



Montana Fish, Wildlife & Parks



Photo by Helga Pac

Montana 2006 Avian Influenza Surveillance Project Report

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INTRODUCTION

Influenza is a respiratory disease that has infected animals and humans throughout recorded history (Webster et al. 2006). Avian influenza (AI) is a type A influenza virus enzootic in wild populations of more than 100 bird species that rarely is expressed clinically (AHAW Panel 2006). Waterfowl and shorebirds in particular have been identified as reservoirs for the virus in nature (Olsen et al. 2006).

Influenza viruses are classified by two proteins expressed on the surface of the virus, hemagglutinin and neuraminidase. There are currently 16 subtypes of hemagglutinin (H1-H16) and 9 subtypes of neuraminidase (N1-N9) that have been detected in bird populations worldwide (Munster et al. 2005). Pathogenicity, the ability to cause disease, in AI viruses may be distinguished as low pathogenic (LPAI) and highly pathogenic (HPAI) based on genetic features of the virus and the severity of the illness they cause in poultry (Centers for Disease Control and Prevention 2007). Most AI strains are classified as LPAI because they typically cause little or no clinical sign of disease in infected birds (Munster et al. 2005, Brown et al. 2006, Olsen et al. 2006). While influenza viruses are normally highly species-specific (World Health Organization 2007b), HPAI causes severe illness and death in poultry, and can also cause disease in humans and some mammals (Olsen et al. 2006, Webster et al. 2006). LPAI viruses containing hemagglutinin of subtypes H5 and H7 may become highly pathogenic after introduction to poultry (Munster et al. 2005).

The strain of avian influenza currently causing global concern is the highly pathogenic H5N1 (hereafter referred to as “HP-H5N1”) Asian strain. The emergence and recent spread of HP-H5N1 into Asia, the Middle East, Europe, and Africa has resulted in impacts to the poultry industry and presents an important threat to human health. Concern has elevated about the potential expansion of HP-H5N1 to North America that could cause illness and mortality in wild bird populations, introduce disease that could negatively affect the poultry industry, and be potentially dangerous to humans on a large scale through mutation or recombination (World Health Organization 2007b). While HP-H5N1 infections in humans are rare, they can cause severe illness and death, currently at a rate of approximately 60% of all human infections (World Health Organization 2007a). Though H7 infection in humans is also extremely rare, conjunctivitis can occur among people who have direct contact with infected birds (Webster et al. 2006).

The role of wild birds in the movement and transmission of HP-H5N1 is poorly understood. Circumstantial evidence suggests wild waterfowl may introduce AI viruses in the low pathogenic form to poultry flocks and some species of migratory waterfowl may carry HP-H5N1 to new geographical areas during migration (World Health Organization 2007b). The pathways by which HP-H5N1 has and will spread between countries have been debated extensively. Surveillance of wild ducks in the Northern Hemisphere showed a high prevalence of LPAI virus in primarily juvenile birds (~60%) in early fall before southbound migration, which then fell sharply. Waterfowl and shorebird influenza genetic data from the Americas indicate interplay between these host species. Molting, migration stopovers, and wintering grounds allow birds to exist in high densities and provide opportunities for the transmission of LPAI viruses between wild and captive birds and between species (Olsen et al. 2006). Research on wild bird migration in combination with movements in the poultry and

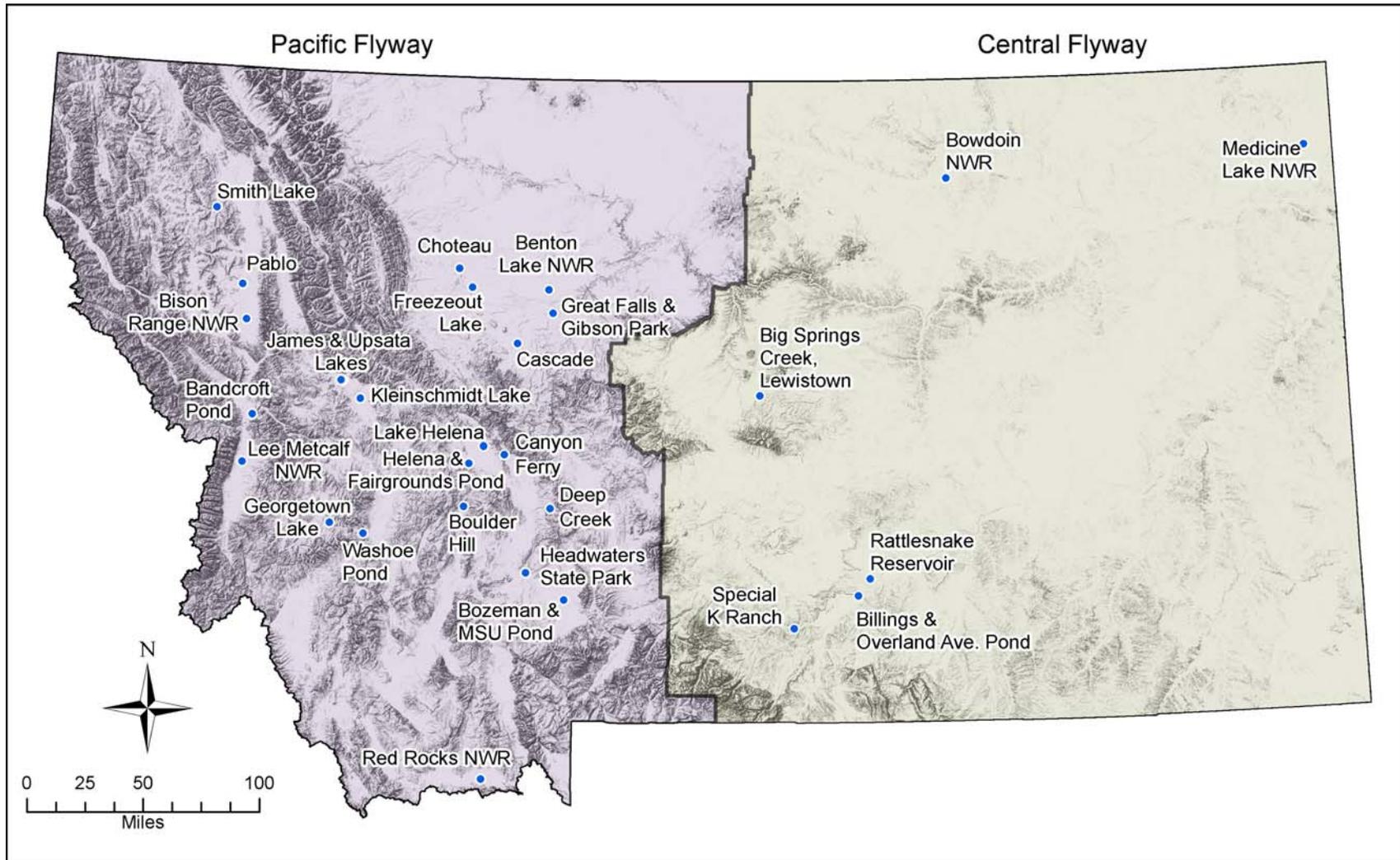
wild bird trade showed that most HP-H5N1 introductions to Asia were likely through poultry, most spread to Europe was likely through migratory birds, and movement in Africa was likely caused by both poultry and migrating wild birds. While many expect HP-H5N1 to enter North America from the north through the migration of wild birds from eastern Siberia, surveillance in Alaska shows very low AI infection rates (0.06%), which suggests that frequency of intercontinental virus transfer is likely low (Winker et al. 2007). Given the unregulated importation of poultry in Mexico and Brazil, Kilpatrick et al. (2007) predict HP-H5N1 may be introduced to the Western Hemisphere through infected poultry and to mainland United States by subsequent movement of migrating birds from southern neighboring countries. Local North American bird populations may amplify the disease and act as wild sentinel birds from which the arrival of HP-H5N1 may be detected (Brown et al. 2006).

