Surveillance Plan for Highly Pathogenic Avian Influenza in Waterfowl in the United States
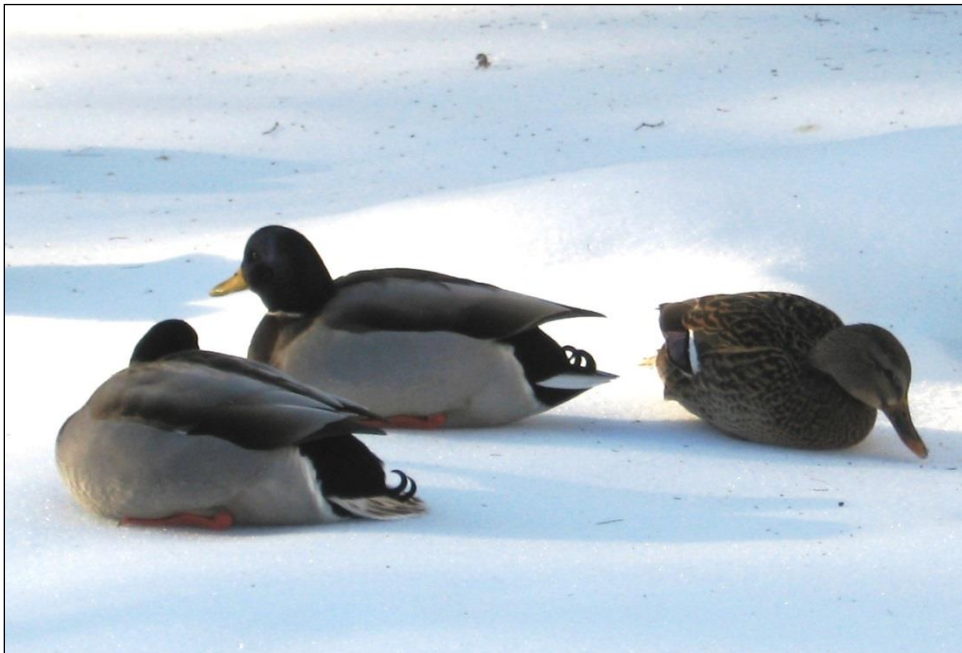
June 2015

Table of Contents

Executive Summary

Objectives

The purpose of this document is to detail the essential components of a national surveillance system for influenzas of interest in wild, migratory birds in order to provide HPAI infection risk information to domestic poultry producers and to the greater agricultural and USDA community. A priority is to determine the areas where HPAIV is located so poultry producers can be alerted and subsequently increase their biosecurity measures to help prevent direct or indirect introduction of HPAIV from wild birds. If HPAI-infected areas are detected, additional sampling efforts can then be undertaken to estimate prevalence in high risk bird species. An estimation of prevalence will be useful for determining the probability of HPAI being introduced into the domestic poultry population.
This plan is intended to provide guidance for Federal agencies and other cooperators to conduct influenza surveillance in wild birds. Collaborating entities include the USDA Animal and Plant Health Inspection Service (APHIS) Wildlife Services (WS) and Veterinary Services (VS); the United States Geological Survey (USGS); U.S. Fish and Wildlife Service (USFWS); and the National Flyway Council. The plan supports the collection, diagnostic testing, data management, and analysis of data obtained by USDA-APHIS-Wildlife Services (WS), other Federal agencies, State wildlife agencies, and tribal cooperators. Specific Objectives of the plan are:

Specific Objectives

1) Identify the distribution of influenzas of interest by U.S. flyways and through select, high priority watersheds
2) Detect spread of influenzas of interest to new areas of concern
3) Provide a flexible surveillance framework that can be modified to monitor wild waterfowl populations for re-assortments of influenzas, introductions of new viruses, and to estimate apparent prevalence of important influenzas once detected in an area of concern.

The goal is for this sampling effort to provide information in order to improve management actions that are taken to address the multitude of issues associated with HPAIVs. This includes risks to commercial poultry, backyard poultry, game bird farms, wild birds, wild bird rehabilitation facilities, falconry birds, and captive bird collections in zoos/aviaries.

Recommendations

This plan integrates analyses describing dabbling duck movements/flyways, dabbling duck mixing areas, environmental constraints for persistence of influenza virus, and historic influenza prevalence in waterfowl to identify, using a risk based approach, priority regions for surveillance activities. These analyses identified a minimum of 136 watersheds as important for sampling with 95 watersheds identified as critical for surveillance. Surveillance was recommended across three biologically and epidemiologically important seasons – summer, fall and winter.

Introduction

This document describes a plan to conduct national level surveillance for highly pathogenic avian influenza virus (HPAI) in wild migratory birds. Collaborating entities include the USDA Animal and Plant Health Inspection Service (APHIS) Wildlife Services (WS) and Veterinary Services (VS); the United States Geological Survey (USGS); U.S. Fish and Wildlife Service (USFWS); and the National Flyway Council. The plan supports the collection, diagnostic testing, data management, and analysis of data obtained by USDA-APHIS-Wildlife Services (WS), other Federal agencies, State wildlife agencies, and tribal cooperators. Implementation of national level surveillance directly supports the U.S. Interagency Strategic Plan for the Monitoring of Avian Influenzas of Significance in Wild Birds (Interagency Strategic Plan).

Highly Pathogenic Avian Influenza in the United States

The first report of H5N1 HPAI occurred in Asia in 1996 and now a diverse viral gene pool exists in the world due to co-circulation of many avian influenza viruses (AIVs) in domestic and wild birds. The Asian H5N1 HPAI is the predecessor of multiple viral reassortants, including H5N2, H5N5, and H5N8. Since May 2013, nearly twice as many H5 and H7 AIVs have been reported in domestic and wild bird samples across the world compared to the previous 18 months.

In the fall of 2014, H5N2 was identified in commercial poultry in the Fraser Valley region of southern British Columbia, Canada. Subsequent samples collected from wild birds in the United States, combined with mortality events associated with captive raptors, revealed at least two HPAIs in circulation. WS, in coordination with State agencies, USGS, and other Federal agencies, continues to collect wild bird samples to define the extent of HPAI infection in specific avian species groups (Appendix 1). Surveillance activities targeting wild bird samples from hunter-killed animals revealed that by January 2015, every State sampled in the Pacific Flyway had at least one HPAI positive. Detections continue to be reported in additional flyways, along with multiple outbreaks in backyard and commercial poultry.

Specific Goals and Objectives

The purpose of this document is to detail the essential components of a national surveillance system for influenzas of interest in wild, migratory birds in order to provide HPAI infection risk information to domestic poultry producers and to the greater agricultural and USDA community. A priority is to determine the areas where HPAIV is located so poultry producers can be alerted and subsequently increase their biosecurity measures to help prevent direct or indirect introduction of HPAIV from wild birds. If HPAI-infected areas are detected, additional sampling efforts can then be undertaken to estimate prevalence in high risk bird species. An estimation of prevalence will be useful for determining the probability of HPAI being introduced into the domestic poultry population.

This plan is intended to provide guidance for Federal agencies and other cooperators to conduct influenza surveillance in wild birds. This plan will be evaluated and refined after 1 year to reflect new findings.

Objectives

1) Identify the distribution of influenzas of interest by U.S. flyways and through select, high priority watersheds
2) Detect spread of influenzas of interest to new areas of concern
3) Provide a flexible surveillance framework that can be modified to monitor wild waterfowl populations for re-assortments of influenzas, introductions of new viruses, and to estimate apparent prevalence of important influenzas once detected in an area of concern.

The goal is for this sampling effort to provide information in order to improve management actions that are taken to address the multitude of issues associated with HPAIVs. This includes risks to commercial poultry, backyard poultry, game bird farms, wild birds, wild bird rehabilitation facilities, falconry birds, and captive bird collections in zoos/aviaries.

