

Montana Fish, Wildlife & Parks 2008 Avian Influenza Surveillance Project Report

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EXECUTIVE SUMMARY

The emergence and spread of the highly pathogenic avian influenza (AI) H5N1-Asian strain (HP-H5N1) in Asia, the Middle East, Europe, and Africa has elevated concern about potential expansion of HP-H5N1 to North America. Such an event could have negative affects on the poultry industry, humans, and wild bird populations. 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 in 2006, which was continued through 2008. Montana was considered a high priority state because the Pacific and Central Flyways divide the state and it borders Canada.

This report covers the work performed by Montana Fish, Wildlife and Parks (FWP) during the 2008 surveillance period. The objectives of the project were to employ multiple sampling strategies to maximize the chance of detecting HP-H5N1, including sampling live and hunter-harvested waterfowl throughout fall migration and collecting samples from wild bird mortality/morbidity events. To achieve the 2008 objectives, personnel from FWP collected 860 swab samples from live and hunter-harvested birds and 42 mortality/morbidity samples. Six weekly prospective mortality transects (n=128) were also conducted on lakes and wetlands throughout the state to systematically record the presence of target bird populations and mortality events. Avian influenza virus in low pathogenic form was detected in Montana samples as expected, while HP-H5N1 was not found during the three years of surveillance in Montana or elsewhere in North America.

The 2009 avian influenza surveillance is underway. FWP added spring mortality/morbidity transects to the 2009 sample design, which began in May, while sampling of live birds in Montana will begin in August with refuge banding operations. Opportunistic mortality/morbidity samples are collected throughout the year.

INTRODUCTION

The emergence and spread of the HP-H5N1 Asian strain in Asia, the Middle East, Europe, and Africa has elevated concern about potential expansion of the disease to North America. Such an event could have negative affects on the poultry industry, humans, and wild bird populations (World Health Organization 2007). The role of wild migratory birds in the movement and transmission of HP-H5N1 is poorly understood and strongly contested (Krauss *et al.* 2007, Peterson *et al.* 2007, van Gils *et al.* 2007). Circumstantial evidence suggests wild waterfowl may introduce AI viruses in the low pathogenic form to poultry flocks (World Health Organization 2007) and some species of waterfowl may asymptotically carry HP-H5N1 to new geographical areas during long distance migration (Chen *et al.* 2006, Lvov *et al.* 2006, but see Weber *et al.* 2007). Molting, migration stopovers, and wintering grounds allow birds to exist in high densities and provide opportunities for the transmission of low pathogenic avian influenza (LPAI) viruses between species, and wild and captive birds (Olsen *et al.* 2006), which then may recombine or mutate into a highly pathogenic form (Scholtissek *et al.* 1978, Ungchusak *et al.* 2005, Dugan *et al.* 2008).

The USDA and the USFWS initiated a nationwide avian influenza surveillance project for the early detection of HP-H5N1 in 2006. Montana was considered a high priority state for sampling because it contained both the Pacific and Central Flyways and bordered Canada. FWP and USDA-Animal and Plant Health Inspection Service (APHIS) Wildlife Services (WS) conducted the Montana AI surveillance project sampling during the last three years and the Montana Department of Livestock (MDoL) and the U.S. Geological Survey National Wildlife Health Center (NWHC) laboratories tested the samples. The Department of Public Health and Human Services and the Tribal Nations were also collaborators in the nation-wide effort. The objectives of this project were to employ multiple sampling strategies to maximize the chance of detecting HP-H5N1. This report is designed to record the AI surveillance performed by FWP during 2008 and across years to track methodologies. WS was responsible for half of the AI live and hunter-harvested bird swab sampling, as well as environmental sampling, as designated by the national AI surveillance plan. The statewide 2008 Montana Avian Influenza Surveillance Project report was prepared by WS and may be found at USDA-APHIS-Wildlife Services, U.S. States and Tribes 2009.

Sample Design

The Montana AI surveillance sampling strategy was a step-down approach from the U.S. Interagency Strategic Plan (Interagency Asian 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 $\leq 1.5\%$. FWP surveillance methods included the investigation of avian morbidity and mortality, conducting mortality transects, and sampling wild live and hunter-harvested waterfowl throughout fall migration. Surveillance efforts were accomplished through the extensive cooperation of FWP, WS, USFWS, and city and county managers where urban trapping was conducted.

2006 FWP Surveillance Effort

The criteria outlined in the 2006 Montana Sampling Plan (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006) stated that FWP should collect 1000 of the

total 2000 statewide cloacal samples from birds identified as species of concern. Cloacal swab samples were collected from live wild waterfowl during banding operations at the Benton Lake, Bison Range/Ninepipes, and Medicine Lake National Wildlife Refuges and wild and semi-domestic waterfowl at six urban ponds across the state. Cloacal swab samples were also collected from hunter-harvested waterfowl at Benton Lake, Lee Metcalf, and Red Rocks Lakes National Wildlife Refuges, Canyon Ferry, Headwaters State Park, Freezeout Lake, and Lake Helena. Testing protocol included combining up to five individual cloacal samples in a sample pool that was screened to detect all influenza A viruses. Mortality/morbidity sampling was performed by collecting opportunistic samples statewide throughout the year. For the complete 2006 Montana AI surveillance annual report, see Jaffe *et al.* 2007.

2007 FWP Surveillance Effort

Changes from 2006 AI surveillance protocols included initially screening all swab samples individually rather than pooling samples, and the addition of an oropharyngeal swab placed in the same vial with a cloacal swab to amplify the sample (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). To adjust for the increased cost of the initial screening, total swab sampling criteria was reduced from 2000 to 1500 samples statewide, thereby reducing FWP's sampling goal to 750 samples. Cloacal and oropharyngeal sampling on live birds was performed in conjunction with USFWS National Wildlife Refuge waterfowl banding operations at Benton Lake and by trapping wild and semi-domestic waterfowl on seven urban ponds across the state. Hunter-harvested waterfowl was sampled at Benton Lake and Red Rocks Lakes National Wildlife Refuges and at Freezeout Lake.

In 2007, FWP added weekly prospective mortality/morbidity surveillance as an AI detection method at six sites to systematically survey species of concern throughout the state of Montana (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). Surveillance was conducted from summer through freeze-up on bodies of water supporting species capable of demonstrating clinical symptoms and/or dying due to HPAI infection (U.S. Department of the Interior Fish and Wildlife Service 2008). A total of 10 sites were used as weekly transects and 18 additional sites were explored for possible future surveillance. Opportunistic mortality/morbidity samples were collected statewide throughout the year. For the complete 2007 Montana AI surveillance annual report, see Jaffe *et al.* 2008.

2008 FWP Surveillance Effort

The Montana Sampling Plan Supplement for 2008 followed modifications made in 2007 (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). The 2008 sampling criteria included a total of 1600 cloacal-oropharyngeal statewide samples, 800 of which were to be collected by FWP. The three strategies FWP employed for cloacal and oropharyngeal sampling were coordinating with USFWS National Wildlife Refuge waterfowl 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. FWP also continued mortality/morbidity transects and the collection of opportunistic mortality/morbidity samples throughout the state (Figure 1).