During the summer of 2006, the U.S. Department of Agriculture (USDA) and the U.S. Fish and Wildlife Service (USFWS) initiated a nationwide avian influenza surveillance project for the early detection of HP-H5N1 that included all 4 flyways, all states, and tribal lands in the United States. The Pacific Flyway was considered a top priority to sample waterfowl and shorebirds potentially en route from Russia during the fall migration. Montana was considered a top priority state because the Pacific and Central Flyways divide the state and it borders Canada. Montana Fish, Wildlife and Parks (MFWP) and USDA-APHIS-Wildlife Services (WS) were lead agencies in the 2006 Avian Influenza Surveillance Project in collaboration with USFWS, Montana Department of Livestock (MT DoL), the Department of Public Health and Human Services, and the Tribal Nations. The objectives of this project were to sample live and hunter-harvested waterfowl for the potential early detection of HP-H5N1 throughout fall migration, collect environmental samples from areas of high waterfowl concentration, and collect mortality/morbidity samples from wild bird mortality events in the state of Montana as part of the national interagency surveillance.

STUDY AREA

Montana is the fourth largest of the 50 states with a total of over 93 million acres. Elevations range from 1,900 feet along the Missouri River to the highest point, Granite Peak in south-central Montana, at 12,850 feet. Topography is highly varied across the state ranging from the coniferous forests of the Rocky Mountains and associated foothills in the western third to expansive prairies of the Great Plains in the eastern two-thirds of the state (Figure 1). Land ownership is comprised of over 60 million acres of private and tribal lands (65%) and nearly 28 million acres (30%) of federal lands, while state owned lands account for over 5 million acres (5%; Montana Fish and Game Department 1971). Ecotypes also vary and include montane forests, intermountain and foothill grasslands, shrub grasslands, and plains grasslands and forests, each of which includes aquatic and riparian zones.

Figure 1. The Pacific and Central Flyways in Montana, and sampling sites for the 2006 Montana AI Surveillance Project. National Wildlife Refuge is referred to as “NWR”.



The Pacific and Central Flyways divide Montana; the Pacific Flyway contains Hill, Chouteau, Cascade, Meagher, and Park counties and all counties west, while the Central Flyway includes Blaine, Fergus, Judith Basin, Wheatland, Sweet Grass, Stillwater, and Carter counties and all counties east. Of the 413 birds species documented in the state, 268 breed and 145 use stopover sites in Montana during seasonal migrations or occasionally occur in the state.

METHODS

Sample Design

The 2006 Montana AI surveillance sampling strategy was a step-down approach from the U.S. Interagency Strategic Plan (Interagency HPAI Early Detection Working Group 2006) and the Pacific and Central Flyway plans (Pacific Flyway Council 2006, Central Flyway Council 2006). The above plans suggested that ≥ 200 samples would be required to detect one positive HP-H5N1 sample in a defined bird population of >1000 individuals with a 95% confidence interval at a disease prevalence of $\leq 1.5\%$. Sample design assumptions included 1) the populations of birds to be sampled were homogeneous and accessible, 2) HP-H5N1 was uniformly distributed across bird populations, and 3) representative sampling would be random and unbiased. Because these assumptions could not be met for wild migratory waterfowl, sampling was increased in an attempt to account for biases and sample sizes were extrapolated across large landscapes for multi-state and flyway sampling efforts (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006). The national and flyway plans placed emphasis on particular species in specific areas. Most sampling during 2006 was conducted in the Pacific Flyway due to the availability of the target waterfowl species and known suitable sampling sites.

Multiple sampling strategies were employed to maximize the chance of detecting HP-H5N1. Investigating disease events in dead or dying birds is considered one of the best opportunities to detect the potential introduction of HP-H5N1 into Montana by wild migratory birds (Wobeser 2006). Environmental sampling allows for the analysis of fecal material from waterfowl habitats because viable AI virus can be detected in feces for a period of time in cool temperatures (Interagency Asian HPAI Early Detection Working Group 2006). Wild live and hunter-harvested bird surveillance enables the selection of species that represent the highest risk of exposure to HP-H5N1, which includes birds that migrate directly between Asia and North America (primary species) and/or mix in Alaska staging areas with species that could bring HP-H5N1 from Asia (secondary species). Species of primary concern for the 2006 AI surveillance in Montana included Tundra Swan (TUSW), Lesser Snow Goose (SNGO), and Northern Pintail (NOPI). These species move between Asia and North America and could contact the Asian HP-H5N1 directly (Alaska Interagency HPAI Bird Surveillance Working Group 2006). Secondary and wild sentinel species included Mallard (MALL), American Widgeon (AMWI), Gadwall (GADW), and Northern Shoveler (NSHO). Additional priority species were Blue-winged Teal (BWTE), Green-winged Teal (AGWT), and Common Goldeneye (COGO). High numbers of these species migrate through the state and provide opportunity for sampling through banding operations, waterfowl hunting, and urban trapping (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006). Cloacal sampling targeted specific species spatially distributed across Montana and temporally distributed from August through December. Environmental sampling was also spatially and temporally distributed throughout the sampling period, while mortality/morbidity samples were taken opportunistically. Surveillance efforts were

accomplished through the extensive cooperation of MFWP, WS, USFWS, and city and/or county managers where the urban trapping was conducted.

Cloacal Sampling

Field

The criteria outlined in the 2006 Montana Sampling Plan (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006) stated that MFWP and WS should each collect 1000 cloacal samples from birds identified as species of concern for a total of 2000 samples statewide. The two agencies collaborated in their sampling efforts to achieve these objectives. The three strategies employed for cloacal sampling were coordinating with USFWS National Wildlife Refuge banding operations, sampling hunter-harvested waterfowl at National Wildlife Refuges and on state-owned lands, and trapping wild and semi-domestic waterfowl on urban ponds across the state.

Of the refuges that maintained waterfowl banding programs in Montana, the Bison Range/Ninepipes, Benton Lake, and Medicine Lake National Wildlife Refuges participated in the 2006 live bird AI surveillance. Refuge banding efforts in the state were conducted during August-September and used methods approved by the U.S. Fish and Wildlife Service and Canadian Wildlife Service (1977). Swim-in traps were used to capture waterfowl in six locations at the Bison Range/Ninepipes and four locations at Medicine Lake, while net-launchers were used at three sites at Benton Lake. Trapping sites were rotated throughout the week at each refuge. Waterfowl were first banded by USFWS and tribal biologists, cloacal samples were then taken by AI sampling teams consisting of MFWP, WS, and USFWS personnel, and the birds were released.

