SEASONAL VARIATION IN THE HEALTH OF HIGH-LATITUDE WINTERING MALLARDS (ANAS PLATYRHYNCHOS)

By

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Abstract

Mallards (*Anas platyrhynchos),* the most abundant species of dabbling duck in North America, are increasingly wintering in urban centers at latitudes north of their traditional wintering grounds. We captured mallards throughout the non-breeding period in Fairbanks, Alaska in 2012/13 and 2013/14, as well as in Anchorage, Alaska, in 2014/15, to assess seasonal patterns in forage selection and body condition, as well as the influenza A virus (IAV) dynamics within these urban wintering mallard populations. Using stable isotope data ($\delta^{13}C$ and $\delta^{15}N$ values) from serum and whole blood, we identified seasonal shifts in diet from invertebrates and aquatic vegetation in autumn to anthropogenic food subsidies (i.e. corn and bread) during winter by mallards in both populations. Additionally, mallards wintering in Fairbanks maintained higher body mass levels throughout the winter period than mallards wintering in Anchorage, which declined in mass from autumn to late winter. To study the associated health conditions mallards wintering at these high-latitude locations experience, we examined infection dynamics of influenza A viruses (IAVs), as mallards are considered a natural reservoir host of IAV viruses. We screened mallards for both active infections and prior exposure to IAVs. Molecular screening indicated both IAV prevalence and seroprevalence varied by each season at each site/year. Age differences were pronounced for both infection and immune responses, with juvenile mallards having higher IAV prevalence and adults having higher IAV seroprevalence. Evidence for active infections and antibodies to IAVs were detected throughout each sampling year at both locations. Variability in mallard immune responses, suggests individual heterogeneity in the timing of infections and duration of immune responses to IAVs across the non-breeding period. Thus, the combination of these findings provide valuable information about when mallards may be relying most on anthropogenic food subsidies and the potential for these populations to serve as biotic

reservoirs for IAVs throughout the non-breeding period. Wildlife management agencies may consider these data when developing management objectives or regulations concerning these urban wintering mallard populations.

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General Introduction

Urbanization continues to provide ecologists with new opportunities to better understand the use of anthropogenic habitats by a multitude of wildlife species (DeStefano and DeGraaf 2003, Newsome et al. 2015). Human induced changes to the environment may negatively impact certain species (DeStefano and DeGraaf 2003), often reducing biodiversity in favor of more resilient or non-native wildlife species (Chace and Walsh 2006, Taylor et al. 2013). Nevertheless, urban wildlife species likely face both direct and indirect consequences by occupying urban habitats. Consequently, providing baseline knowledge of the ecological conditions faced by urban-dwelling species may be necessary for wildlife management agencies to effectively conserve local biodiversity (McKinney 2002).

Occupation of urban areas may not be relegated to one particular group of taxa, and avian populations are consistently present and easily recognized in many urban environments (Savard et al. 2000, Taylor et al. 2013). Specifically, documentation of avian population assemblages during winter through the annual Audubon Christmas Bird Count (CBC; National Audubon Society 2016) has provided a valuable tool for researchers identifying habitat colonization and mid-winter distribution patterns across North America (La Sorte and Thompson III 2007). Results from these surveys have identified a variety of avian species wintering in urban environments of North America; however, the effects contributing to the presence of these populations during winter are not fully understood. Avian species may benefit from creation of proper habitat and generally warmer winter temperatures in urban environments (McKinney 2002, Ciach and Fröhlich 2016). However, increased access to high-energy supplementary foods may represent the most important ecological effect associated with persistence of wild birds at urban locations during winter (Chace and Walsh 2006).

Feeding wild birds, especially during the winter, has increased wildlife-human interactions and despite constant provisioning of high-energy supplementary foods, the ability of urban bird populations to make use of anthropogenic diet items is likely context-dependent (Jones and Reynolds 2008, Robb et al. 2008). Provisioning of cereal crops such as corn and wheat may not benefit all avian species, yet waterfowl have increasingly made use of such subsidies at both urban and agricultural habitats in North America (Baldassarre and Bolen 1984, Alisauskas and Hobson 1993, Haramis et al. 2001). In particular, mallards (*Anas platyrhynchos*) may remain at these habitats, which may be considered unfavorable to other waterfowl (Brodsky and Weatherhead 1984, Heusmann 1988). Corn, wheat, or bread provided to mallards wintering in urban city centers may supplement dietary lipids but may be insufficient in dietary protein, vitamins, and minerals (Jorde et al. 1983, Loesch and Kaminski 1989). In addition to lipid-rich food items, mallards may also acquire high-protein diet items to maintain adequate body condition during winter (Miller et al. 2009).

Aside from field observations, the use of supplementary foods and the associated effects on the health of mallards wintering in urban environments of Alaska have yet to be determined. In addition to remarkable dietary plasticity, dabbling ducks such as mallards may save energy through modifications in both resting and loafing postures (Brodsky and Weatherhead 1984). Consequently, through mechanisms of energy conservation and focused foraging effort, mallards may avoid performing energetically expensive behavior during daily minimum temperature periods (Smith and Prince 1973, Jorde et al. 1984). Thus, mallards may be able to acquire adequate nutritional reserves even at northern locations (Olsen and Cox 2003); however if winter diet item availability is limited, such circumstances may result in negative effects on body condition throughout the non-breeding period (Pawlina et al. 1993).

In addition to increased metabolic constraints on body condition, mallards wintering at high-latitude urban locations may experience an increased risk of pathogen infection, as high densities of wildlife at supplementary feeding locations may contribute to increased transmission (Ditchkoff et al. 2006, Bradley and Altizer 2007). Mallards and other dabbling ducks species (family Anatidae, tribe Anatini) are natural reservoir hosts of influenza A viruses (IAVs; Slemons 1974) and are commonly included in IAV investigations in both North America and Eurasia (Olsen et al. 2006, Munster et al. 2007). While many studies regarding IAV dynamics in mallards have been from rural locations or through captive studies (Hinshaw et al. 1980, Latorre-Margalef et al. 2009, Tolf et al. 2013), relatively fewer investigations have focused on urban populations, despite the potentially higher risk for zoonotic disease transmission (Verhagen et al. 2012). In relation to other waterfowl species, mallards have been shown to have some of the highest IAV infection rates (Hinshaw et al. 1985, Runstadler et al. 2007, Wilcox et al. 2011), exhibit wide antigenic diversity (Wilcox et al. 2011, Hill et al. 2012), and produce a long-lasting immune response in the form of detectable antibodies (Fereidouni et al. 2010). However, the migratory nature of most wild mallard populations reduces the success of repeat sampling of birds during winter (Latorre-Margalef et al. 2013, Tolf et al. 2013).

Therefore, high-latitude wintering mallard populations provide an opportunity to identify both ecological conditions they experience while wintering in Alaska. The objectives of this study were to assess: (1) use of supplementary foods by mallards persisting at urban locations in Alaska, (2) seasonal patterns in body mass indicative of the condition of these birds, and (3) the potential role of wintering mallards in the maintenance of IAVs at high-latitude wintering locations.

In Chapter 1, I investigated fine-scale resource use patterns by mallards wintering in urban environments of Fairbanks and Anchorage, Alaska. I collected serum and whole blood samples from wintering mallards during monthly trapping periods during the non-breeding period (Aug – Apr). I collected natural diet items from Anchorage capture locations and diet items from dabbling ducks harvested on the Anchorage Coastal Wildlife Refuge (ACWR) and at the Twentymile/Placer River valleys. I examined the $\delta^{15}N$ and $\delta^{13}C$ ratios of all blood samples and diet items, including anthropogenic foods (e.g. corn, bread, wheat). Stable isotope analyses provide an effective means of reconstructing consumer diets, using non-lethal procedures (Hobson and Clarke 1993). I examined the influence of year, season, and sex on the stable isotope ratios of mallards overwintering at urban habitats in both Fairbanks and Anchorage. Additionally, I quantified the proportion of natural and anthropogenic food items in the diet of Anchorage mallards using SIAR a stable isotope mixing model (Parnell et al. 2008). I predicted that wintering mallard diets would shift from largely natural to mostly anthropogenic food subsidies, due to higher energy and availability of supplementary foods (Jorde et al. 1984). In addition, I compared seasonal changes in mallard body mass, representing the overall condition of individuals wintering in Fairbanks and Anchorage, two cities with distinctly different winter climates. The results from chapter 1 will help inform waterfowl managers and regulatory agencies when mallards may rely most heavily on supplementary foods during the non-breeding period and the potential life-history costs and benefits associated with wintering at high-latitude urban habitats in Alaska.

In Chapter 2, I evaluated IAV dynamics within mallard populations wintering at urban locations of Anchorage and Fairbanks, Alaska. Previous IAV investigations have identified the potential perpetuation of IAVs in Alaskan wetlands (Ito et al.1995, Lang et al. 2008, Reeves et

al. 2011), however it is currently unknown whether mallards wintering in Alaska harbor IAVs throughout the non-breeding season. Thus, I assessed sources of variation including age, sex, season, and year relative to IAV prevalence and seroprevalence at the population level. Additionally, through the combination of several types of infection data, I estimated the probability of developing or losing antibodies to an IAV infection during the 2014/15 sampling year in Anchorage. Finally, I included subtype-specific antibody information to provide a more comprehensive index of which IAV subtypes may be circulating in un-sampled birds and temporarily outside of the duration of our sampling effort. My goal was to identify whether wintering mallards may act as a biotic reservoir maintaining IAVs at high-latitude locations such as Alaska during winter. This chapter provides new data regarding IAV dynamics in Alaskan wintering waterfowl, and may contribute to improving surveillance-sampling efforts. As many large cities in North America have wintering populations of mallards, the results from this chapter may serve as a basis for understanding IAV dynamics in understudied populations.

In summary, while both of my thesis chapters are unique, they each address factors affecting the health conditions of urban wintering mallards in Alaska. This thesis provides baseline information for future studies assessing life-history costs and benefits of waterfowl wintering at high-latitude locations.

Chapter 1. Use of anthropogenic food subsidies by two high-latitude wintering mallard (*Anas platyrhynchos*) populations[1]

1.1 Abstract

Understanding how species respond to increasing anthropogenic change is an important step towards identifying effective management strategies of urban wildlife populations. Multiple species of North American waterfowl are increasingly wintering in urban centers at latitudes north of their traditional wintering grounds, presumably due to anthropogenic alterations providing suitable habitat. We captured mallards (*Anas platyrhynchos*) throughout the non-breeding period in Fairbanks and Anchorage, two urban centers in Alaska which support two of the northernmost concentrations of wintering mallards in North America. Using stable isotope data (δ^{13}C and δ^{15}N values) from serum and whole blood, we assessed seasonal patterns in forage selection for both populations and used an isotopic mixing model to quantify the relative contribution of anthropogenic subsidies to diet of Anchorage mallards. Additionally, we examined seasonal changes in body condition at both sites. Stable isotope analysis revealed that mallards captured at both locations exhibited shifts in diet from invertebrates and aquatic vegetation in autumn to anthropogenic subsidies (i.e., corn and bread) during winter. For mallards wintering in Anchorage, the mean proportional contributions of anthropogenic subsidies increased from ~60% of the diet in autumn (September – October) to ~86% of the diet by the late winter (February – April) period. Despite similar seasonal patterns of δ^{13}C values for individuals in both populations, variation in δ^{15}N values suggests mallards in Anchorage

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consumed relatively larger quantities of natural foods. Trends in body mass also varied by location, with Fairbanks mallards maintaining higher body mass levels throughout the winter period, whereas mass of Anchorage mallards declined from autumn to late winter. Greater loss of body mass for Anchorage mallards may reflect an inconsistent level of subsidy or a response to milder local climate conditions requiring less nutrient reserves. Winter diets consisting predominantly of anthropogenic foods suggest that supplementary diet items may have contributed to recent increases in the number of wintering mallards in Fairbanks and Anchorage.

1.2 Introduction

Responses by wildlife populations to anthropogenic alterations to the environment are progressively more evident and overlap of wildlife and humans in urban areas poses a suite of unique management implications (Drapeau et al. 2000, Prange and Gehrt 2004). In particular, an increasing number of avian species are colonizing new urban habitats (La Sorte and Boecklen 2005), and the impacts of supplementary foods provided to these species have become a topic of increased debate (Jones and Reynolds 2008, Robb et al. 2008). Supplementary feeding provides high-energy food sources during physiologically challenging winter months, and provides outdoor enthusiasts with consistent wildlife viewing opportunities. However, feeding birds can induce "reliance" upon such food subsidies, and may potentially increase transmission of diseases (Fischer et al. 1997, Jones and Reynolds 2008). Although many studies of supplementary feeding have focused on northern-wintering passerine species (Robb et al. 2008), waterfowl have also benefitted from human cultivated foods through consumption of excess agricultural crops (e.g., Clark et al. 1986, Abraham et al. 2005). Unlike passerines however, waterfowl are rarely provided subsidies directly and most waterfowl species breeding at high-

latitude locations migrate annually to warmer latitudes with reliable open water habitat and food availability (Bellrose 1980). However, throughout North America some waterfowl species are increasingly wintering in or near urban population centers further north than traditional wintering locations (La Sorte and Thompson III 2007). Human induced alterations to the landscape (e.g., warm water effluents) at these locations provide waterfowl with suitable habitat which may be enhanced through direct food subsidies.

Mallards (*Anas platyrhynchos*), the most abundant dabbling duck species in North America (USFWS Survey 2016), are adept at making use of a variety of habitat types and are increasingly opting to overwinter at locations within major urban population centers of Alaska (National Audubon Society 2016). In Fairbanks, Alaska, wintering mallards were practically nonexistent prior to 1992, but have increased from ~27 in 2003 to 400 – 600 in recent years, based on the Christmas Bird Count conducted annually each December (National Audubon Society 2016). In Anchorage, Alaska's largest city, wintering mallard abundance is currently 1200 – 1500, an increase from 10 – 20 in the early 1970s (National Audubon Society 2016). Mallards wintering in Anchorage, a coastal location, have access to open-water ponds and marine habitats in comparison to the small (~2km) section of open water on the Chena River available to mallards in Fairbanks. Despite the potential for increased transmission of pathogens within these wintering populations, large anthropogenic food subsidies are consistently made available at these locations. However, the quantity and quality of such foods may vary and it is unknown to what extent such subsidies are required to support urban Alaska-wintering mallards.

Mallards are known for their ability to consume a wide variety of natural diet items, including aquatic and terrestrial invertebrates, aquatic vegetation, and natural seeds (Krapu and Reinecke 1992). During winter, mallards consume high-energy foods to offset increased

metabolic costs resulting from a number of physiological processes (e.g., thermoregulation, feather molt, pair formation; Hohman et al. 1992). However, in addition to consumption of high-energy diet items, mallards must also consume food items sufficient in protein to maintain good condition during winter (Miller et al. 2009). If food items are abundant, birds may opportunistically acquire extra nutrients in preparation for extreme weather events (Lovvorn 1994). However, if severe weather conditions perpetually inhibit foraging at wintering locations, birds may maintain high levels of excess nutrients, relying on stored reserves to balance the costs of nutrient depletion (Boos et al. 2002, Jorde et al. 1983). Mallards have been shown to minimize foraging effort in an attempt to maintain nutrient reserves, if wintering at a nutritionally favorable location (Smith and Prince 1973, Jorde et al. 1984). Consequently, fluctuations in body mass may be a reliable indicator of the overall condition of mallards remaining at northern locations during winter (Boos et al. 2000). In support of this correlation, northern wintering mallards have been shown to exhibit greater body mass than populations wintering on the southern extent of their distribution (Whyte and Bolen 1984, Pawlina et al. 1993, Olsen and Cox 2003).