Migratory Waterfowl Movement

Waterfowl and water bird migration in North America generally consists of north-south seasonal movements between breeding grounds and wintering areas. There are four administrative flyways in North America (Figure 1). These flyways are broadly defined corridors where the migratory paths of many species of interest tend to converge.   They are associated with major topographical features in North America, which also tend to be aligned along a north-south axis. The four flyways—Atlantic, Mississippi, Central, and Pacific—have areas of overlap and convergence, particularly at the north and south ends. The administrative flyway boundaries are defined based on known bird migration routes, but the biological flyway boundaries are not geographically fixed or sharply defined.

Figure 1: The four administrative flyways in the US

North American flyways represent the predominant pathways of migratory bird movements within broad geographic areas. Many migratory bird species use specific flyways; however, many species migrate across flyways during the fall and spring. The Pacific Flyway is thought to be the most likely area of introduction for the HPAI viruses detected in Canada and the United States in December of 2014.
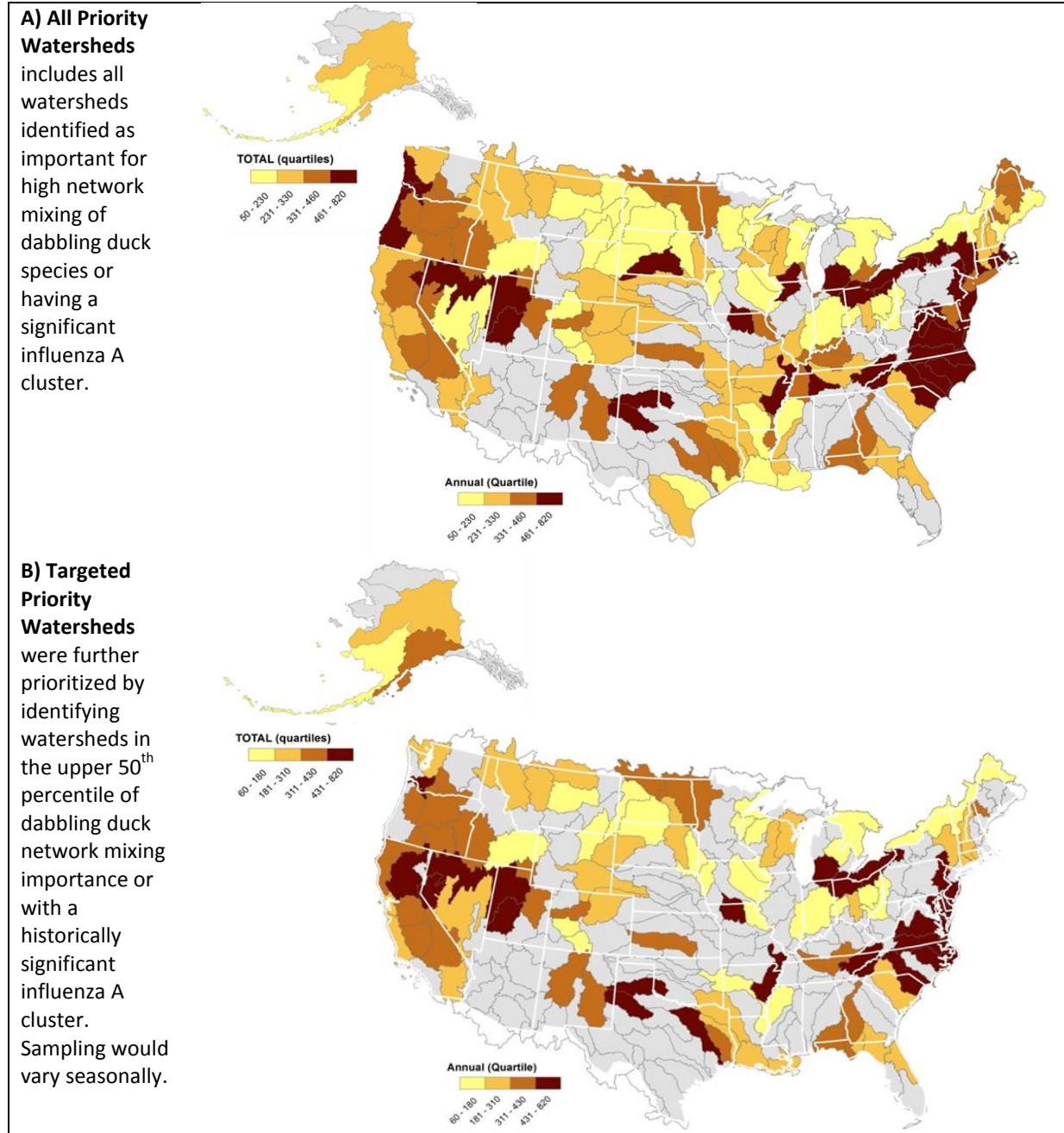
Species Identified for Collection

In 2006, the U.S. Departments of Agriculture and Interior, along with multiple State and tribal agencies, implemented a nationally coordinated avian influenza surveillance effort in wild birds. This large-scale surveillance system has provided an unprecedented amount of data on avian influenza viruses in U.S. wild bird populations and can inform current surveillance efforts. This dataset, along with many others, has identified dabbling ducks as the primary reservoir for avian influenzas, including H5s and H7s [1,2]. Therefore, the primary focus of sampling will continue to be on dabbling ducks species including American Green-Winged Teal, Mallard, Northern Pintail, American Black Duck, Wood Duck, Blue-Winged Teal, Cinnamon Teal, Northern Shoveler, Gadwall, and American Wigeon. Close coordination should occur at the local level to ensure complementary and additive surveillance and sampling approaches. Coordinating sampling with planned banding activities, waterfowl hunts, or other similar projects would provide an opportunity to increase efficiency and provide valuable cost-saving measures.

Identification of Priority Watersheds

With the intended goal of identification within a biologically relevant scale and not at the State level, watersheds were selected as the sampling unit for the surveillance design. Watersheds are used to identify key factors important to waterfowl and influenza biology and ecology. To identify the baseline HPAI distribution across U.S. flyways and constituent watersheds and to detect early spread of HPAI to new flyways and regions, sample units were defined geographically using the USGS 4-digit hydrologic unit code (HUC4). There are approximately 222 HUC4s in the United States and they represent an area drained by a river system, a reach of a river and its tributaries in that reach, a closed basin(s), or a group of streams forming a coastal drainage area [3].

As a result, all States may not be represented in the design. Also, Hawaii was not included in this initial analysis, because the data needed to identify priority surveillance areas were not available; however, sample sizes can be developed for Hawaii using more limited data in the future if needed. To identify which watersheds increase the likelihood of detection for influenzas of interest, we used a combination of two measures and two constraints to capture the underlying biological aspects for influenza. The measures included: 1) watersheds with significant historic influenza clusters (Appendix 2) and 2) watersheds identified as having high inter- and intra-flyway mixing of dabbling ducks within the lower 48 States (Appendix 3). The two constraints were based on median annual number of days above 0°C and below 0°C in a watershed (Appendix 4). These were used in concert to develop targeted surveillance.

Figure 2. Targeted watersheds that ranked as a significant priority (colored) or low priority (gray/white) using network movements of dabbling ducks and influenza A clusters. Legend indicates the estimated number of samples required.

A) All Priority Watersheds includes all watersheds identified as important for high network mixing of dabbling duck species or having a significant influenza A cluster.

