

Original Article—

# Host Correlates of Avian Influenza Virus Infection in Wild Waterfowl of the Sacramento Valley, California

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**SUMMARY.** Avian influenza viruses (AIVs) are distributed globally in members of the family Anatidae (waterfowl), and significant disease may occur when these viruses infect commercial poultry or humans. Early detection of AIV through surveillance of wild waterfowl is one measure to prevent future disease outbreaks. Surveillance efforts that are designed to account for host and environmental determinants of susceptibility to infection are likely to be most effective. However, these determinants have not been clearly delineated and may vary with location. Because some regions are at greater risk for AIV outbreaks, the factors that contribute to AIV infection of waterfowl in these areas are of interest. We investigated the prevalence of AIVs in hunter-killed waterfowl at wintering sites in California's Central Valley. Overall, AIV prevalence was 10.5% and, after controlling for age and sex, was greatest in northern shovellers (*Spatula clypeata*) and lowest in wood ducks (*Aix sponsa*). Overall, AIV prevalence was higher in females than in males, but this trend was driven by one sampling year and one waterfowl species (green-winged teal, *Anas crecca*). AIV prevalence in waterfowl was lower in samples collected from brackish wetlands compared with those collected from freshwater wetlands, suggesting that wetland type or other environmental factors contribute to AIV prevalence. This study adds to our understanding of the ecology of AIV infection in waterfowl and may assist in developing more efficient, targeted surveillance efforts for the detection of potentially harmful viruses circulating in North American waterfowl.

**RESUMEN.** Correlación de hospedadores en la infección por el virus de la influenza aviar en aves acuáticas silvestres del Valle de Sacramento en California.

Los virus de la influenza aviar se distribuyen globalmente en miembros de la familia *Anatidae* (aves acuáticas) y pueden ocurrir enfermedades importantes cuando estos virus infectan aves comerciales o a los humanos. La detección temprana de los virus de influenza mediante la vigilancia de aves acuáticas silvestres es una medida para prevenir futuros brotes de enfermedades. Es probable que los esfuerzos de vigilancia diseñados para tener en cuenta los determinantes ambientales y del huésped para la susceptibilidad a la infección sean más eficaces. Sin embargo, estos determinantes no se han delineado claramente y pueden variar según la ubicación. Debido a que algunas regiones tienen un mayor riesgo de brotes de influenza aviar, los factores que contribuyen a la infección de las aves acuáticas en estas áreas son de interés. Se investigó la prevalencia de virus de influenza en aves acuáticas muertas por cazadores en sitios de estancia invernal en el Valle Central de California. En general, la prevalencia de los virus de influenza fue del 10.5% y, después de controlar por edad y sexo, fue mayor en los patos cuchara comunes del norte (*Spatula clypeata*) y más baja en los patos joyuyo (*Aix sponsa*). En general, la prevalencia de influenza fue mayor en las hembras que en los machos, pero esta tendencia fue influenciada por un año de muestreo y una especie de ave acuática (cerceta común, *Anas crecca*). La prevalencia de influenza aviar en aves acuáticas fue menor en las muestras recolectadas de humedales salobres en comparación con las recolectadas de humedales de agua dulce, lo que sugiere que el tipo de humedal u otros factores ambientales contribuyen a la prevalencia de los virus de influenza. Este estudio contribuye al conocimiento de la ecología en la infección por influenza aviar en las aves acuáticas y puede ayudar a desarrollar esfuerzos de vigilancia más eficientes y específicos para la detección de virus potencialmente dañinos que circulan en las aves acuáticas de América del Norte.

**Key words:** avian influenza virus, waterfowl, Sacramento Valley, Central Valley, California

**Abbreviations:** AIV = avian influenza virus; AMWI = American wigeon; CL = confidence limits; CNWR = Colusa National Wildlife Refuge; GADW = Gadwall; GIWA = Grizzly Island Wildlife Area; GWTE = green-winged teal; HPAI = highly pathogenic avian influenza; LPAI = low pathogenicity avian influenza; MALL = mallard; NOPI = northern pintail; NOSH = northern shoveler; RNDU = ring-necked duck; rRT-PCR = reverse transcription PCR; UBB = Upper Butte Basin; WODU = wood duck

Wild birds of the orders Anseriformes (ducks, geese, and swans) and Charadriiformes (shorebirds and gulls) are globally distributed and are the major natural reservoir for nearly all influenza A virus subtypes. Although avian influenza virus (AIV) infection in waterfowl does not typically result in observable disease symptoms,

outbreaks with public health or economic impacts can occur when the virus crosses the species barrier to infect poultry and humans (1).

Although the extent and direction of movements vary between species, wild waterfowl often complete annual long-distance migrations along loosely defined routes from nesting grounds in northern latitudes to temperate overwintering regions in the south. North America is divided into four major flyways: the Atlantic, Mississippi, Central, and Pacific flyways. The Pacific Flyway extends

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from Alaska to South America, with many species nesting and overwintering in California. AIV prevalence among waterfowl is reported to be highest in the Pacific Flyway (2).

The distribution and comingling of large populations of waterfowl at nesting sites and migratory routes provides opportunities for the emergence and transmission of novel virus strains. Because of its extensive overlap with other flyways, the Pacific Flyway is thought to be an important location for cross-species transmission of multiple AIV subtypes and for mixing of AIVs of North American and Eurasian lineages (3,4,5). Whereas the rate of AIV genetic migration is greater within a flyway than between flyways (6), Alaska is connected to multiple flyways, making it a potential site for the introduction of Eurasian-lineage AIV genes into North America. Northern pintails (*Anas acuta*; NOPI) are known to migrate between Asia and the Pacific Flyway and contribute to interflyway genetic migration (7,8). Moreover, a highly pathogenic avian influenza (HPAI) H5N8 virus of Asian origin was introduced into North America by transcontinental migratory waterfowl in 2014–2015 (5). Ultimately, this virus gave rise to several reassortant AIVs, including an H5N2 strain that spread rapidly within the United States, resulting in the loss of millions of turkeys and egg-laying chickens, with the greatest effect in the Upper Midwest (9). The economic impact of this outbreak on the U.S. poultry industry was estimated at \$879 million (10). Although no human fatalities were reported during the 2014–2015 outbreak, HPAI H5N1 is endemic in poultry in several countries in Asia and North Africa. Reported human fatalities due to H5N1 infection number nearly 500 since 2003 and have nearly all been associated with exposure to infected poultry (11).

These recent outbreaks of HPAI viruses highlight the need to prioritize a continued investment in waterfowl surveillance and pandemic preparedness programs. Numerous surveillance studies have investigated the ecology of AIV in waterfowl, identifying key host or environmental factors that determine AIV prevalence in waterfowl. Exposure risk and susceptibility to AIV is inversely correlated with age and positively correlated with population densities at molting and wintering sites (12,13). Prevalence of AIV is generally highest on premigration staging areas at northern latitudes (14,15). Although prevalence of infections has been reported to be higher in males than females, there is no general consensus regarding differences in low pathogenicity avian influenza (LPAI) virus susceptibility between sexes (16,17). Additionally, one study demonstrated that migratory birds are more likely than local resident birds to be exposed to AIV, whereas other studies report similar infection rates between migratory and resident birds on wintering grounds (18,19,20). Determining the relative AIV prevalence in different waterfowl species has been an area of intense interest. Whereas dabbling ducks (tribe Anatini) are consistently shown to have a higher AIV prevalence than waterfowl belonging to other tribes (14), mallards (*Anas platyrhynchos*; MALL), northern pintails, northern shovelers (*Spatula clypeata*; NOSH), cinnamon teal (*Spatula cyanoptera*), blue-winged teal (*Spatula discors*), and American wigeon (*Mareca americana*; AMWI) have each been identified as having the highest AIV prevalence in individual studies carried out at different locations (2,14,21,22,23,24,25,26,27,28).