Effective management of northern-wintering mallards requires information on the factors driving their non-migratory behavior, and determining the relative importance of anthropogenic food subsidies is an important step in formulating potential management actions. Rather than relying on analysis of foregut and fecal contents alone to make inference on foraging preferences, an increasing number of dietary investigations are making use of naturally occurring stable isotopes to reveal patterns in foraging activity (Hobson 2011). Dietary reconstruction through the use of stable isotopes may provide less biased estimates of dietary proportions and can be accomplished via non-lethal procedures (Hobson and Clarke 1993). Tissues vary with

regard to isotopic integration and by incorporating multiple tissue types, such as serum and whole blood into dietary analyses, both recent (7–10 days for plasma/serum) and longer term (~4 weeks for whole blood) shifts in diet may be quantified (Schmutz et al. 2006, Hahn et al. 2012). Carbon and nitrogen stable isotope ratios can often be used to distinguish a shift between natural and anthropogenic food subsidies (Alisauskas and Hobson 1993, Van Hemert et al. 2012); for example, anthropogenic foods derived from corn have carbon isotope ratios which are distinct from high-latitude plants and animals due to differences in photosynthetic pathway (C4 vs. C3) (Savory et al. 2014), whereas animal diet items may vary in nitrogen isotope ratio according to trophic level (Post 2002, Fry 2006).

Our primary objective was to examine fine-scale dietary patterns and changes in body mass of mallards overwintering at two urban locations in Alaska. Specifically, we used an isotopic mixing model and generalized linear models to: (1) quantify the contribution of anthropogenic food subsidies to Anchorage mallard diet, (2) assess variation in diet composition relative to season, year, and location, and (3) examine seasonal variation of body mass relative to age, sex, season, and year. Because availability of natural diet items may be limited and mallards require high-energy food subsidies during cold winter months (Jorde et al. 1984), we predicted that diets would shift from natural food items during autumn to anthropogenic foods like corn and bread during winter (Nov-Apr). Additionally, we expected body mass of Fairbanks mallards to be higher as compared to mallards wintering in Anchorage relative to consistently lower temperatures throughout winter (Nov-Apr) in Fairbanks.

1.3 Methods

1.3.1 Study Area

We captured mallards from August – April of 2012/13 and 2013/14, in Fairbanks, Alaska (64°50'N, 147°45'W) and from September – April of 2014/15 in Anchorage, Alaska (61°11'N, 149°52'W). Located in the interior boreal region of Alaska, Fairbanks experiences harsh winters, with mean December daily temperatures frequently falling well below -20°C (National Oceanic and Atmospheric Administration 2016), which drastically limits the availability of open water. During winter in Fairbanks, 400 – 600 mallards concentrate along the ~2 km section of the Chena River (National Audubon Society 2016), where warm water discharge from a power plant maintains open-water habitat. Each day, from mid-October through April, an organized volunteer group provides large quantities of feed (~90 kg) in the form of a corn, wheat, and protein pellet mixture. In comparison, the maritime climate of Anchorage experiences relatively warmer winter temperatures (mean December 2014 daily temperature -3°C; National Oceanic and Atmospheric Administration 2016) and provides a wider availability of open-water habitat for 1200 – 1500 overwintering mallards. Capture locations in Anchorage included both natural and artificial wetlands in close proximity to residential areas, where supplementary foods were presumably provided in smaller quantities by a larger number of people.

1.3.2 Sample Collection and Years

We attempted to capture mallards over ~7-day trapping periods during each month of each year during the three-year project. We captured/recaptured a total of 320 mallards in 2012/2013 and 175 mallards in 2013/2014 in Fairbanks, and 700 mallards in 2014/2015 in Anchorage. We used swim-in bait traps, walk-in bait traps, whoosh nets, and net guns for capture; traps were baited with corn and checked at 30 minute intervals. We used plumage and

cloacal examination to determine sex and categorize age as being either hatched the previous summer (hatch year; HY) or more than one year prior (after hatch year; AHY) (Hochbaum 1942, Carney 1992). To obtain an index of body condition, mallards were weighed ± 1.0 g using a digital scale and scored for the amount of grain in their crop through esophageal palpation. Each bird was banded with a U.S. Geological Survey (USGS) metal leg band. We collected 1.5 ml of whole blood from the jugular vein of ducks before release at original capture locations. Blood samples were not obtained from recaptured individuals if they were bled within the previous 7-day period. After sample collection, aliquots of blood samples were separated into serum and plasma through centrifugation and stored along with whole blood at -20°C until analysis.

1.3.3 Foregut Analysis

We obtained hunter-harvested mallard, northern pintail (*Anas acuta*), and american green-winged teal (*Anas crecca*) carcasses (n = 36) to identify natural diet items birds were selecting at nearby locations. Carcasses were collected between September – November 2014 from various locations within the Anchorage Coastal Wildlife Refuge (ACWR) (61°10'N, 150°2'W), Alaska and the Twentymile/Placer River Valley (60°48'N, 148°59'W), ~49 km southeast of Anchorage. All diet items present in the mouth were collected and separately frozen at -20°C for later analysis. The esophagus, proventriculus, and gizzard were immediately removed in the laboratory, and frozen at -20°C, until dissection. Dissection of the esophagus and proventriculus yielded both plant and animal dietary items which were sorted into categories (invertebrates, fish, and seeds) and subsequently frozen at -20°C for stable isotope analysis. Invertebrates and natural seeds from gut content samples were identified to the level of order (Borror and White 1970, Pennak 1989). Depending upon the quantity of diet items present in an individual carcass, samples were stored as either singular (n = 1) or pooled (n < 20) samples.

1.3.4 Dietary Item Collection

In addition to diet items collected from hunter-shot birds, we opportunistically collected potential diet items during the winter of 2014/15 at capture locations in Anchorage, Alaska and from natural habitats at the nearby ACWR. Probable diet items were collected using a mesh D-frame dip net or small diameter mesh sieve from areas where mallards were observed feeding. We collected arthropods, seeds, and berries from capture locations and other small water bodies that marked individuals frequented during the winter. Additionally, we collected coho salmon (*Oncorhynchus kisutch*) eggs from a local fish hatchery on Ship Creek in Anchorage, where marked individuals had been re-sighted. We also sampled a variety of types of bread (e.g., white, whole wheat) and cracked corn from local stores, as these were the most common diet items identified through field observations. All diet items were sorted by category and identified to the level of order; singular (n = 1) or pooled (n < 20) samples were frozen at -20°C for stable isotope analyses.

1.3.5 Stable Isotope Analysis

Tissue (n = 943) and diet item samples (n = 92; Appendix A) were analyzed for carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values) at the University of Alaska Anchorage, Environmental and Natural Resources Institute Stable Isotope Lab. Samples were individually freeze dried for a minimum of 48 h then ground with mortar and pestle. Dried, homogenized samples between 0.8 – 1.0 mg were inserted into tin capsules, which were crushed and placed into a zero-blank autosampler. Samples were combusted to CO_2 and N_2 in a Costech ECS 4010 elemental analyzer (Costech, Valencia CA), and carbon and nitrogen isotope ratios were measured via continuous flow isotope ratio mass spectrometry using a Thermofinnigan

DeltaPLUS XP mass spectrometer with a ConFlo III interface. Stable carbon and nitrogen isotope ratios are given as δ – values, representing relative abundance of heavy isotope in parts per thousand (‰), according to the equation: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$ ‰. In this equation, X represents the heavy isotope (^{13}C or ^{15}N), R represents the ratio of heavy/light isotope ($^{13}C/^{12}C$ and $^{15}N/^{14}N$), and $R_{standard}$ represents the international standards of Vienna PeeDee Belemnite for carbon and atmospheric N_2 for nitrogen. We included internal standards of purified methionine (Alfa Aesar, %C = 40.3, %N = 9.4, $\delta^{13}C$ = -34.6‰, $\delta^{15}N$ = -0.9‰), homogenized peach leaf (NIST 1547, %C = 46.8, %N = 2.94, $\delta^{13}C$ = -25.9‰, $\delta^{15}N$ = 1.9‰), and homogenized chinook salmon muscle (University of Alaska, $\delta^{13}C$ = -19.3‰, $\delta^{15}N$ = 15.5‰) with all samples as quality controls. Long-term records of internal standards yield an analytical precision of 0.1‰ for $\delta^{15}N$ and 0.1‰ for $\delta^{13}C$.

1.3.6 Statistical Analysis

We assessed variation in the carbon and nitrogen isotope ratios of the whole blood and serum of wintering mallards using generalized linear models (R Core Team 2016). To avoid the confounding effects of ontogenetic dietary change and growth on stable isotope ratios (Gaye-Siessegger et al. 2004, Barnes et al. 2007, Sears et al. 2008), we limited the scope to analyses of adult birds. For our analysis of isotope ratios, we included one capture occasion and randomly selected one capture occasion from the entire sampling year for individuals captured on multiple occasions. Because monthly sample sizes were variable, we combined capture events into 3 seasons: autumn (August – October), early winter, (November – January), and late winter (February – April). Samples were not collected from both locations during the same year, prohibiting our ability to assess site-specific variation within a given year. For each isotope ratio ($\delta^{15}N$ and $\delta^{13}C$) and sample type (whole blood and serum) we considered 13 models assessing

support for variation relative to year, season, sex, and their interactions. To assess temporal patterns in body condition (mass adjusted for weight of bait in the esophagus), we included the original capture occasion and one capture occasion from each season for individuals captured on multiple occasions. We conducted analyses on individuals with complete covariate information and we considered 14 models which allowed adjusted mass to vary relative to year, season, sex, and their interactions. We present the mean and standard error for estimates from models predicting variation in isotope ratios and body condition as (mean ± SE). For all analyses, models were evaluated using an information-theoretic approach with model rankings based on Akaike's Information Criterion corrected for sample size (AICc; Burnham and Anderson 2002). We examined all data for departures from normality by assessing homogeneity of variance through residual plots and probability distribution plots in the R environment (R Core Team 2016).

We did not use a mixing model to estimate dietary proportions for the Fairbanks population because of the lack of natural dietary endpoints. However, to investigate the proportional contribution of anthropogenic foods to the diet of adult mallards wintering in Anchorage, we used the SIAR (stable isotope analysis in R) package in R (Parnell et al. 2008, 2010; Jackson et al. 2009). Dietary proportions were estimated for adult mallards grouped by season. Using a Bayesian approach, the SIAR package provides maximum likelihood estimates of proportional contributions of diet items to a given tissue, and allows for inclusion of informative priors (Parnell et al. 2008). We present the mean and 95% credible intervals for the proportion of each dietary item estimated in the model (mean; 95% credible interval). Because mallards are opportunistic feeders, we included concentration dependence to account for variation in C and N of diet items and used mean δ^{15}N and δ^{13}C values (± SD) from both natural

and anthropogenic food sources. We grouped diet items into broad categories in an attempt to limit the number of dietary endpoints. These groups included seeds and berries, fish and salmon eggs, and freshwater and marine invertebrates (Table 1.1). We used bread and corn to represent C3 and C4 anthropogenic food sources (O'Leary 1988), respectively, as these are the food items most commonly provided to mallards (Table 1.1). Discrimination factors previously estimated for mallard red blood cells ($\Delta\ \delta^{13}C = -0.5 \pm 0.6‰$ and $\Delta\ \delta^{15}N = 3.6 \pm 0.5‰$) and plasma ($\Delta\ \delta^{13}C = 0.3 \pm 0.5‰$ and $\Delta\ \delta^{15}N = 4.4 \pm 0.6‰$; Hahn et al. 2012) were used to account for differences between isotopic ratios of the source and the consumer due to trophic enrichment (Hobson and Clark 1992). We included a post-hoc sensitivity analysis in which each trophic enrichment factor for $\delta^{15}N$ and $\delta^{13}C$ in both whole blood and serum was varied by ± 1 SD to assess the reliability of our proportional dietary contributions (Jones et al. 2010, Van Hemert et al. 2012).

1.4 Results

1.4.1 Variation in Isotope Ratios

We captured and bled a total of 1061 mallards between August and April of 2012 – 2015. Censoring of samples obtained from hatch-year birds, and limiting inclusion of recaptured birds to a single sampling event, yielded 471 blood and 472 serum samples for stable isotope analyses. Our top approximating model for $\delta^{13}C$ values in serum ($w = 0.64$) indicated support for variation by year, season, and their interaction (Table 1A-1). The top approximating models for $\delta^{13}C$ values in whole blood ($w = 0.88$), $\delta^{15}N$ values in serum ($w = 0.84$) and $\delta^{15}N$ values in whole blood ($w = 0.91$) supported variation of isotope ratios by year and season with an additive relationship of sex (Tables 1A-2, 1A-3, 1A-4). Across years, serum $\delta^{13}C$ values increased from autumn to late winter, with highest values from adult mallards during late winter of 2013/14 in

Fairbanks (Figure 1.1A; Table 1A-5). Whole blood $\delta^{13}C$ values followed a similar pattern (Figure 1.1A, 1.1B). Autumn whole blood $\delta^{13}C$ values were lower during both years in Fairbanks (Figure 1.1B; Table 1A-6) than in Anchorage (Figure 1.1B; Table 1A-6); whereas early- and late winter values were similar between years and sites (Figure 1.1B; Table 1A-6). Serum $\delta^{15}N$ values for Fairbanks mallards were lower and less variable than those for Anchorage mallards (Figure 1.1C, 1.1D; Table 1A-7). Whole blood $\delta^{13}N$ values followed a similar pattern (Figure 1.1C, 1.1D; Table 1A-8). Males had higher whole blood $\delta^{13}C$ values than females across seasons and years (Figure 1.1B; Table 1A-6). While males had higher serum and whole blood $\delta^{15}N$ values than females, the sex-specific differences were less pronounced for Fairbanks mallards in comparison to mallards wintering in Anchorage (Figure 1.1C, 1.1D; Tables 1A-7, 1A-8).

1.4.2 Foregut Dietary Items

We examined 36 hunter-harvested Mallards (n = 30), Northern Pintails (n = 3), and American Green-winged Teal (n = 3) carcasses collected between September 2014 and November 2014 for the presence of natural dietary items. Foregut samples representing the seeds and berries group were largely dominated by Alismatales and Poales, whereas invertebrates mostly comprised the orders Amphipoda, Odonata, and Diptera. Several mallards had fish of the order Gasterosteiformes in their proventriculus. Among the diet groups, corn had the highest $\delta^{13}C$ value. The other groups had similar $\delta^{13}C$ values but differed substantially in their $\delta^{15}N$ values (Figure 1.2; Table 1.1). The fish group had the highest $\delta^{15}N$ value followed by the invertebrate group, including both freshwater and marine invertebrates (Figure 1.2; Table 1.1).

1.4.3 Dietary Analysis of Anchorage Mallards

Stable isotope ratios of adult mallard whole blood (n = 269) and serum (n = 279), suggests diet of the Anchorage population largely shifted from natural diet items to

anthropogenic foods by the late winter season (presented as mean, 95% credible interval) (Figure 1.3). SIAR proportional estimates of autumn diet from serum indicate that corn/corn products were lowest during autumn (36%, 31 – 41%). In combination with corn, the estimate for bread (24%, <1 – 46%) contributed to a diet comprised of ~60% anthropogenic food subsidies during the autumn season. By late winter, the contribution of anthropogenic foods increased to ~86% of the diet with more corn/corn products (55%, 51 – 58%) than bread (31%, 19 – 43%). The contributions of invertebrates and seeds/berries to the diet declined seasonally (Table 1B-1). The contribution from invertebrates was highest in autumn (16%, <1 – 28%) in comparison to late winter (4%, 0 – 8%), declining similarly to the contribution from seeds/berries in autumn (23%, <1 – 45%) and late winter (8%, 0 – 19%). Mean estimates for fish/salmon eggs were low and stable remaining below ~5% of the diet across all seasons (Table 1B-1).

Estimates from whole blood yielded a lower contribution of anthropogenic foods during autumn than serum (~54%), with similar contributions from corn and bread. The contribution from corn/corn products increased from autumn (27%, 21 – 33%) to late winter (63%, 60 – 66%). In comparison to estimates from serum, estimates from whole blood indicated a decline in the proportion of bread between autumn (27%, <1 – 53%) and late winter (15%, <1 – 30%). Combined contributions from invertebrates and seeds/berries in whole blood were higher than estimates from serum and declined from ~44% in autumn to ~19% of the diet by late winter (Table 1B-2). As indicated from serum samples, estimates of the contribution of fish/salmon eggs were highest during the early winter season (~6%), but did not make up a large proportion of the diet during any period of sampling (Table 1B-2).