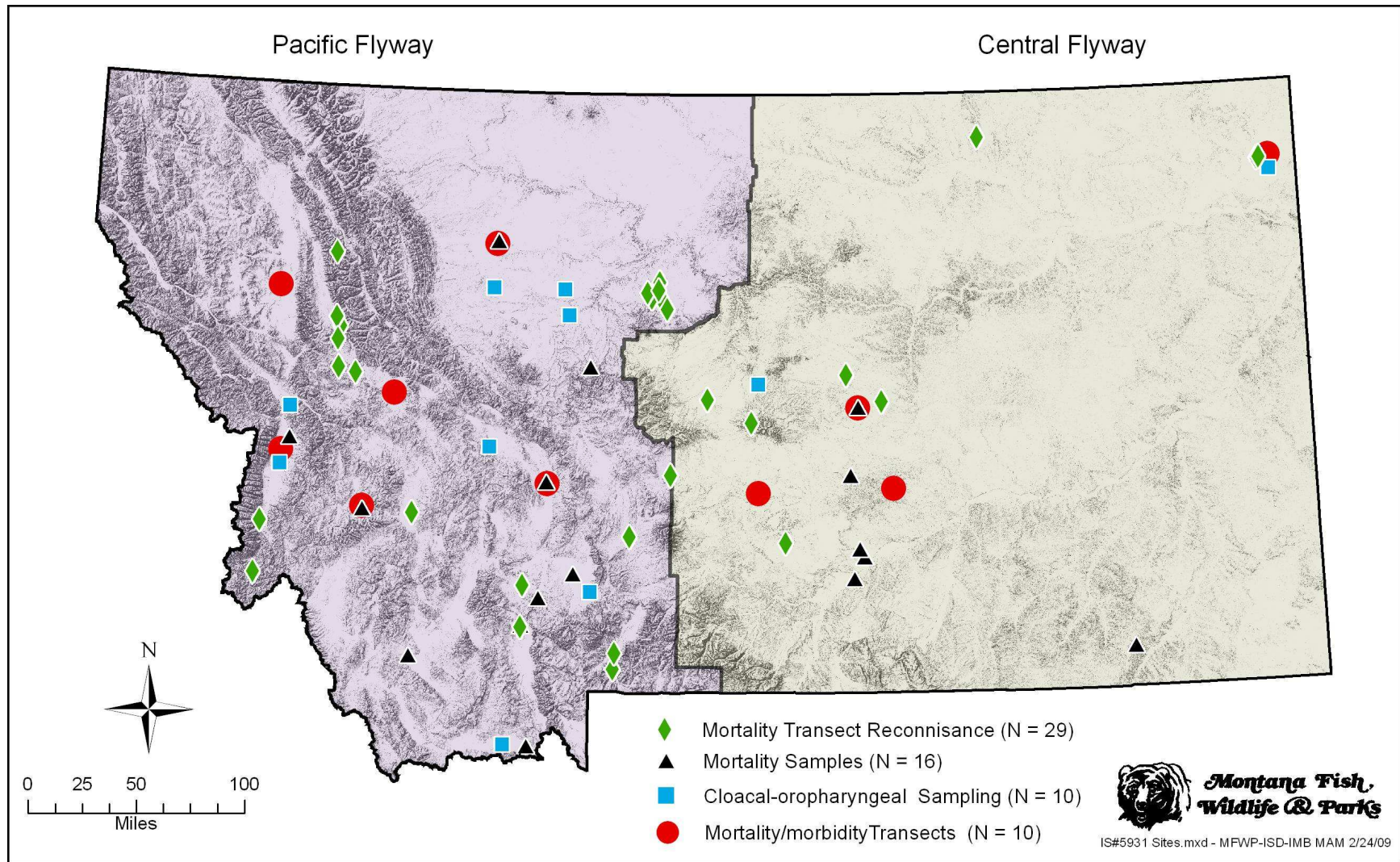


Figure 1. The Pacific and Central Flyways in Montana, and sampling sites for the 2008 FWP Montana AI surveillance.

METHODS

Cloacal and Oropharyngeal Sampling

Cloacal and oropharyngeal 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, sample sizes were increased as well as extrapolated across large landscapes for multi-state and flyway sampling efforts in an attempt to account for biases (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006). Cloacal and oropharyngeal sampling was spatially distributed across Montana and temporally distributed from August through December. According to USDA-APHIS-Wildlife Services, U.S. States and Tribes (2008), approximately 30% of swab samples should be collected from resident or non-migrating waterfowl and the remaining 70% should be collected from migratory species upon arrival in fall through freeze-up. Specific species identified as potential carriers of HPAI but not exhibiting clinical disease were targeted for surveillance. Species of primary concern for the 2008 live and hunter-harvested bird surveillance in Montana included tundra swan (TUSW), lesser snow goose (LSGO), northern pintail (NOPI), and Ross's goose (ROGO). These species move between Asia and North America and could contact Asian HP-H5N1 directly (Alaska Interagency HPAI Bird Surveillance Working Group 2006). Secondary and wild sentinel species included mallard (MALL), American wigeon (AMWI), gadwall (GADW), and northern shoveler (NSHO). Additional secondary species considered a priority were blue-winged teal (BWTE), common goldeneye (COGO), canvasback (CANV), green-winged teal (AGWT), redhead (REDH), and wood duck (WODU). High numbers of most 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). Hybrid semi-domestic geese and ducks served as sentinel species and were sampled at urban ponds.

Field Effort

Live bird AI sampling was conducted in conjunction with National Wildlife Refuge waterfowl banding at Benton Lake and Medicine Lake during September using methods approved by the U.S. Fish and Wildlife Service and Canadian Wildlife Service (1977). Swim-in traps were employed at four locations at Medicine Lake while net-launchers were used at three sites at Benton Lake. Trapping efforts were rotated between sites at both refuges. Waterfowl were banded by USFWS biologists and cloacal and oropharyngeal samples were taken by FWP AI personnel. Sampled birds were then released.

Urban wild and semi-domestic bird sampling began in mid-September and ran throughout the sampling period. AI personnel used swim-in traps at four urban ponds across the state to collect cloacal and oropharyngeal samples. Because swim-in traps required a flat surface covered by ≤ 1.5 feet of water, traps were set in water only at Bancroft Pond in Missoula and Gibson Pond in Great Falls. Swim-in traps modified for use on land were utilized at the Lewis and Clark Fairgrounds Pond in Helena and the MSU Pond in Bozeman. Permission to trap was granted by city and/or county managers, while FWP Information and Education personnel and city managers worked together to notify the public of the trapping activities.

Hunter-harvested waterfowl sampling began in the beginning of October and ran concurrently with urban trapping through early December. Hunter-harvested waterfowl were sampled at Benton Lake, Lee Metcalf, and Red Rocks Lakes National Wildlife Refuges, Freezeout Lake, and a site on Spring Creek near Lewistown. Hunter participation was voluntary and information about AI and the surveillance was distributed to hunters onsite and at FWP offices. Sampling concluded when hunting diminished and as lakes froze.

Lab Testing

Cloacal-orpharyngeal samples were submitted to the MDoL and were tested using real-time reverse transcription-polymerase chain reaction (rRT-PCR). All samples were screened individually 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 (Spackman *et al.* 2002, Munster *et al.* 2009). 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 a standard rRT-PCR for N1. Virus isolation (VI) was also performed by NVSL on all samples to confirm AI virus isolates 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 Fish and Wildlife Service 2006).