AIV surveillance efforts have frequently been carried out on the scale of entire flyways, continents, or countries. These studies draw conclusions across large geographic areas containing numerous and varied habitats. The ecology of AIV is likely to vary with location,

however, with some areas and timeframes being more likely to support AIV transmission and outbreaks (29).

California's Central Valley provides wintering habitat for 20% of waterfowl in North America and 60% of birds migrating within the Pacific Flyway (21). Additionally, California is unique in that it contains a large local breeding population of mallard, gadwall (*Mareca strepera*, GADW), and cinnamon teal (30), such that the majority of harvested ducks for these three species are produced locally within the state (20,31). The northern portion of the Central Valley (i.e., the Sacramento Valley) has been described as a high-risk area for AIV outbreaks, yet it has frequently been overlooked in surveillance studies (32,33). Human interactions with animals have increased dramatically in recent history, increasing the risk of cross-species transmission of new and re-emerging zoonotic pathogens. This state of affairs can be seen not just in the global dissemination of HPAI H5N1 viruses (34,35), but in new threats such as SARS-CoV-2. The allocation of resources by funding agencies for pathogen detection and pandemic preparedness may be increasingly challenging. By employing surveillance strategies on the basis of an understanding of the host and environmental factors that determine AIV prevalence in waterfowl, AIV surveillance programs can more efficiently detect viruses of possible concern to human and poultry health. In this study, we characterized the prevalence of AIV in wild waterfowl of California's Central Valley and described the host features that correlated with virus carriage.

## MATERIALS AND METHODS

**Sample collection.** For assessing correlations between waterfowl host features (i.e., species, age, sex) and AIV infection, cloacal swabs were collected from hunter-harvested wild waterfowl at four locations in California's Central Valley between 2014 and 2019. Sample collection sites included the Colusa National Wildlife Refuge (CNWR) in Colusa County, CA, and the Upper Butte Basin Wildlife Area (UBB) in Butte and Glenn counties, CA. Sampling occurred during the hunting season between October and January.

For the purpose of comparing AIV prevalence between two Central Valley locations, CNWR/UBB and the Suisun Marsh, data from cloacal samples collected from northern shovelers at Grizzly Island Wildlife Area (GIWA) in Solano County, CA, during winter 2018–2019 were combined with 2014–2019 data from GIWA and private duck clubs in Solano County, CA (combined sample set referred to as GIWA) retrieved from the National Institute of Allergy and Infectious Diseases Influenza Research Database (36).

Cloacal samples were collected with a Dacron polyester-tipped swab (Becton, Dickinson and Company, Sparks, MD). Samples were preserved in cryovial tubes containing 1 ml of cold viral transport medium (Medium 199 supplemented with  $2 \times 10^6$  units/L penicillin, 200 mg/L streptomycin,  $2 \times 10^6$  units/L polymyxin B, 250 mg/L gentamicin,  $5 \times 10^5$  units/L nystatin, 60 mg/L ofloxacin HCl, 0.5% bovine serum albumin Fraction V) and kept on ice for no longer than 6 hr before being secured in a  $-80^\circ\text{C}$  freezer until analysis. At the time of sample collection, waterfowl species and sex were determined. Where appropriate, the standard four-letter abbreviation for species names as delineated by the American Ornithological Society is used. Because of a lack of solid distinguishing features in females, the blue-winged teal and cinnamon teal species were combined. When possible, age determination was made as either immature or adult on the basis of plumage according to the method of Carney (37).

**Screening for AIV.** Viral RNA was extracted from cloacal swab samples on the KingFisher Duo purification system (Thermo Fisher

Scientific, Waltham, MA) with the MagMAX-96 AI/ND Viral RNA Isolation Kit (Thermo Fisher Scientific) according to manufacturer's suggested protocol. Extracted RNA was screened for AIV by a real-time reverse transcription PCR (rRT-PCR) assay with the AgPath-ID One-Step RT-PCR kit (Thermo Fisher Scientific) and a primer/probe set targeting the viral matrix (M) gene (Life Technologies, Carlsbad, CA). The primer and probe sequences were (38): matrix forward (5'-ARA TGA GTC TTC TRA CCG AGG TCG-3'); matrix reverse (5'-TGA AAA GAC ATC YTC AAG YYT CTG-3'); matrix probe (5'-/56-FAM/TCA GGC CCC CTC AAA GCC GA/3BHQ\_1/-3'). The thermocycling protocol was 45°C for 10 min, 95°C for 10 min, then 45 cycles of 95°C for 15 sec and 60°C for 30 sec. Samples with a cycle threshold value  $\leq 40$  were considered positive. Matrix gene-positive samples were further screened by an HPAI-specific rRT-PCR assay that used the following primers and probe: H5 EA+1462 (5'-TGG GTA ATG GTT GTT TCG AG-3'); H5 EA-1565 (5'-ATT GCT TCT TCT GAA TAC TTA GGG-3'); and H5EA 1487 probe [(6-FAM) 5'-TCA CAA ATG/ZEN/TGA TAA CGA ATG TAT GGA GAG C-3' (IBFQ)].

**Statistical analysis.** Tables, summary statistics, and graphics were used extensively to explore and uncover patterns in bird sampling characteristics (i.e., the distribution of sex or species across year) and the relationship of AIV prevalence across the varying bird characteristics. Statistical analyses were conducted on the 2362 samples obtained from the eight most frequently sampled species (AMWI, GADW, green-winged teal [*Anas crecca*, GWTE], MALL, NOPI, NOSH, wood duck [*Aix sponsa*, WODU], ring-necked duck [*Aythya collaris*, RNDU]), accounting for 95% of all samples collected. Because of the small sample sizes for certain combinations of bird characteristics, relative risk ratios and chi-square tests were used to make bivariate comparisons of AIV prevalence across levels of categorical measures such as species or year with a Bonferroni correction for multiple comparisons as appropriate. A multivariable logistic regression model was used to assess the association of AIV prevalence across independent measures such as year, sex, and species.

## RESULTS

**Sampling effort and AIV prevalence.** To determine the overall prevalence and correlates of AIV infection in Central Valley waterfowl, we collected a total of 2492 cloacal swabs from hunter-harvested waterfowl. Samples were collected between 2014 and 2019 during annual fall/winter hunting seasons at the CNWR and UBB (Fig. 1A). A reasonable effort was made to collect a similar number of samples each year. However, because of inconsistent cooperation from hunters and unpredictable conditions that resulted in the periodic closing of sampling sites (e.g., portions of UBB were closed for much of fall 2015 because of flooding), sample collection was not evenly distributed across the entire study period (Fig. 1B). Our sampling effort represents 18 species in total: nine species of dabbling ducks (Anatini), representing 90% of all samples collected, three species each of geese (Anserini; 3.6% of samples) and pochards (Aythyini; 5.1% of samples), and two species of sea ducks (Mergini; 1% of samples). The eight most frequently sampled species (AMWI, GADW, GWTE, MALL, NOPI, NOSH, WODU, RNDU) accounted for 95% of all samples collected (Table 1). Harvested birds of a majority of species showed an age and sex bias toward immature birds and male birds, respectively (Table 1). Age data were not collected for ~40% of our samples because a waterfowl biologist was unavailable to make an accurate determine of waterfowl age. One-half of all samples for which age data are lacking are in the 2014–2015 sample set. The data for a majority of samples from the