1.4.4 Sensitivity Analysis

Increasing or decreasing the carbon trophic enrichment factors ($\Delta^{13}C$) for serum and whole blood by ± 1 SD resulted in <5% changes in dietary estimates. Increasing or decreasing the nitrogen trophic enrichment factors ($\Delta^{15}N$) by ± 1 SD resulted in slightly larger changes in dietary estimates. While most changes were <5%, decreasing the ($\Delta^{15}N$) by 1 SD increased autumn serum by 9%, and decreased the carbon residual error term (SD2G1) estimate for autumn whole blood by 10%. Additionally, increasing the ($\Delta^{15}N$) by 1 SD led to a 21% increase in the SD2G1 for autumn whole blood, providing less precise dietary estimates for that season.

1.4.5 Body Condition

The top AICc approximating model (w = 0.99) allowed body mass to vary by age, sex, season, year, and the interactions of age and year with season (Table 1.2). Body mass estimates were higher for males than females and higher for adults than juveniles (Figure 1.4; Table 1C-1). Across seasons and years, estimates of body mass were highest in adults (M: 1514 ± 25, F: 1330 ± 22) during mid-winter of 2013/14 in Fairbanks and lowest in juveniles (M: 1110 ± 23, F: 948 ± 24) during late winter of 2014/15 in Anchorage (Figure 1.4; Table 1C-1). During both years of sampling in Fairbanks, body mass of both sex/age classes increased from autumn to early winter and decreased during late winter to near autumn levels (Figure 1.4; Table 1C--1). Age-specific differences in body mass of Fairbanks mallards were most pronounced for males in autumn of 2012/13 (AHY: 1316 ± 13, HY: 1183 ± 14) and 2013/14 (AHY: 1280 ± 19, HY: 1115 ± 24), as well as for females in autumn of 2012/13 (AHY: 1153 ± 14, HY: 1021 ± 14) and 2013/14 (AHY: 1118 ± 19, HY: 953 ± 24) (Figure 1.4; Table 1C-1). However, by late winter of 2012/13 and 2013/14, age-specific differences in body mass for both sexes were less prominent.

Body mass of mallards captured in Anchorage declined from autumn through the late winter season for both sexes (Figure 1.4; Table 1C-1). Similar to mallards captured in Fairbanks, age-specific differences in body mass of mallards wintering in Anchorage were most distinct in autumn for both males (AHY: 1377 ± 12, HY: 1263 ± 12) and females (AHY: 1214 ± 13, HY: 1101 ± 13), becoming less pronounced through late winter (Figure 1.4; Table 1C-1).

1.5 Discussion

Adult mallards wintering at two urban city centers in Alaska exhibited seasonal increases in anthropogenic food consumption from autumn through late winter, suggesting that supplementary food items contribute to the observed patterns in body condition of these populations during the coldest months of the year. Whereas the seasonal shift toward greater anthropogenic food consumption was consistent among study sites, body mass was lower and declined seasonally for mallards wintering in Anchorage in comparison to Fairbanks mallards which maintained body mass throughout the winter.

For the Anchorage population, sampling of a broad range of natural and anthropogenic food items permitted analysis of diet composition for the winter of 2014/15 using dietary stable isotope mixing models. The proportional contribution of anthropogenic foods such as corn and bread products increased steadily from autumn to late winter. The combined contributions of natural diet items from the invertebrate, fish/salmon eggs, and seeds/berries groups, declined by ~26% from autumn through late winter. Reduced consumption of natural food items suggests that either availability of these items decreased, or that anthropogenic subsidies were favored. We lack broad-scale data on food availability, but suspect that ice and snow greatly reduce the availability of diet items such as invertebrates and natural seeds during winter at freshwater locations in Alaska. Because mallards and other waterfowl species have been shown to seek out

high-energy cereal grains to offset the depletion of energy stores during inclement weather (Jorde et al. 1983, Haramis et al. 2001), anthropogenic foods may therefore be favored over naturally occurring food items. Because successful trapping of birds required competition with anthropogenic foods being provided, we used corn as a pre-bait/bait food item during all three years of the study. Nevertheless, we suggest pre-bait/baiting of traps had a minimal effect on stable isotope ratios, as consumption of our bait took place within several days of discrete trapping periods and was predominantly placed within the traps themselves on capture dates.

Although we did not use a mixing model to estimate dietary proportions for the Fairbanks population due to the lack of well-constrained dietary endpoints, the stable isotope ratios of blood fractions representing both more recent (serum) and more integrated (whole blood) diet of Fairbanks mallards support field observations of consumption of significant amounts of C4 (corn-based) and some C3 supplementary food items (e.g., wheat). Nevertheless, despite greatly increasing $\delta^{13}C$ values of Fairbanks mallards from autumn (Aug – Oct) through late winter (Feb – Apr), serum and whole blood $\delta^{15}N$ values remained relatively high and stable, suggesting consumption of some natural diet items elevated in $\delta^{15}N$ in addition to the commercial poultry feed (mixture of corn, wheat, mineral pellet) provided daily by a local volunteer group.

In light of direct observations of the supplementary foods offered to Fairbanks mallards, prior invertebrate sampling upstream from our capture locations on the Chena River during mid-winter yielded multiple species of macroinvertebrate larvae (Irons 1988, Irons et al. 1993), and a variety of other benthic macroinvertebrates have been collected between the months of May-September (Benson et al. 2012). Additionally, the Chena River represents a highly productive sub-arctic Alaskan river due to the annual influx of several Pacific salmon species (Oswood et al. 1992). Both invertebrate and fish/salmon eggs taxonomically similar to those collected in

Anchorage are present in the Chena River, yet the relatively lower δ^{15}N values from mallards in Fairbanks suggests less consumption of these food items during the non-breeding season. Conversely, since mallards have been shown to consume large quantities of fish/fish parts at lower latitude locations when agricultural grains became unavailable during winter (Olsen et al. 2011), the lower δ^{15}N values from Fairbanks mallards may simply reflect less availability of fish or fish parts in the open-water portion of the Chena River, rather than a difference in foraging strategy.

We consistently documented provisioning of energy-rich anthropogenic foods (i.e. corn and bread) to mallards at both locations. Assuming supplementary foods offered at these two locations contain relatively similar isotope values, higher δ^{15}N values from Anchorage mallards may reflect consumption of natural diet items obtained across a more complex assemblage of habitat types. The unusually warm winter of 2014/15 in Anchorage (National Oceanic and Atmospheric Administration 2016) may have provided mallards with increased access to habitats not normally available during winter, and we frequently observed individuals moving among capture locations across a broader area as compared to mallards in the Fairbanks population. Additionally, the proximity of capture locations to the marine environment may also have contributed to the elevated δ^{15}N values from mallards wintering in Anchorage. Since recoveries of mallards banded in Anchorage occurred on the ACWR and Susitna Flats State Game Refuge near Anchorage during autumn, marine-derived nutrients typically enriched in ^{15}N (Gende et al. 2002) may provide additional nutrient supplements deficient in anthropogenic foods such as corn and bread (Jorde et al. 1983, Loesch and Kaminski 1989, Miller et al. 2009).

In addition to variation in isotope values and differences in the number of available habitat types, mallards in Fairbanks and Anchorage also displayed disparate trends in body mass

throughout the non-breeding period. Body mass estimates of mallards during early winter (Nov – Jan) in Fairbanks were higher than early winter mass estimates of Anchorage mallards. Furthermore, in Fairbanks both sexes increased in body mass and male estimates from early winter (Nov – Jan) were higher than all other early winter (Nov – Jan) mass estimates reviewed by Olsen and Cox (i.e. North Dakota, Nebraska, Arkansas; 2003). As a result, Fairbanks mallard body condition improved or remained stable between autumn and late winter, whereas mallards wintering in Anchorage during 2014/15 were lighter during late winter than during autumn.

Seasonal patterns in body mass by mallards in our sample populations may be indicative of variation in food availability or winter climate between site-years. In Fairbanks, a local volunteer group provides 400 – 600 mallards with 90 kg of high carbohydrate, energy-rich foods on a daily basis, which equates to roughly 180 g of supplementary food per bird each day. However, field observations indicate substantial amounts of leftover foods, suggesting the daily amount of subsidies provided in Fairbanks were greater than the amount needed to satisfy daily energy expenditure requirements. Conversely, Anchorage supplementary foods are less consistently provided and probably proportionally less (i.e., mass of feed/bird) as compared to foods provided in Fairbanks. Thus, through consumption of large quantities of supplementary foods and minimization of travel between foraging locations, Fairbanks mallards may have had adequate subsidies to build endogenous reserves for maintenance of body condition.

Alternatively, the high level of supplementary feeding at both Fairbanks and Anchorage capture locations could suggest variation in body condition observed in our study represents a physiological response to differing climates. As winter temperatures frequently reach -20°C or lower in Fairbanks (National Oceanic and Atmospheric Administration 2016), mallards must retain sufficient reserves to mitigate the effects from extreme negative temperatures. In

comparison, winter temperatures are warmer in coastal regions of Alaska such as Anchorage (National Oceanic and Atmospheric Administration 2016). In Anchorage, where a higher number of mallards overwinter (Figure 5), increased competition for high-energy food subsidies in combination with a milder local climate, likely contributed to differences in body mass patterns from mallards wintering in Fairbanks. However, additional research is needed to parse out the mechanisms underlying body mass patterns in our sample populations.

The highly plastic foraging behavior of mallards choosing to remain at high-latitude wintering locations may allow these populations to take advantage of several ecologically important life-history benefits. Overwintering at high-latitude locations may allow mallards to enter the breeding season in relatively better body condition than their migratory counterparts, and increasing evidence suggests factors influencing body condition during winter have the potential to affect subsequent breeding success (Heitmeyer and Fredrickson 1981, Devries et al. 2008, Sedinger and Alisauskas 2014). Additionally, early recognition of environmental cues signaling when to depart to nesting locations from nearby wintering areas may allow high-latitude wintering mallards to secure prime nesting locations before migrating birds arrive (Arzel et al. 2006). As a result, early nest-initiating pairs can use stored nutrients for energetically expensive activities such as egg laying, nest incubation, and the pre-alternate molt period (Krapu 1991, Devries et al. 2008), rather than competing for preferential nest site locations.

Intraspecific variation in the scale of life-history benefits likely exists, and mallards wintering at urban locations in Alaska may also experience potential carry-over effects from such behavior. In the absence of many natural predators and the need to acquire additional reserves prior to long-distance migration (Biebach 1996, Bond and Esler 2006), mallards wintering at urban locations in Alaska may experience minimal tradeoff consequences for

maintaining elevated body mass levels throughout the non-breeding period. We suggest the greatest costs incurred from wintering at urban locations may be long-term effects from diet quality and the potential for increased disease transmission. While we did not measure the nutritional quality of anthropogenic foods provided to mallards at supplementary feeding locations, we observed mallards in Anchorage consuming a higher proportion of bread, a commonly provided, but not nutritionally characterized supplementary diet item (Jones and Reynolds 2008). Moreover, consistently foraging on anthropogenic diet items at limited open-water habitats, often with fecal material present on or near food items, may have increased the risk for transmission of pathogens. As mallards are considered a natural host of influenza A viruses (IAVs; Slemons et al. 1974), and transmission generally occurs through fecal contaminated water (Webster et al. 1992), extended foraging under such circumstances throughout the non-breeding period may have increased the potential for perpetuation of IAVs within these populations (Spivey 2017).

Our findings indicate that anthropogenic food subsidies make up a large and seasonally increasing component of the diet of mallard populations wintering at several urban locations in Alaska. Despite location-specific differences in the frequency of supplementary provisioning, mallards consuming supplementary foods were able to reach sufficient body condition to mitigate local climate conditions faced during winter. Our data documenting dietary shifts from natural to anthropogenic foods by abundant avian species may help researchers identify variables contributing to such behavior in less ubiquitous avian species. Inclusion of citizen science data such as historical records from Christmas Bird Counts (National Audubon Society 2016) may help researchers determine long-term distribution changes by bird species wintering at the northern extent of their range. Furthermore, longitudinal studies identifying patterns in body

condition of wintering waterfowl may allow researchers to forecast the response of other waterfowl populations to climate and human-related changes in resource availability. Finally, wildlife managers and resource agencies may consider information from this study when determining management objectives including setting population goals and managing disease risk for species dependent on anthropogenic foods in urban environments.

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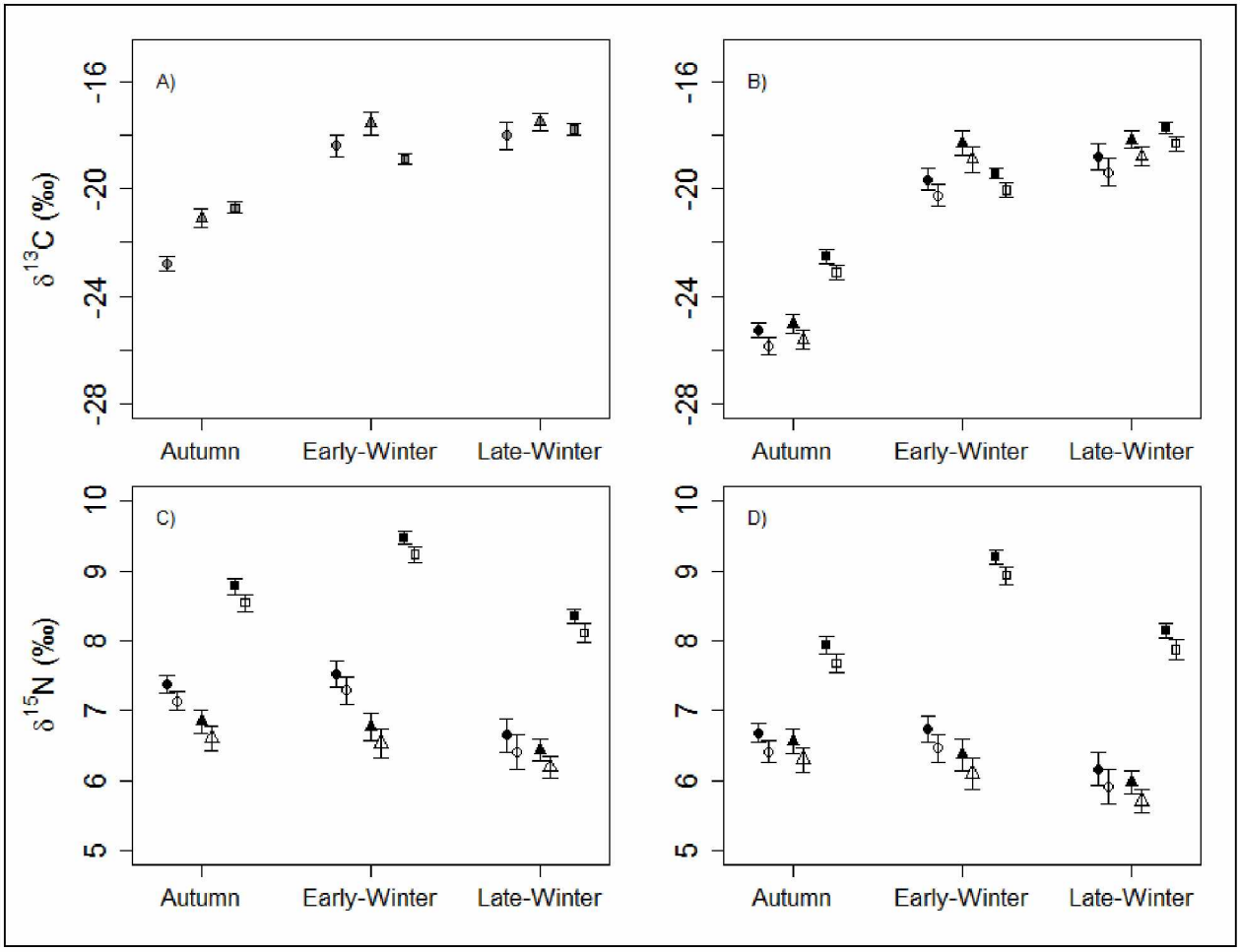

Figure 1.1. The least square means (±SE) of δ^{13}C values from serum (**A**) and whole blood (**B**), as well as δ^{15}N values of serum (**C**) and whole blood (**D**), from adult mallards wintering in Fairbanks and Anchorage, Alaska (2012-2015). Variation by sex was not supported for δ^{13}C values of serum (**A**) and combined adults are represented by gray filled shapes. Males are represented by black filled shapes and females are represented by open shapes. Circles represent samples from 2012/13 (Fairbanks), triangles represent samples from 2013/14 (Fairbanks), and squares represent samples from 2014/15 (Anchorage).