Hunter-harvested waterfowl and urban wild bird sampling began as banding operations ended and ran concurrently October through December. Hunter-harvested waterfowl were sampled at Benton Lake, Bowdoin, Lee Metcalf, and Red Rocks National Wildlife Refuges, Canyon Ferry, Headwaters State Park, Freezeout Lake, and Lake Helena. Sampling concluded daily and seasonally as hunting diminished or lakes froze completely. Hunters were asked if they would allow MFWP and WS AI personnel to sample their harvested birds, and pamphlets containing information about AI and the surveillance were distributed to hunters onsite and at MFWP offices. AI personnel also used swim-in traps at six urban ponds across the state to collect cloacal samples from wild and semi-domestic waterfowl. Because swim-in traps required a flat surface covered by ≤ 1.5 feet of water, traps were set in water only at Bandcroft Pond in Missoula and Gibson Pond in Great Falls. Swim-in traps modified for use on land were also utilized at Bandcroft Pond and at Lewis and Clark Fairgrounds Pond in Helena, MSU Pond in Bozeman, Overland Pond in Billings, and Washoe Pond in Anaconda. Permission to trap was granted by city and/or county managers and MFWP Information and Education personnel and city managers worked together to notify the public of the urban trapping activities.

The date, collector, county and site, location in WGS 84 decimal degrees, as well as the three most abundant species at each site were recorded on USDA datasheets for all cloacal sampling. Species, sex, age, condition, and band number, when present, for each bird sampled were also recorded. Species, sex, and approximate age were identified via plumage (Carney 1992). Specific age class was confirmed with the maturity of sex organs. Cloacal

samples were taken from live and hunter-harvested birds by gently swabbing the cloacal lining to obtain mucosa. The swab was then placed in a glass vial containing brain-heart infusion broth to preserve the sample. A pre-printed barcode with a sample identification number was placed on the vial and corresponding datasheet and lab submission form to track each sample. Samples were shipped overnight to the National Animal Health Laboratory Network laboratory at Colorado State University (CSU) in Styrofoam-lined boxes with cold packs within 24-48 hours of when the samples were collected. A sample batch referral number, the submitter, and number of samples in each shipment were recorded on the datasheet and corresponding lab submission form. Lab submission forms were sent to CSU with the samples while all datasheets were sent from the field to the MFWP AI Coordinator and immediately faxed to the WS national database manager. An additional MFWP datasheet was used during hunter-harvest sampling to record the hunter's name, Montana license number (ALS#), contact information, bird species, and the sample barcode number to connect hunters with the birds sampled.

Lab

CSU combined up to five individual cloacal samples to form sample pools that were tested by real-time reverse transcription-polymerase chain reaction (rRT-PCR). All pools were initially screened with a matrix gene primer/probe set designed to detect all influenza A viruses. Samples testing positive were further analyzed to identify H5 and H7 subtypes. Samples that screened positive or suspect for H5 or H7 were then sent to the National Veterinary Services Laboratory (NVSL) in Ames, Iowa. NVSL performed confirmatory testing for H5 and H7 subtypes using rRT-PCR, and 2 side-by-side molecular assays, standard rRT-PCR and WHO-RT-PCR, for N1. Virus isolation (VI) tests were performed on all samples in order to isolate AI viruses and determine whether or not H5 and N1 were linked in the same viral strain. All samples that produced positive results using VI were then tested for pathogenicity using chicken inoculation studies and/or, if enough RNA was present in the clinical sample, a target amino acid sequence analysis was performed to determine virulence potential of the virus (U.S. Department of the Interior and Wildlife Service 2006).

Environmental Sampling

Field

WS was responsible for the collection of 1000 environmental samples across the state from August through December. Hence, environmental sampling ran concurrently with cloacal sampling. According to the 2006 Montana Sampling Plan, batches of 20-30 individual specimens per sampling session were to be collected at pond levees, boat docks, dikes or dams, and shorelines. Samples taken from the same location were to be collected at least three weeks apart to avoid duplicating specimens from the same birds (USDA-APHIS-Wildlife Services et al. 2006). Environmental sampling sites included Bandcroft, Gibson, Lewis and Clark Fairgrounds, MSU, Overland, and Washoe ponds, Bowdoin, Medicine Lake, Cascade, Freezeout Lake, and the Special K Ranch southeast of Columbus. Fresh feces (<24 hours old) were collected with swabs and placed in cryovials containing bovine albumin diluent to preserve the samples and virus particles, if present. As with the cloacal samples, pre-printed barcodes were placed on the vials and corresponding USDA lab submission forms. The date, collector, county and site, location in WGS 84 decimal degrees, and the three most abundant species at each site were recorded, as well as a sample batch referral number, submitter, and number of samples in each shipment. Samples and related lab

submission forms were shipped overnight to the USDA-APHIS-WS National Wildlife Research Center in Fort Collins, CO, in Styrofoam-lined boxes with cold packs within 48 hours of sample collection.

Lab

Up to five individual environmental samples were combined to form sample pools that were treated with an inhibitex compound to remove natural inhibitors in the fecal samples. Pooled samples were tested using rRT-PCR following the same protocols as described for the cloacal samples to detect AI viruses. If positive, pools were tested again with rRT-PCR for H5 and H7. Presumptive and suspect H5 and H7 positive pools were then sent within 48 hours to NVSL for confirmatory testing following cloacal sample testing protocols.

Mortality/Morbidity Sampling

The 2006 Montana Sampling Plan specified the collection ≤ 400 mortality/morbidity samples during the 2006 sampling period. In August, MFWP established a toll-free number and a web-based reporting system on the MFWP website through which the public could report dead or sick birds. The MFWP AI Coordinator, Wildlife Lab Supervisor, and Wildlife Veterinarian determined which of the reports made by the public were investigated according to criteria set forth in the 2006 Montana Sampling Plan. These criteria included consideration of the reported species as a potential concern for the presence of HP-H5N1 and the circumstances under which the dead or sick birds were found. Morbid birds were euthanized in accordance with the Guidelines for Euthanasia of Non-domestic Animals (AAZV 2006) and entire carcasses were shipped within 24 hours for necropsy and disease testing at the USFWS National Wildlife Health Center (NWHC) in Madison, WI. Birds found within 24 hours of death were also shipped to NWHC for testing when AI personnel determined the carcasses were suitable for disease testing. Some samples were sent to the MT DoL lab in Bozeman to expedite the reporting of cause of death for mortality events. If shipment within 48 hours of death was not possible, carcasses were frozen and shipped as soon as possible. The NWHC lab submission form contained the submitter, date of carcass collection, location data recorded in WGS 84 decimal degrees, whether the bird was euthanized or found dead, and environmental factors where the bird was found. The species, age, sex, condition, and clinical signs of disease of the bird were also recorded. If a mortality event occurred, the onset and end date, known and estimated numbers of dead birds, bird populations at-risk, and movements of the at-risk populations were recorded.