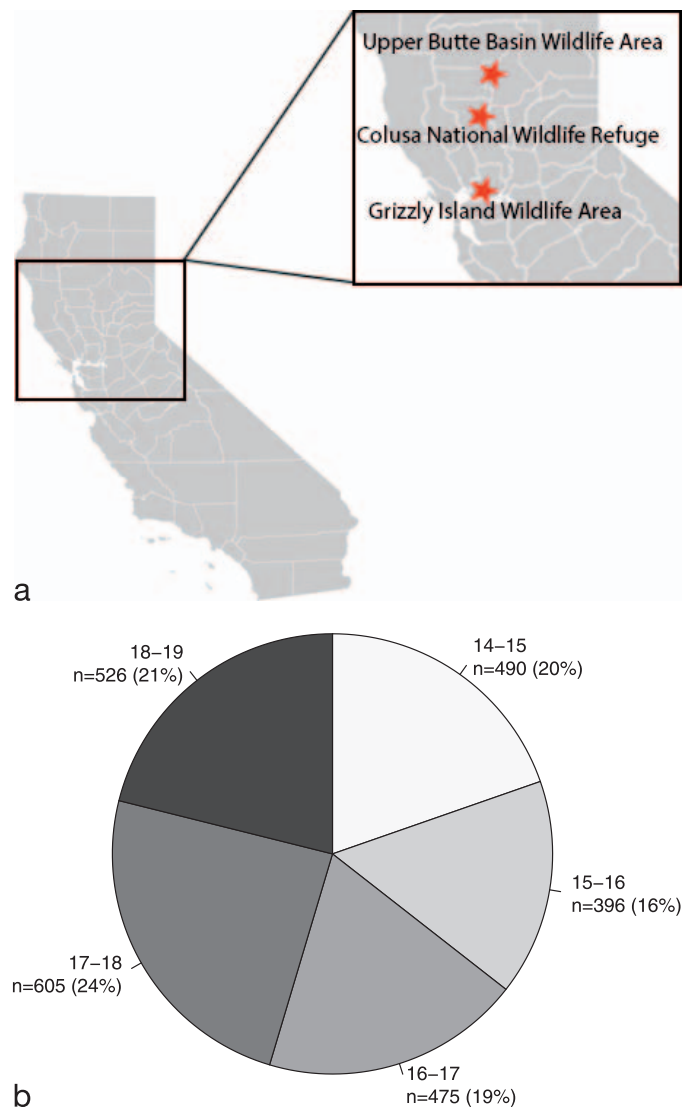


Fig. 1. (A) Locations of wild waterfowl influenza A virus surveillance in California's Central Valley and (B) number of samples collected each year.

other years of our study include age determination. Despite the lack of age data for a large number of samples, trends in our data are consistent with other published reports of hunter harvests in Central California (21).

Overall, 261 birds tested positive for AIV, resulting in a prevalence estimate of 10.5% (Table 1). All samples that tested positive for AIV by matrix gene PCR also tested negative in the H5 HPAI-specific PCR assay. Virus prevalence was roughly between 5% and 10% in 4 of the 5 yr. AIV prevalence was lowest during 2014–2015 (6.7%) and highest during the 2015–2016 season (19.9%; Fig. 2A). A multivariable logistic regression model accounting for species and sex indicated that, of our most frequently sampled species (hereafter referred to as the analysis sample), a bird was 3.2 times (95% confidence limits [CL] 1.2, 1.5;  $P < 0.0001$ ) more likely to test positive for AIV in 2015–2016 than in 2014–2015. Without regard to species or sex, immature birds were 1.2 times (95% CL 1.2, 1.5;  $P < 0.0001$ ) more likely to test positive for AIV than adult birds.



Table 1. AIV surveillance in wintering waterfowl at CNWR and UBB, 2014–2019.

	No. sampled	No. AIV-positive	AIV prevalence (%)	Age bias <sup>A</sup> (immature/adult)	Sex bias <sup>A</sup> (M/F)
Tribe Anatini (dabbling ducks)					
Wood duck ( <i>Aix sponsa</i> ; WODU)	151	2	1.3	2.4	1.4
Green-winged teal ( <i>Anas crecca</i> ; GWTE)	357	23	6.4	0.6	1.4
Mallard ( <i>Anas platyrhynchos</i> ; MALL)	420	44	10.5	1.9	2.2
Northern pintail ( <i>Anas acuta</i> ; NOPI)	184	20	10.9	0.7	3.8
American wigeon ( <i>Mareca americana</i> ; AMWI)	532	57	10.7	1.8	1.2
Eurasian wigeon ( <i>Mareca penelope</i> ; EUWI)	1	0	0.0	—	—
Gadwall ( <i>Mareca strepera</i> ; GADW)	239	20	8.4	1.9	1.7
Cinnamon teal/blue-winged teal ( <i>Spatula cyanoptera</i> ; CITE/ <i>Spatula discors</i> ; BWTE)	40	5	12.5	1.8	1.2
Northern shoveler ( <i>Spatula clypeata</i> ; NOSH)	320	67	20.9	1.3	0.9
Tribe Anserini (geese)					
Cackling goose ( <i>Branta hutchinsii</i> ; CAGO)	1	0	0	—	—
Greater white-fronted goose ( <i>Anser albifrons</i> ; GWFG)	76	6	7.9	1.6	2.2
Snow goose ( <i>Anser caerulescens</i> ; SNGO)	13	1	7.7	1.0	4.0
Tribe Aythyini (pochards)					
Canvasback ( <i>Aythya valisineria</i> ; CANV)	2	0	0.0	—	—
Lesser scaup ( <i>Aythya affinis</i> ; LESC)	1	0	0.0	—	—
Ring-necked duck ( <i>Aythya collaris</i> ; RNDU)	123	11	8.9	0.5	1.2
Tribe Mergini (sea ducks)					
Bufflehead ( <i>Bucephala albeola</i> ; BUFF)	12	2	16.7	1.2	2
Hooded merganser ( <i>Lophodytes cucullatus</i> ; HOME)	14	3	21.4	0.1	0.2
Other water bird taxa					
American coot ( <i>Fulica americana</i> ; AMCO)	6	0	0.0	—	—
Total	2,492	261	10.5	1.4	1.5

<sup>A</sup>— = Age and sex bias are not reported for species with  $n < 10$ .

In the analysis sample, AIV prevalence was 10.5% (244 positive birds/2326 birds in the analysis sample). AIV prevalence was higher in northern shovelers at 20.9%, compared with 8.8% for the other seven species combined ( $\chi^2 = 41.9$   $P < 0.0001$ ; Table 1; Fig. 2B). Furthermore, an all-pairwise comparison demonstrated that AIV prevalence was higher in northern shovelers than in each of the other species individually (Bonferroni corrected  $P < 0.01$ ), except for northern pintails ( $P = 0.16$ ) and ring-necked ducks ( $P = 0.13$ ). Overall, northern shovelers were 2.2 times more likely (95% CL 1.8, 2.9;  $P < 0.0001$ ) to test positive for AIV than other species in the analysis sample (data not shown). The relative risk of AIV infection in northern shovelers was highest in 2017–2018 when they were three times more likely (95% CL 1.6, 5.6;  $P < 0.003$ ) to test positive for AIV than other species (Fig. 2C). Increased relative risk of AIV infection and AIV prevalence in northern shovelers compared with the other seven species combined was observed for each of the five sampling years and, after correcting for multiple comparisons, reached statistical significance in each year except 2016–2017 (Figs. 2C, 3,  $P < 0.125$ ). Furthermore, a multivariable logistic regression model accounting for the additional confounders of year and sex demonstrated that shovelers were significantly more likely to test positive for AIV compared with the other species in our analysis sample (Table 2).