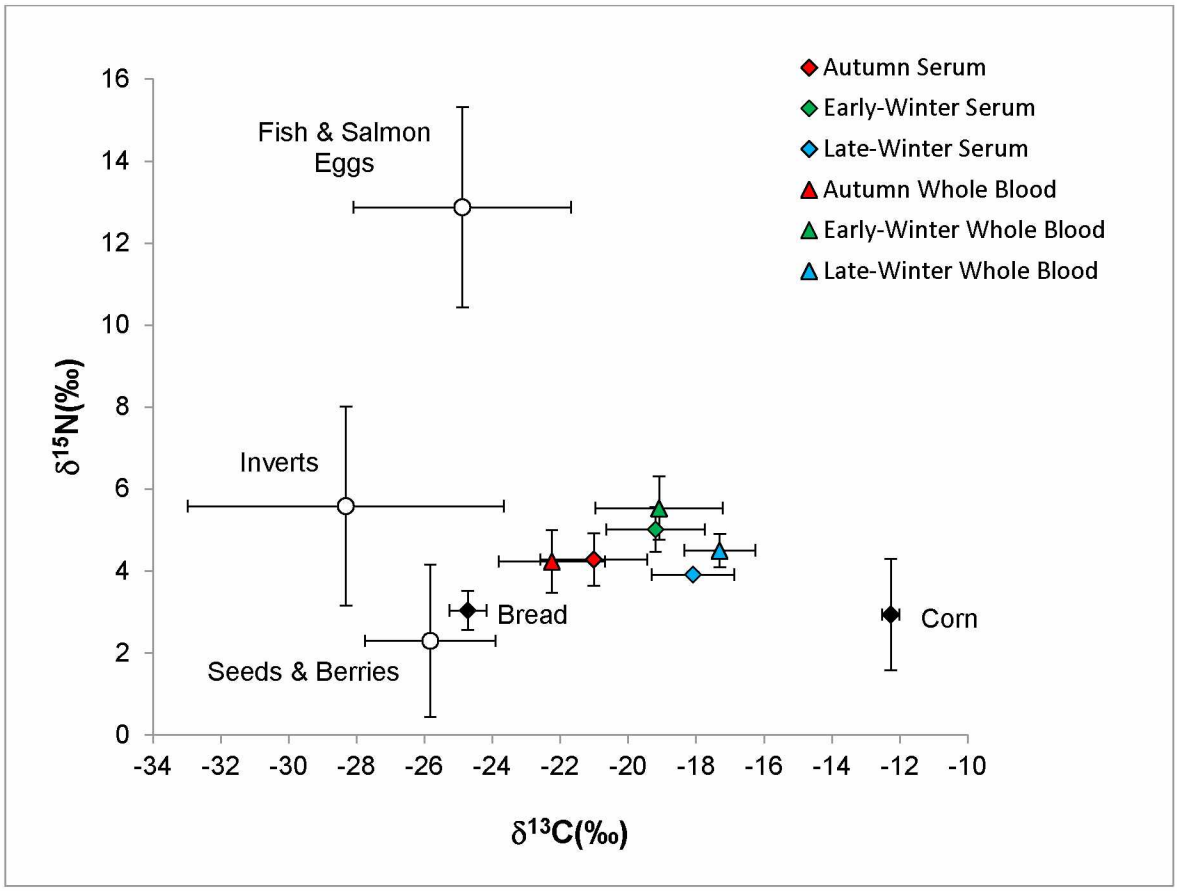

Figure 1.2. Mean $\delta^{13}C$ and $\delta^{15}N$ (±SD) of 2 anthropogenic food groups (black filled circles) and 3 natural food groups (white filled circles), as well as serum and whole blood (color-filled shapes) from mallards wintering in Anchorage, Alaska (2014-2015). All serum (diamonds) and whole blood (triangles) samples are grouped by season and have been adjusted with trophic enrichment factors of ($\Delta\ \delta^{13}C = 0.3\pm0.5‰$ and $\Delta\ \delta^{15}N = 4.4\pm0.6‰$) for serum and ($\Delta\ \delta^{13}C = -0.5\pm0.6‰$ and $\Delta\ \delta^{15}N = 3.6\pm0.5‰$) for whole blood.

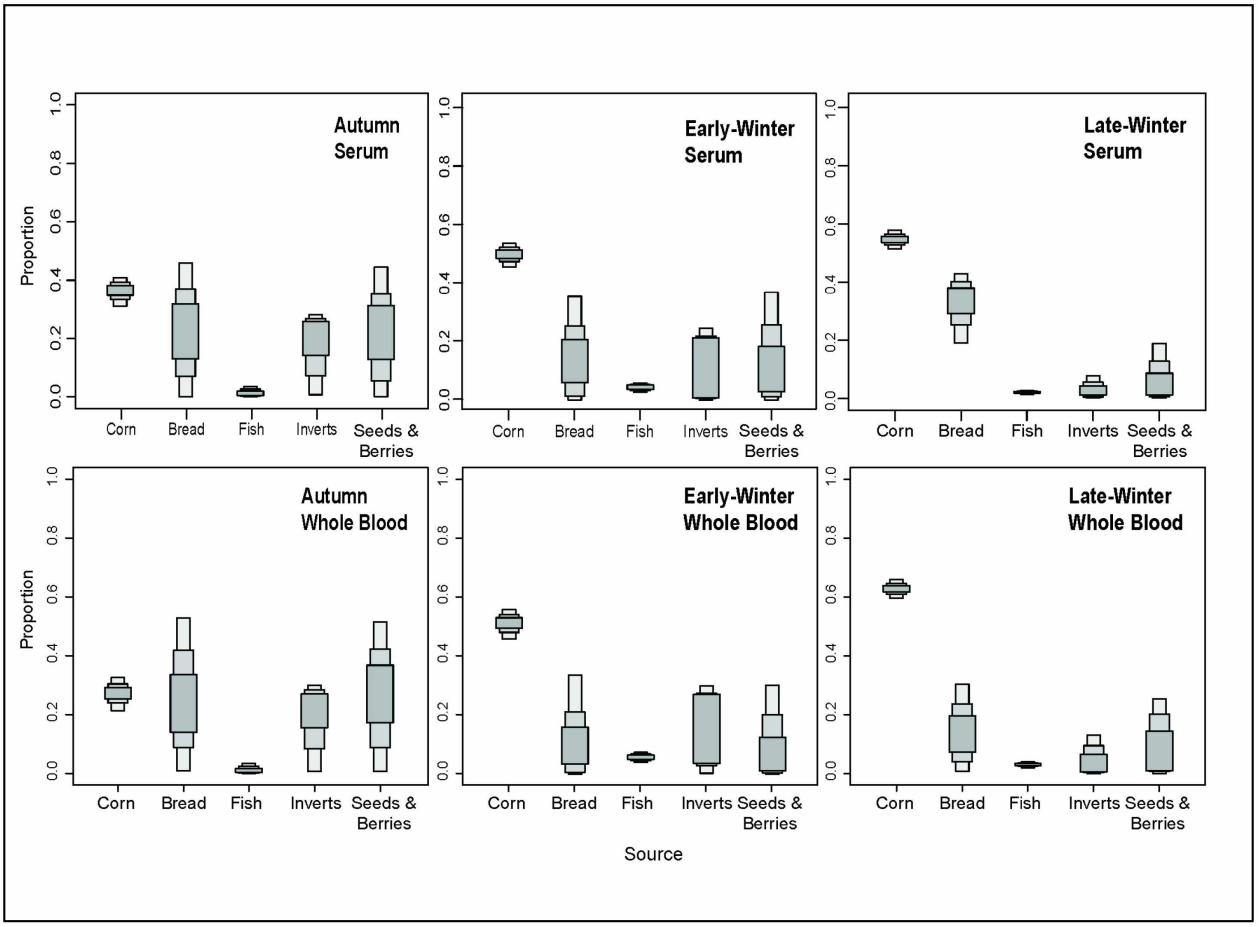

Figure 1.3. Relative proportional contributions of anthropogenic and natural dietary items in the diet of mallards wintering in Anchorage, Alaska (2014-2015). Proportions indicate estimates for whole blood (n=269) and serum (n=279), providing 95th (light gray), 75th (gray) and 25th (dark gray) Bayesian credible intervals for dietary endpoints.

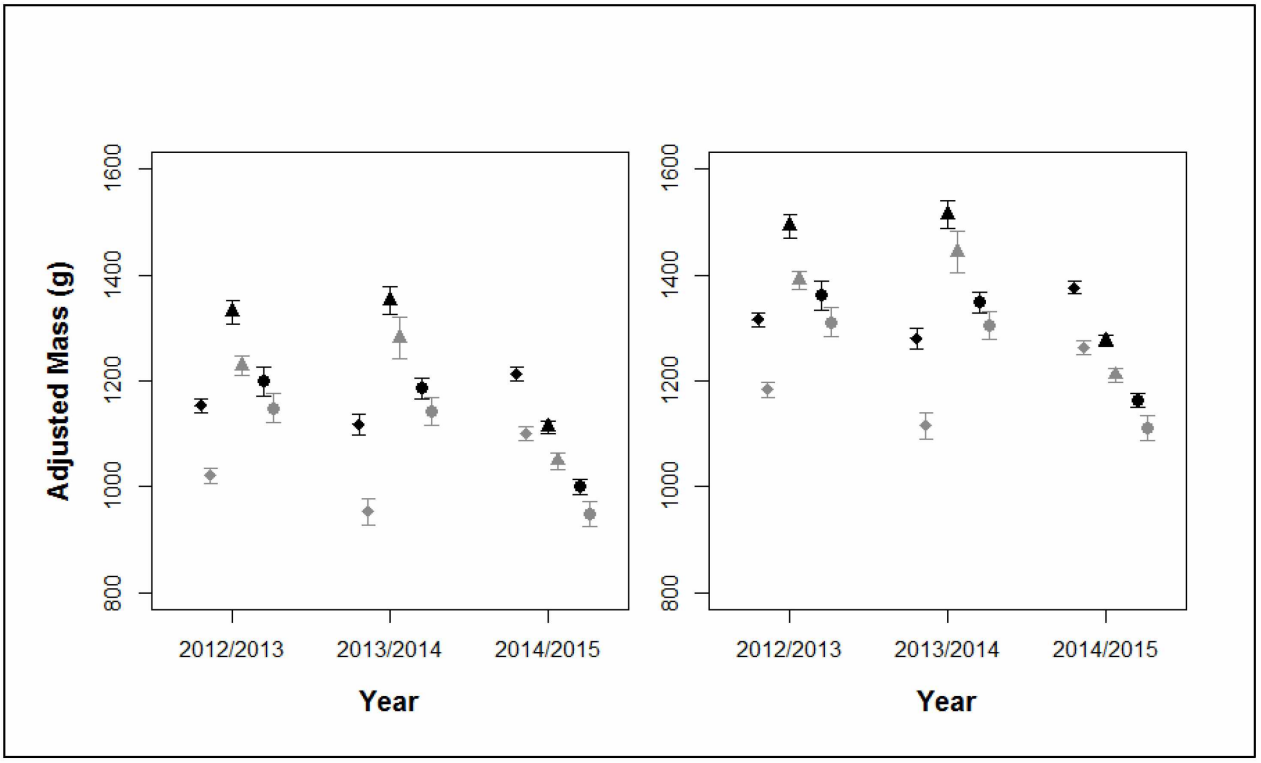

Figure 1.4. Least square mean (±SE) estimates of body mass (g) for female (left plot) and male (right plot) mallards wintering in Fairbanks and Anchorage, Alaska (2012-2015). Black symbols represent adults (AHY) and gray symbols represent juveniles (HY). Diamonds represent autumn (Aug – Oct) estimates, triangles represent early winter (Nov – Jan) estimates, and circles represent late winter (Feb – Apr) estimates.

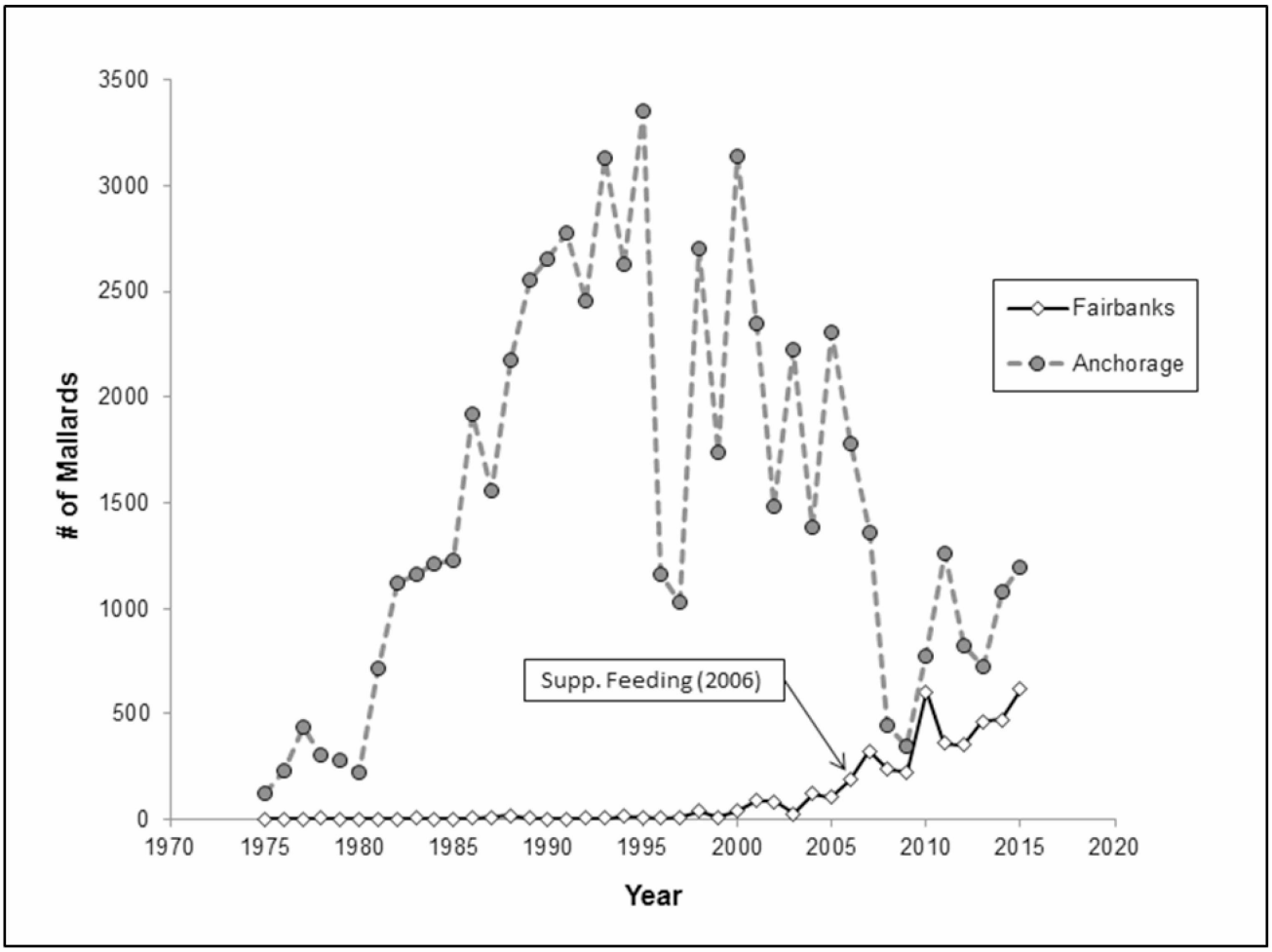

Figure 1.5. The number of mallards observed during annual Christmas Bird Counts wintering in Anchorage and Fairbanks, Alaska from 1975-2015. The box represents the initiation year of a large-scale supplementary feeding program on the Chena River in Fairbanks.