Sampling Effort

FWP AI personnel collected 860 cloacal-orpharyngeal samples toward the total 1600 sampling objective for Montana during 2008. Banding operations yielded 216 samples (25%) and urban trapping efforts produced 126 samples (15%) for a total of 342 live bird samples (40%). Hunter-harvested samples totaled 518 (60%). Sampling effort consisted of 47 total sampling days; refuge banding produced 6 sample days, urban trapping yielded 10, while hunter-harvest produced 31. Sampling effort across all swab sampling methods resulted in overall means of 4.3 days/site and 18.3 samples/sample day at 11 sites. Banding operations produced the highest mean number of samples/sampling day (36.0) while urban trapping and hunter-harvest sampling yielded close to the same mean number of samples/sampling day (12.6 and 16.7, respectively; Table 1). Though Benton Lake banding operations produced the highest mean of samples/sampling day (33.0), the most productive site was Freezeout Lake, which yielded more than one-third of the total swab samples collected (41.2%; Table 2).

Table 1. 2008 FWP Montana AI surveillance swab sampling effort according to method.

	Sampling Method			
	Banding	Urban	Hunter-harvest	Total
Number of sites	2	4	5	11
Total samples	216	126	518	860
Percentage of total samples	25	15	60	100
Total sample days	6	10	31	47
Mean sample days/number of sites	3.0	2.5	6.2	4.3
Mean samples/sample day	36.0	12.6	16.7	18.3

Table 2. Number of sample days, and number and percentage of samples per site across cloacal and oropharyngeal sampling methods during the 2008 FWP Montana AI surveillance.

Method	Site	Sample days	Total number of samples	Percentage samples per method
Banding (live bird)	Benton Lake	5	165	76.4
	Medicine Lake	1	51	23.6
Total		6	216	100.0
Urban (live bird)	MSU Pond	6	65	51.6
	Lewis & Clark Pond	2	23	18.2
	Bancroft Pond	1	19	15.1
	Gibson Pond	2	19	15.1
Total		10	126	100.0
Total live bird		16	342	100.0
Hunter-harvest (dead bird)	Freezeout Lake	21	354	68.3
	Red Rocks Lakes	2	63	12.2
	Benton Lake	2	45	8.7
	Lee Metcalf	4	39	7.5
	Spring Creek	2	17	3.3
Total		31	518	100.0
Sampling Total		47	860	100.0

The highest proportion of cloacal-oropharyngeal samples was collected in the northeastern section of the Montana Pacific Flyway at Freezeout Lake and Benton Lake. Sampling was distributed quite evenly across the rest of the Pacific Flyway both spatially and temporally. Cloacal and oropharyngeal sampling occurred at two sites in the Central Flyway, mostly at Medicine Lake. While sampling peaked statewide during the opening weekend of waterfowl hunting (10/2-10/3), the peak for a single site occurred at Freezeout Lake in the end of October and beginning of November with lesser snow goose sampling (Figure 2).

The 2008 Montana Sampling Plan called for cloacal-oropharyngeal samples from 100 tundra swans, 150 lesser snow geese, and 120 northern pintails (50 from banding operations and 70 from hunter-harvest sampling) as primary species of concern, whereas the majority of secondary species samples were to come from mallards (n=510). The FWP AI team collected 62 tundra swan, 119 lesser snow goose, 147 northern pintail, and 19 Ross's goose samples from available birds. Primary species comprised 40.3% of the total samples collected. The 210 mallard samples collected were approximately one quarter of all FWP cloacal-oropharyngeal samples collected. Additional secondary species of concern, gadwall (n=69), green-winged teal (n=63), and northern shoveler (n=43), comprised 20.3% of the total cloacal-oropharyngeal samples, while the rest of the species sampled combined yielded 14.9% of the total samples (Table 3).

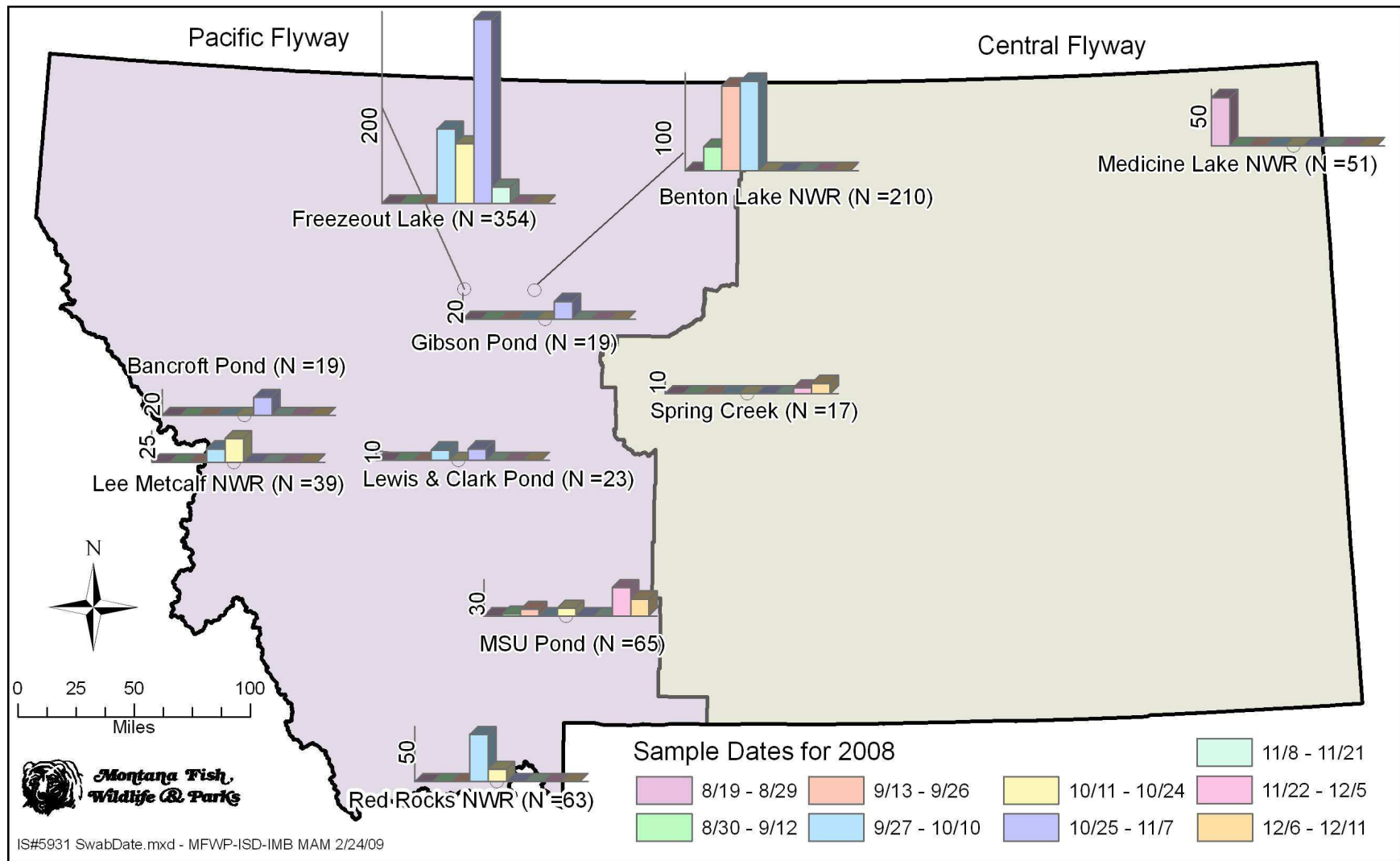


Figure 2. Temporal distribution of the 2008 FWP Montana AI cloacal and oropharyngeal sampling; sites with <25 total samples were excluded. Scale bar numbers are the maximum number of samples collected during a two-week sample period. National Wildlife Refuge is referred to as “NWR”.