Notably, AIV prevalence in wood ducks (1.3%) was significantly lower than in all other species in the analysis sample combined ( $\chi^2 = 13.4$ ,  $P = 0.0002$ ; Fig. 2B). Indeed, AIV was detected in only two individual wood ducks during this study, one each in 2018 and 2019 (Table 1). Wood ducks were 0.32 times (95% CL 0.04, 2.3;  $P = 0.35$ ) and 0.19 times (95% CL 0.03, 1.34;  $P = 0.07$ ) as likely to test positive for AIV as other birds in the analysis sample in 2017–2018 and 2018–2019, respectively (Fig. 2C). An all-pairwise

comparison demonstrated that AIV prevalence was significantly lower (all Bonferroni corrected  $P < 0.05$ ) in wood ducks than in all other frequently sampled species, except for gadwall ( $P = 0.19$ ), ring-necked ducks ( $P = 0.22$ ), and green-winged teal ( $P = 0.75$ ).

**AIV prevalence and sex.** Sex has been associated with AIV infection status of wild waterfowl, with AIV prevalence being greater in males than in females (12,17). However, this result has not been observed consistently across different studies. A bivariate comparison of sex and AIV prevalence demonstrated that AIV prevalence was lower, overall, in males than in females ( $\chi^2 = 4.7$ ;  $P = 0.03$ ; data not shown). However, this trend was likely driven by the result of our sampling in 2014–2015, when AIV prevalence in females (12%) was about four times higher than in males (3.5%; Fig. 4A). Indeed, a multivariable logistic regression model demonstrated that confounding variables (year, species, etc.) could not be ruled out as an explanation for our observation of lower AIV prevalence in males (odds ratio = 0.83;  $P = 0.196$ ; Table 2). The female bias of AIV infection was observed most prominently for green-winged teal, with a 10.9% AIV-positive rate in females compared with 3.4% in males (Fig. 4B). This observation did not reach statistical significance after a Bonferroni adjustment for multiple comparisons. For other species in our analysis sample, a sex bias either could not be determined (AMWI, MALL, NOPI, NOSH, WODU) or AIV infection showed a slight (statistically insignificant) male bias (GADW; Fig. 4B).

**Effect of location on AIV prevalence.** Our results demonstrate that AIV prevalence in the Central Valley is consistently higher in northern shovelers relative to other waterfowl species. Although our result is consistent with several other studies of Pacific Flyway waterfowl (21,22,26,28), others report contrasting results when sampling across greater geographic distances within the Pacific Flyway (2). Because waterfowl habitats can vary significantly across large areas, it is reasonable, if not anticipated, that the host correlates

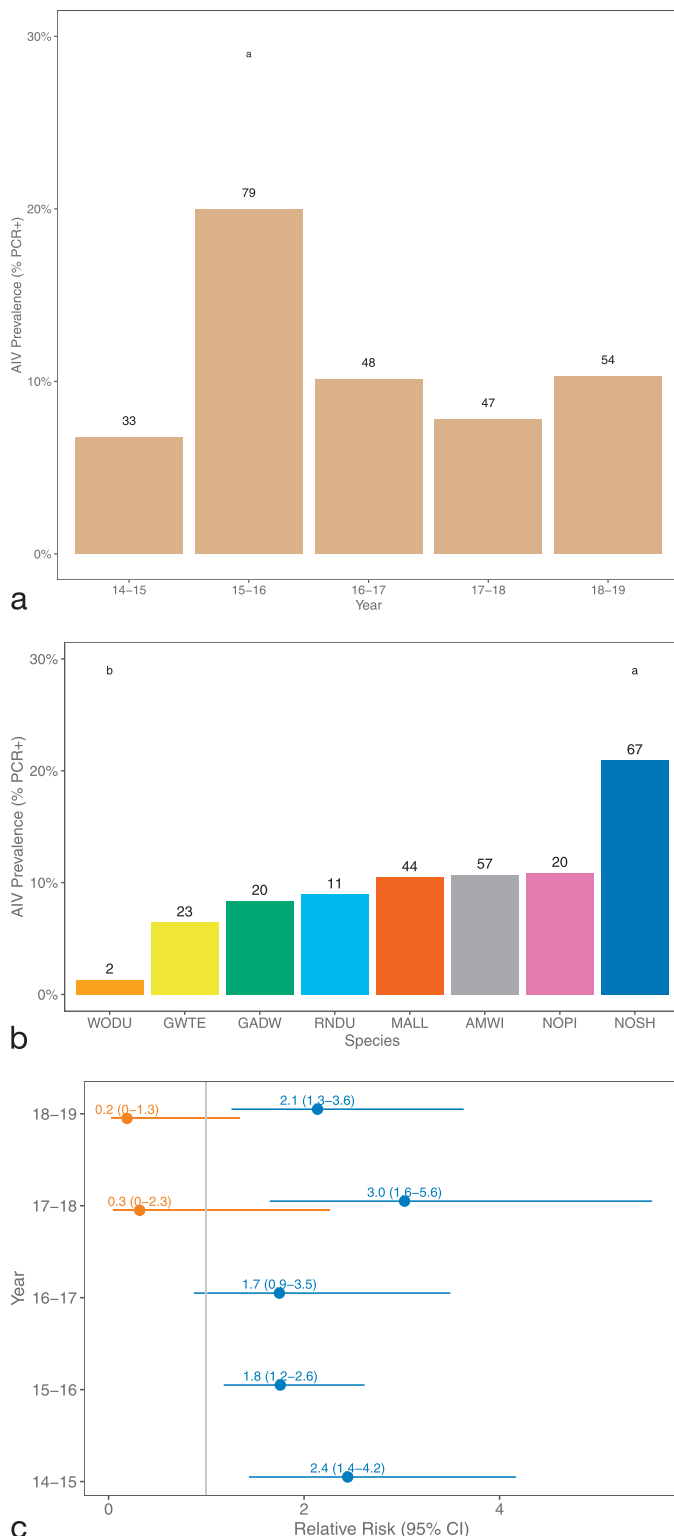


Fig. 2. AIV prevalence in hunter-killed waterfowl. (A) Total RNA was extracted from cloacal swab samples, and AIV prevalence for each year of the study was determined by rRT-PCR for the matrix (M) gene. AIV prevalence is presented as percent PCR-positive. The number of birds that tested positive for AIV in each year is displayed above the corresponding bar. “a” indicates  $P < 0.0001$  as determined by a chi-square test of equal proportions. (B) Overall AIV prevalence in the most frequently sampled species was determined through the same methods as in panel A. The number of birds for each species that tested positive is displayed above each bar. “a” and “b” indicate the statistical significance

of AIV infection will vary within an entire flyway or at the continental scale. To determine the extent to which the ecology of AIV can vary across smaller geographic areas within a flyway, we compared AIV prevalence in waterfowl at CNWR and UBB with data from samples collected at GIWA and nearby private duck clubs in the Suisun Marsh. GIWA is located approximately 50 miles (~80 km) northeast of San Francisco at the confluence of the Sacramento and San Joaquin rivers in the San Francisco estuary. The Suisun Marsh is the largest contiguous estuarine wetland in the United States. The GIWA cloacal swab samples that were analyzed for comparison with CNWR/UBB were a combination of samples collected from northern shovelers for this purpose during the 2018–2019 season and surveillance data from the same period of time as our study that are available on the Influenza Research Database (IRD, <https://www.fludb.org/brc/home.spg?decorator=influenza>). Overall, AIV prevalence was significantly higher at UBB than at GIWA (10.5% vs. 3.4%;  $\chi^2 = 57$ ;  $P < 0.0001$ ; data not shown), and this trend held for each year of our study period (Fig. 5; no data from GIWA for 2017–2018 were available on the Influenza Research Database). Importantly, among species for which a sufficient number of samples were collected (AMWI, GADW, GWTE, MALL, NOPI, NOSH), AIV prevalence was higher in birds of all species, with the exception of green-winged teal, at UBB compared with GIWA (data not shown).