Table 1.1. Sample size and mean isotopic values (±SD) for anthropogenic and natural diet items, as well as serum and whole blood used in SIAR mixing model analysis of diet of mallards wintering in Anchorage, Alaska (2014 – 2015).

Source	Type	*n*	$\delta^{13}C$	SD	$\delta^{15}N$	SD
Anthropogenic	Corn	7	-12.3	0.3	2.9	1.4
	Bread	16	-24.7	0.6	3.0	0.5
Natural	Fish & Salmon Eggs[a]	14	-24.9	3.2	12.9	2.5
	Invertebrates[b]	30	-28.3	4.7	5.6	2.4
	Seeds & Berries[c]	25	-25.8	1.9	2.3	1.9
Mallard	AUT serum[d]	83	-21.3	2.1	8.7	1.2
	E-W serum[d]	113	-19.5	1.9	9.4	1.1
	L-W serum[d]	83	-18.4	1.7	8.3	1.2
	AUT wb[d]	65	-22.7	2.2	7.8	1.3
	E-W wb[d]	111	-19.6	2.5	9.1	1.3
	L-W wb[d]	93	-17.8	1.6	8.1	0.9

[a]Includes: Gasterosteiformes and Salmoniformes; [b]Includes: Odonata, Amphipoda, Diptera, Hemiptera, Annelida, and Polychaeta; [c]Includes: Poales, Alismatales, Rosales. [d]Includes: mallard serum and whole blood (wb) from autumn (AUT; Sept – Oct), early winter (E-W; Nov – Jan), and late winter (L-W; Feb – Apr).

Table 1.2. Model selection results predicting variation in adjusted body mass of mallards wintering in Fairbanks (2012/13; 2013/14) and Anchorage (2014/15), Alaska. We ranked models with lowest Akaike's Information Criterion corrected for sample size (ΔAIC_c) and highest model weight (w_i) relative to others in the candidate model set. We also report the number of parameters (K) and we did not make inference from top approximating models with equivalent structure and one additional parameter.

Model Name	K	Δ AICc	w_i
AdjMass ~ Sex+Age*Season*Year	20	0.00[b]	0.99
AdjMass ~ Sex+Age+Season*Year	12	10.42	0.01
AdjMass ~ Sex*Age+Season*Year	13	12.27	0.00
AdjMass ~ Sex+Season*Year	11	157.93	0.00
AdjMass ~ Sex*Season*Year	19	162.73	0.00
AdjMass ~ Sex*Age*Season+Year	15	279.90	0.00

1.8 Appendix

Appendix 1.A. Model selection results from models evaluating variation in isotope values ($\delta^{13}C$ and $\delta^{15}N$) from serum and whole blood of mallards wintering in Fairbanks and Anchorage, Alaska (2012 – 2015).

Table 1.A-1. Model selection results predicting variation in the $\delta^{13}C$ values of mallard serum collected from mallards wintering in Fairbanks (2012/13; 2013/14) and Anchorage (2014/15), Alaska. We ranked models with lowest Akaike's Information Criterion corrected for sample size (ΔAIC_c) and highest model weight (w_i) relative to others in the candidate model set. We also report the number of parameters (K) and we did not make inference from top approximating models with equivalent structure and one additional parameter.

Model Name	K	Δ AICc	w_i
$\delta^{13}C$ ~ Year*Season	10	0.00	0.64
$\delta^{13}C$ ~ Year*Season+Sex	11	1.20	0.35
$\delta^{13}C$ ~ Year*Season*Sex	19	8.71	0.01
$\delta^{13}C$ ~ Year+Season	6	17.88	0.00
$\delta^{13}C$ ~ Year+Season*Sex	9	23.44	0.00
$\delta^{13}C$ ~ Season	4	33.39	0.00

Table 1.A-2. Beta estimates and standard errors (SE) from the top AICc approximating model assessing variation in the $\delta^{13}C$ values of mallard serum collected from mallards wintering in Fairbanks (2012/13; 2013/14) and Anchorage (2014/15), Alaska.

Covariate	β	SE
Intercept	-22.82	0.27
2013/14	1.72	0.44
2014/15	2.11	0.35
Early winter	4.42	0.49
Late winter	4.79	0.57
(2013/14)*Early winter	-0.89	0.74
(2014/15)*Early winter	-2.61	0.57
(2013/14)*Late winter	-1.20	0.74
(2014/15)*Late winter	-1.87	0.65

Table 1.A-3. Model selection results predicting variation in the $\delta^{13}C$ values of mallard whole blood collected from mallards wintering in Fairbanks (2012/13; 2013/14) and Anchorage (2014/15), Alaska. We ranked models with lowest Akaike's Information Criterion corrected for sample size (ΔAIC_c) and highest model weight (w_i) relative to others in the candidate model set. We also report the number of parameters (K) and we did not make inference from top approximating models with equivalent structure and one additional parameter.

Model Name	K	Δ AICc	w_i
$\delta^{13}C \sim$ Year*Season+Sex	11	0.00	0.88
$\delta^{13}C \sim$ Year*Season*Sex	19	4.91	0.08
$\delta^{13}C \sim$ Year*Season	10	5.81	0.05
$\delta^{13}C \sim$ Year+Season*Sex	9	31.83	0.00
$\delta^{13}C \sim$ Year+Season	6	36.29	0.00
$\delta^{13}C \sim$ Season+Sex	5	59.85	0.00

Table 1.A-4. Beta estimates and standard errors (SE) from the top AICc approximating model assessing variation in the $\delta^{13}C$ values of mallard whole blood collected from mallards wintering in Fairbanks (2012/13; 2013/14) and Anchorage (2014/15), Alaska.

Covariate	β	SE
Intercept	-25.86	0.31
2013/14	0.25	0.43
2014/15	2.75	0.37
Early winter	5.61	0.46
Late winter	6.46	0.55
Males	0.59	0.21
(2013/14)*Early winter	1.11	0.73
(2014/15)*Early winter	-2.54	0.56
(2013/14)*Late winter	0.37	0.72
(2014/15)*Late winter	-1.66	0.64

Table 1.A-5. Model selection results predicting variation in the $\delta^{15}N$ values of mallard serum collected from mallards wintering in Fairbanks (2012/13; 2013/14) and Anchorage (2014/15), Alaska. We ranked models with lowest Akaike's Information Criterion corrected for sample size (ΔAIC_c) and highest model weight (w_i) relative to others in the candidate model set. We also report the number of parameters (K) and we did not make inference from top approximating models with equivalent structure and one additional parameter.

Model Name	K	Δ AICc	w_i
$\delta^{15}N \sim$ Year*Season+Sex	11	0.00	0.84
$\delta^{15}N \sim$ Year*Season	10	4.03	0.11
$\delta^{15}N \sim$ Year+Season*Sex	9	6.90	0.03
$\delta^{15}N \sim$ Year+Season	6	8.28	0.01
$\delta^{15}N \sim$ Year*Season*Sex	19	9.15	0.01
$\delta^{15}N \sim$ Year+Sex	5	67.57	0.00

Table 1.A-6. Beta estimates and standard errors (SE) from the top AICc approximating model assessing variation in the $\delta^{15}N$ values of mallard serum collected from mallards wintering in Fairbanks (2012/13; 2013/14) and Anchorage (2014/15), Alaska.

Covariate	β	SE
Intercept	7.14	0.14
2013/14	-0.54	0.20
2014/15	1.40	0.16
Early winter	0.15	0.22
Late winter	-0.73	0.26
Males	0.23	0.10
(2013/14)*Early winter	-0.22	0.34
(2014/15)*Early winter	0.55	0.26
(2013/14)*Late winter	0.31	0.34
(2014/15)*Late winter	0.31	0.30

Table 1.A-7. Model selection results predicting variation in the $\delta^{15}N$ values of mallard whole blood collected from mallards wintering in Fairbanks (2012/13; 2013/14) and Anchorage (2014/15), Alaska. We ranked models with lowest Akaike's Information Criterion corrected for sample size (ΔAIC_c) and highest model weight (w_i) relative to others in the candidate model set. We also report the number of parameters (K) and we did not make inference from top approximating models with equivalent structure and one additional parameter.

Model Name	K	Δ AICc	w_i
$\delta^{15}N$ ~ Year*Season+Sex	11	0.00	0.91
$\delta^{15}N$ ~ Year*Season	10	4.75	0.08
$\delta^{15}N$ ~ Year*Season*Sex	19	10.90	0.00
$\delta^{15}N$ ~ Year+Season*Sex	9	26.15	0.00
$\delta^{15}N$ ~ Year+Season	6	28.71	0.00
$\delta^{15}N$ ~ Year+Sex	5	78.43	0.00

Table 1.A-8. Beta estimates and standard errors (SE) from the top AICc approximating model assessing variation in the $\delta^{15}N$ values of mallard whole blood collected from mallards wintering in Fairbanks (2012/13; 2013/14) and Anchorage (2014/15), Alaska.

Covariate	β	SE
Intercept	6.41	0.15
2013/14	-0.12	0.21
2014/15	1.26	0.18
Early winter	0.05	0.23
Late winter	-0.51	0.27
Males	0.27	0.10
(2013/14)*Early winter	-0.25	0.36
(2014/15)*Early winter	1.21	0.27
(2013/14)*Late winter	-0.08	0.35
(2014/15)*Late winter	0.71	0.31

Appendix 1.B. Results of SIAR Bayesian stable isotope diet models for adult mallards wintering in Anchorage, Alaska (2014 – 2015).

Table 1.B-1. SIAR Bayesian mixing model dietary proportions for pooled diet item groups, using stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) from mallard serum. Mallards were captured from August – April of 2014/15 in Anchorage. Season refers to the proportion of diet items for mallards captured during autumn (Sept – Oct), early winter (Nov – Jan), and late winter (Feb – Apr). Residual error terms representing nitrogen (SD1) and carbon (SD2) are provided for each season.

Source	Season	LCI	UCI	Mode	Mean
Corn	Autumn	0.31	0.41	0.36	0.36
Bread	Autumn	<1	0.46	0.24	0.24
Fish	Autumn	<1	0.04	0.01	0.02
Inverts	Autumn	<1	0.28	0.22	0.16
Seeds & Berries	Autumn	<1	0.45	0.22	0.23
SD1G1	Autumn	0.04	1.20	0.93	0.40
SD2G1	Autumn	1.41	2.32	1.87	1.86
Corn	Early winter	0.45	0.54	0.50	0.50
Bread	Early winter	0.00	0.35	0.10	0.17
Fish	Early winter	0.3	0.06	0.5	0.04
Inverts	Early winter	0.00	0.24	0.02	0.13
Seeds & Berries	Early winter	0.00	0.37	0.07	0.17
SD1G2	Early winter	0.33	0.99	0.74	0.59
SD2G2	Early winter	1.43	2.15	1.79	1.80
Corn	Late winter	0.51	0.58	0.55	0.55
Bread	Late winter	0.19	0.43	0.34	0.31
Fish	Late winter	0.01	0.03	0.02	0.02
Inverts	Late winter	0.00	0.08	0.02	0.04
Seeds & Berries	Late winter	0.00	0.19	0.05	0.08
SD1G3	Late winter	0.00	0.31	0.03	0.13
SD2G3	Late winter	1.41	1.98	1.66	1.69

Table 1.B-2. SIAR Bayesian mixing model dietary proportions for pooled diet item groups, using stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) from mallard whole blood. Mallards were captured from August – April of 2014/15 in Anchorage. Season refers to the proportion of diet items for mallards captured during autumn (Sept – Oct), early winter (Nov – Jan), and late winter (Feb – Apr). Residual error terms representing nitrogen (SD1) and carbon (SD2) are provided for each season.

Source	Season	LCI	UCI	Mode	Mean
Corn	Autumn	0.21	0.33	0.27	0.27
Bread	Autumn	<1	0.53	0.23	0.27
Fish	Autumn	<1	0.03	<1	0.02
Inverts	Autumn	<1	0.30	0.22	0.17
Seeds & Berries	Autumn	<1	0.52	0.25	0.27
SD1G1	Autumn	0.04	1.25	0.96	0.73
SD2G1	Autumn	1.38	2.45	1.92	1.92
Corn	Early winter	0.46	0.56	0.51	0.51
Bread	Early winter	0.00	0.34	0.08	0.15
Fish	Early winter	0.04	0.07	0.05	0.06
Inverts	Early winter	<1	0.30	0.25	0.16
Seeds & Berries	Early winter	0.00	0.30	0.06	0.13
SD1G2	Early winter	0.07	1.15	0.89	0.72
SD2G2	Early winter	1.86	2.75	2.33	2.32
Corn	Late winter	0.60	0.66	0.63	0.63
Bread	Late winter	<1	0.30	0.11	0.15
Fish	Late winter	0.02	0.04	0.03	0.03
Inverts	Late winter	0.00	0.13	0.03	0.06
Seeds & Berries	Late winter	0.00	0.25	0.06	0.13
SD1G3	Late winter	0.00	0.57	0.12	0.27
SD2G3	Late winter	1.29	1.84	1.52	1.56

Appendix 1.C. Regression output from models evaluating variation in body condition of mallards wintering in Fairbanks and Anchorage, Alaska (2012 – 2015).

Table 1.C-1. Beta estimates and standard errors (SE) from the top AICc approximating model assessing variation in adjusted body mass of mallards wintering in Fairbanks (2012/13; 2013/14) and Anchorage (2014/15), Alaska. We ranked models with lowest Akaike's Information Criterion corrected for sample size (ΔAIC_c) and highest model weight (w_i) relative to others in the candidate model set. We also report the number of parameters (K) and we did not make inference from top approximating models with equivalent structure and one additional parameter.

Covariate	β	SE
Intercept	1155.04	13.49
Males	159.70	8.12
HY	-132.95	18.08
Early winter	176.99	23.98
Late winter	45.9	29.19
2013/14	-10.52	22.03
2014/15	61.00	16.58
HY*Early winter	31.16	31.73
HY*Late winter	82.78	41.21
HY*(2013/14)	-57.65	34.65
HY*(2014/15)	19.14	23.99
Early winter*(2013/14)	31.75	38.66
Late winter*(2013/14)	-2.38	39.10
Early winter*(2014/15)	-277.49	28.13
Late winter*(2014/15)	-259.06	33.44
HY*Early winter*(2013/14)	88.07	62.28
HY*Late winter*(2013/14)	62.14	59.47
HY*Early winter*(2014/15)	17.66	38.93
HY*Late winter*(2014/15)	-22.17	50.96

Chapter 2. Maintenance of low-pathogenic influenza A viruses and antibody response in high-latitude urban wintering mallards (*Anas platyrhynchos*)[2]

2.1 Abstract

Prevalence of influenza A virus (IAV) infections in northern-breeding waterfowl has previously been reported to reach an annual peak during late summer or autumn; however, little is known about IAV infection dynamics in waterfowl populations persisting at high-latitude regions, such as Alaska, during winter. We captured mallards (*Anas platyrhynchos*) throughout the non-breeding season (August – April) of 2012 – 2015 in Fairbanks and Anchorage, two major cities in Alaska, to assess patterns of IAV infection and antibody production using molecular methods and a standard serologic assay. In addition, we used virus isolation, genetic sequencing, and a virus microneutralization assay to characterize viral subtypes and to evaluate the immune response of mallards captured on multiple occasions through time. We captured 923 mallards during three successive sampling years: Fairbanks in 2012/13 and 2013/14, and Anchorage in 2014/15. Prevalence varied by age, season, and year/site with high and relatively stable estimates throughout the non-breeding season. Infected birds were detected in all locations/seasons except early winter in Fairbanks during 2013/14. IAVs with 17 combinations of hemagglutinin (H1 – 5, H7 – 9, H11, H12) and neuraminidase (N1 – 6, N8, N9) subtypes were isolated. Antibodies to IAVs were detected throughout the autumn and winter for all sampling locations and years; however, seroprevalence was higher among adults and varied among years. Mallards exhibited individual heterogeneity with regard to immune response, providing instances

[2]Prepared for submission to PLOS ONE as Spivey, T. J., M. S. Lindberg, B. W. Meixell, K. R. Smith, W. B. Puryear, K. R. Davis, J. A. Runstadler, D. E. Stallknecht, and A. M. Ramey. 2017. Maintenance of Low-Pathogenic Influenza A Viruses and Antibody Response in High-Latitude Wintering Mallards (*Anas platyrhynchos*)

of both seroconversion and seroreversion to detected viral subtypes. The probability than an individual transitioned from one serostatus to another varied by age, with juvenile mallards having higher rates of seroconversion and seroreversion than adults. Our study provides evidence that a diversity of IAVs circulate in populations of mallards wintering at urban locations in Alaska, and we suggest waterfowl wintering at high latitudes may play an important role in maintenance of viruses across breeding seasons.