Table 3. Number of 2008 FWP Montana AI cloacal-oropharyngeal samples according to species and method, and percentage of total samples according to species.

Species	Banding	Urban	Hunter-harvest	Total	Percentage of total samples
Mallard	66	101	43	210	24.4
Northern Pintail	123	0	24	147	17.1
Lesser Snow Goose	0	0	119	119	13.8
Gadwall	1	0	68	69	8.0
Green-winged Teal	14	0	49	63	7.3
Tundra Swan	0	0	62	62	7.2
Northern Shoveler	0	0	43	43	5.0
Blue-winged Teal	5	0	29	34	4.0
American Wigeon	6	0	26	32	3.7
Ross's Goose	0	0	19	19	2.2
Redhead	0	0	14	14	1.6
Hybrid Goose	0	14	0	14	1.6
Hybrid Duck	0	10	0	10	1.2
Ring-necked Duck	0	0	7	7	0.8
Lesser Scaup	0	0	6	6	0.7
Ruddy Duck	0	0	5	5	0.6
Canvasback	1	0	2	3	0.3
Wood Duck	0	1	1	2	0.2
Common Goldeneye	0	0	1	1	0.1
Total	216	126	518	860	100.0

Age and sex classes were divided into hatch-year, after-hatch-year, female, male, and undetermined. Nearly half of all birds sampled were classified as hatch-year (n=422, 49.1%) while the other half were classified after-hatch-year birds (n=418, 48.6%). Sex and age were not determined for 18 birds sampled and only age was not determined for two additional birds (2.3%). Within species sampled in numbers greater than 30, gadwall, northern shoveler, American wigeon, and blue-winged teal hatch-year birds were sampled in highest numbers (>65%) while northern pintail, lesser snow goose, and green-winged teal age classes were sampled quite evenly. Tundra swan and mallard after-hatch-year birds were sampled in higher numbers (>65%) than hatch-year birds (Table 4).

Table 4. Number of the 2008 FWP Montana AI cloacal-oropharyngeal samples according to species, age, and sex classes; “Unkn” denotes undetermined sex. Data from undetermined aged birds (2 male hybrid ducks) and undetermined sex and aged birds (14 hybrid geese, 3 hybrid ducks, 1 tundra swan) were excluded.

Species	Number of hatch-year				Number of after-hatch-year			
	Male	Female	Unkn	Total	Male	Female	Unkn	Total
Mallard	27	39	0	66	99	45	0	144
Northern Pintail	39	49	0	88	13	46	0	59
Lesser Snow Goose	0	0	58	58	3	1	57	61
Gadwall	16	37	0	53	8	8	0	16
Green-winged Teal	9	26	0	35	11	17	0	28
Tundra Swan	0	0	5	5	5	14	37	56
Northern Shoveler	13	18	0	31	4	8	0	12
Blue-winged Teal	9	16	0	25	5	3	1	9
American Wigeon	9	12	0	21	7	4	0	11
Ross’s Goose	0	0	11	11	0	1	7	8
Redhead	2	4	0	6	8	0	0	8
Ring-necked Duck	2	4	0	6	0	1	0	1
Lesser Scaup	0	4	1	5	1	0	0	1
Hybrid Duck	2	0	0	2	2	1	0	3
Ruddy Duck	1	4	0	5	0	0	0	0
Canvasback	0	2	0	2	1	0	0	1
Wood Duck	1	1	0	2	0	0	0	0
Common Goldeneye	0	1	0	1	0	0	0	0
Total	130	217	75	422	167	149	102	418

Most primary species of concern, tundra swan, lesser snow goose, Ross’s goose, and northern pintail samples were collected in northwestern Montana at Freezeout Lake and Benton Lake (Figure 3) while secondary species sampling was distributed throughout western and central Montana. Hunter-harvested birds provided the greatest species diversity for sampling, whereas urban trapping allowed for little diversity given nearly all birds available for trapping at ponds were mallards and hybrid geese and ducks.