## DISCUSSION

Our multiyear study of AIV ecology in California’s Central Valley demonstrates an overall AIV prevalence estimate of 10.5%, with the single-year prevalence ranging between ~5% and 10% for 4 of the 5 yr of the study (Table 1; Fig. 2A). This result is consistent with the overall prevalence rate of AIV in overwintering birds of the Family Anatidae as reported by others (2,14,24,27). In our study, as in other published reports, AIV prevalence was higher in immature birds than in adult birds and likely represents an annual influx of immunologically naïve birds onto wetlands (17,18,39). Furthermore, we demonstrate that northern shovelers have the highest AIV prevalence among species that were commonly sampled with the exception of northern pintails and ring-necked ducks, for which estimates of prevalence were not statistically different. The lack of statistical significance in AIV prevalence between northern pintails and northern shovelers may be explained by a strong male bias in our pintail sample (Table 1). No such bias was found in our ring-necked duck sample set. Given their abundance on Central Valley wetlands (40) and the high AIV prevalence rates we found in our study, shovelers may be an important species to target for future AIV surveillance sampling in this region. Although this study is not the first to indicate that northern shovelers have the highest AIV prevalence in the Central Valley (26), it is the first study of such duration as ours to demonstrate that increased AIV prevalence in northern shovelers is an annual trend.

of AIV prevalence in NOSH ( $P < 0.0001$ ) and WODU ( $P = 0.0002$ ), respectively, relative to the other seven species combined. (C) The relative risk of AIV infection for NOSH (blue) and WODU (orange) is shown for each year of the study. Ninety-five percent confidence intervals are shown in parentheses. Data for WODU is shown only for 2017–2018 and 2018–2019 because no wood duck samples tested positive in other years.

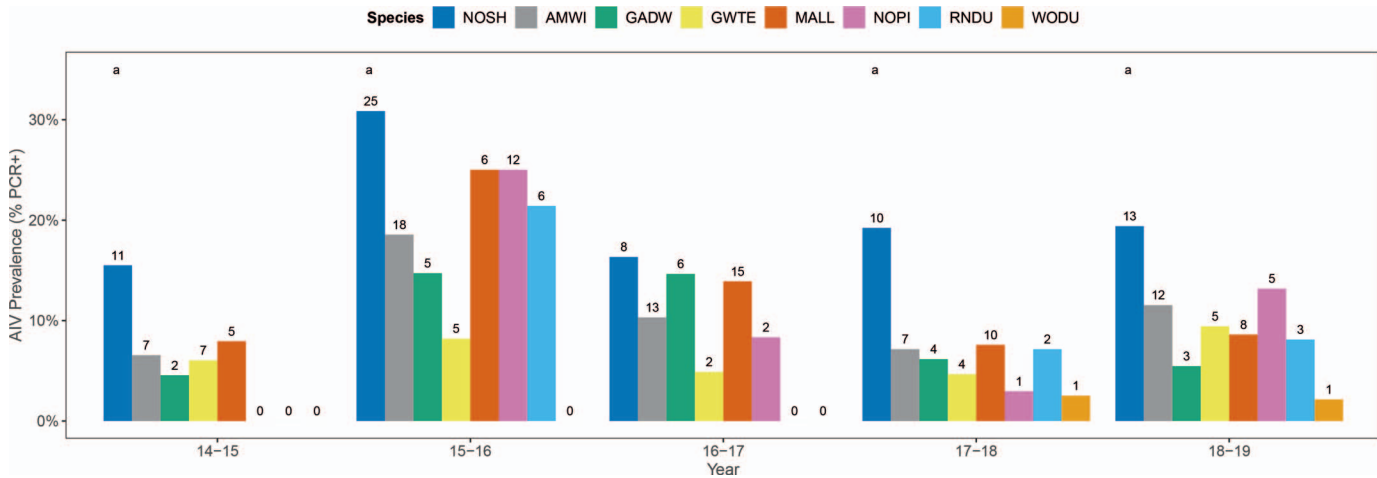


Fig. 3. AIV prevalence in the most frequently sampled species for each year of the study. Total RNA was extracted from cloacal swab samples and AIV prevalence was determined by rRT-PCR for the matrix (M) gene. AIV prevalence is presented as percent PCR-positive. The number of samples from each species that tested positive in each year are shown above each bar. “a” indicates statistical significance ( $P < 0.05$ ) of AIV prevalence in NOSH relative to the other seven species combined.

Although the mechanism of increased AIV prevalence in northern shovelers is beyond the scope of this study, several compelling hypotheses emerge as one considers the unique morphologic features and foraging behaviors of shovelers. The large, spoon-shaped bill of northern shovelers is lined by comb-like projections called lamellae, which function to filter food out of mud and water. Lamellar density is higher for northern shovelers relative to other ducks, allowing them to take crustaceans and other aquatic invertebrates as a greater portion of their diet (41,42,43). For instance, *Daphnia* spp. (commonly called water fleas) are small, filter-feeding planktonic crustaceans that make up a critical part of the northern shoveler diet (44). Daphnids are themselves filter-feeders and *Daphnia magna* is capable of accumulating influenza A virus RNA within its tissue (45,46). Although it has been suggested that virus accumulated in daphnids is not infectious, future research is warranted to test whether other aquatic invertebrates act as vectors, increasing AIV exposure in ducks that consume them in large numbers.

In addition to the potential for directly increasing exposure to AIV, greater consumption of planktonic crustaceans may indirectly enhance susceptibility of northern shovelers to AIV by affecting the normal intestinal microbiota. Susceptibility to influenza virus

infection in humans was recently shown to be associated with differences in the bacterial community of the nose and throat (47). Although less is known about the role of the microbiome in waterfowl health and disease, one study reports that the bacterial community identified in the cloaca of northern shovelers contrasts with that of mallards in several metrics of composition and diversity (48). Further work is necessary to better understand the relationship between the waterfowl cloacal microbiome and AIV infection and the extent to which the microbiome contributes to AIV prevalence in nature.

An alternative (although not mutually exclusive) explanation to our observation of high AIV prevalence in northern shovelers is that the average duration of virus shedding in this species is longer than it is for other species. Previous studies have demonstrated species-dependent variation in the duration of virus shedding in experimentally infected ducks (49,50). However, these studies did not include northern shovelers, and the results are unlikely to represent the duration of virus shedding in naturally infected ducks. Future studies could compare the duration of virus shedding in shovelers with that of a sympatric species. Evidence that northern shovelers shed virus for a longer period of time would have important implications for AIV transmission dynamics and surveillance programs because it would mean that northern shovelers could potentially be a source of virus spread across greater distances within migratory flyways.

In contrast to the relatively high AIV prevalence that we observed in northern shovelers at UBB, prevalence at GIWA during the same period of time was significantly lower (Fig. 5). This observation may be explained by differences in water type at the two locations. Grizzly Island, part of the Suisun Marsh, is located between the San Francisco Bay and the Sacramento–San Joaquin River Delta and is an important waterfowl use area with salinity levels highly variable in this estuarine system (30,51). Consistent with the hypothesis that water type contributes to AIV prevalence in waterfowl, Stallknecht *et al.* (52) and Brown *et al.* (53) demonstrated that influenza virus stability in water decreases ~10-fold with increasing salinity. Our result suggests that comparative analysis of water chemistry, even at locations in close proximity to one another, may be one important consideration for determining where AIV surveillance studies are

Table 2. Multivariable logistic regression model of AIV prevalence on species, year, and sex.