2.2. Introduction

Extensive surveillance sampling for influenza A viruses (IAVs) and IAV antibodies at high-latitude regions such as Alaska throughout summer and autumn has helped to elucidate patterns of viral dynamics in migratory birds [1–3]. For waterfowl, a major reservoir host of IAVs [4], late summer/early autumn is generally considered the period of peak IAV prevalence in dabbling duck (family Anatidae, tribe Anatini) populations, as birds congregate in high densities prior to migration [5, 6]. During this time, a seasonal increase in the proportion of immunologically naïve juvenile birds, and increased bird-to-bird interactions during staging, leads to efficient transmission and dispersal of viruses through migration [7]. However, as migrating waterfowl reach lower-latitude wintering areas, prevalence declines as the proportion of individuals with antibody protection increases [8, 9]. Since most waterfowl species depart to more southerly staging and wintering areas, considerably less attention has focused on surveillance of waterfowl remaining at high-latitude locations during late autumn and winter. Consequently, limited empirical data exist to assess the potential maintenance of IAVs in biotic (wild birds) or abiotic (waterbird habitats) viral reservoirs in high-latitude regions during the non-breeding season.

Several previous investigations have explored the perpetuation of IAVs in Alaska between breeding seasons; however, the potential for resiliency of viruses to overwinter is still poorly understood. In an investigation conducted in interior and coastal wetlands of Alaska, Ito et al. [10] isolated IAVs from lake water, and therefore speculated that IAVs could potentially be maintained in Alaskan lakes overwinter. Yet, most of the lakes were sampled in late summer/autumn when ducks were present at wetlands and therefore the ability of viruses to remain viable overwinter in Alaskan wetlands was not clearly demonstrated. Lang et al. [11] amplified IAV gene segments from pond sediment samples collected monthly throughout winter at an interior Alaskan wetland. However, despite identifying a diverse array of hemagglutinin (HA) viral subtypes from RNA extracted from sediment samples, the authors were unable to isolate viable IAVs [11]. Using genetic approaches, two investigations [7, 12] identified highly similar IAVs infecting dabbling ducks across years at multiple locations in Alaska, suggesting viral persistence may take place between breeding seasons. Furthermore, the relative importance of viral maintenance in Alaska during winter versus the introduction of IAVs by migrating waterfowl arriving in spring remains unclear. Thus, these studies collectively provide support for potential interannual persistence of IAVs in Alaska; however the mechanism(s) maintaining viruses between breeding seasons at high-latitude locations remain unresolved.

Waterfowl wintering in Alaska have only recently been targeted as part of IAV surveillance efforts, despite the potential for viral maintenance in these populations [13]. Ecological alterations, such as warm water discharges and anthropogenic food subsidies provided in two major cities of Alaska have created open-water habitats with sufficient resources for birds to overwinter. For example, the number of mallards (*Anas platyrhynchos*) observed during winter in Fairbanks and Anchorage, Alaska, has increased substantially in recent years

[14]. Our principal objective was to better understand the potential role of wintering waterfowl in the maintenance of IAVs at high-latitude regions of North America and to gain insights into how population immunity may relate to viral dynamics. We collected swab and serum samples from mallards over the course of three non-breeding seasons at two urban locations in Alaska to quantify: (1) seasonal rates of IAV infection and antigenic diversity of viruses infecting birds, (2) variation in population IAV seroprevalence, (3) the individual immune response of recaptured individuals previously exposed to an IAV, and (4) probabilities of individuals to develop (seroconversion) or lose (seroreversion) detectable antibodies to an IAV during the non-breeding season.

2.3 Materials and Methods

2.3.1 Ethics Statement

Capture and processing of wild mallards was approved by the Institutional Animal Care and Use Committee at the University of Alaska Fairbanks (UAF; 358515-11/662280-3) and was authorized by U.S. Federal Bird Banding and Marking Permits (#08350 and #23191). All mallards were released at original capture locations after handling.

2.3.2 Sample Locations and Years

During August – April of 2012/13 and 2013/14, mallards were captured on the Chena River in Fairbanks, Alaska. This section of the Chena River remains open throughout winter due to warm water effluent from a local power plant. Approximately 400 – 600 mallards wintered in this location during the study period [14]. We also captured mallards at Westchester Lagoon and Cuddy Midtown Park in Anchorage, Alaska, from September – April of 2014/15. An estimated 1200 – 1500 mallards overwintered in Anchorage during our study [14], where they frequently

concentrated in small areas of open freshwater on municipal parklands and coastal marsh habitats. Mallards were captured using swim-in bait traps, walk-in bait traps, whoosh nets, and net guns. We determined the sex and age of captured birds based on cloacal and feather examination [15, 16], and marked all birds with U.S. Geological Survey metal leg bands.

2.3.3 Influenza A Virus RNA Detection and Virus Isolation

Cloacal and oropharyngeal swabs were collected from mallards during initial and subsequent captures using sterile polyester tipped swabs. Swabs samples were placed into viral transport media (VTM) (M4RT from Remel Inc., Lenexa, KS, USA) and immediately stored at -80°C (or below) until processing. Viral RNA was extracted from 50 µl of VTM sample using the Omega Mag-Bind Viral DNA/RNA kit (Omega Bio-Tek, Norcross, GA, USA) and a Kingfisher Magnetic Particle Processor (Thermo Scientific, Waltham, MA, USA). RNA was screened using qScript XLT One-Step RT-qPCR ToughMix (Quanta Biosciences, Gaithersburg, MD, USA) and analyzed for fluorescence on an ABI 7500 real-time PCR System (Applied Biosystems, Foster City, CA, USA) for a conserved IAV matrix gene segment (MA) target, as previously described [17]. Samples producing cycle threshold (Ct) values ≤ 45 were considered positive for IAV RNA and individual birds were considered infected if either a cloacal or oropharyngeal swab was determined as being positive for IAV RNA. Positive samples were inoculated into the allantoic cavity of 10 day old embryonated chicken eggs (ECEs) (Charles River, CT, USA), and incubated at 37°C for 72 hours. RNA was extracted from 50 µl of amnio-allantoic fluid (AAF) and screened for the IAV MA gene as described above. Whole genome sequencing was performed on RNA from IAV positive AAF ($Ct \leq 45$) at either the Massachusetts Institute of Technology BioMicro Center in Cambridge, MA or the J. Craig Venter Institute in Rockville, MD, as previously described [18]. Each isolate was assigned an HA and neuraminidase (NA) subtype

based on the highest percentage identity for respective gene segments using the nucleotide BLAST function on GenBank.

2.3.4 Serum Collection and Analysis

We obtained approximately 1.5 ml of whole blood from the jugular vein of mallards during initial capture and on re-capture occasions if birds had not been bled within the previous 7 days. Blood samples were separated through centrifugation and serum was stored at -20°C until analysis. Sera samples were screened for antibodies to the IAV nucleoprotein (NP) gene segment [19, 20] using a commercially available blocking enzyme-linked immunosorbent assay (bELISA; AI MultiS-Screen Avian Influenza Virus Antibody Test Kit; IDEXX Laboratories, Westbrook, Maine, USA) following the manufacturer's instructions. We considered sample to negative control ratio (S/N) values less than 0.5 as positive based on the manufacturer's recommendations. Alternative threshold values (0.6 – 0.7) may increase the sensitivity of the assay [19, 21], however this change is accompanied by a slight decrease in specificity, and we prioritized the 100% specificity of this assay for detection of antibodies to IAVs.

We used virus microneutralization (MN) assays to H1 – H12 IAV subtypes to further characterize the individual immune response of mallards found to be seropositive on multiple capture occasions. Antigens for MN assays were prepared in Madin Darby Canine Kidney cells (MDCK; American Type Culture Collection, Manassas VA, USA). During virus propagation, and in all MN assay procedures, cells were maintained in minimal essential media (MEM; Sigma-Aldrich, St. Louis MO, USA) containing TPCK-trypsin (final concentration of 1µg/ml; Worthington Biochemical Corporation, Lakewood, NJ, USA) and antibiotics (final concentration of 100 units penicillin, 0.1mg streptomycin, and 0.25 µg amphotericin B/ml; Sigma-Aldrich). Antigen was stored at -80°C until used. For antibody testing, sera were diluted 1:10 in MEM and

heat inactivated at 57°C for 30 minutes. Serum samples were screened at a 1:20 dilution against all antigens. For the screen, 25 µl of the diluted serum (1:10) were placed in a single well of a 96-well v-bottom plate corresponding to each antigen. An additional well for each serum sample served as a serum control to determine potential toxicity. A positive control well using chicken antisera to each antigen (provided by the National Veterinary Services Laboratory, APHIS, USDA) and a negative control well using MEM were also included. Each antigen (25 µl containing 100 median tissue culture infective doses [$10^{2.0}$ $TCID_{50}$]) was added to each well, not including the serum control wells, which received 25 µl MEM. Plates were incubated for 2 hr at room temperature after which 25 µl from each well was transferred to a second 96-well tissue culture plate with a confluent monolayer of MDCK cells. Prior to transfer, the tissue culture plate containing the MDCK cells was washed two times with Dulbecco's phosphate buffered saline (Sigma-Aldridge) and 150 µl of trypsin supplemented MEM was added to each well. The inoculated tissue culture plate was incubated at 5% CO_2 at 37°C and was visually read at 72 hours. For the test result to be considered valid, all controls (serum, positive, and negative) had to meet their expected negative or positive status. In addition, based on back titration in MDCK cells (four replicates per dilution), the viral titer of the antigen had to fall within $10^{1.5}$ and $10^{2.5}$ $TCID_{50}$/25 µl. Sera were considered positive on the screen if no cytopathic effect (CPE) was observed. All positive serum samples were titrated. Each positive serum sample was diluted two-fold in MEM on a 96 well v-bottom plate (final volume of 25 µl. well at dilutions 1:20 to 1:640) and tested as described above. If CPE was observed at the minimum 1:20 dilution, the sample was classified as negative; if not, the positive titer was recorded as the highest dilution at which no CPE was observed. Viruses used as antigens in the MN assays included A/mallard/MN/AI12-4297/2012 (H1N1), A/mallard/MN/AI08-2755/2008 (H2N3), A/mallard/MN/AI10-2593/2010

(H3N8), A/mallard/MN/AI10-3208/2010 (H4N6), A/mallard/MN/AI11-3933/2011 (H5N1), A/mallard/MN/AI08-2721/2008 (H6N1), A/mallard/MN/AI08-3770/2009 (H7N9), A/mallard/MN/SG-01048/2008 (H8N4), A/RUTU/DE/AI11-809/2011 (H9N2), A/mallard/MN/SG-00999/2008 (H10N7), A/mallard/MN/SG-00930/2008 (H11N9), and A/mallard/MN/SG-3285/2007 (H12N5).

2.3.5 Statistical Analyses

We used generalized linear logistic regression models and an information-theoretic approach to assess patterns of variation in IAV prevalence and seroprevalence. We considered variation relative to month and season, with seasons defined as: autumn (August – October), early winter (November – January), and late winter (February – April). In each analysis, we randomly selected one capture occasion from each season for individuals captured on multiple occasions. To assess differences in IAV infection status and seroprevalence associated with host age, we defined two distinct age classes for mallards, with birds characterized as being either juvenile (hatched the previous summer; HY) or adult (> 1 year old; AHY). Our candidate model sets assessing sources of variation in IAV prevalence and seroprevalence consisted of 56 models which contained various additive and multiplicative effects of the variables age, sex, monthly trend, season, and year. Model support was evaluated using Akaike's Information Criterion corrected for sample size [AICc; 22]. We eliminated models with equivalent structure and one additional parameter from consideration and in the case of model selection uncertainty, we present model-averaged estimates from supported models within 4 ΔAIC of the top approximating model [23].

We used multi-state models in Program MARK [24, 25] to estimate the probability of seroconversion or seroreversion to IAVs for mallards sampled in Anchorage (the location with

the largest sample size). This analysis included capture histories of the 82 individuals captured on at least 2 distinct occasions. We estimated state transition probabilities (Ψ_i) and capture probability (p_i), using a two-stage approach. In the first stage, we constrained Ψ to a highly parameterized structure and considered 4 models explaining variation in p; these models allowed p to vary relative to age, sex, and a monthly trend. In the second stage, we fixed p to the top supported structure from stage 1 and considered 4 models to assess competing hypotheses regarding sources of variation in Ψ. Survival was held constant in all models. Estimates were back-transformed from the logit link and are presented ± SE unless otherwise specified.

To gain inference on trends in antibody titers through time, we plotted the change in titer for antibodies to H1 – H12 HA subtype viruses for recaptured individuals inferred as being seropositive on two or more occasions. We randomly selected two capture occasions to assess subtype-specific antibody changes for seropositive individuals with more than two captures during the 2014 – 2015 sampling year. We plotted trendlines for specific HA subtype antibodies if detections occurred in multiple months across the study period and considered antibody titers changing ≤ 2 log titers as "stable" and titers changing by > 2 log titers as either increasing or decreasing. Associated coefficient of determination (R^2) values and (Δlog) titer values were estimated using the R environment [26].

2.4 Results

2.4.1 IAV Prevalence and Subtype Diversity

We detected IAV RNA in 134 of 1182 cloacal swab samples and 67 of 1186 oropharyngeal swabs collected from 923 mallards captured in Fairbanks and Anchorage over the course of our study. After reducing our dataset to samples from initial captures and one randomly

selected recapture event in other seasons, our summary of IAV prevalence included 1062 paired swab samples with an apparent prevalence of 17% (Table 2.1). In our analysis of IAV prevalence, the top AICc approximating model ($w = 0.43$) allowed prevalence to vary by age, season, year, and the interaction between season and year (Table 2.2). A model with the same variables, but containing an age*season interaction term, also received substantial support ($w = 0.17$; Table 2.2); we present model averaged predictions from these two models.

Estimated IAV prevalence was higher in juveniles than adults, especially during the autumn season (Fig 2.1; Table 2A-1). During 2012/13 in Fairbanks, estimated IAV prevalence remained relatively stable between autumn and late winter (Fig 2.1). Prevalence estimates for this sampling location/year were lower for both age classes during autumn (AHY: 0.11 ± 0.03, HY: 0.21 ± 0.04) as compared to late winter (AHY: 0.24 ± 0.10, HY: 0.30 ± 0.11). During 2013/14 field season in Fairbanks, estimated IAV prevalence for both age classes was similar in autumn (AHY: 0.13 ± 0.04, HY: 0.24 ± 0.06) and late winter (AHY: 0.12 ± 0.04, HY: 0.22 ± 0.06) but we did not detect any positive samples from the 74 birds sampled during early winter (Fig 2.1). In Anchorage during 2014/15 sampling year, IAV prevalence declined from autumn to early and late winter (Fig 2.1). Prevalence was higher for juveniles (HY: 0.39 ± 0.04) than adults (Fig 2.1; AHY: 0.22 ± 0.03) during autumn, but this difference was less pronounced during early winter (AHY: 0.07 ± 0.02, HY: 0.13 ± 0.03) and late winter (AHY: 0.10 ± 0.03, HY: 0.18 ± 0.06).