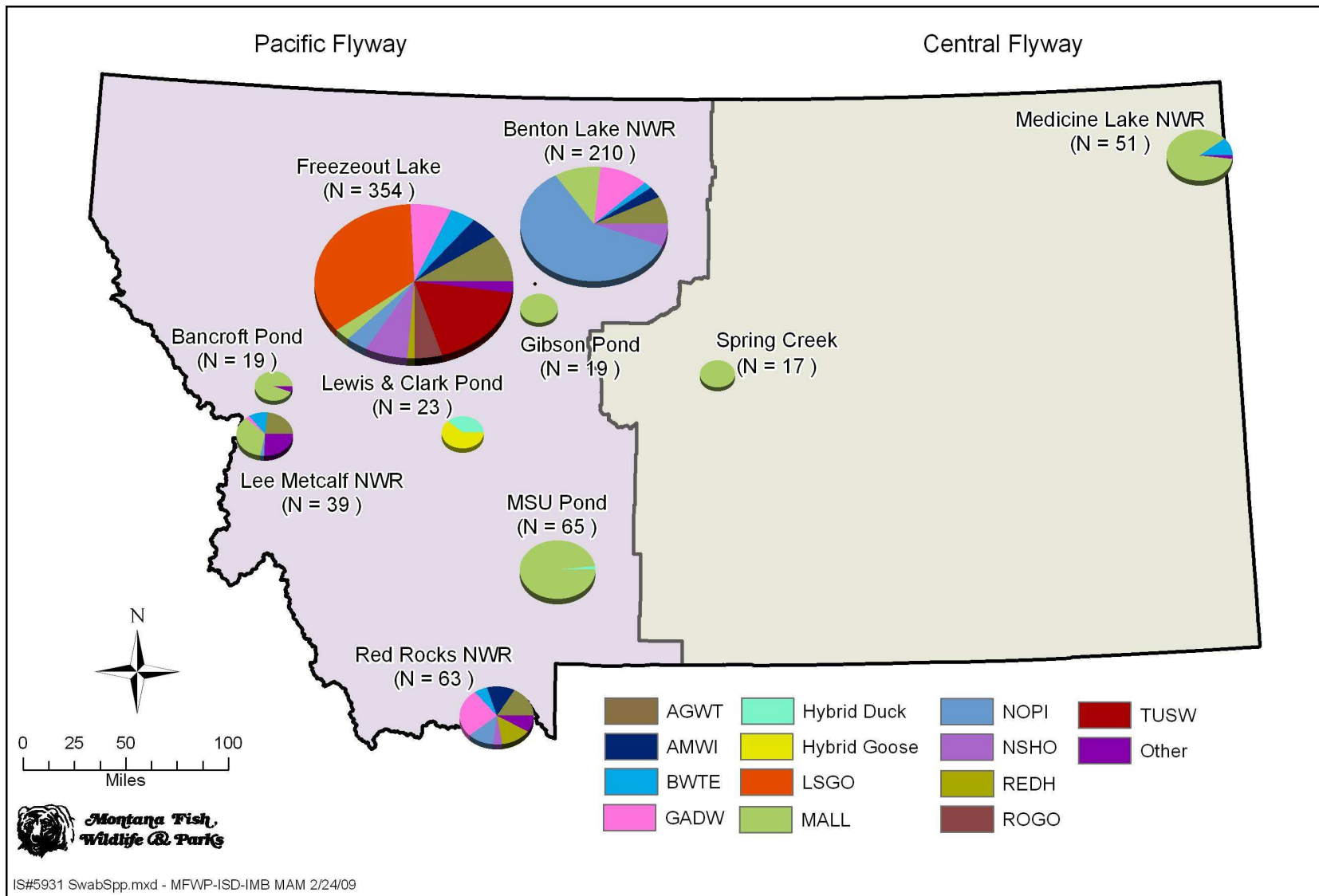


Figure 3. Spatial distribution of the 2008 FWP Montana AI cloacal and oropharyngeal sampling according to species. The “Other” category combines all species from which ≤ 11 samples were collected (n= 67, Table 3). National Wildlife Refuge is referred to as “NWR”.

FWP cloacal and oropharyngeal sampling effort was spread temporally throughout fall in conjunction with refuge banding operations 8/19 – 10/1, and during the harvest of waterfowl 9/27 – 12/8 and urban wild bird sampling 9/12 – 12/11. Sampling peaked on 10/4, the opening day of waterfowl hunting in Montana, and ended in early December as fall migration subsided (Figure 4). The collection of samples from primary species began with northern pintails conducted during refuge banding and peaked 11/1. Ross's goose, lesser snow goose, and tundra swan sampling was conducted during the waterfowl hunting season and peaked 11/3, 11/8, and 11/17, respectively (Figure 5). The sampling of secondary species began at urban ponds in August and peaked on 10/4 during the opening day of waterfowl hunting. Mallard sampling was quite consistent throughout the sampling season and across methods, while samples from other secondary species, gadwall, American wigeon, and northern shoveler, were mostly collected from hunter-harvested birds later in the season (Figure 6). Additionally, blue-winged teal and green-winged teal were sampled in numbers >30; sampling began during refuge banding and peaked on 10/4. Sentinel species (hybrid geese and ducks) were sampled at urban ponds consistently throughout October and November.

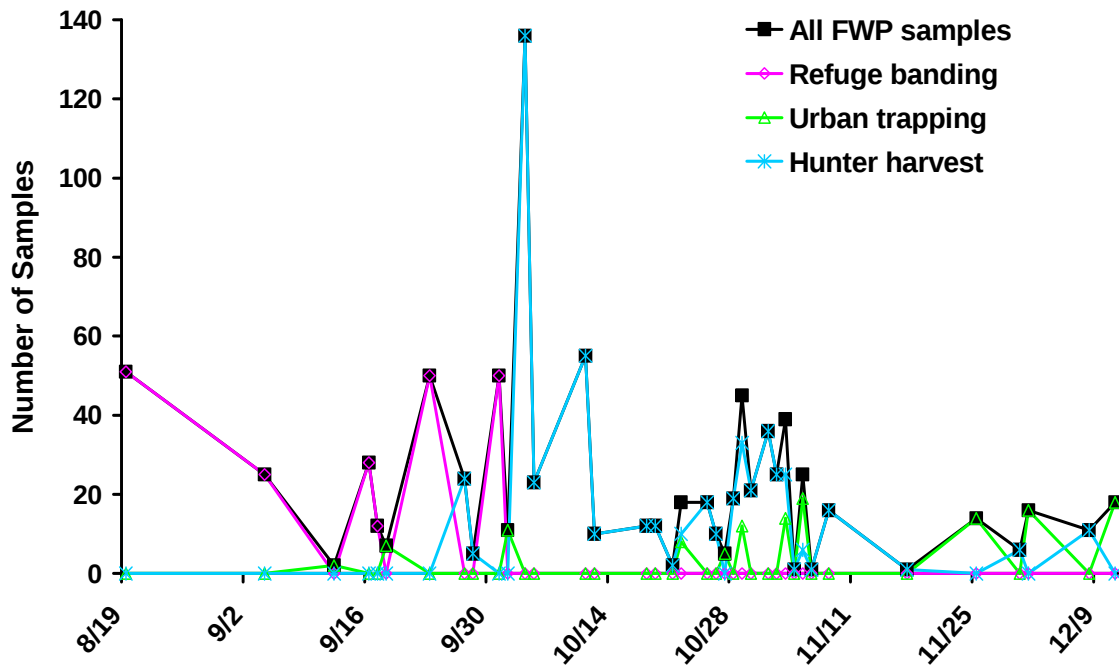


Figure 4. Temporal distribution of 2008 FWP Montana AI cloacal and oropharyngeal sampling.

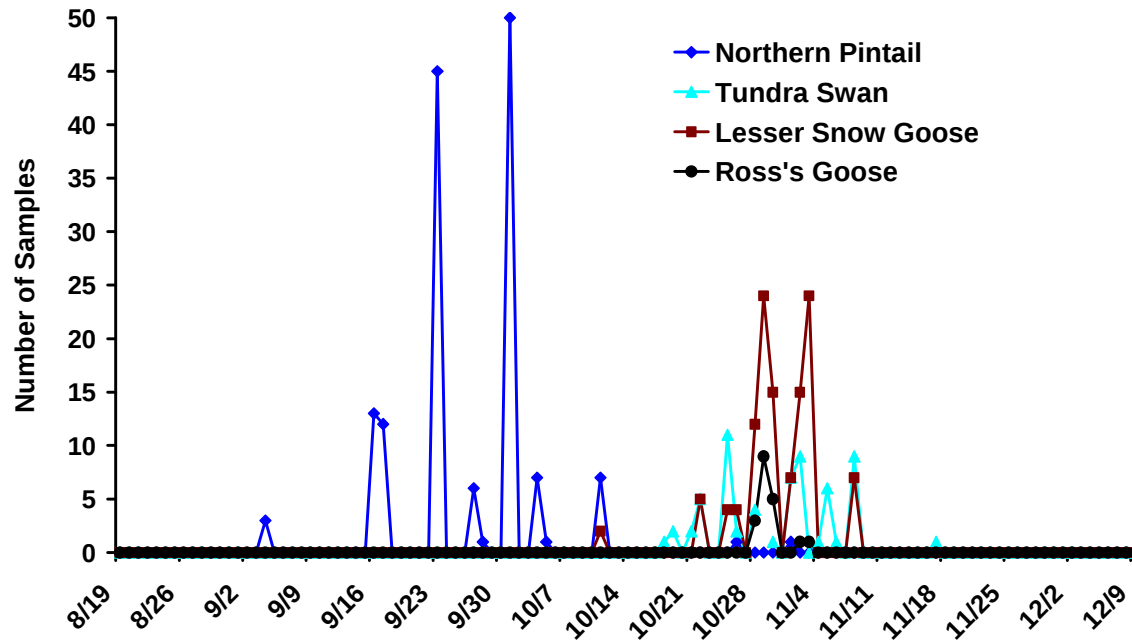


Figure 5. Temporal sampling distribution of primary species for the 2008 FWP Montana AI surveillance.