Data Management and Reporting of Results

MFWP maintained databases for all AI mortality/morbidity and cloacal data collected throughout the 2006 Montana AI surveillance. MFWP AI personnel also entered cloacal data into a USDA national web-based database system. USDA personnel entered Montana environmental data into a separate USDA national database where they were cleaned and results were reported according to sample pools tested for the AI matrix and county; no results were reported for H5, H7, or N1 testing. Mortality/morbidity results were reported directly to MFWP by NWHC and MT DoL, and contained cause of death and results of AI and additional disease testing. USDA first reported cloacal sampling results via Excel database spreadsheet and later through the USDA web-based database, and included H5, H7, and N1 screening results, as well as LPAI subtype and pathogenicity. USDA is currently importing

AI cloacal and environmental data and results into the HPAI Early Detection Data System database, which is managed by the U.S. Geological Survey.

SAMPLING EFFORT

Cloacal Sampling

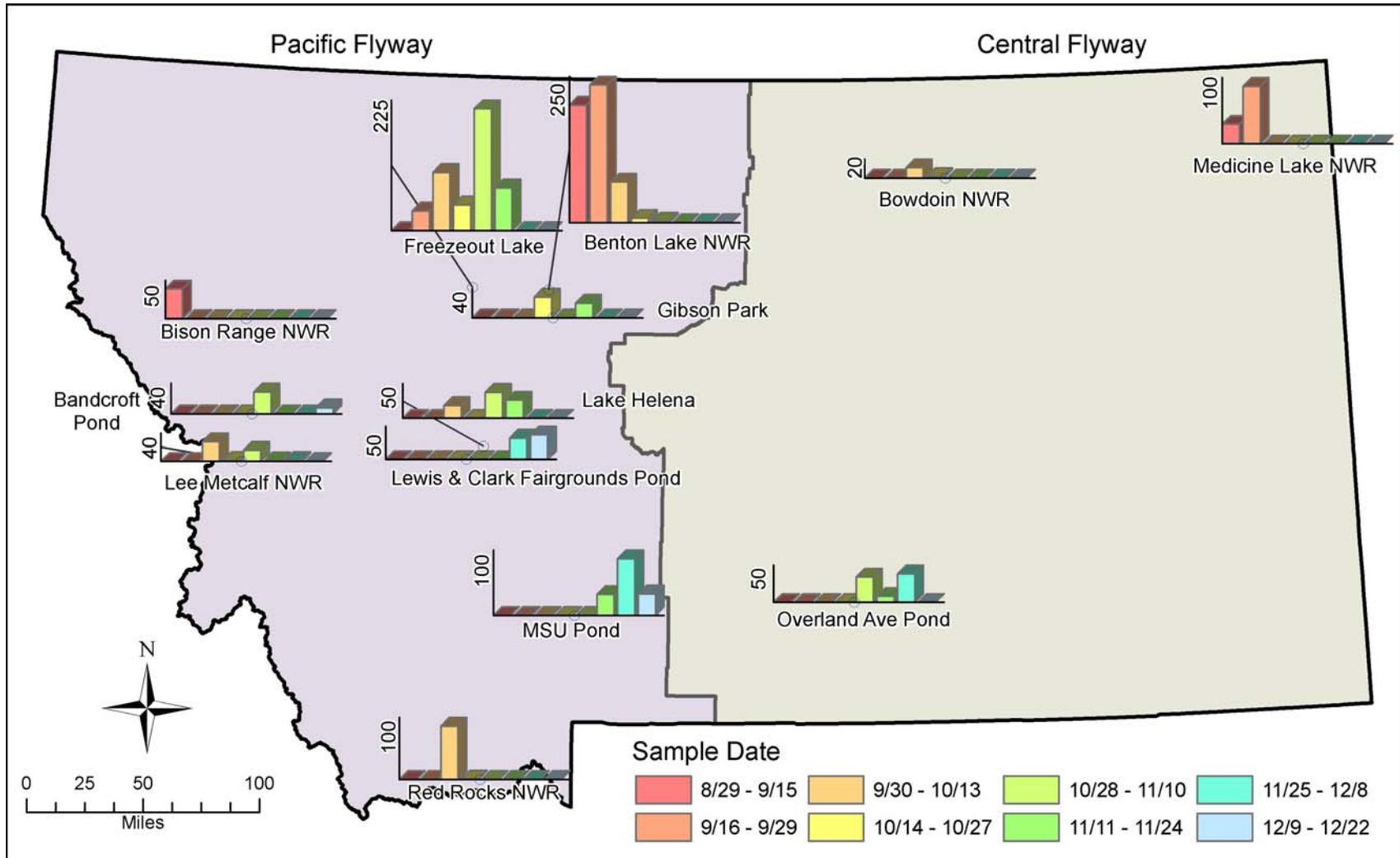
Cloacal sampling was performed in conjunction with refuge banding operations 8/29/06 – 9/28/06, hunter-harvested waterfowl sampling was conducted 9/23/06 – 11/24/06, ending as fall migration subsided, and urban wild bird sampling was conducted 10/17/06 – 12/14/06. A total of 139 sampling days were comprised of 21 days from refuge banding operation sampling and 35 days from urban pond sampling for a total of 56 days of live bird sampling, and 83 days from hunter-harvest sampling. Sampling effort resulted in overall means of 8.2 days/site and 14.5 samples/sample day at 17 sites across all cloacal sampling methods (Table 1). MFWP collected a total of 1012 cloacal samples by 11/9/06 and continued to collect samples in collaboration with WS through December to fulfill the total cloacal sampling objective of 2000 samples during the 2006 surveillance period. A total of 2022 cloacal samples were collected; banding operations yielded 660 samples (33%) and urban trapping efforts produced 494 samples (24%) for a total of 1154 live bird samples (57%). Hunter-harvested samples totaled 868 (43%; Table 2). Banding operation sampling at Benton Lake and hunter-harvest sampling at Freezeout Lake each yielded approximately one-quarter of the total samples collected (n=465, n=490, respectively). Banding operations produced the highest mean number of samples/sampling day (31.4) while urban trapping and hunter-harvest sampling yielded close to the same mean number of samples/sampling day (urban = 14.1, hunter-harvest = 10.5; Table 1). Benton Lake banding operations were the most productive with a mean of 35.8 samples/sampling day. The least productive sampling sites were Canyon Ferry, Headwaters State Park, and Washoe Pond, (Table 2).

Table 1. 2006 Montana AI Surveillance Project cloacal sampling effort.

	Sampling Method			Total
	Banding	Urban	Hunter-harvest	
Number of sites	3	6	8	17
Total samples	660	494	868	2022
Percent of total samples	33	24	43	100
Total sample days	21	35	83	139
Mean sample days/number of sites	7.0	5.8	10.4	8.2
Mean samples/sample day	31.4	14.1	10.5	14.5

The highest proportion of samples was collected in the northwest section of the Montana Pacific Flyway at Benton Lake during September and Freezeout Lake peaking in mid-November. Sampling was distributed fairly evenly across the rest of the Pacific Flyway both spatially and temporally. Cloacal sampling occurred at three sites in the Central Flyway; most samples were collected during refuge banding operations at Medicine Lake during September (Figure 2).