Virus isolation yielded isolates from 6% of the 1062 mallards included in the analysis of IAV prevalence, with isolates from 3% of mallards in 2012/13, 5% of mallards from 2013/14, and 8% of mallards during 2014/15 (Table 2.1). Across sampling years, only H3N8 was isolated in every year of the study and H4N6 and H12N5 were isolated from mallards in Fairbanks during

2013/14 and Anchorage in 2014/15 (Fig 2.1). In Fairbanks, additional IAV subtypes isolated included H2N4, H2N9, and H8N4 during 2012/13 and H11N3 during 2013/14 (Fig 2.1). In Anchorage during 2014/15, additional IAV subtypes isolated included H1N1, H2N1, H2N3, H5N2, H7N3, H9N2, and H11N9 (Fig 2.1).

2.4.2 Antibody Prevalence

We detected antibodies to IAVs in 495 of 1061 serum samples from mallards in Fairbanks and Anchorage collected over three successive sampling years. After reducing our dataset to samples from initial captures and a randomly selected recapture event in other seasons, our summary of IAV seroprevalence included 984 serum samples from 897 individuals, yielding an apparent seroprevalence of 46%. Antibodies to IAV were detected in 43% of the samples from 2012/13, 35% of the samples from 2013/14, and 51% of samples from 2014/15 (Table 2.1).

Our top AICc approximating model ($w = 0.67$) indicated support for variation in seroprevalence relative to age, sex, season, year, and the interaction between season and year (Table 2.3). Seroprevalence estimates were higher for adults than juveniles and higher for males than females (Fig 2.2; Table 2A-2). Across seasons and years, estimates of IAV seroprevalence were highest in adults (M: 0.72 ± 0.04, F: 0.64 ± 0.05) during autumn of 2012/13 and lowest in juveniles (M: 0.05 ± 0.03, F: 0.03 ± 0.02) during early winter of 2013/14. During 2012/13 in Fairbanks, IAV seroprevalence declined through late winter in both adult (M: 0.36 ± 0.09, F: 0.27 ± 0.08) and juvenile (M: 0.36 ± 0.09, F: 0.27 ± 0.08) mallards (Fig 2.2). During 2013/14 in Fairbanks, IAV seroprevalence estimates were higher in both autumn (M: 0.20 ± 0.05, F: 0.14 ± 0.04) and late winter (M: 0.38 ± 0.07, F: 0.29 ± 0.06) as compared to early winter (Fig 2.2). During 2014/15 in Anchorage, estimates of IAV seroprevalence remained higher and more stable across all seasons than during two autumn/winters in Fairbanks (Fig 2.2).

2.4.3 Serostatus State Transitions

Of the 82 mallards recaptured in Anchorage, 63 individuals maintained the same serostatus, whereas 19 individuals transitioned from one serostatus to the other (seroconversion = 8, seroreversion = 11; Table 2.4). The top AICc approximating model indicated support for variation in capture probability by a monthly trend; the probability of state transition varied relative to age (Tables 2B-1). The probabilities of seroconversion (0.19 ± 0.07) and seroreversion (0.54 ± 0.14) for juveniles were considerably higher than for adults (seroconversion = 0.05 ± 0.03, seroreversion = 0.05 ± 0.03; Table 2.5, 2B-2).

2.4.4 Virus MN Assay

Of 284 birds recaptured over the course of our study, 39 were seropositive on at least two capture occasions within the same year. Eight of these individuals were sampled in Fairbanks during 2012/13 and 31 individuals were sampled in Anchorage during 2014/15. The single individual recaptured during 2013/14 in Fairbanks was excluded from analysis. For recaptured mallards sampled in Fairbanks in 2012/13, we had sufficient data to assess trends in antibody titers for six HA viral subtypes (Fig 2.3). Antibody titers to H5 subtype IAVs appeared to decrease between capture occasions, whereas antibody titers to other IAV subtypes (H3, H4, H7, H11, H12) were inferred to be stable (≤ 2 log titer change) through time. A larger number of recaptured mallards in Anchorage in 2014/15 allowed us to assess trends in antibody titers to 12 HA subtype viruses (Fig 2.4). For Anchorage mallards, antibody titers appeared to remain stable to the majority (9/12) of H1 – 12 HA viral subtypes between capture occasions. The three exceptions were an apparent decline in antibody titers to H3 HA subtype viruses and increases in antibody titers to H7 and H8 HA subtype viruses over the course of the 2014/15 sampling period (Fig 2.4).

2.5 Discussion

2.5.1 Viral Prevalence

In this study, we present information on the dynamics of IAVs within mallard populations at the northern extent of their winter distribution, providing evidence for perpetuation of IAVs throughout the non-breeding period. Our estimates of IAV prevalence for mallards during autumn (Aug – Oct) are similar to those reported by previous studies of dabbling ducks in Alaska [2, 27, 28], however we also provide evidence for relatively high rates of IAV infection throughout early winter (Nov – Jan) and late winter (Feb – Apr). Thus, waterfowl wintering at high latitudes may be infected with IAVs in late winter (Feb – Apr) similar to or perhaps even higher than reported in previous studies of North American and European waterfowl at lower latitudes [29–32].

In agreement with previous studies investigating IAV dynamics in post-breeding waterfowl [5, 6], our results indicate that prevalence during autumn was higher for juvenile mallards than adults, which is congruent with susceptibility of immunologically naïve juvenile ducks to IAVs post-fledging [33]. The effect of age remained throughout the non-breeding season, although age-specific differences in prevalence estimates were less pronounced during early (Nov – Jan) and late winter (Feb – Apr). This suggests that IAV prevalence approaches equivalence among waterfowl age classes through time which may be a function of adaptive immune responses of juvenile birds more closely resembling those of adults as they are repeatedly exposed to locally circulating IAVs throughout the non-breeding period [34]. Site-specific prevalence estimates were comparable, but we isolated a broader diversity of IAV subtypes in Anchorage, where our rate of virus isolation was higher as compared to Fairbanks. This may be a function of differences in the populations in which IAVs are maintained or

environmental conditions facilitating resiliency of IAVs throughout the winter period. In Fairbanks, 400 – 600 mallards used a ~2 km section of open water on the Chena River, and tended to concentrate at the location where supplemental feed was provided daily. In Anchorage, a larger number of mallards (1200 – 1500) used a number of small ponds and several riverine locations over a much broader area, and birds were frequently observed traveling between locations throughout the day. The larger population of mallards in Anchorage combined with a more variable distribution of individuals among habitats may have supported a greater diversity of viruses. Furthermore, the proximity to marine habitats and direct connectivity to other areas with wintering mallard populations may have increased the likelihood for introduction of viruses in the Anchorage population. Alternatively, differences in environmental conditions may have contributed to the seasonal/geographic variation in virus isolation results. Because we did not record variables such as water temperature, pH, and salinity, which may affect the perpetuation of IAVs in aquatic reservoirs [35–37], our strength of inference regarding the role of environmental factors in perpetuation and transmission of IAVs at the Chena River in Fairbanks and capture locations in Anchorage is limited.

2.5.2 Antibody Prevalence

Seroprevalence was consistently elevated during autumn (Aug – Nov) in comparison to other seasons (early & late winter) across all three years of the study. Additionally, seasonal variation was more pronounced in Fairbanks than in Anchorage and geographic variation in seroprevalence may reflect location-specific differences in viral dynamics between the two sample populations. For example, our estimates of IAV prevalence in mallards were lower in both years in Fairbanks during autumn than for mallards in Anchorage. Thus, we might expect that population immunity in Fairbanks birds following the presumptive peak in prevalence would

also be lower during the early winter and late winter seasons. Alternatively, we may have missed the peak in IAV prevalence during our discrete sampling efforts, resulting in the mismatch between prevalence and seroprevalence patterns during both years in Fairbanks. Consequently, IAV seroprevalence did not exhibit an expected increase following autumn peaks in viral prevalence in Fairbanks, whereas seroprevalence remained high in both early and late winter during the final year of the study in Anchorage.

Seroprevalence estimates were higher for adults than juveniles, consistent with findings of other investigations of IAV seroprevalence in waterfowl [3, 38, 39]. Previous exposure to IAVs, likely resulting in a long-lasting immune response, presumably contributed to age specific differences in seroprevalence [40–42]. We also found support for sex-specific variation in seroprevalence, with higher estimates for males than females. Because of differences in the timing and location of our study in comparison to other IAV seroprevalence studies of waterfowl [3, 38, 39], seasonal and geographical variation may have contributed to our higher seroprevalence estimates for males rather than females. Furthermore, our higher seroprevalence estimates from adult male mallards in this study are consistent with a limited sample obtained during late-summer from a nearby interior Alaska breeding location [28]. Therefore, sex-specific seroprevalence patterns in dabbling ducks may ultimately be influenced by life-history characteristics of the population sampled. For example, during winter, females must acquire sufficient nutrient stores in preparation for each potential breeding effort. Despite consistent availability of anthropogenic foods at our capture locations, which may potentially afford more energy for immune responses to circulating IAVs [43], lower seroprevalence estimates of females in our study may reflect a trade-off between increased immunity and increased body condition required for the upcoming breeding season.

2.5.3 Probability of Seroconversion/reversion

We found support for age-specific differences in the probabilities of seroconversion and seroreversion, with juveniles having higher probabilities for both rates than adults. Because most recaptured individuals maintained their serostatus across capture occasions, the elevated rates of seroconversion/reversion for juvenile mallards suggests age-specific differences in the duration of antibody production. This finding support results from captive studies suggesting that immunologically naïve juvenile mallards produce a shorter-duration immune response than adults previously exposed to a similar antigen [44]. Furthermore, low estimates of seroconversion/seroreversion rates for adults suggest that antibody responses in mature birds may be relatively long-lived. Sample size restrictions limited our ability to test for geographic and sex variation relative to seroconversion/reversion rates, yet our age-specific estimates for seroconversion and seroreversion are lower in comparison to most within-year estimates reported for other species of waterfowl such as pink-footed geese [*Anser brachyrhynchos*; 38] and lesser snow geese [*Chen caerulescens*; 39]. As IAV dynamics have been shown to vary considerably among tribes of waterfowl [8], discrepancies in our rates of seroconversion/reversion may be attributed to specific differences in IAV dynamics within reservoir host species [8, 45, 46].

2.5.4 Trends in Antibody Titers

We identified changes in antibody titers to H1 – H12 HA subtype viruses through MN between capture occasions; however, we only identified four subtypes (H3, H5, H7, H8) that exhibited a directional trend, suggesting potential variation in the timing of epidemiological peaks of infection for various HA subtypes circulating in our sample populations. In Fairbanks in 2012/13, we found evidence of declining antibody titers to H5 subtype IAVs; however, we did not isolate IAVs of this subtype during our sampling period. It is possible that H5 viruses were

circulating among birds during our period of sampling but were undetected or that H5 infections occurred prior to initiation of our sampling efforts in August. Alternatively, seroreactivity observed to H5 subtype IAVs may have represented heterosubtypic immune responses to several closely related antigens (H2N4, H2N9) isolated from Fairbanks mallards during autumn sampling [41, 42, 47].

For Anchorage mallards sampled in 2014/15, we detected declining antibody titers to H3 subtype IAVs, which appears to be consistent with the timing of circulation for H3 HA subtype viruses in the Anchorage population. That is, isolation of H3 subtype viruses occurred only during autumn, and therefore declining titers to H3 HA subtypes is consistent with seroreversion in birds after the epidemiological peak of infection in our study population. Additionally, we identified increasing antibody titers to H7 and H8 subtype viruses. Although we did not detect any H8 subtype viruses, we did isolate H7 subtype viruses during early and late winter. Thus, our data comparing trends in antibody titers in wintering populations of mallards relative to results of virus isolation suggests that antibody profiling of wild birds has utility as a supplement to traditional viral sampling by providing inference on the circulation of viral subtypes which may be undetected or under-represented through periodic sampling regimes.

2.5.5 Conclusions

Our study demonstrates that low-pathogenic IAVs circulate within populations of waterfowl wintering at urban locations in Alaska. Therefore, it is possible that resident waterfowl play a role in IAV maintenance during the non-breeding season at high-latitude locations in North America. As the influx of spring migrating dabbling ducks typically occurs in late April and May at high-latitude locations, our relatively high estimates of late winter (Feb – Apr) IAV prevalence suggests transmission among individuals in our sample populations may continue in

light of increasing population immunity. Hence, mallards wintering at urban locations in Alaska may serve as a reservoir of IAVs at high-latitude locations, supplementing viruses seeded by spring-migrating waterfowl. Additionally, our study demonstrates the utility of including subtype-specific antibody information for identifying viral subtypes not identified through virus isolation during discrete sampling periods. Inclusion of MN data may provide wildlife managers and regulatory agencies with a more informed snapshot of recently circulating viruses if limited surveillance funding prohibits continuous sampling.

2.6 Acknowledgments

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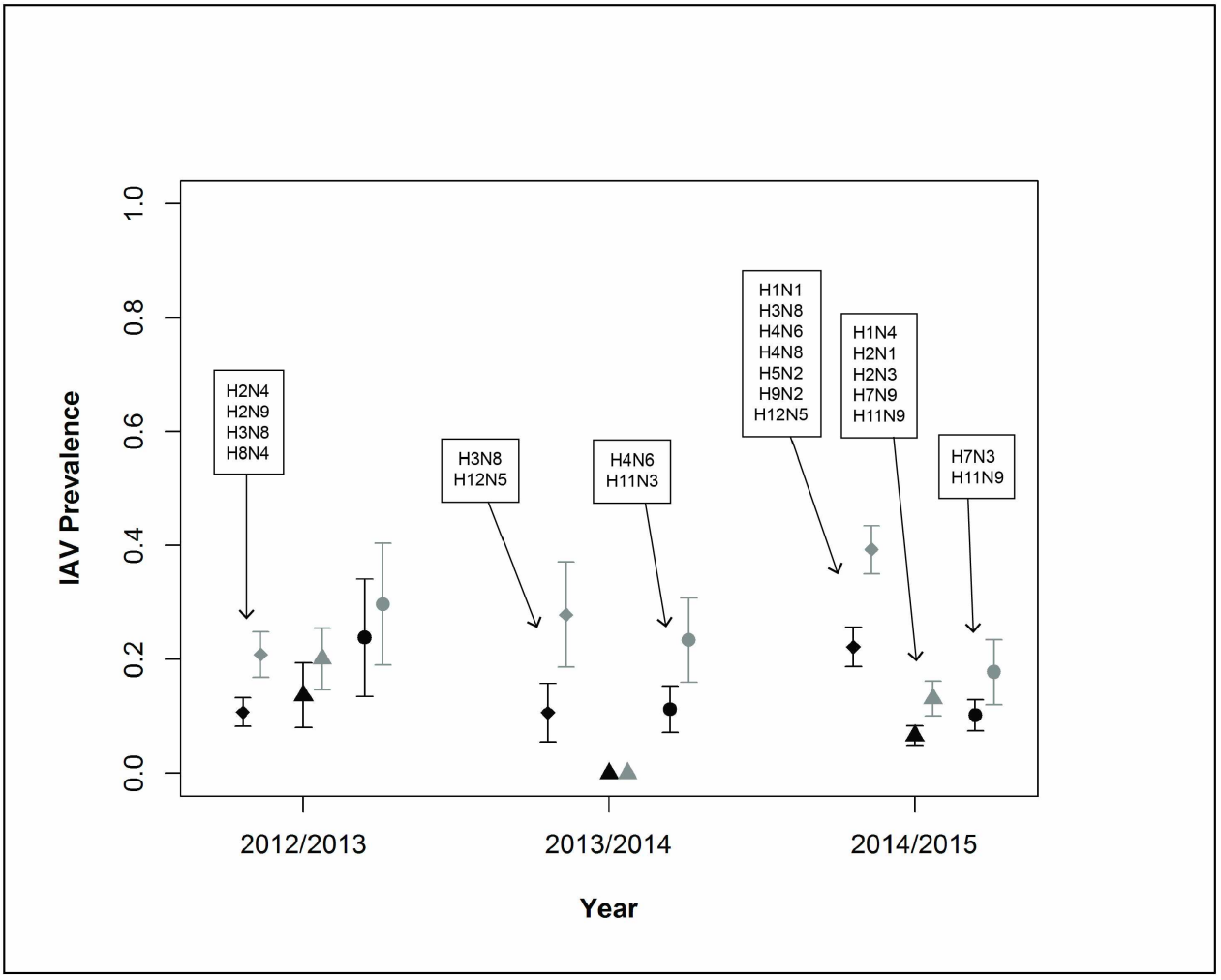

Figure 2.1. Estimated prevalence (± SE) of influenza A virus (IAV) for adult (black) and juvenile (gray) mallards captured in Fairbanks and Anchorage, Alaska during August – April of 2012 – 2015. Diamonds represent autumn (Aug – Oct), triangles represent early winter (Nov – Jan), and circles represent late winter (Feb – Apr). Boxes indicate viral subtype combinations isolated during a given season.