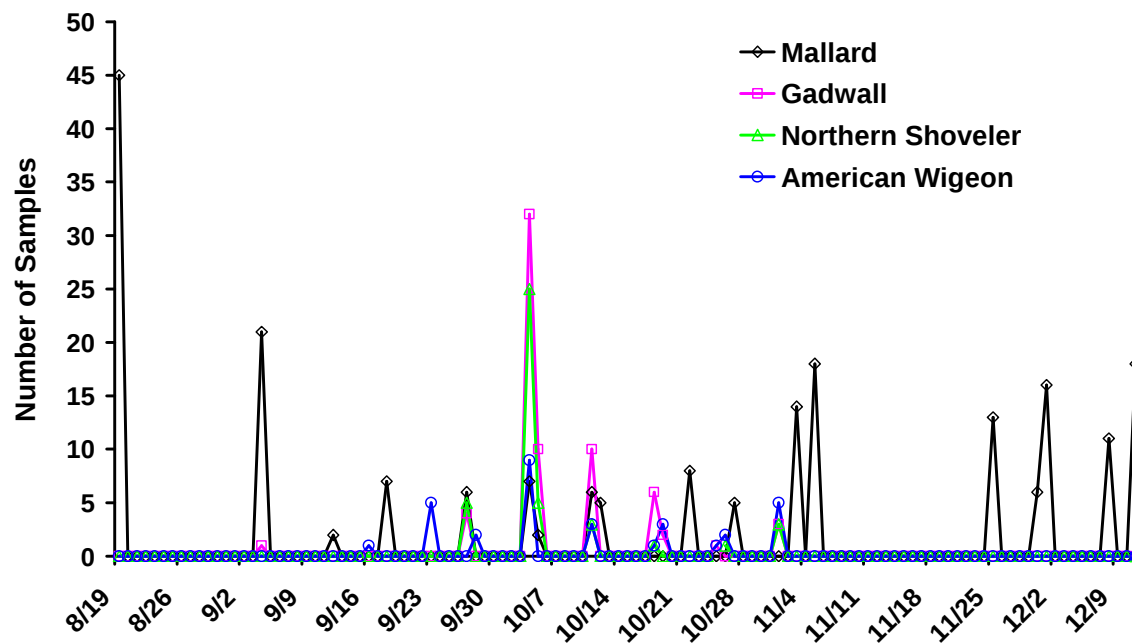


Figure 6. Temporal sampling distribution of secondary species for the 2008 FWP Montana AI surveillance.

Mortality/Morbidity Sampling

The 2008 Montana Sampling Plan Supplement specified the collection of ≤ 200 opportunistic mortality/morbidity samples during the 2008 sampling period. Reports made by the public were investigated according to the AI sampling criteria, which 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). Bird carcasses suitable for disease testing found within 24 hours of death and euthanized birds were shipped for necropsy and disease testing at NWHC in Madison, WI.

Lab Testing

NWHC tested tracheal and cloacal swab samples and tissues by direct extraction. Testing procedures followed those described for cloacal-oro-pharyngeal sample testing and samples that tested positive for either H5 or H7 were sent to NVSL for confirmation (Spackman 2002, Munster et al. 2009).

Sampling Effort

A total of 47 mortality/morbidity samples were collected by FWP and USFWS from 28 species that included birds from 32 mortality events reported statewide (Table 6). The 40 calls received on the FWP toll-free reporting system and three website reports of dead and dying birds yielded five mortality/morbidity sampling events. Multiple-bird mortality events at Belgrade, Benton Lake, Billings, Bowdoin, Canyon Ferry Lake, Choteau, Dillon, Ennis Lake, north of Norris, and Shepherd, as well as single-bird mortalities across the state were investigated. All carcasses were submitted to NWHC for AI testing. Of the 29 birds categorized by age and sex, 17 were classified as hatch-year birds (5 females, 11 males, 1 undetermined), 10 were classified as after-hatch-year birds (3 females, 7 males), and two were classified as undetermined age (1 female, 1 male).

Mortality/Morbidity Transects

FWP AI personnel conducted six weekly prospective mortality transects 2-10 kilometers long to systematically survey species of concern throughout the state of Montana for morbidity and mortality (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). Species identified as sensitive to HPAI infection that resulted in clinical disease and death were targeted for surveillance from arrival during fall migration until freeze-up (U.S. Department of the Interior Fish and Wildlife Service 2008). Priority species included tundra and trumpeter swans, American wigeon, canvasback, lesser scaup, northern shoveler, redhead, ring-necked duck, and wood duck, as well as shorebirds, grebes, terns and gulls (Becker 1966, Brown *et al.* 2006, Brown *et al.* 2008). Reconnaissance was conducted throughout the Pacific and Central Flyways on lakes and wetlands in early and late fall to find sites for surveillance based on location, water conditions, access, and target species abundance. Once surveillance sites were established, transects were conducted every 5-9 days and continually evaluated based on the presence of priority species. Transects were performed consistently at six sites across the state and alternate locations were substituted when target species abundance declined in October and November due to migration. Surveillance was terminated at a site when total target species numbered ≤ 200 , a site was inaccessible due to winter conditions, or the lake or wetland froze over.

Mortality/morbidity transects contoured within ten feet of the shoreline to detect morbidity and mortality events either by canoeing or walking. To record target species presence and an index of abundance, censuses were conducted with spotting scopes and high-powered binoculars from a single point on each transect that allowed maximum visibility to the observer. To avoid double counting during the performance of individual transects, only numbers of each species counted upon initial sighting were recorded to yield a minimum number, and only counts of additional target species not seen during the initial census were added during the transect. Because it is likely bird populations were resampled across consecutive surveys, census data were reported as “bird observations”. All symptomatic or dead birds of suitable quality were collected and tested for AI by submission of intact carcasses to NWHC following the protocols described above.

Table 6. 2008 Montana AI mortality/morbidity samples submitted to NWHC according to species.

Species	Number of samples
Trumpeter Swan	6
Lesser Snow Goose	5
Eared Grebe	4
Mallard	4
California Gull	2
Gadwall	2
Mourning Dove	2
Red-winged Black Bird	2
American Coot	1
American Robin	1
American White Pelican	1
Bald Eagle	1
Black-capped Chickadee	1
Brown-headed Cowbird	1
Brewer’s Blackbird	1
Cassin’s Finch	1
Common Nighthawk	1
Gray Partridge	1
House Finch	1
Northern Flicker	1
Northern Shoveler	1
Ring-billed Gull	1
Sharp-shinned Hawk	1
Tundra Swan	1
Unknown Duckling	1
Western Bluebird	1
Yellow-headed Blackbird	1
Yellow-rumped Warbler	1
Total	47

Sampling Effort

Reconnaissance began 6/26 on mortality/morbidity transects used during the 2007 surveillance and an additional 33 lakes and wetlands across the state (Figure 1). Eleven sites were chosen for the 2008 mortality/morbidity transects based on the presence of target species, four of which differed from the previous year. A total of 128 weekly transects were conducted between 7/8 and 12/5 throughout the state of Montana. Transect routes ranged from 2 to 10 km in length for a total of 55 km and averaged 5 (± 2.61) km. Completed surveys ranged from 30 to 270 minutes and averaged 137 (± 46.65) minutes for a total of 302 hours (Table 7). A total of 28,837 bird observations were recorded upon initial sighting of target species during the transects, over half of which were ducks, geese, and swans. One quarter of the birds observed were gulls and terns, and the remaining fifth were comprised of grebes, shorebirds, and cranes (Table 8). Dead and sick birds found on transects totaled 33 and 1, respectively. The 15 identifiable carcasses were comprised of one ring-billed gull, two American coots, three Canada geese, and nine American white pelicans, eight of which were found at Eyraud Lakes. Seven of the carcasses collected on transects were sent to NWHC to test for AI and determine cause of death.