Figure 2. Temporal distribution of the 2006 Montana AI cloacal sampling. Sites with <10 total samples were excluded. Scale bar numbers are the maximum number of samples collected during a two-week sample period, National Wildlife Refuge is referred to as “NWR”.



The 2006 Montana Sampling Plan called for samples from 170 Tundra Swans, 150 Lesser Snow Geese, and 310 Northern Pintails (150 from banding operations, 160 from hunter-harvest sampling) as the primary species of concern, whereas the majority of secondary species samples were to come from Mallards (n=890). The Montana AI team collected 52 Tundra Swan, 151 Lesser Snow Goose, and 219 Northern Pintail samples from available birds. The 1072 Mallard samples collected comprised approximately half of all cloacal samples. The other secondary species of concern, Gadwall (n=152), Northern Shoveler (n=100), and American Widgeon (n=97), comprised 17.3% of the total cloacal samples, while the rest of the species sampled combined comprised 8.9% of the total samples (Table 3).

Table 2. Number of sample days, and number and percent of samples per site and across cloacal sampling methods during the 2006 Montana AI Surveillance Project.

Method	Site	Sample days	Total number of samples	Percent samples per method
Banding (live bird)	Benton Lake	13	465	70.5
	Medicine Lake	4	141	21.3
	Bison Range	4	54	8.2
Total		21	660	100
Urban (live bird)	MSU Pond	7	180	36.4
	Overland Pond	8	109	22.1
	Lewis & Clark Pond	5	82	16.6
	Gibson Pond	8	65	13.2
	Bandcroft Pond	4	51	10.3
	Washoe Pond	3	7	1.4
Total		35	494	100
Total live bird		56	1154	100
Hunter-harvest (dead bird)	Freezeout Lake	29	490	56.5
	Lake Helena	16	103	11.9
	Red Rocks	6	98	11.3
	Benton Lake	9	85	9.8
	Lee Metcalf	15	60	6.9
	Bowdoin	3	22	2.5
	Canyon Ferry	4	7	0.8
	Headwaters State Park	1	3	0.3
Total		83	868	100
Sampling Total		139	2022	100

Age class was divided into hatch-year and after-hatch-year birds. Slightly more than half of all birds sampled were hatch-year (n=1076, 53.2%) while after-hatch-year birds (n=900, 44.5%) yielded fewer samples. Age for 46 birds sampled (2.3%) was not determined. Within species, Northern Pintail, Gadwall, Northern Shoveler, American Widgeon, and Blue-winged Teal hatch-year birds were sampled in highest numbers (~70-75%) while Lesser Snow Goose, Mallard, and Green-winged Teal age classes were sampled quite evenly. Tundra Swan and Common Goldeneye after-hatch year birds were sampled in higher numbers (~70%) than hatch-year birds (Table 4).

Table 3. Number of sample days, and number and percent of the 2006 Montana AI cloacal samples according to species and method.

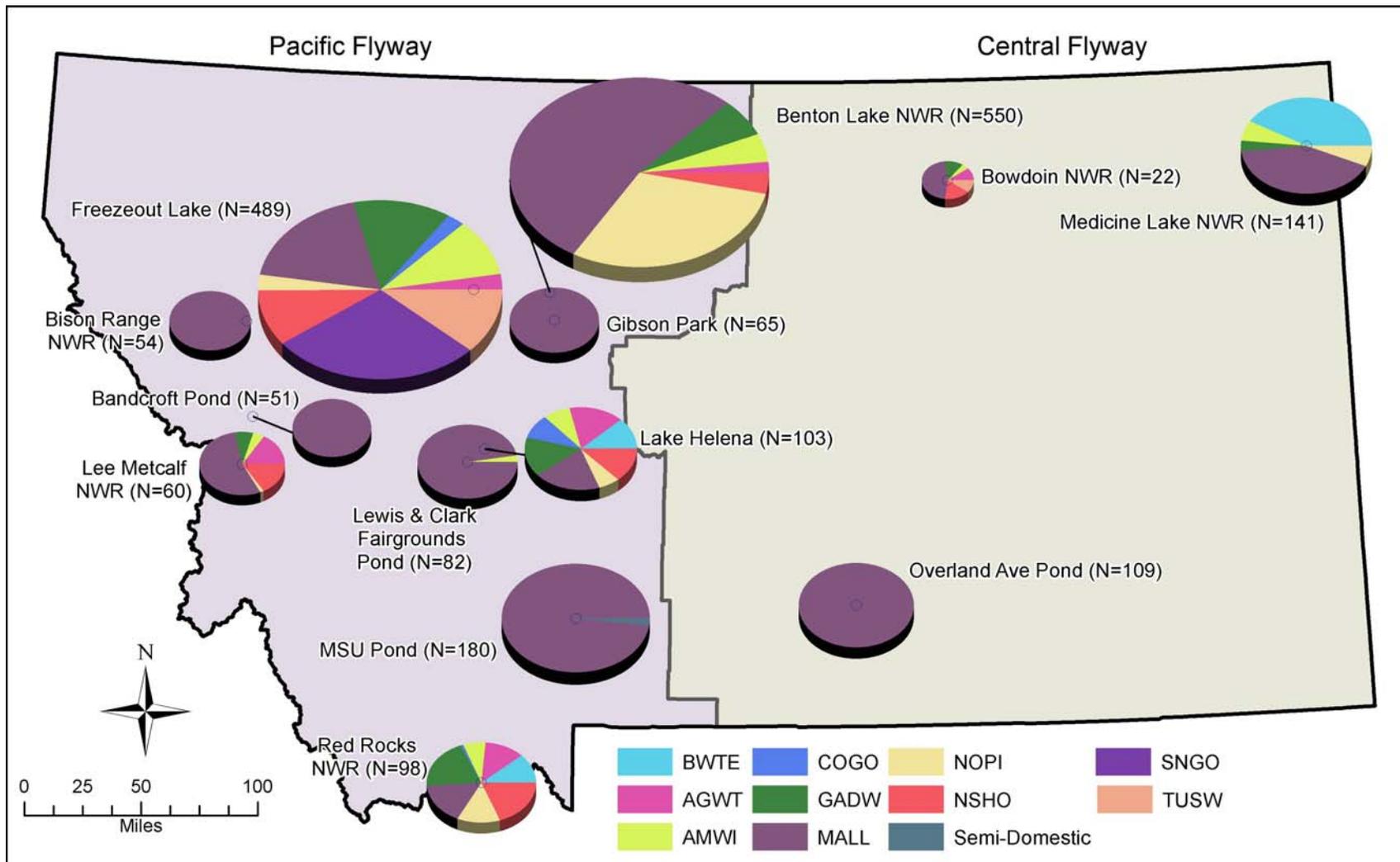
Species	Sample days	Banding	Urban	Hunter-harvest	Total	Percent of total samples
Mallard	51	407	488	177	1072	53.0
Northern Pintail	21	169	0	50	219	10.8
Gadwall	15	4	0	148	152	7.5
Lesser Snow Goose	14	0	0	151	151	7.5
Northern Shoveler	14	0	0	100	100	4.9
American Wigeon	24	19	2	76	97	4.8
Blue-winged Teal	6	59	0	22	81	4.0
Green-winged Teal	12	2	0	62	64	3.2
Tundra Swan	12	0	0	52	52	2.6
Common Goldeneye	10	0	0	25	25	1.2
Trumpeter Swan	3	0	0	3	3	0.1
Semi-domestic	1	0	3	0	3	0.1
Wood Duck	1	0	1	0	1	>0.1
Bufflehead	1	0	0	1	1	>0.1
Lesser Scaup	1	0	0	1	1	>0.1
Total	185	660	494	868	2022	100

Table 4. Number and percent of the 2006 Montana AI cloacal samples according to species and age class.