B) Targeted Priority Watersheds were further prioritized by identifying watersheds in the upper 50th percentile of dabbling duck network mixing importance or with a historically significant influenza A cluster. Sampling would vary seasonally.
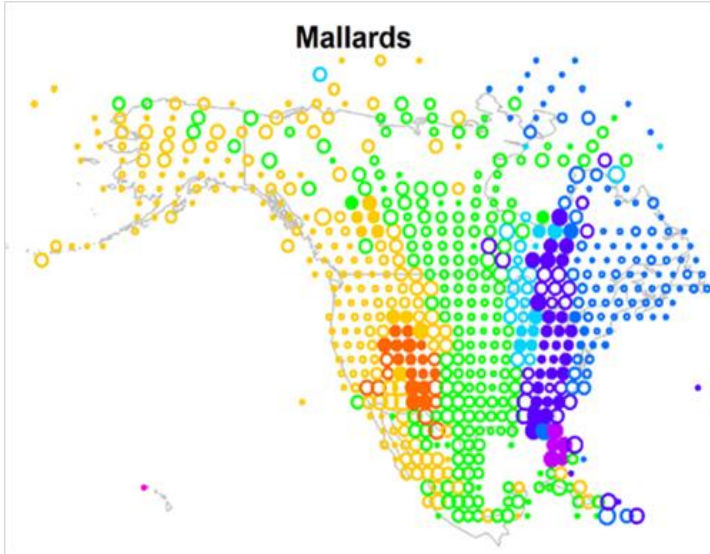
1) Historic influenza clusters

To identify watersheds with significant influenza clusters, we used data collected from dabbling ducks during the surveillance effort conducted from 2006-2011 for the early detection of HPAI H5N1. To encompass known seasonal differences in influenza prevalence in waterfowl and important biological periods for waterfowl migration, the analysis considers three periods. Briefly, influenza in waterfowl has a well-documented seasonal pattern which increases through the breeding season, peaks in late summer-early fall, and then typically declines through the fall breeding period and is at its lowest during the over wintering period [4]. With this pattern in mind, our periods represent the summer breeding season (May-August), fall migratory season (August-December), and the over-wintering season (December-February). The spring northern migration was not included for three reasons. First, little is known about spring migration and data to estimate spring populations is not available. Second, data describing spring influenza is limited. Finally, logistical constraints on sampling during this time of year are significant.

All dabbling duck samples tested for the influenza matrix gene were separated by three biologically relevant seasons and analyzed using the Getis-Ord Gi* spatial statistic to identify historic "hot spots" of influenza activity in dabbling ducks at a regional watershed scale. This resulted in identifying specific watersheds as having or not having significant influenza clusters (see Appendix 2). This analysis revealed geographic regions where influenza virus in sampled waterfowl was higher than expected [5]. A majority of these influenza clusters occur in northern latitudes, a finding supported by previous studies related to location of major waterfowl breeding grounds and additional research on the environmental persistence of influenzas at cold temperatures [6]. These results offer an important data stream to inform high priority areas for wild bird avian influenza surveillance. One limitation of this approach is that earlier surveillance was collected conveniently and did not have a random design. However, there are no randomly collected avian influenza datasets, thus this information represents the best data to date describing influenza at a national scale. In addition, the recent introduction of HPAI is the first and only recorded evidence of HPAI in North America. Consequently, there are no national scale data available on HPAI. Our assumption is that because dabbling ducks are the natural reservoir for AI and are typically asymptomatically infected, LPAI serves as a suitable proxy for HPAI. However, it is unclear at this time if LPAI and HPAI will behave differently in North America.

Figure 3. Example map showing nodes that depict the spatial distribution of mallards. Color represents flyway membership and the size of the node is scaled to the CF metric, representing regions important to flyway structure and thus potential areas of increased mixing. Filled circles are statistically significant CF nodes at α=0.05 .

2) Inter and intra-flyway mixing of dabbling ducks

To identify watersheds important for mixing of waterfowl populations, we used the consolidation factor(CF), a recently developed metric that pinpoints geographic areas of high mixing for multiple dabbling duck species [7]. Specifically, this metric is a measure of within- and between-flyway movement processes important for influenza, and is based on historic U.S. Fish and Wildlife Service banding data [7]. The metric was used to identify watersheds within each flyway that account for the majority of within-flyway mixing of waterfowl and to identify watersheds important for linking watersheds (identifying watersheds where mixing of birds from multiple flyways occurs.) See Appendix 3 for detailed methods.

3) Temperature constraints

These watersheds were then constrained to address biological processes for both the host and the pathogen. Temperature is associated with waterfowl migration and influences avian influenza persistence in the environment. There is a well-documented pattern of reduced avian influenza prevalence in waterfowl at southern latitudes, especially during the breeding season [6]. This is largely attributed to viral degradation at higher temperatures [8]. To address this within the framework of a targeted surveillance effort, we incorporated the documented decay of virus at increasing temperatures above 0˚C to identify watersheds during the breeding period in which AI was less likely to persist. Because water salinity and pH also influence avian influenza, we used a conservative approach—removing watersheds with a 30-year median annual number of days above 0˚C of 365. See Appendix 4 for detailed methods.

Ambient temperature and number of consecutive days below 0˚C have direct consequences for waterfowl [9,10], and can affect their energy requirements. These cumulative effects also influence water temperature and ice formation. Increasing ice coverage can decrease availability of wetland foods, reducing nutrient acquisition by wetland-obligate waterfowl (e.g., gadwall, northern shoveler) and resulting in migration. We addressed this in surveillance targeting by removing watersheds from surveillance during the overwintering period in which waterfowl were unlikely to

be present. Because migration can be influenced by a myriad of other factors such as snow and ice cover precluding foraging for field-feeding waterfowl (e.g., mallard), we removed watersheds with an estimated 30-year median number of days below 0˚C of at least 180 from the winter surveillance effort.

Sample Size Estimation

1) Waterfowl Population Estimates

Currently, seasonal population estimates for waterfowl at a watershed or regional level are not available for the entire United States. To mitigate this, we developed a Bayesian Gamma-Poisson Mixture model to estimate a 15-year (1998-2013) median seasonal population within each watershed. The approach integrated bird band recovery data and the estimated annual continental dabbling duck population available from the U.S. Fish and Wildlife Service. The result was an estimate of the median population of ducks (with 95 percent credible intervals) that might be expected in the watershed during the fall and winter. We used an alternative method to derive breeding season (May-August) population estimates because bird banding data at the continental scale is not available for the spring or summer periods. To estimate summer populations, we used the breeding bird survey data, one of the only large-scale datasets available, to develop an aggregate estimate of relative abundance at the watershed scale [11]. We did not estimate spring populations because band recovery data or other survey data are not available. Our approach for developing course population estimates ensures that the influenza surveillance effort accounts for the movement of migratory dabbling duck species across space and time. While these approaches have limits, they provide a relative understanding of the distribution and potential populations across the United States. Appendix 4 describes the method for estimating populations and the corresponding assumptions in greater detail.

2) Avian Influenza Occurrence in U.S. Waterfowl Populations

To estimate true seasonal expected influenza A prevalence within watersheds, we used prior dabbling duck surveillance data from 2006 to 2011. A Bayesian Beta-Binomial model was used to estimate the true seasonal and monthly prevalence. The Bayesian model accounted for uncertainties in the diagnostic test process (sensitivity and specificity), differences in seasonal and monthly sampling efforts, and observed variability from year to year in influenza A prevalence. This method resulted in estimates for each watershed for the seasonal median prevalence (with 95 percent credible intervals) expected during the fall, winter, and breeding periods, should influenza be introduced. Appendix 4 describes specific details about the model.

3) Estimation of Watershed Sample Sizes

Sample sizes required to identify influenzas of interest were developed for each season within each priority watershed. Using the seasonally expected dabbling duck population (part 1) and the expected influenza prevalence (part 2) given introduction, we estimated the number of samples

required to detect influenzas of interest for each season. We assumed that the influenza of interest (i.e. any highly pathogenic influenza) represented 20 percent of the expected influenzas in a watershed, which is within the range of previously reported H5 prevalence values in dabbling ducks. This provided the data needed to compute detection prevalence thresholds.