Table 7. 2008 Montana AI mortality/morbidity transect start and end dates, length and average survey times for complete surveys.

Transect	Date		Transect length (km)	Average survey time (min)	Number of surveys
	start	end			
Brown's Lake	7/9	11/3	9	200	18
Canyon Ferry, Pond 2	7/8	12/5	6	195	22
Deadmans Basin Reservoir	11/18	12/4	3	55	2
Eyraud Lakes	7/16	11/1	5	115	17
Georgetown Lake	7/10	10/30	4	150	17
Lake Mason	11/10	12/2	4	100	4
Lee Metcalf	11/19	12/3	2	140	3
Medicine Lake, Sayer Bay	7/16	11/12	4	105	18
Pablo Reservoir	11/20	12/04	10	175	3
Warm Springs Ponds	10/29	11/25	6	175	2
Yellow Water Reservoir	7/9	12/1	2	95	22
Total	7/8	12/5	55	130	128
Transect reconnaissance	6/26	12/3	---	70	33

Table 8. Montana 2008 mortality/morbidity transect bird observations according to family.

Family	Number counted (%)	
Anatidae (ducks, geese, swans)	15,901	(55)
Laridae (gulls, terns)	7,022	(24)
Podicipedidae (grebes)	2,500	(9)
Scolopacidae (sandpipers, phalaropes)*	2,021	(7)
Charadriidae (plovers, killdeer)	760	(3)
Recurvirostridae (avocets, stilts)	538	(2)
Gruidae (cranes)	95	(<1)
Total	28,837	(100)

*Includes curlews, dowitchers, godwits, sanderlings, willets, yellowlegs.

Data Management, Reporting of Results, Statistics

FWP AI personnel entered cloacal and oropharyngeal sampling data into a USDA national web-based database system. USDA reported cloacal-oropharyngeal sample results through the USDA web-based database, which included H5, H7, and N1 screening results, as well as LPAI subtype and pathogenicity. All 2008 cloacal and oropharyngeal data and results were then uploaded to FWP's existing AI database. NWHC reported mortality/morbidity results directly to FWP, which contained the outcome of AI and additional disease testing, and cause of death when possible. AI mortality/morbidity transect and carcass data and results were entered into FWP databases. Confidence intervals were calculated for the proportion of matrix positive cloacal-oropharyngeal swab samples according to species (R Core Development Team, 2006). Using the Agresti-Coull interval, the assumptions were 1) sampling was random or at least representative of the entire population, 2) LPAI rates were the same temporally, spatially and across trapping methods, and 3) there was no measurement error. Confidence intervals for matrix positive cloacal-oropharyngeal swab samples by sex and age classes for individual species were not calculated due to the large differences in the proportion of matrix positives within each sex and age class.

RESULTS

While AI virus was found in samples, HP-H5N1 was not detected in Montana during the 2008 surveillance. Because the AI surveillance did not focus on the detection of LPAI, samples that tested matrix positive but H5 and H7 negative were not tested with VI to determine AI subtype.

Cloacal-oropharyngeal Samples

Matrix Results

Of the 860 FWP cloacal-oropharyngeal samples submitted for AI testing, 134 (16%) samples tested positive on the AI matrix. While hunter-harvest yielded the highest percentage of samples for testing (60%) and matrix positive samples (51%), refuge banding produced the highest percentage of matrix positive samples by collection method (26%: Table 9).

Table 9. 2008 FWP Montana matrix positive cloacal-oropharyngeal sample numbers and percentage according to method of sample collection.

Method	Number of samples	Number of matrix positives	Percentage matrix positives of sample total
Hunter-harvest	518	68	13%
Refuge banding	216	57	26%
Urban trapping	126	7	7%
Total	860	134	16%

Due to sample size limitations, samples were pooled for temporal analysis by sex and age class in the following time increments, August - September, October, November – December. The proportion of hatch-year females and males that tested matrix positive during August - September was highest among all sex and age classes, and then decreased throughout the season. The proportion of matrix positive after-hatch-year females and males increased from September to October and then decreased again in November (Figure 7). Known sex and age classes across all sampled species and methods were also pooled for species-specific analysis. The highest proportion of matrix positive samples within the primary species of concern was northern pintail (0.44, n=147). The proportion of matrix positive samples varied among the other primary species; Ross's goose proportion of matrix positive samples (0.21) was above the 0.16 average for all species, while lesser snow goose and tundra swan were below the average for all species (0.10 and 0.03, respectively, Table 10).