	Odds ratio	95% CI	P-value
Species (reference group: northern shoveler)			
American wigeon (AMWI)	0.47	0.32–0.70	<0.0001
Gadwall (GADW)	0.39	0.22–0.66	<0.0001
Green-winged teal (GWTE)	0.30	0.18–0.49	<0.0001
Mallard (MALL)	0.55	0.35–0.84	0.006
Northern pintail (NOPI)	0.48	0.27–0.82	0.009
Ring-necked duck (RNDU)	0.36	0.17–0.69	0.004
Wood duck (WODU)	0.06	0.01–0.19	<0.0001
Year (Reference year: 2014–2015)			
2015–2016	3.24	2.10–5.12	<0.0001
2016–2017	1.61	1.00–2.62	0.053
2017–2018	1.14	0.70–1.88	0.592
2018–2019	1.59	1.00–2.57	0.054
Sex: male (reference female)	0.83	0.63–1.10	0.196

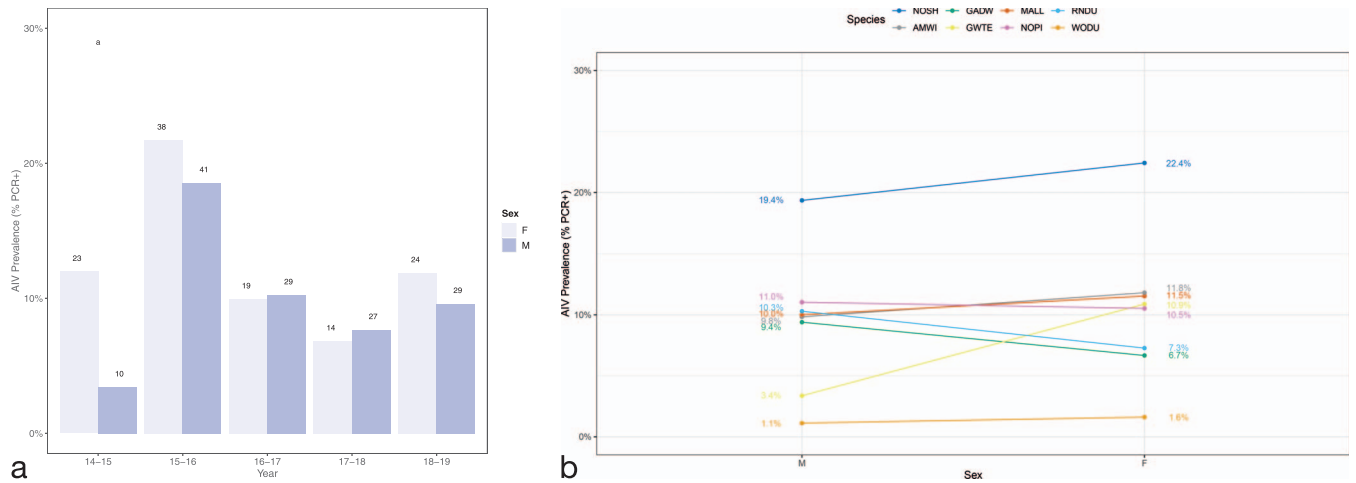


Fig. 4. Comparison of AIV prevalence in male and female ducks. (A) Overall AIV prevalence in male and female ducks for each year of the study was determined as previously described. (B) AIV prevalence in male and female ducks of the most frequently sampled species. “a” indicates statistical significance ( $P < 0.05$ ) of AIV prevalence in female ducks relative to male ducks in 2014–2015.

more likely to result in the detection and isolation of the greatest number of viruses. Additional comparisons of AIV infection rates of species collected in saline *vs.* freshwater wetlands is warranted.

In 2015–2016, ~70% of California’s land area was at “exceptional” or “extreme” levels of drought, a condition that may contribute to increased incidence of infectious disease. For instance, drought was determined to be a major driver of West Nile virus epidemics (54). As a result of decreased water availability in California in 2015, some state wetlands, including our major sampling site at UBB, did not receive the expected water allotment, driving birds to congregate at greater density on fewer flooded areas. Increased AIV transmission risk is positively correlated with waterfowl density (39). Although our data do not definitively demonstrate a role for drought in the elevated AIV prevalence that

we observed in 2015–2016, it is possible that drought conditions leading to a lack of water may have led to overcrowding and enhanced transmission of AIV in waterfowl. As of June 2021, 85% of California (including the entire Central Valley) is at “exceptional” or “extreme” drought level, up from 12% at the beginning of the water year, suggesting that California may be slipping into another multiyear drought event (55). Over the next several years, we plan to monitor closely AIV prevalence trends in relation to population density and water availability. Climate models predict future droughts to be of increasing frequency and severity across the western United States. Further investigation of a potential link between drought and increased incidence of AIV transmission is warranted because of the effect this could have on poultry and human health, especially in areas that are at high risk for both drought and AIV outbreaks.

In our study, AIV prevalence in wood ducks was significantly lower than for all other species in our analysis sample except for gadwall, green-winged teal, and ring-necked ducks (Fig. 2B). The lack of statistical significance in our comparison of AIV prevalence in wood ducks with green-winged teal and ring-necked ducks may be explained by a strong female sex bias in our sample of the latter two species (Table 1). Our result suggests that wintering wood ducks are rarely infected with LPAI viruses in the wild. Low AIV detection in wood ducks during the fall/winter may be a result of long-lasting protective immunity after exposure and infection during the spring/summer breeding season. Consistent with this notion, a survey of AIV in wild birds in Canada reported virus prevalence of 68% in wood ducks sampled between July and September (25). Work is ongoing in our laboratory to determine the AIV infection rate and seroprevalence of antibodies to AIV in wood ducks sampled during the spring/summer.

Additionally, Central Valley wood ducks are largely a resident population and LPAI virus is believed to circulate in resident birds before the arrival of migrants, after which migratory birds act as amplifiers of locally circulating virus strains (18,56). Because habitat preferences of wood ducks differ from many more common dabbling ducks, frequent interactions with large, multispecies congregations of waterfowl is likely reduced (57). Low AIV prevalence in samples collected from wood ducks during the fall/

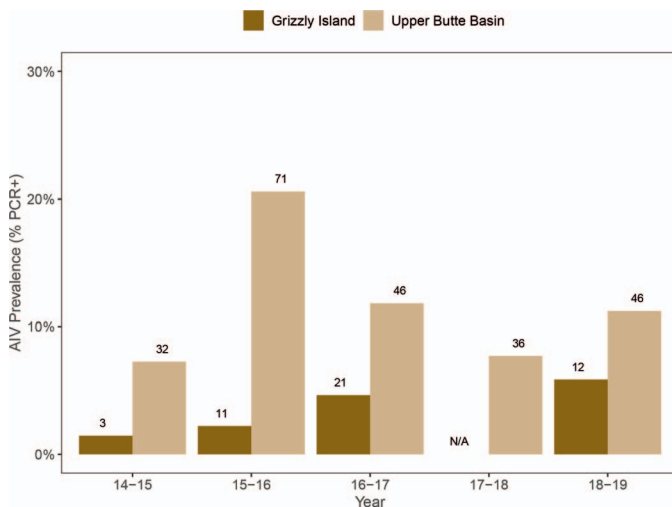


Fig. 5. Comparison of AIV prevalence in wild waterfowl at the Upper Butte Basin Wildlife Area/Colusa National Wildlife Refuge and Grizzly Island Wildlife Area. AIV prevalence was determined by rRT-PCR for the M gene on cloacal swabs collected from hunter-killed waterfowl at UBB/CNWR (tan) and GIWA (brown). Species included in this analysis are AMWI, GADW, GWTE, MALL, NOPI, and NOSH. Except for the 2018–2019 NOSH samples, all GIWA data were pulled from the Influenza Research Database.



winter in our study may reflect a relative lack of exposure to migrant waterfowl that drive AIV transmission in the fall.