The numbers in Tables 1 and 2 represent the total annual number of samples [12] needed across watersheds to detect the presence of influenza A of interest at a 95 percent confidence level within a given watershed if the prevalence meets or exceeds the detection prevalence threshold. Detection prevalence thresholds for influenzas of interest vary among and within each sampled watershed by season, but have a minimum level of detection of 1%. The sample sizes also assume 86.3 percent diagnostic sensitivity for the matrix rRT-PCR (Janice Pedersen, personal communication; Mia Torchetti, personal communication). Sampling all watersheds requires extremely large sample sizes. In contrast, targeting sampling of high priority watersheds (Figure 4) lowers the sample sizes required while still allowing us to infer distribution across the United States for influenzas of interest, because the targeting criteria preferentially selects watersheds with greatest connectivity and potential for spread (i.e., those most at risk for introduction of influenzas of interest).

Table 1. Estimated annual sample sizes needed for detections at two different scales

	Number of Watersheds	Sample Sizes for Detection[1]
All Priority Watersheds[2]	136	47,730
Targeted Priority Watersheds[3]	95	30,340

Table 2. Estimated seasonal sample sizes needed by watershed ranking aggregated to the flyway

All Priority Watersheds	No. of Watersheds		**Sample Sizes**					
			Pacific	Central	Mississippi	Atlantic	Total	Percent
Summer	102		3,050	3,410	4,450	5,260	**16,170**	**33%**
Fall	128		4,900	3,590	4,360	2,880	**15,730**	**32%**
Winter	93		4,010	3,250	4,880	4,500	**16,640**	**35%**
All Seasons	**139**	Total	**11,960**	**10,250**	**13,690**	**12,640**	48,540	

Targeted Priority Watersheds	No. of Watersheds		**Sample Sizes**					
			Pacific	Central	Mississippi	Atlantic	Total	Percent
Summer	62		2,690	2,560	2,220	2,310	**9,500**	**31%**
Fall	82		4,180	2,530	2,170	1,520	**9,870**	**33%**
Winter	59		3,360	1,870	2,770	2,970	**10,970**	**36%**
All Seasons	**98**	Total	**10,230**	**6,960**	**7,160**	**6,800**	**31,150**	

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[1] Sample sizes are computed at 95% confidence assuming a diagnostic test sensitivity of 86.3% and are a function of detection threshold with a minimum of 1% and estimated population sizes per watershed in each season. The range of detection level is based on the observed influenza A in the watersheds and assuming influenzas of concern account for 20% of all influenzas.
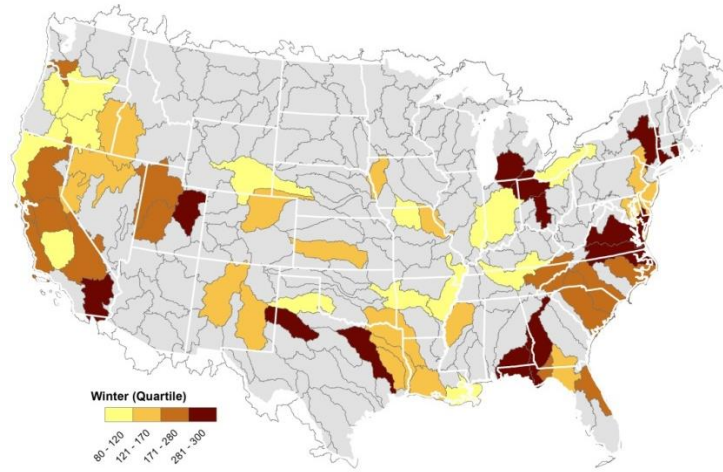
[2] This assumes surveillance in all priority watersheds in all seasons (Figure 2a).

[3] Watersheds that are in the upper 50% of network importance or have an influenza A cluster in a specific season (Figure 2b).

Figure 4. Number of samples to collect annually by watershed and season for surveillance based on targeted priority watersheds

Summer

Summer (Quartile)
60 - 90 91 - 150 151 - 260 261 - 300

Fall

Fall (Quartile)
60 - 80 81 - 110 111 - 170 171 - 260
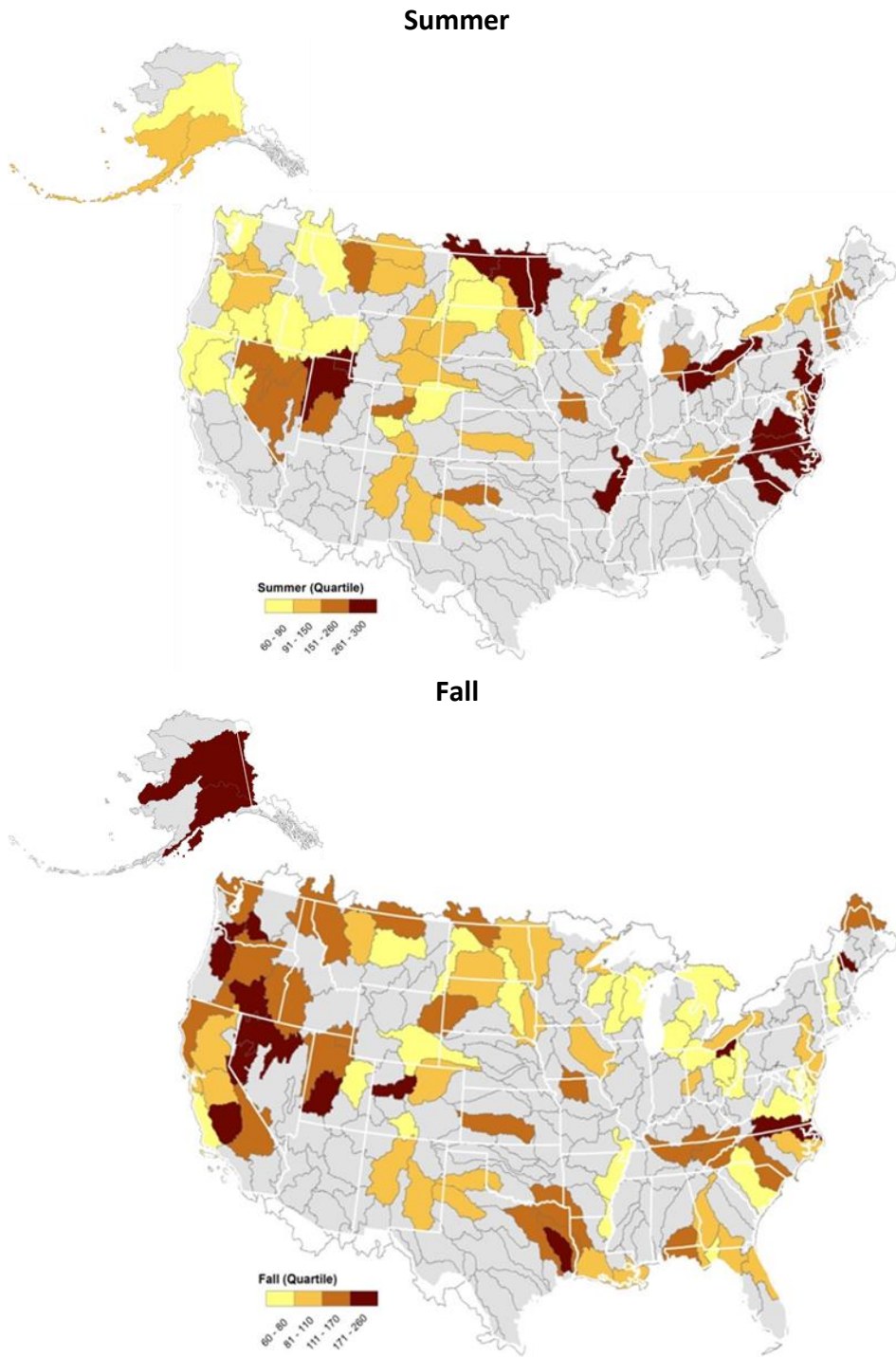
Winter

Sample Collection

National-level surveillance for HPAI in wild, migratory birds will focus on targeted priority watersheds (a minimum of 31,150 samples) and follow a biological year beginning April 1, 2015, through March 31, 2016. During the fall period samples should be collected when dabbling ducks or other species of interest are migrating into or through a specific State. Timing of seasonal migratory movements can vary widely depending on species, region, and current weather patterns. Sample collection efforts should be coordinated within each State and include efforts by Federal, State, local, university, and non-governmental participants. Coordination will allow for efficient and cost-effective collection of wild bird samples. Local expertise should be used to attempt to collect informative samples from areas and species of high importance.

