A virus. Journal of Virology 82: 3769-3774.

YAMNIKOVA, S. S., A. S. GAMBARYAN, A. B. TUZIKOV, N. V. BOVIN, M. N. MATROSOVICH, I. T. FEDYAKINA, A. A. GRINEV, V. M. BLINOV, D. K. LVOV, D. L. SUAREZ, and D. E. SWAYNE. 2003. Differences between HA receptor-binding sites of avian influenza viruses isolated from Laridae and Anatidae. Avian Diseases 47: 1164-1168.

ZAMARIN, D., M. B. ORTIGOZA, and P. PALESE. 2006. Influenza A virus PB1-F2 protein contributes to viral pathogenesis in mice. Journal of Virology 80: 7976-7983.

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.