Avian influenza viruses continue to represent a significant threat to human and poultry health. As one of a growing list of viral zoonoses to affect human health, resources for surveillance and pathogen detection could become stressed, highlighting the importance of targeted surveillance efforts that account for our growing understanding of the host and environmental factors that determine the exposure and infection rates of waterfowl to AIV. California's Central Valley has been described as an area where AIV outbreaks are likely (32,33). Our data provide information on the ecology of AIV in the Central Valley that can be used to design targeted AIV surveillance studies in the region going forward.

## REFERENCES

- Alexander DJ. An overview of the epidemiology of avian influenza. *Vaccine* 25:5637–5644; 2007.
- Groepner SR, DeLiberto TJ, Vrtiska MP, Pedersen K, Swafford SR, Hygnstrom SE. Avian influenza virus prevalence in migratory waterfowl in the United States, 2007–2009. *Avian Dis.* 58:531–540; 2014.
- Pearce JM, Ramey AM, Flint PL, Koehler AV, Fleskes JP, Franson JC, Hall JS, Derksen DV, Ip HS. Avian influenza at both ends of a migratory flyway: characterizing viral genomic diversity to optimize surveillance plans for North America. *Evol Appl.* 2:457–468; 2009.
- Winker K, McCracken KG, Gibson DD, Pruett CL, Meier R, Huettmann F, Wege M, Kulikova IV, Zhuravlev YN, Perdue ML, *et al.* Movements of birds and avian influenza from Asia into Alaska. *Emerg Infect Dis.* 13:547–552; 2007.
- Lee DH, Torchetti MK, Winker K, Ip HS, Song CS, Swayne DE. Intercontinental spread of Asian-origin H5N8 to North America through Beringia by migratory birds. *J Virol.* 89:6521–6524; 2015.
- Fourment M, Darling AE, Holmes EC. The impact of migratory flyways on the spread of avian influenza virus in North America. *BMC Evol Biol.* 17:118; 2017.
- Miller MR, Fleskes JP, Takekawa JY, Orthmeyer DL, Casazza ML, Perry WM. Satellite tracking of northern pintail spring migration from California, USA: the route to Chukotka, Russia. *Casarca* 7:229–233; 2001.
- Pearce JM, Reeves AB, Ramey AM, Hupp JW, Ip HS, Bertram M, Petrula MJ, Scotton BD, Trust KA, Meixell BW, *et al.* Interspecific exchange of avian influenza virus genes in Alaska: the influence of trans-hemispheric migratory tendency and breeding ground sympatry. *Mol Ecol.* 20:1015–1025; 2011.
- U.S. Department of Agriculture–Animal and Plant Health Inspection Service. *Epidemiologic and other analyses of HPAI-affected poultry flocks*. Fort Collins CO: USDA-APHIS. Doc 300.0615, Version 5; Sep 2015.
- Ramos S, MacLachlan M, Melton A. Impacts of the 2014–2015 highly pathogenic avian influenza outbreak on the U.S. poultry sector. Washington, DC: USDA Economic Research Service. <https://www.ers.usda.gov/publications/pub-details?pubid=86281>. [modified 2017 December 7; accessed 2021 March 11]. URL; 2017.
- World Health Organization. Cumulative number of confirmed human cases of avian influenza A (H5N1) reported to WHO. Geneva, Switzerland; WHO Emergency Situational Updates [Modified 2021 April 15; Accessed 2021 June 21]. [https://www.who.int/publications/m/item/cumulative-number-of-confirmed-human-cases-for-avian-influenza-a\(h5n1\)-reported-to-who-2003-2021-15-april-2021](https://www.who.int/publications/m/item/cumulative-number-of-confirmed-human-cases-for-avian-influenza-a(h5n1)-reported-to-who-2003-2021-15-april-2021) URL; 2020.
- van Dijk JG, Verhagen JH, Wille M, Waldenstrom J. Host and virus ecology as determinants of influenza A virus transmission in wild birds. *Curr Opin Virol.* 28:26–36; 2018.
- Maxted AM, Luttrell MP, Goekjian VH, Brown JD, Niles LJ, Dey AD, Kalasz KS, Swayne DE, Stallknecht DE. Avian influenza virus infection dynamics in shorebird hosts. *J Wildl Dis.* 48:322–334; 2012.
- Bevins SN, Pedersen K, Lutman MW, Baroch JA, Schmit BS, Kohler D, Gidlewski T, Nolte DL, Swafford SR, DeLiberto TJ. Large-scale avian influenza surveillance in wild birds throughout the United States. *PLoS One* 9:e104360; 2014.
- Runstadler JA, Happ GM, Slemons RD, Sheng ZM, Gundlach N, Petrula M, Senne D, Nolting J, Evers DL, Modrell A, *et al.* Using RRT-PCR analysis and virus isolation to determine the prevalence of avian influenza virus infections in ducks at Minto Flats State Game Refuge, Alaska, during August 2005. *Arch Virol.* 152:1901–1910; 2007.
- Zuk M, McKean KA. Sex differences in parasite infections: patterns and processes. *Int J Parasitol.* 26:1009–1023; 1996.
- Nallar R, Papp Z, Epp T, Leighton FA, Swafford SR, DeLiberto TJ, Dusek RJ, Ip HS, Hall J, Berhane Y, *et al.* Demographic and spatiotemporal patterns of avian influenza infection at the continental scale, and in relation to annual life cycle of a migratory host. *PLoS One* 10:e0130662; 2015.
- van Dijk JGB, Hoyer BJ, Verhagen JH, Nolet BA, Fouchier RAM, Klaassen M. Juveniles and migrants as drivers for seasonal epizootics of avian influenza virus. *J Anim Ecol.* 83:266–275; 2014.
- Gaidet N, Caron A, Cappelle J, Cumming GS, Balanca G, Hammoumi S, Cattoli G, Abolnik C, Servan de Almeida R, Gil P. Understanding the ecological drivers of avian influenza virus infection in wildfowl: a continental-scale study across Africa. *Proc R Soc Biol Sci Ser B* 279:1131–1141; 2012.
- Hill NJ, Takekawa JY, Ackerman JT, Hobson KA, Herring G, Cardona CJ, Runstadler JA, Boyce WM. Migration strategy affects avian influenza dynamics in mallards (*Anas platyrhynchos*). *Mol Ecol.* 21:5986–5999; 2012.
- Hill NJ, Takekawa JY, Cardona CJ, Ackerman JT, Schultz AK, Spragens KA, Boyce WM. Waterfowl ecology and avian influenza in California: do host traits inform us about viral occurrence? *Avian Dis.* 54:426–432; 2010.
- Siembieda JL, Johnson CK, Cardona C, Anchell N, Dao N, Reisen W, Boyce W. Influenza A viruses in wild birds of the Pacific flyway, 2005–2008. *Vector Borne Zoonotic Dis.* 10:793–800; 2010.
- Diskin ER, Friedman K, Krauss S, Nolting JM, Poulson RL, Slemons RD, Stallknecht DE, Webster RG, Bowman AS. Subtype diversity of influenza A virus in North American waterfowl: a multidecade study. *J Virol.* 94:e02022–19; 2020.
- Farnsworth ML, Miller RS, Pedersen K, Lutman MW, Swafford SR, Riggs PD, Webb CT. Environmental and demographic determinants of avian influenza viruses in waterfowl across the contiguous United States. *PLoS One* 7:e32729; 2012.
- Parmley EJ, Bastien N, Booth TF, Bowes V, Buck PA, Breault A, Caswell D, Daoust PY, Davies JC, Elahi SM, *et al.* Wild bird influenza survey, Canada, 2005. *Emerg Infect Dis.* 14:84–87; 2008.
- Hill NJ, Takekawa JY, Cardona CJ, Meixell BW, Ackerman JT, Runstadler JA, Boyce WM. Cross-seasonal patterns of avian influenza virus in breeding and wintering migratory birds: a flyway perspective. *Vector Borne Zoonotic Dis.* 12:243–253; 2012.
- Munster VJ, Baas C, Lexmond P, Waldenstrom J, Wallensten A, Fransson T, Rimmelzwaan GF, Beyer WE, Schutten M, Olsen B, *et al.* Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. *PLoS Pathog.* 3:e61; 2007.
- Montalvo-Corral M, Lopez-Robles G, Hernandez J. Avian influenza survey in migrating waterfowl in Sonora, Mexico. *Transbound Emerg Dis.* 58:63–68; 2011.
- Ramey AM, Reeves AB, Drexler JZ, Ackerman JT, De La Cruz S, Lang A, Leyson C, Link P, Prosser DJ, Robertson GJ, *et al.* Influenza A viruses remain infectious for more than seven months in northern wetlands of North America. *Proc R Soc Biol Sci Ser B* 287:20201680; 2020.
- Ackerman JT, Herzog MP, Yarris GS, Casazza ML, Burns E, Eadie JM. Waterfowl ecology and management. In: Moyle PB, Manfree A, Friedler PL, editors. *Suisun Marsh: ecological history and possible futures*. Berkeley (CA): University of California Press. p. 103–132; 2014.
- De Sobrino CN, Feldheim CL, Arnold TW. Distribution and derivation of dabbling duck harvests in the Pacific Flyway. *Calif Fish Game* 103:118–137; 2017.
- Belkhiria J, Alkhamis MA, Martinez-Lopez B. Application of species distribution modeling for avian influenza surveillance in the United States considering the North America migratory flyways. *Sci Rep.* 6:33161; 2016.