Multiple collection strategies are identified in the Interagency Strategic Plan: live wild bird, hunter harvest, morbidity/mortality investigation, and environmental sampling. This surveillance plan supports using a combination of strategies to increase the probability of detecting HPAI. Sampling morbidity and mortality events may be informative, but to date, the HPAI viruses circulating in wild birds in the United States cause limited morbidity and mortality in most dabbling duck species. There is evidence that multiple raptor species and owl species may die from HPAI infections (see Appendix 5). States should remain flexible to incorporate opportunistic sampling of morbidity/mortality events in highly susceptible species as needed. We recommend contacting the appropriate personnel from raptor rehabilitation or related outreach programs when signs of infection are detected in raptors and briefing them on appropriate communication, sampling, and biosecurity responses.

Opportunistic sampling of any morbidity or mortality event in these species could provide highly relevant information. In the case of large numbers of sick or dying birds, a subset of wild birds should be sampled depending on the circumstances surrounding the morbidity/mortality event (including the number of wild birds involved and potential causes of death). Investigations related to morbidity/mortality events should be conducted regardless of time of year, number of species involved, or number of samples already collected in the State. In addition, as more data become available on how these novel influenzas impact avian species, managers and Federal agencies may be interested in sampling areas that intersect with species of conservation concern (see Appendix 6). A similar approach can be used in regions of poultry production. Targeted priority watersheds identified in this surveillance effort inherently include areas of poultry production. However, in specific regions of concern, we recommend sampling that also informs poultry producers of local influenza risk so they can increase biosecurity measures to help prevent direct or indirect introduction of HPAIV from wild birds (see Appendix 7).

Sample collection should be redistributed to additional watersheds if the number of samples targeted for collection within a watershed is not met, or if a watershed is not accessible for sample collection. These additional replacement watersheds will be taken from priority watersheds identified in the Targeted Priority Watersheds plan. The decision of which watershed to sample will be determined by proximity to the watershed originally slated for sampling. Sample sizes for the new watershed will differ from the original watershed and will be based on watershed-specific calculations.

WS and its cooperators will continue to collect one cloacal and one oropharyngeal swab from each wild bird sampled by hunter harvest, and live wild bird collection strategies. Cloacal and oropharyngeal swabs will be combined in the same tube of media. All samples will be submitted to an approved National Animal Health Laboratory Network (NAHLN) laboratory. The NAHLN laboratory will screen samples to determine if type A influenza virus is present; if the test is positive, the sample will be further analyzed by H5 and H7 specific assays. Samples testing H5 or H7 positive at a NAHLN laboratory will be sent to the National Veterinary Services Laboratory (NVSL) for confirmatory testing and final diagnosis.

Appendix 1. Wild Bird Species That Tested Positive for HPAI in the United States (December 2014-May 20, 2015)

1. American Green-winged Teal *(Anas crecca)*
2. American Wigeon *(Anas americana)*
3. Gadwall *(Anas strepera)*
4. Mallard *(Anas platyrhynchos)*
5. Northern Pintail *(Anas acuta)*
6. Northern Shoveler *(Anas clypeata)*
7. Wood Duck *(Aix sponsa)*
8. Cooper's Hawk *(Accipiter cooperii)*
9. Peregrine Falcon (*Falco peregrinus)*
10. Red-tailed Hawk (*Buteo jamaicensis)*
11. Bald Eagle *(Haliaeetus leucocephalus*)
12. Canada Goose (*Branta canadensis)*
13. Cinnamon Teal (*Anas cyanoptera*)
14. Lesser Snow Goose (*Chen caerulescens)*
15. Ring-necked Duck (*Aythya collaris*)
16. Snowy Owl (*Bubo scandiacus*)

Appendix 2. Methods for Historical AI Clusters in Wild Birds

Dabbler matrix infection data were stratified by date collected (Fall=August-December, Winter=December-February, Breeding=May-August; time periods overlap because of variability across migrations) and related spatially on USGS 4-digit hydrologic unit code (HUC4). Clusters of matrix positive wild birds were identified using the Getis-Ord Gi* spatial statistic in ArcGIS. The analysis examines wild bird data (the number of samples and the AI status of each sample) that has been aggregated at the HUC4 watershed scale. Avian influenza activity within a watershed is analyzed in the context of neighboring watersheds. To be considered a statistically significant hotspot, the watershed will have a higher value and the surrounding watersheds will also have a higher value that would be expected due to random chance.

We ran the analysis for both number of positive birds aggregated at the HUC4 watershed scale (count data) and on matrix prevalence aggregated at the HUC4 scales (proportion data). Watersheds identified as having a significant cluster of AI matrix positive dabblers or watersheds with a significant cluster of high matrix positive prevalence values were identified as high priority sample targets. Analyses used a previously calculated distance band of 100,000 meters (Bevins et. al, 2014).

Appendix 3. Development of Watershed Level Consolidation Factor (Waterfowl Mixing) Metrics

Buhnerkempe (2012) developed network metrics for network regions at a resolution of 200km. To assign these metrics to watersheds, a method for disaggregation and assignment of the measures to watersheds was developed. The network nodes represent band recoveries for distinct square regions in North America extending 100km out from a central reference location. In order to disaggregate and assign measures to watersheds, the network measure of interest for each HUC4 watershed, CF_i^{HUC4}, were estimated using the method described in Equation 1 and Equation 2.

The network measure for three dabbling duck species (northern pintail [NOPI], American green-winged teal [AGWT], and mallards [MALL]) is $\boldsymbol{W_i^{HUC4}}$ for each HUC4 watershed. This is the weighted sum of the network metric M_{jk} for HUC8 watershed j (within 100 km of the central reference location for network node k.) P_{jk}^{HUC8} is a weighting factor representing the 20-year mean proportion of dabbling duck band recoveries, njk, in HUC8 watershed j within the neighborhood of network node k. The CF was first assigned to the HUC8 watershed because the course spatial resolution of the HUC4 watersheds contained multiple network nodes. The HUC8 watersheds provided a resolution that allowed representation of the neighborhoods of each network node (Figure 5).

Equation 1. Assignment of network measures to watersheds

$$W_i^{HUC4} = \sum_{i=1}^{n} M_{jk} \, P_{jk}^{HUC8}$$

$$\text{Where, } P_{jk}^{HUC8} = \frac{n_{jk}}{\sum_{k=i,j=1}^{J} n_{jk}}$$

To estimate the consolidation factor for dabbling ducks, we used an aggregate measure for mallards, American green-winged teal, and northern pintails. These three species are assumed to represent the majority of mixing and connectivity within North American waterfowl; thus, this aggregate metric can be considered a proxy for dabbling ducks. The aggregate measure assumed a linear relationship across the three species and was calculated as:

**Equation 2. Aggregate measure for consolidation factor.**

$$CF_i^{HUC4} = \sum_{i=1}^{n} W_{jk}^{MALL} + W_{jk}^{AGWT} + W_{jk}^{NOPI}$$

Figure 5. Example of the neighborhood for one network node (red dot and black line) using HUC8 watersheds. Colors represent the number of band recoveries in each watershed. Gray indicates no band recoveries.
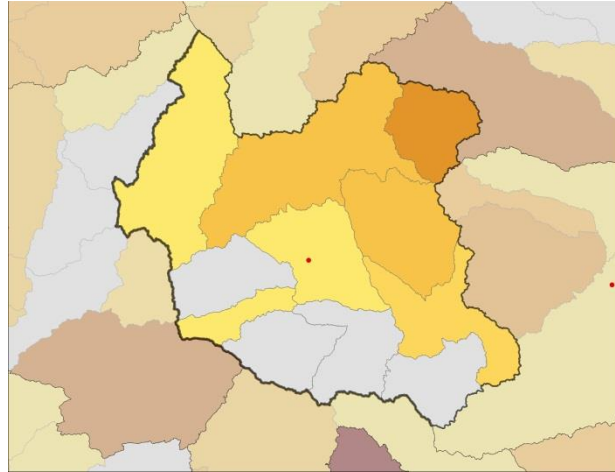
This method of disaggregation allowed the identification and weighting of watersheds by their contribution to the CF. Figure 5 illustrates this point and represents the neighborhood of watersheds and the band recoveries that would have contributed to the network metric. There is obvious heterogeneity across the neighborhood from watershed to watershed. Figure 6 illustrates the HUC8 level aggregate CF and the HUC4 aggregated CF. As presented in Figure 6, for some HUC4 watersheds only a small fraction of sub-watersheds (HUC8) were important for mixing. For this reason we only considered HUC4 watersheds that had a minimum proportion of 0.3 represented by HUC8 watersheds important for mixing in the ranking of watersheds for surveillance. We acknowledge that the minimum proportion of 0.3 is somewhat arbitrary, but it allows the identification of priority HUC4 watersheds. It also serves to address logistical considerations of allocating sampling to a HUC4 watershed with only a small proportion important for waterfowl mixing.

Figure 6. Aggregate consolidation factor for HUC8 and HUC4 watersheds. Note some HUC8 watersheds with CF values only represent a small proportion of HUC4 watersheds.
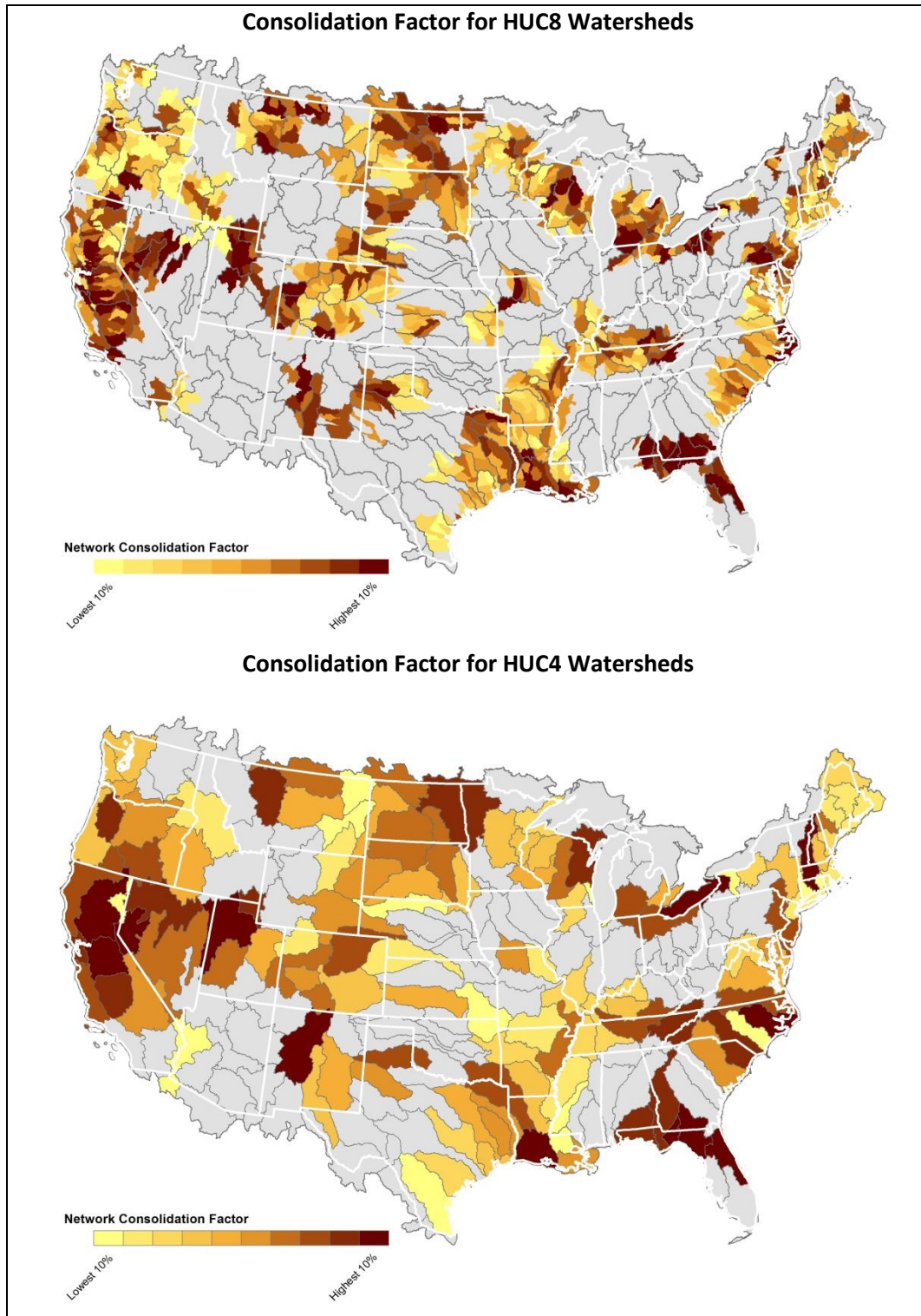
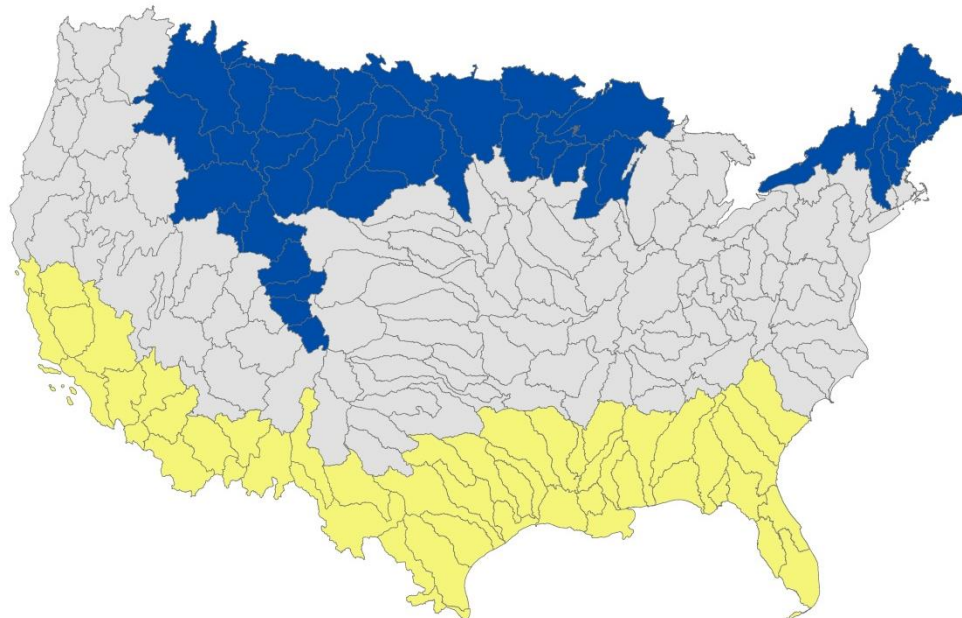# Appendix 4. Dabbling Duck Population Estimation and Temperature Constraints

Temperature constraints, the cumulative number of days above 0˚C and below 0˚C, were derived for each watershed in a given year from the National Oceanic and Atmospheric Administration (NOAA) weather station data. We identified weather stations within 250 km of each watershed centroid (up to 10 closest stations), then calculated the number of days each station had an observed maximum temperature above 0˚C and the number of days with an observed minimum temperature below 0˚C. We then adjusted for the difference in elevation between each weather station and the watershed centroid using the average adiabatic lapse rate temperature correction formula [13]:

$$\Delta T = 6.49\text{˚C}/1000 \text{ m}$$

where ΔT represents a change in temperature of 6.49˚C for every 1,000 meters of elevation gained or lost between the weather station location and the watershed centroid. We averaged across the selected weather stations and over 30 years of observations, or all years in a 30-year time period for which data were available. The results of the temperature constraint are presented in

Figure 7.