Species	Hatch-year		After-hatch year		Undetermined		Total
	Number	Percent	Number	Percent	Number	Percent	Number
Mallard	476	44.4	566	52.8	30	2.8	1072
Northern Pintail	161	73.5	58	26.5	0	0	219
Gadwall	104	68.4	48	31.6	0	0	152
Lesser Snow Goose	71	47.0	79	52.3	1	0.7	151
Northern Shoveler	72	72.0	21	21.0	7	7.0	100
American Wigeon	75	77.3	20	20.6	2	2.1	97
Blue-winged Teal	60	74.1	21	25.9	0	0	81
Green-winged Teal	37	57.8	24	37.5	3	4.7	64
Tundra Swan	13	25.0	38	73.1	1	1.9	52
Common Goldeneye	7	28.0	16	64.0	2	8.0	25
Trumpeter Swan	0	0	3	100	0	0	3
Semi-domestic	0	0	3	100	0	0	3
Wood Duck	0	0	1	100	0	0	1
Bufflehead	0	0	1	100	0	0	1
Lesser Scaup	0	0	1	100	0	0	1
Total	1076	53.2	900	44.5	46	2.3	2022

Most Tundra Swan, Lesser Snow Goose, and Northern Pintail samples were collected in north-central Montana at Freezeout Lake and Benton Lake (Figure 3). Mallards were sampled at nearly all sites across the state; most samples were distributed throughout western

Figure 3. Spatial distribution of the 2006 Montana AI cloacal sampling according to species. Trumpeter Swan and species from which one sample was collected were excluded. National Wildlife Refuge is referred to as “NWR”.



and central Montana. The majority of the remaining species sampled were spread across the western and central parts of the state with the exception of Blue-winged Teal, which was mostly sampled in the northeastern corner of the state at Medicine Lake. Hunter-harvest birds provided the greatest diversity of species for sampling, whereas urban trapping allowed for little diversity given nearly all birds available for trapping at ponds were Mallards.

The collection of samples from primary species began with Northern Pintails on 9/6/06 and peaked 9/14/06; the majority of samples were collected during refuge banding operations. Lesser Snow Goose sampling began 10/7/06 and peaked 11/3/06, while Tundra Swan sampling began 10/21/06 and peaked 10/28/06; samples for both species were collected from only hunter-harvested birds. Sampling of all three species ended in mid-late November (Figure 4). Sampling of secondary species began with Mallards on 8/29/06 and peaked twice, on 9/6/06 during refuge banding and 12/4/06 during urban trapping. American Wigeon and Gadwall sampling began 9/6/06 and peaked the first day of waterfowl hunting on 9/30/06; Gadwall sampling peaked a second time on 10/28/06. Northern Shoveler sampling began and peaked on 9/30/06. Gadwall and Northern Shoveler sampling ended in mid-late November while Mallard and American Wigeon sampling ended during mid December (Figure 5). Additional species sampled in numbers ≥ 25 included Blue-winged Teal (began 9/18/06, peaked 9/19/06, ended 11/5/06), Green-winged Teal (began 9/19/06, peaked 9/30/06, ended 11/24/06), and Common Goldeneye (began 9/30/06, peaked 11/5/06, ended 12/13/06).

Figure 4. Temporal sampling distribution of primary species for the 2006 Montana AI Surveillance Project.

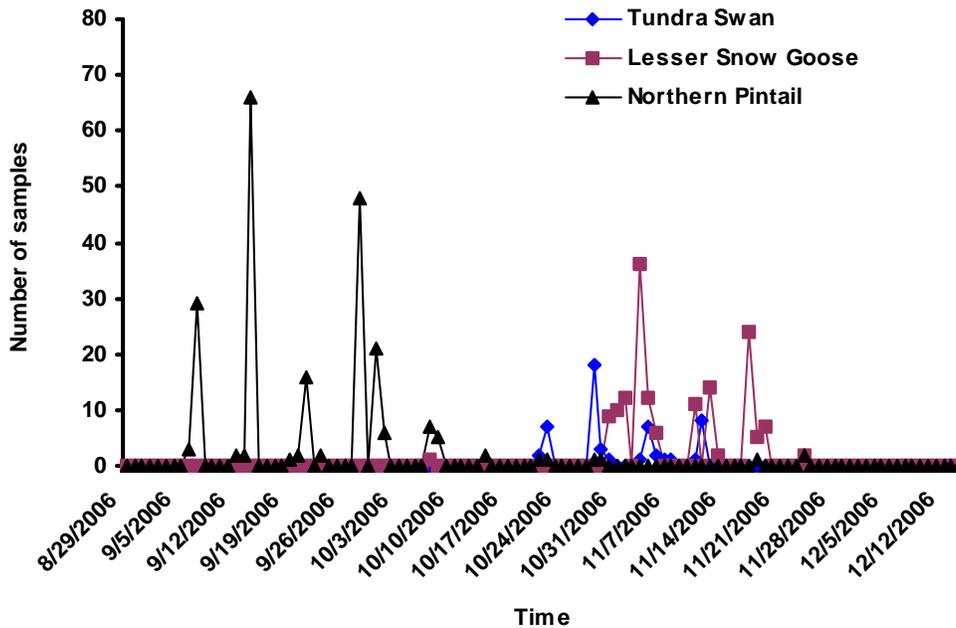
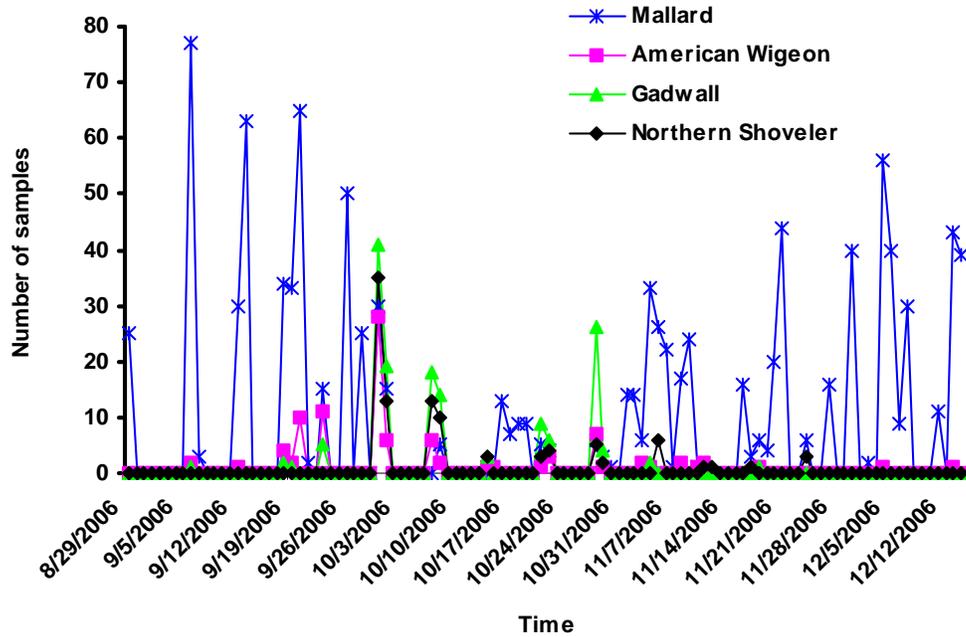


Figure 5. Temporal sampling distribution of secondary species for the 2006 Montana AI Surveillance Project.