33. Belkhiria J, Hijmans RJ, Boyce W, Crossley BM, Martinez-Lopez B. Identification of high risk areas for avian influenza outbreaks in California using disease distribution models. *PLoS One* 13:e0190824; 2018.
34. Fusaro A, Zecchin B, Vrancken B, Abolnik C, Ademun R, Alassane A, Arafa A, Awuni JA, Couacy-Hymann E, Coulibaly MB, *et al.* Disentangling the role of Africa in the global spread of H5 highly pathogenic avian influenza. *Nat Commun.* 10:5310; 2019.
35. Li YT, Linster M, Mendenhall IH, Su YCF, Smith GJD. Avian influenza viruses in humans: lessons from past outbreaks. *Br Med Bull.* 132:81–95; 2019.
36. Zhang Y, Aevermann BD, Anderson TK, Burke DF, Dauphin G, Gu Z, He S, Kumar S, Larsen CN, Lee AJ, *et al.* Influenza research database: an integrated bioinformatics resource for influenza virus research. *Nucleic Acids Res.* 45:D466–D474; 2017.
37. Carney SM. *Species, age, and sex identification of ducks using wing plumage.* Washington DC: U.S. Department of the Interior, U.S. Fish and Wildlife Service; 1992.
38. Ramey AM, Hill NJ, Cline T, Plancarte M, De La Cruz S, Casazza ML, Ackerman JT, Fleskes JP, Vickers TW, Reeves AB, *et al.* Surveillance for highly pathogenic influenza A viruses in California during 2014–2015 provides insights into viral evolutionary pathways and the spatiotemporal extent of viruses in the Pacific Americas Flyway. *Emerg Microbes Infect.* 6:e80; 2017.
39. Papp Z, Clark RG, Parmley EJ, Leighton FA, Waldner C, Soos C. The ecology of avian influenza viruses in wild dabbling ducks (*Anas* spp.) in Canada. *PLoS One* 12:e0176297; 2017.
40. Skalos D, Weaver M. *Central valley midwinter waterfowl survey 2020 results.* Sacramento, CA: California Department of Fish and Wildlife Waterfowl Program [Modified 2020 August 13; Accessed 2021 June 22]. <https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=137485&inline;2020>.
41. Gurd DB. Predicting resource partitioning and community organization of filter-feeding dabbling ducks from functional morphology. *Am Nat.* 169:334–343; 2007.
42. Tietje WD, Teer JG. Winter feeding ecology of northern shovelers on freshwater and saline wetlands in south Texas. *J Wildl Manage.* 60:843–855; 1996.
43. Guillemain M, Fritz H, Guillon N. Foraging behavior and habitat choice of wintering northern shoveler in major wintering quarter in France. *Waterbirds* 23:355–363; 2000.
44. DuBow P. Feeding ecology and behavior of post-breeding male blue-winged teal and northern shovelers. *Can J Zool.* 63:1292–1297; 1985.
45. Abbas MD, Nazir J, Stumpf P, Marschang RE. Role of water fleas (*Daphnia magna*) in the accumulation of avian influenza viruses from the surrounding water. *Intervirology* 55:365–371; 2012.
46. Meixell BW, Borchardt MA, Spencer SK. Accumulation and inactivation of avian influenza virus by the filter-feeding invertebrate *Daphnia magna*. *Appl Environ Microbiol.* 79:7249–7255; 2013.
47. Lee KH, Gordon A, Shedden K, Kuan G, Ng S, Balmaseda A, Foxman B. The respiratory microbiome and susceptibility to influenza virus infection. *PLoS One* 14:e0207898; 2019.
48. Hird SM, Ganz H, Eisen JA, Boyce WM. The cloacal microbiome of five wild duck species varies by species and influenza A virus infection status. *mSphere* 3:e00382–18 2018.
49. Brown JD, Stallknecht DE, Beck JR, Suarez DL, Swayne DE. Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. *Emerg Infect Dis.* 12:1663–1670; 2006.
50. Henaux V, Samuel MD. Avian influenza shedding patterns in waterfowl: implications for surveillance, environmental transmission, and disease spread. *J Wildl Dis.* 47:566–578; 2011.
51. Enright C. Physical processes and geomorphic features. In: Moyle PB, Manfree A, Friedler PL, editors. *Suisun Marsh: ecological history and possible futures.* Berkeley (CA): University of California Press. p. 45–64; 2014.
52. Stallknecht DE, Kearney MT, Shane SM, Zwank PJ. Effects of pH, temperature, and salinity on persistence of avian influenza viruses in water. *Avian Dis.* 34:412–418; 1990.
53. Brown JD, Stallknecht DE, Valeika S, Swayne DE. Susceptibility of wood ducks to H5N1 highly pathogenic avian influenza virus. *J Wildl Dis.* 43:660–667; 2007.
54. Paull SH, Horton DE, Ashfaq M, Rastogi D, Kramer LD, Diffenbaugh NS, Kilpatrick AM. Drought and immunity determine the intensity of West Nile virus epidemics and climate change impacts. *Proc Biol Sci.* 284:20162078; 2017.
55. National Drought Mitigation Center, U.S. Department of Agriculture, National Oceanic and Atmospheric Administration. *U.S. drought monitor.* Lincoln (NE): National Drought Mitigation Center [accessed 2021 Jun 30]; 2021.
56. Lisovski S, van Dijk JGB, Klinkenberg D, Nolet BA, Fouchier RAM, Klaassen M. The roles of migratory and resident birds in local avian influenza infection dynamics. *J Appl Ecol.* 55:2963–2975; 2018.
57. Baldassarre G. *Ducks, geese, and swans of North America.* Baltimore (MD): Johns Hopkins University Press; 2014.

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