Figure 7. Watersheds identified with a 30-year median number of 365 days above 0˚C (yellow) and 180 days below 0˚C (blue). Data derived from National Oceanic and Atmospheric Administration weather station data.

Expected Dabbling Duck Populations

To estimate seasonal expected population that might be present in a watershed, we used two available datasets: bird band and recovery data and the estimated annual continental dabbling duck population available from the U.S. Fish and Wildlife Service from 1998 to 2012. These data were used to estimate the expected proportion of the continental dabbling duck population present within each watershed in each season. This proportion was then used in concert with the estimated annual continental dabbling duck population to estimate the median population within each watershed. We used a Poisson-Gamma mixture model to model the median population in each watershed. The model structure for the population of dabbling ducks in HUC4 watershed *j* in time *t* and year *k* is estimated using the hierarchical model in Equation 3. The watershed population is modeled using the deterministic model in Equation 4.

Equation 3.

$$\left[\lambda_{jtk}, \sigma_k^2 | b_{jtk}, t_{tk}, \theta_k\right]$$
$$\propto Poisson\left(\lambda_{jtk}\right) Gamma\left(\lambda_{jtk} | \frac{g(b,t,\theta)}{\sigma_k^2}, \frac{g(b,t,\theta)}{\sigma_k^2}\right) InverseGamma\left(\sigma_k^2 | .001, .001\right)$$

Where $g(b, t, \theta)$ is the expected proportion of the population in watershed *j* in time *t* and year *k.* This is estimated using the band recoveries for watershed *j* in month *t* and year *k* and the total recoveries for North America in month *t* and year *k,* $\left(\frac{b_{jtk}}{t_{tk}}\right)$. This proportion is multiplied by the estimated annual population of dabbling ducks θ_k.

Equation 4.

$$g(b, t, \theta) = \left(\frac{b_{jtk}}{t_{tk}}\right)\theta_k$$

Aggregating the data by month, year, and watershed allowed for including multiple forms of uncertainty in the estimates. These include but are not limited to variation in hunter effort, band reporting, recovery, and banding effort. While we did not model these explicitly, our intent is to provide an estimate of the expected population that may be present during the fall and winter periods. Our assumption is that these uncertainties do not greatly influence the median estimates for the population that might be present. However, this assumption might be violated in regions with low hunter effort or small populations. Our method also assumes that the band recoveries are proportional to the continental population at a given time and represent the spatial distribution of the continental population. This assumption may be violated early in the fall during the teal seasons, before the primary waterfowl seasons have opened. An example is if migration begins early and species such as mallards have begun to move prior to the season opening, which is typically in October. We have attempted to address this variation by including multiple years of data.

Expected Influenza A Prevalence

To estimate seasonal true expected prevalence within watersheds, we used prior dabbling duck surveillance data from 2006 to 2011. The data were aggregated by month and year for each

watershed, resulting in the count of sampled birds and the diagnostic test results. Aggregating the data by month, year, and watershed allowed for the inclusion of uncertainty in the estimates from differences in sampling effort and annual variation in prevalence. Our model structure used a binomial sampling distribution that accounted for uncertainty in the diagnostic test process. The expected seasonal prevalence was estimated using Equation 5.

Equation 5.

$$[\pi_{jtk}, \text{Se}, \text{Sp}, \theta \,|\, y_{jtk}] \propto Binomial(n, g(\pi_{jtk}|\text{Se}, \text{Sp}, \theta))\text{Beta}(Se|\alpha, \beta)\text{Beta}(Sp|\alpha, \beta)Beta(\theta|\alpha, \beta)$$

Equation 6.

$$g(Se, Sp, \theta) = \theta\text{Se} + (1 - \theta)(1 - \text{Sp})$$

Where n is the number of birds tested and $g(Se, Sp, \theta)$ is the estimated true probability of an individual bird is infected with AI. θ is the unknown prevalence of AI in the watershed in the month and year. Se and Sp are the sensitivity and specificity, respectively, of the diagnostic test. We used an uninformative Beta prior for the unknown prevalence:

$$\theta \sim Beta(\alpha = 1, \beta = 1)$$

Uncertainty of sensitivity (Se) and specificity (Sp) was modeled using independent informative beta prior distributions [14] using known estimates for the diagnostic test Se (86.3%) and Sp (99.99%) (Janice Pedersen, personal communication; Mia Torchetti, personal communication). Specifically these priors were:

$$Se \sim Beta(\alpha = 20.833, \beta = 4.148)$$

$$Sp \sim Beta(\alpha = 8.403, \beta = 1.001)$$

Model Fitting and Evaluation

The models for expected population and prevalence were fit for each watershed and biological season using Markov chain Monte Carlo (MCMC) techniques and implemented in WinBUGS software [15]. Posterior inferences were based on 100,000 iterations with a sampling lag of 5, after a burn-in of 20,000 iterations was discarded. We assessed convergence by running five chains from dispersed starting values, observing autocorrelation among samples and investigating the Brooks-Gelman-Rubin convergence statistic [16]. We used the median of the posterior distributions as an estimate for the parameters of interest, and the 2.5 and 97.5 percent points as estimates of the 95 percent credible intervals.

Appendix 5. Morbidity and Mortality Surveillance

The technical guidance in this plan has focused on active surveillance of live or hunter-harvested waterfowl species to achieve the stated surveillance objectives. However, passive surveillance through investigation of morbidity and mortality events in wild birds can provide an ancillary source of information or surveillance stream for detection of influenzas of interest. Within a given HUC, a sample collected from surveillance of morbidity and mortality events should provide at least as much information regarding the detection of novel influenzas of interest as a sample collected from hunter harvested or live-bird surveillance. Thus, samples collected during passive surveillance can potentially be used to achieve active surveillance sample size goals. Additionally, if individuals involved in a morbidity or mortality event have a higher probability of being infected with influenzas of interest, including samples from these events may increase the overall probability of detection of the surveillance program. These types of differential probabilities of infection between apparently healthy and sick individuals have been exploited for other wildlife diseases to maximize the efficiency of detection surveillance efforts [17]. However, passive surveillance is only useful when the goal of a surveillance program is detection of a pathogen. It should not be used when intensity metrics (e.g., prevalence) are the focus.

Including morbidity and mortality surveillance directly in the statistical design of this technical plan is not feasible due to the highly stochastic nature in the occurrence and location of these events and the resulting lack of a useful underlying probability model. Therefore, we recommend investigating all significant morbidity and mortality events, particularly if they occur in regions important to species conservation or domestic poultry production. These passive surveillance efforts should focus on wild bird species known or suspected to be susceptible to infection by influenzas of interest. Preliminary information from the current outbreak suggests that Canada geese and raptor species likely to feed or scavenge on waterfowl may be particularly affected, and therefore should be included in any passive surveillance activities. Additionally, morbidity and mortality events of other groups of birds known to be affected or carry influenzas such as waterfowl, wading birds, geese, and swans all represent potential sources of information regarding the geographic and host range expansion of novel influenza viruses.

We recommend conducting passive surveillance as a complementary effort to the active surveillance activities. The integration of these two efforts will provide the highest likelihood of early detection of influenzas of interest in new geographic locations and wildlife species.