Environmental Sampling

WS collected 998 environmental samples on 35 sample days from 9/19/06 through 12/26/06 at 11 sites statewide. The mean number of sample days/total number of sites was 3.5 and the mean number of samples/sample day was 28.5. The largest numbers of samples were collected at Lewis and Clark Pond (163), Overland Pond (160), and Freezeout Lake (160), comprising nearly half of all samples collected (Table 5).

Table 5. Number and percent of the 2006 Montana AI environmental samples according to site.

Site	Sample days	Total number samples	Percent samples per site type
Bowdoin	2	64	6.4
Medicine Lake	1	40	4.0
Lewis & Clark Pond	5	163	16.3
Overland Pond	5	160	16.0
Washoe Pond	4	105	10.5
MSU Pond	2	60	6.0
Gibson Pond	2	51	5.1
Bandcroft Pond	2	50	5.0
Freezeout Lake	6	160	16.0
Special K Ranch	4	96	9.6
Cascade	2	49	4.9
Total	35	998	99.8

Consistent environmental sampling across the state began in mid-October and was distributed quite evenly across the sampling period, peaking in mid-November at Freezeout Lake. Sampling in the Central Flyway was conducted early and late in the surveillance, while sampling in the Pacific Flyway was conducted during mid-late surveillance. Environmental sampling strongly overlapped spatially and temporally with cloacal sampling (Figure 6).

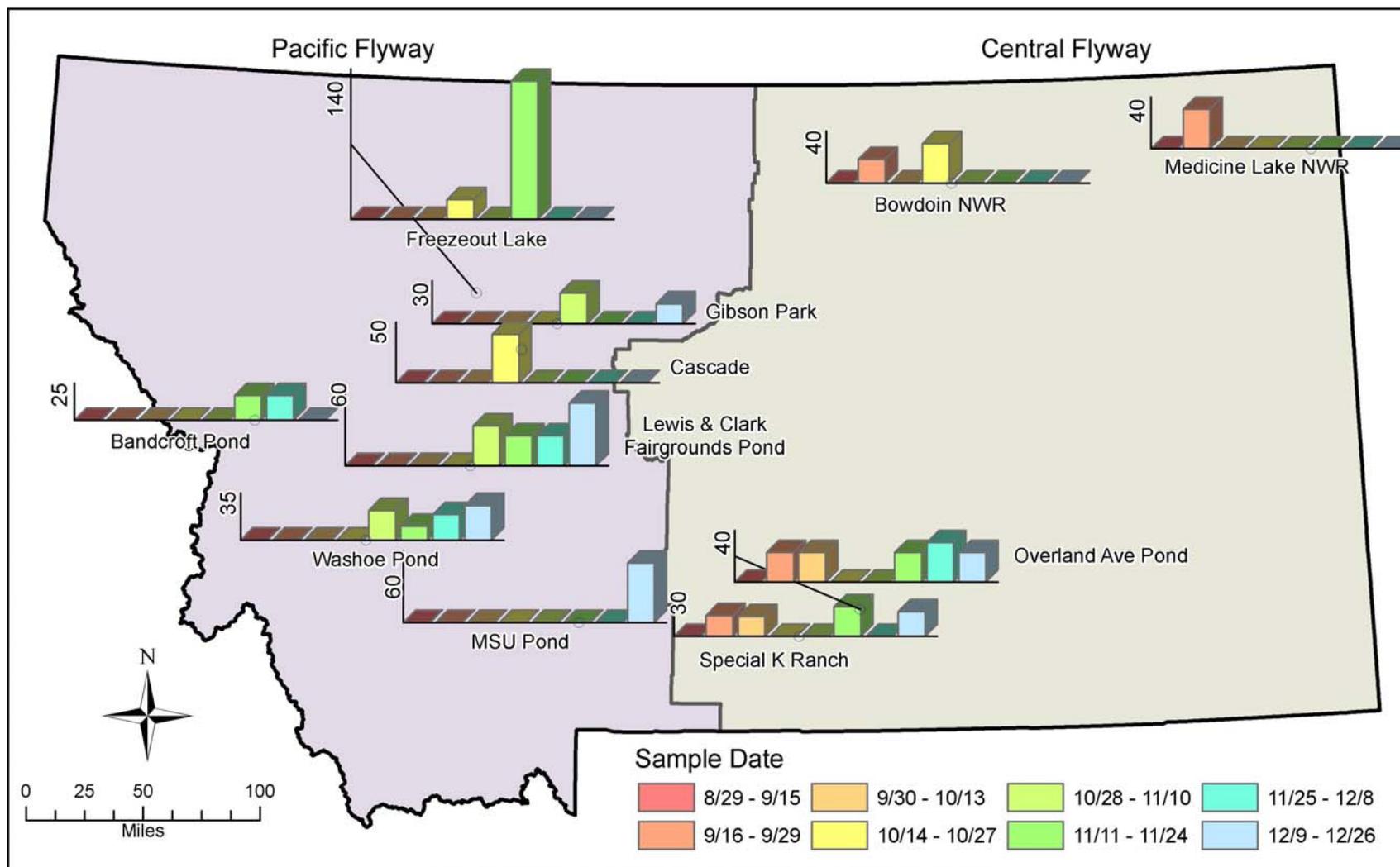
Mortality/Morbidity Sampling

A total of 62 mortality/morbidity samples were collected by MFWP and USFWS from 19 species that included birds from 16 mortality events reported statewide (Table 6). The 31 calls received on the toll-free reporting system and three MFWP website reports of dead and dying birds yielded five mortality/morbidity sampling events which consisted of single birds and a mortality event at Georgetown Lake American Coot. Additional single dead birds and mortality events at Medicine Lake, Smith Lake, and Rattlesnake Reservoir were also investigated and carcasses were collected for AI testing. Five American Coots submitted to MT DoL were sent on to CSU for AI testing and the remaining 57 samples were submitted to NWHC. Of the 48 birds categorized by age and sex at NWHC, 33 were classified as hatch-year birds (12 females, 21 males) and 15 were classified as after-hatch-year birds (7 females, 8 males).

Table 6. 2006 Montana AI mortality/morbidity samples according to species.