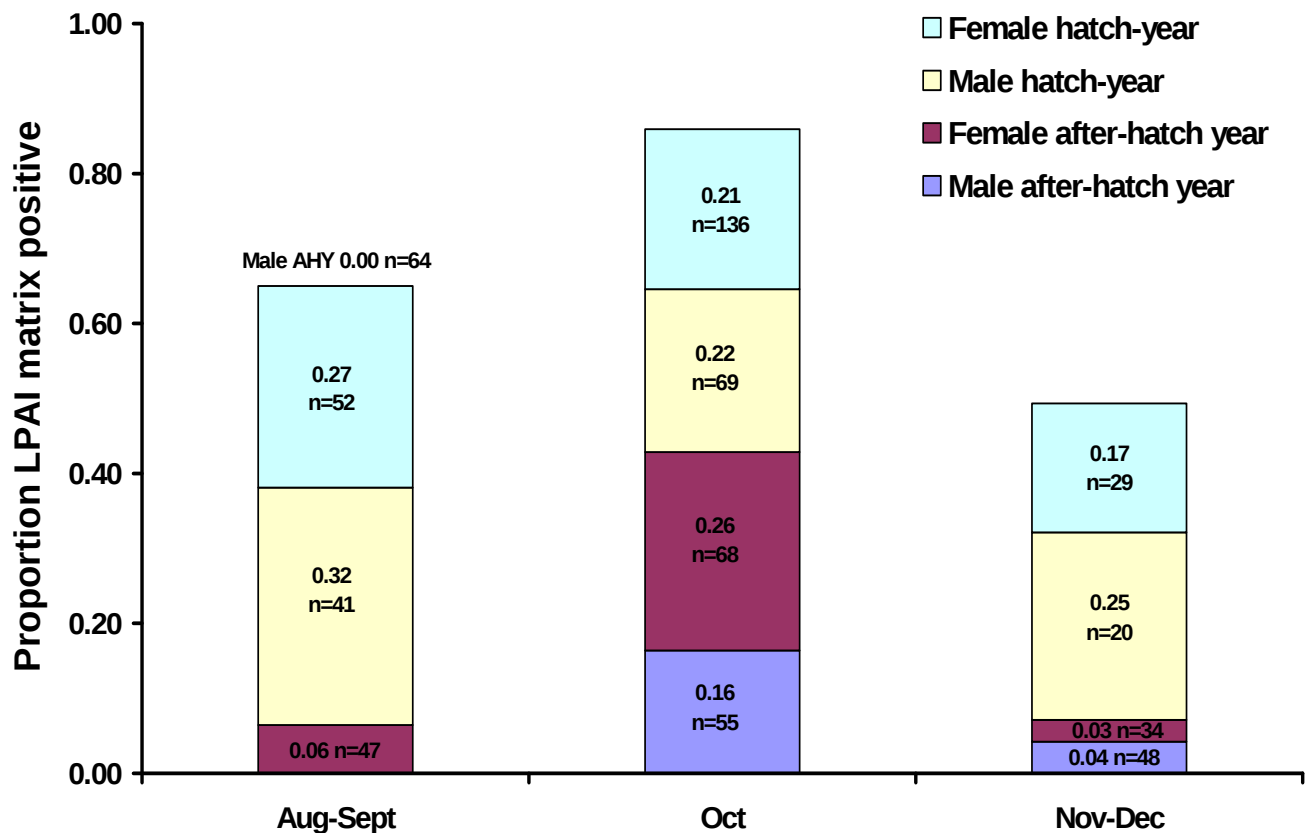


Figure 7. Proportion of 2008 FWP Montana cloacal-oro-pharyngeal swab matrix positives according to known sex and age classes.

Table 10. Proportion of 2008 FWP Montana cloacal-orpharyngeal swab matrix positive samples according to species using the Agresti-Coull interval. X= number of matrix positive samples within species, N= number of birds within species sampled, Mean= proportion of matrix positive samples within species, Lower CI= lower Confidence Interval, Upper CI= upper Confidence Interval.

Species (n=19)	X	N	Mean	Lower CI	Upper CI
Northern Pintail	65	147	0.44	0.36	0.52
Ross's Goose	4	19	0.21	0.08	0.44
Northern Shoveler	9	43	0.21	0.11	0.35
Ruddy Duck	1	5	0.20	0.02	0.64
Lesser Scaup	1	6	0.17	0.01	0.58
Redhead	2	14	0.14	0.03	0.41
Green-winged Teal	7	63	0.11	0.05	0.21
Mallard	22	210	0.10	0.07	0.15
Lesser Snow Goose	12	119	0.10	0.06	0.17
Blue-winged Teal	3	34	0.09	0.02	0.24
Hybrid Goose	1	14	0.07	0.00	0.34
Gadwall	4	69	0.06	0.02	0.14
Tundra Swan	2	62	0.03	0.00	0.12
American Wigeon	1	32	0.03	0.00	0.17
Canvasback	0	3	0.00	0.00	0.62
Common Goldeneye	0	1	0.00	0.00	0.83
Hybrid Duck	0	10	0.00	0.00	0.32
Ring-necked Duck	0	7	0.00	0.00	0.40
Wood Duck	0	2	0.00	0.00	0.71
Total	134	860	0.16	-----	-----

H5 and N1 Results

Nine of the total 860 cloacal-orpharyngeal samples tested positive for H5 during 2008, while all samples tested negative for N1. H7 was not detected in the 2008 FWP samples.

Mortality/Morbidity Samples

All 47 mortality/morbidity carcasses submitted for examination to NWHC were tested for AI virus via rRT-PCR and produced no presumptive matrix positives. None of the carcasses submitted during 2008 were tested using virus isolation due to changes in protocols; only presumptive matrix positive samples were submitted for virus isolation testing. Cause of death for mortality events were reported to individual submitters by FWP and not included in this report.

DISCUSSION

AI virus in low pathogenic form was detected in Montana samples as expected, while HP-H5N1 was not found during the 2008 surveillance in Montana or elsewhere in North America. Nine

birds sampled with cloacal-orpharyngeal swabs tested H5 positive and N1 negative, so low pathogenic H5N1 was also not detected in any FWP samples during 2008.

Within sampling methods, hunter-harvest swab sampling produced the most samples (60%) while refuge banding yielded the highest percentage of matrix positive samples (26%). Hatch-year birds produced higher numbers of matrix positives than adults in early fall, which declined in October. Timing of refuge banding versus hunter-harvest and urban trapping sampling may partially explain this difference. Several studies have shown that AI is more prevalent in early fall and decreases as fall migration proceeds (Stallknecht 2003, Gilbert *et al.* 2006). Changes in LPAI concentration may be due to a combination of premigration density of waterfowl with the high recruitment rate of immunologically naïve juveniles in early fall, while subsequent declines in LPAI may be a result of increased flock immunity and progressive dispersal of bird populations (Stallknecht 2003, Gilbert *et al.* 2006). The use of different trapping methods may also contribute to the differing low pathogenic AI results.

Within species, northern pintails tested in Montana for AI during the 2008 surveillance produced the highest prevalence of matrix positives (44%). Recent studies have shown that northern pintails carry numerous strains of LPAI at some of the highest prevalences among water bird species (Hinshaw *et al.* 1980, Runstadler *et al.* 2007, Ip *et al.* 2008, Parmley *et al.* 2008). In Montana, northern pintail males and females both had prevalences of 44%, while age classes differed. Hatch-year northern pintails produced higher prevalences than adults (49% and 37%, respectively); the highest prevalence was found in hatch-year females (n=49, 51%). While hatch-year northern pintails tested in Alaska produced higher prevalences than the adults, hatch-year males and females differed little (Ip *et al.* 2008).

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. Of the primary target species for cloacal and oropharyngeal sampling, lower numbers of northern pintails were sampled during 2007 than in 2006 and 2008 primarily due to differences in numbers available for banding at Benton Lake. Lesser snow goose sampling decreased consistently after 2006 while tundra swan sampling increased from 2006 to 2007 and decreased again in 2008. The timing of migration 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). 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 2008 Montana AI surveillance. Mallard was the most abundant and available species in Montana and were sampled during refuge banding and urban trapping. However, to maximize sampling of other target species, mallard sampling was continually reduced across the three years of AI sampling. While urban trapping provided the greatest flexibility temporally, as sampling could be conducted according to schedule rather than opportunistically, it afforded the least diversity of species among the methods (n=4). Conversely, hunter-harvest sampling was difficult to allocate temporally while it provided the most species diversity (n=17); 31% of the total hunter-harvest samples were collected during the first weekend of the waterfowl hunting season when the majority of hunting took place, after which sampling tapered. Refuge banding, which provided one-fourth of all cloacal-

oropharyngeal samples among seven species, was concentrated during the month of September and conducted mostly at Benton Lake (19% of total cloacal-oropharyngeal samples). To distribute sample collection temporally during the 2008 surveillance, emphasis was placed on sampling wild sentinel birds at urban ponds and northern pintails during refuge banding, while hunter-harvest sampling was used to target a broad range of specific species.

The 2009 AI surveillance is underway. FWP added spring mortality/morbidity transects to the 2009 sample design, which began in May, while sampling of live birds in Montana will begin in August with refuge banding operations. Opportunistic mortality/morbidity samples are collected throughout the year.

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