Appendix 6. Wildlife Conservation Concerns

The statistical design of this surveillance plan has drawn from current knowledge about the biology and movements of focal waterfowl species (e.g., dabbling ducks) as well as current knowledge about distribution and diversity of influenza viruses within those species to target specific geographic regions for surveillance. This has provided a comprehensive strategy for maximizing the likelihood of detecting influenzas of interest in waterfowl and establishing key locations for monitoring the intensity of infection post-detection. However, given the breadth of movements of migratory waterfowl, they can potentially carry and spread influenza viruses of concern into locations that are home to species of high conservation value and negatively impact their resident populations. Therefore, in addition to the geographic regions selected for surveillance described previously, specific locations within these regions or additional regions with significant species conservation value should be incorporated into the implementation of this surveillance design.

To identify these specific areas of high conservation value, we recommend consulting with tribal, State, and Federal wildlife biologists with specific knowledge of State or Federal species of conservation concern to identify any deficiencies of the surveillance program's current geographic coverage. Such deficiencies should then be remedied prior to final implementation of the surveillance program. Specific groups of conservation species to target are wetland dependent species, wading bird species, gallinaceous birds, and raptors that potentially feed or scavenge on waterfowl. Examples of such species that may be at risk include:

- Whooping cranes (*Grus americana*): wetland dependent ; maintain specific migratory routes and stop-over sites to target for surveillance;
- Piping plovers (*Charadrius melodus*): wading birds that rely solely on gravel or sandy shoals for nesting and make large migratory movements;
- Attwater's prairie chicken (*Tympanuchus cupido attwateri*), lesser prairie chicken (*Tympanchus pallidicinctus*), and Gunnsion sage grouse (*Centrocercus minimus*): gallinaceous conservation species that likely have a low risk of influenza infection, but may be susceptible if waterfowl stop-over sites are also used by these species;
- Peregrine falcons (*Falco peregrinus*): raptor species of high conservation value that potentially feed or scavenge waterfowl where their distributions overlap.

These examples are not exhaustive, but illustrate the important need to address conservation concerns during surveillance activities.

We recommend adding identified areas of conservation concern to the geographic sampling frame if they are not already included within a selected HUC; or, if they are already included in the selection, targeting these locations for focused sampling within the HUC. Including these high-value areas in the surveillance for influenzas of interest will increase the probability of early detection of the pathogen in populations of these critical species, and help mitigate negative impacts while management actions are still feasible.

Appendix 7. Poultry Production Regions

In 2004, the National Animal Health Monitoring System (NAHMS) conducted a study of game-fowl breeder flocks and backyard flocks in the United States (18,19). For this study, a backyard flock was defined as a residence having birds other than pet birds and having fewer than 1,000 birds. The backyard flock study included flocks located within 1 mile of a commercial poultry operation.

On average, 1.9 backyard flocks were located within 1 mile of a commercial operation. The majority of backyard flocks raised table egg breeds (63.2 percent of flocks); 20.6 percent of flocks had ducks; and 8.7 percent had other domestic waterfowl (geese, swans, etc.). The flock size averaged 35 birds. Outdoor access was extremely common, and the majority of flocks had contact with wild birds. Ponds that attract wild waterfowl and wild-bird feeders were present on the property of more than one-third of flocks. Movement of domestic birds off the operation was uncommon.

The most important sources for bird-health information were feed suppliers, followed by extension service personnel and other producers. This finding indicated that feed suppliers may be an effective avenue to reach this population.

In 2010, NAHMS conducted a study to describe the structure of the poultry industry. Participants in this study were company owned or contract farms belonging to large poultry companies. Of the large commercial poultry farms, nearly two-thirds (65.6 percent) were broiler farms. Table-egg production farms accounted for 2.3 percent of farms. Turkey farms accounted for 16.7 percent of farms, and breeder farms accounted for 15.4 percent of farms. Broiler companies had a median of 464 farms, whereas table-egg companies had a median of 8 farms. Nearly all breeder farms (98.8 percent) and three-fourths of turkey farms (73.4 percent) had fewer than 50,000 birds at maximum capacity. The majority of broiler farms (56.3 percent) had 50,000 to 99,999 birds, and the majority of table-egg farms (53.7 percent) had 100,000 or more birds. This study found that <0.1 percent of broiler farms, 11.2 percent of table-egg farms, and 0.1 percent of turkey farms were certified organic farms (20).

In 2013, NAHMS conducted a study of table-egg farms. This study was limited to farms with 3,000 or more layers that were registered and in compliance with the Food and Drug Administration (FDA) egg safety rule. Overall, 27.6 percent of table-egg farms were certified organic. About 7 of 10 organic farms (69.3 percent) were located within 1 mile of other premises with poultry. The majority of organic farms had fewer than 30,000 birds. Organic farms, by definition, must have outdoor access. Nearly all organic farms (95.2 percent) had an uncovered outdoor area for birds. Although wild waterfowl were rarely seen in the layers' outdoor area, nearly two-thirds of farms had seen other wild birds in the outdoor area. However, wild birds did not have access to feed on any organic farms. The primary drinking water was a well on the vast majority of organic farms.

**Figure 8. Distribution of U.S. poultry production in the United States.
(Top) Number of poultry farms per county; (Bottom) Mean number of birds per
farm. Data from National Agricultural Statistics Service (NASS).**
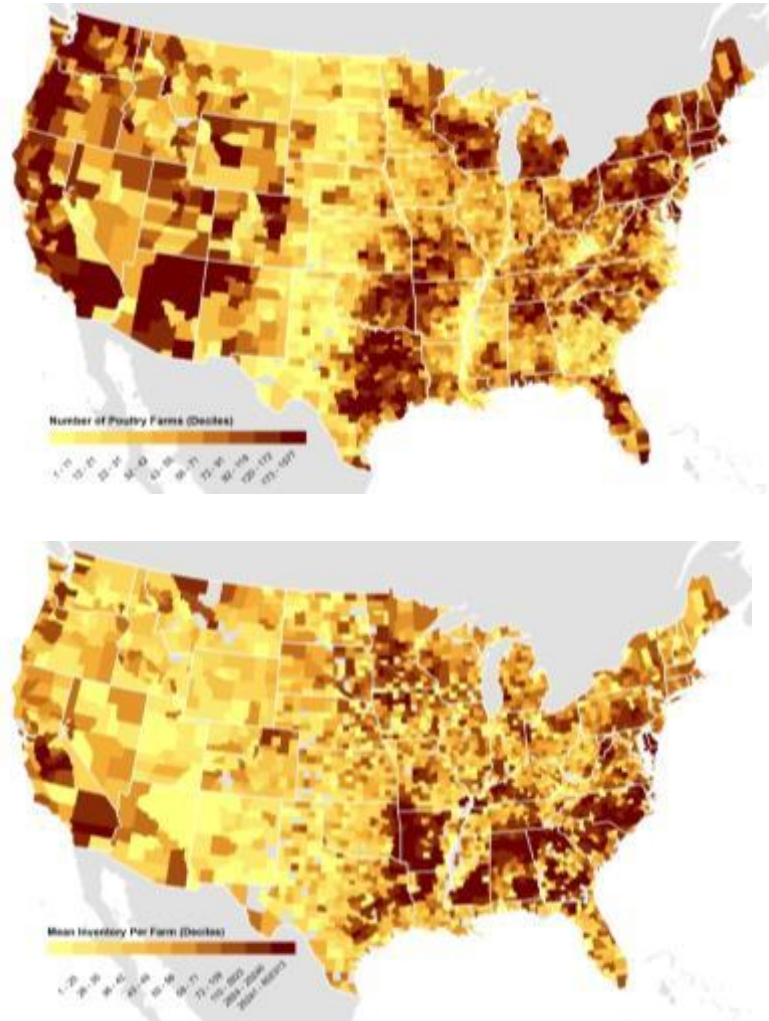
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