Species	Number of samples
American Coot	29
American White Pelican	6
Bufflehead	1
Canada Goose	1
California Gull	2
Common Merganser	1
Evening Grosbeak	2
Gadwall	1
Great Blue Heron	1
Great Horned Owl	1
Mallard	2
Mourning Dove	1
Northern Shoveler	2
Ring-billed Gull	4
Ross' Goose	1
Sanderling	1
Trumpeter Swam	4
Semi-domestic Goose	1
Western Grebe	1
Total	62

Figure 6. Temporal distribution of the 2006 Montana AI environmental sampling. Scale bar numbers are the maximum number of samples collected during a two-week sample period. National Wildlife Refuge is referred to as “NWR”.



RESULTS

While AI virus was found in samples, HP-H5N1 was not detected in Montana or elsewhere in North America during the 2006 surveillance. The surveillance did not focus on the detection of LPAI, but LPAI was specified in nine samples from Montana through the process of testing for HP-H5N1 and included H4N6 (n=2), H5N2 (n=2), H5N3 (n=4), and H6N2 (n=1).

Cloacal Sampling

Of the 2022 cloacal samples submitted for AI testing, 64 (3.2%) tested positive for the AI matrix. Though positive results were detected for H5 using rRT-PCR (n=9) and VI (n=7), and N1 using rRT-PCR (n=3) and WHO- RT-PCR (n=1), H5 and N1 were not found in the same viral strain and thus were not linked. H5 and N1 positive samples were collected during August-September refuge banding at the Bison Range/Ninepipes (n=3) and Benton Lake (n=8) from Mallards (n=8) and Northern Pintails (n=3). Two additional H5-positive samples were collected in November, one from a harvested Lesser Snow Goose at Freezeout Lake and the other from a Mallard trapped live at the MSU Pond. H7 was not detected.

Environmental Sampling

The 204 environmental sample pools produced 12 (5.9%) positive results for AI virus from six counties: Sheridan (Medicine Lake), Lewis and Clark (Lewis and Clark Fairgrounds Pond), Missoula (Bandcroft Pond), Stillwater (Special K Ranch near Columbus), Gallatin (MSU Pond), and Deer Lodge (Washoe Pond). Most positive results occurred in samples collected during December (n=7; Table 5).

Table 5. Number and date of environmental sample pools that tested positive for the AI matrix during the 2006 Montana AI Surveillance Project.

Site	Number of pools	Date sampled
Medicine Lake	2	9/18/06
Lewis & Clark Pond	1	10/31/06
Washoe Pond	2	11/06/06
Bandcroft Pond	1	12/06/06
Special K Ranch	2	12/11/06
MSU Pond	1	12/11/06
MSU Pond	2	12/19/06
Washoe Pond	1	12/20/06
Total	12	-----

Mortality/Morbidity Sampling

The 62 mortality/morbidity samples tested for AI virus produced four presumptive positives based on rRT-PCR, all of which were collected from hatch-year birds at Medicine Lake and tested by NWHC. Samples from one Ring-billed Gull and one Mallard collected on 9/6/06 and 9/7/06, respectively, and one American Coot and one Gadwall collected on 9/18/06 produced the positive results for the AI matrix. Since no positive results for H5 or H7 were detected, NWHC did not test for N1, LPAI subtype, or pathogenicity. Cause of death for mortality events will be reported by MFWP.

DISCUSSION

As expected, AI virus and H5 in low pathogenic form were detected in Montana samples. Results from the cloacal sampling determined that H5 was detected with both rRT-PCR and VI assays and N1 was detected with rRT-PCR and WHO-RT-PCR assays, but not by VI. The rRT-PCR and VI assays testing H5 agreed in five cases and disagreed in six; four samples were rRT-PCR-positive and VI-negative while two samples were H5 rRT-PCR-negative and VI-positive. Differences in the detection of AI virus between the assays may be explained in part by what they detect; PCR detects RNA and VI detects only live virus. Factors that might adversely affect the sensitivity of PCR assays include substances that might inhibit the detection RNA in the sample, inefficient RNA extraction, and the potential of RNA to rapidly degrade before testing. Since VI can detect only live virus, negative results could be due to dead virus in the sample rather than the absence of the virus (Spackman et al. 2002). The sample of particular note from Montana was the female hatch-year Northern Pintail that tested rRt-PCR AI-H5 positive, rRt-PCR AI-N1 positive, WHO-RT-PCR AI-N1 positive, but was typed as H5N3 by VI. There are several possible explanations for this, one of which is that the H5N3 could have out-grown a second AI-N1 virus present in the bird. VI is currently the “gold standard” assay to test for these viruses and the results are therefore considered definitive.

The 2006 AI surveillance effort was a pilot season. The 2006 Montana Sampling Plan called for cloacal samples from 200 shorebirds to be collected in July. Due to funding and time constraints, this was not logistically feasible. As a result, MFWP and WS increased the number of waterfowl samples by 200 to replace the samples that were to be collected from shorebirds. Mallard was the most abundant and available species for sampling in Montana and was therefore sampled strongly during refuge banding and urban trapping. A reduction in Mallard sampling was implemented during hunter-harvest sampling to maximize sampling of all other species specified in the 2006 Montana Sample Plan. Urban trapping success varied depending on the circumstances and was more challenging where flocks of domestic geese and ducks intermingled with wild waterfowl (Gibson, Lewis and Clark Fairgrounds, Washoe ponds). However, urban trapping provided the greatest flexibility since sampling could be conducted according to schedule rather than opportunistically. Hunter-harvest sampling was successfully achieved, but difficult to allocate across time; 42% of the total hunter-harvest samples were collected during the first two weekends of the waterfowl hunting season when the majoring of hunting took place, which then tapered quickly and ended on 12/13/06. The same was true for refuge banding; approximately one-third of all cloacal samples were collected during the month of September, mostly at Benton Lake (23% of total cloacal samples). To spread sample collection temporally, more emphasis could be placed on urban trapping for wild sentinel species while using banding and hunter-harvest sampling to target specific species and augment urban trapping.

Success of wild live and hunter-harvested bird sampling, as well as mortality/morbidity sampling, depended on the availability of the species and numbers of birds during migration. Migration timing can be affected by many factors, including climate and weather patterns (Blokpoel and Richardson 1978, Nichols et al. 1983, Harmata et al. 2000), age of the migrants (Hepp and Hines 1991), population size (Nichols et al. 1983), and bird body mass, especially in hatch-year birds (Owen and Black 1989). The 2006 migration period had unusually mild

weather conditions on average. With respect to July through December averages, temperatures were above average statewide while precipitation for the same period varied across the state; northwest Montana was above average, central Montana was near average, and eastern and southeastern Montana were below average (based on 1985- 2006 averages; National Climactic Data Center 2007). These climactic factors may have impacted the composition of birds available for testing during the 2006 surveillance. It was important to obtain high numbers of hatch-year bird samples because that age class likely contained the highest prevalence of AI viruses during their first fall migration (Olsen et al. 2006); this was accomplished during the 2006 Montana AI surveillance.

Planning for the national 2007 AI surveillance is underway. Changes are being made to sample designs, protocols for sample collection and data recording, database management, as well as laboratory methods. Sampling of live birds in Montana will likely begin in August while mortality/morbidity samples will be collected throughout the year.

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