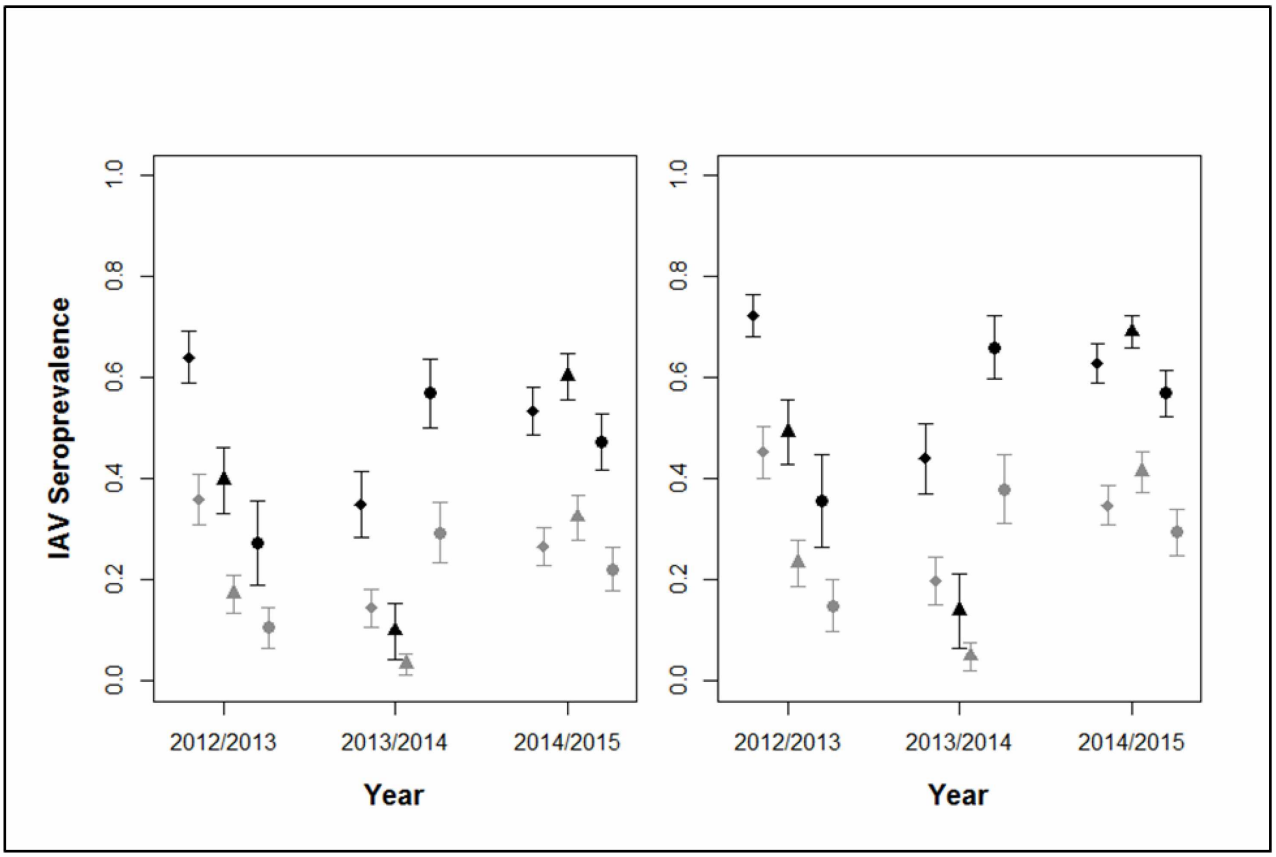

Figure 2.2. Estimated influenza A virus (IAV) seroprevalence (± SE) for female (left) and male (right) mallards captured in Fairbanks and Anchorage, Alaska during August – April of 2012 – 2015. Seroprevalence of adult (black) and juvenile (gray) mallards varied by season and year. Diamonds represent autumn (Aug – Oct), triangles represent early winter (Nov – Jan), and circles represent late winter (Feb – Apr).

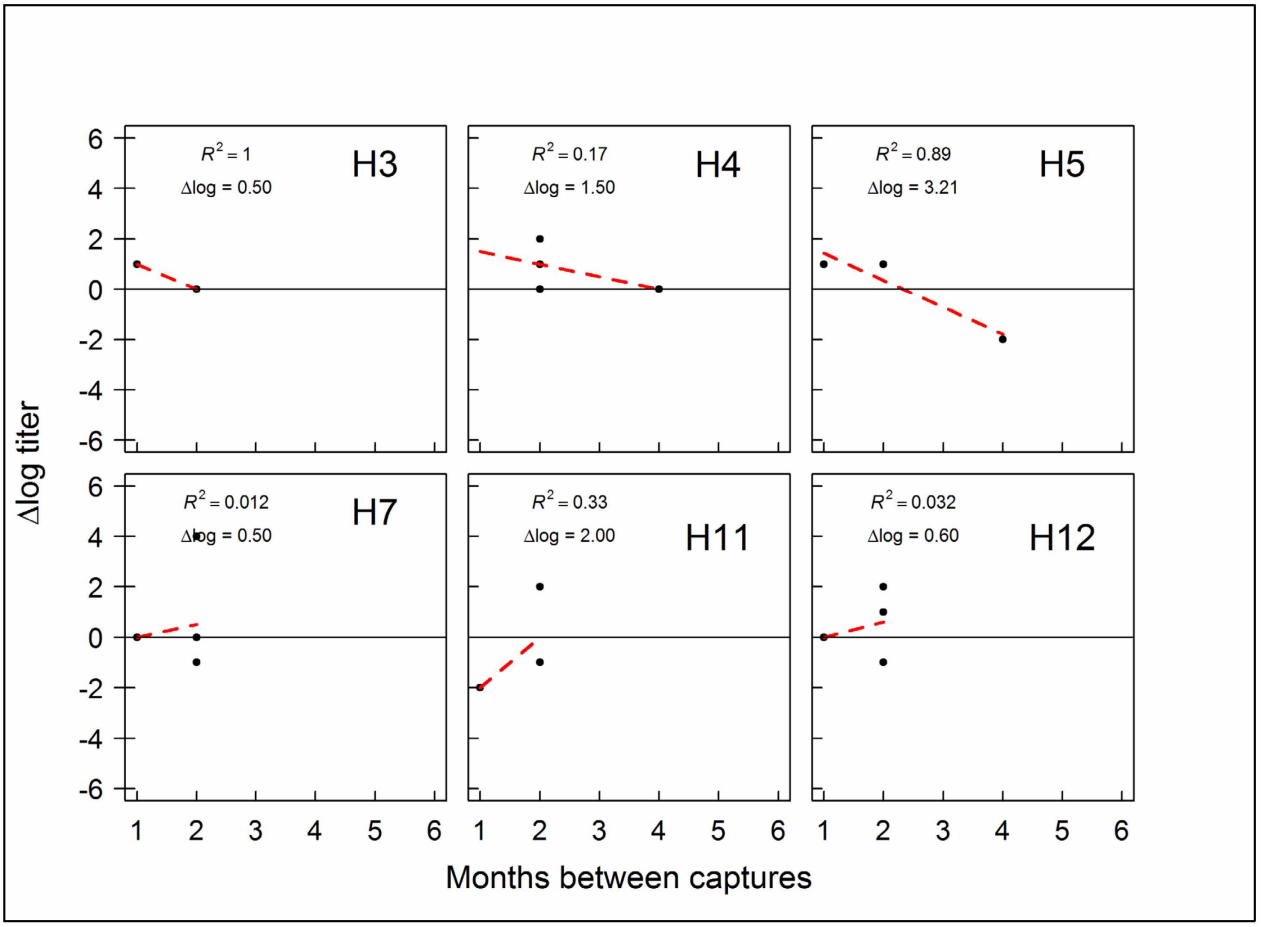

Figure 2.3. Trends in antibody production to HA subtype influenza A viruses (IAVs) by mallards sampled in Fairbanks, Alaska from August 2012 through April of 2013. The change in titer (Δ log titer) of antibodies was obtained from recaptured individuals determined to be seropositive on multiple capture occasions with bELISA. The dashed red line represents a linear regression trend line and associated R-squared value.

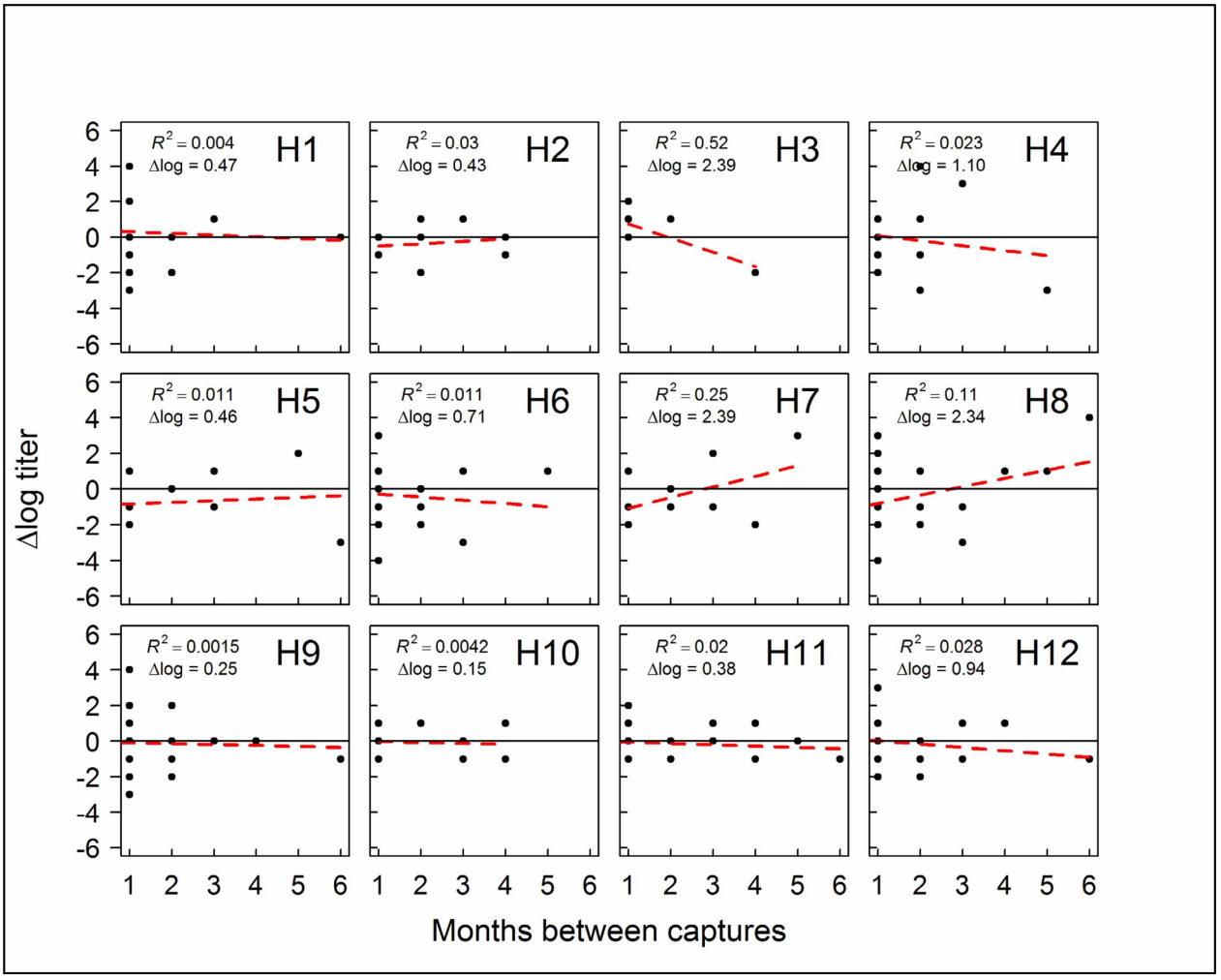

Figure 2.4. Trends in antibody production to HA subtype influenza A viruses (IAVs) by mallards sampled in Fairbanks, Alaska from August 2012 through April of 2013. The change in titer (Δ log titer) of antibodies was obtained from recaptured individuals determined to be seropositive on multiple capture occasions with bELISA. The dashed red line represents a linear regression trend line and associated R-squared value.

Table 2.1. Summary of mallard captures and recapture screening results for molecular detection of viral RNA by rRT-PCR (MA+), virus isolation (VI+), and screening for influenza A virus antibodies using a bELISA (Sero+).

Year	No. Captures	No. Recaptures	% MA+[a]	% VI+[a]	% Sero+[a]
2012/13	277	43	18	3	43
2013/14	162	13	13	5	35
2014/15	484	216	18	8	51
Total	923	272	17	6	46

[a] Estimates from original captures and one randomly selected occasion from each season for birds captured on multiple occasions.

Table 2.2. Results from logistic regression models predicting variation in influenza A virus prevalence for mallards sampled in Fairbanks and Anchorage, Alaska from August through April of 2012 – 2015.

Model Name	K[a]	AICc[b]	Δ AICc	w_i[b]
Age+Season∗Year	10	913.65	0.00	0.43
Age+Sex+Season∗Year	11	915.15	1.51	0.20
Age∗Season∗Year	18	915.48	1.83	0.17
Age∗Sex+Season∗Year	12	915.54	1.90	0.17
Age∗C.Month∗Year	12	919.34	5.69	0.02

[a] Number of parameters in the model.
[b] Akaike's Information Criterion corrected for sample size (AICc) and model weight (w_i) relative to others in the candidate model set.

Table 2.3. Results from logistic regression models predicting variation in influenza A virus seroprevalence for mallards sampled in Fairbanks and Anchorage, Alaska from August through April of 2012 – 2015.

Model Name	K[a]	AICc[b]	Δ AICc	w_i[b]
Age+Sex+Season∗Year	11	1244.46	0.00	0.67
Age∗Sex+Season∗Year	12	1246.49	2.03	0.24
Age+Season∗Year	10	1248.83	4.37	0.07
Age+Sex∗Season∗Year	19	1252.03	7.57	0.02

[a] Number of parameters in the model.
[b] Akaike's Information Criterion corrected for sample size (AICc) and model weight (w_i) relative to others in the candidate model set.

Table 2.4. Serostatus histories for 82 mallards captured on multiple occasions during the winter of 2014/15 in Anchorage, Alaska. Serostatus of juvenile (HY) and adult (AHY) mallards was determined to be either negative (-) or positive (+) through bELISA.

Group	(n)	(-)→(-)	(+)→(+)	(-)→(+)	(+)→(-)
AHY	43	15	24	2	2
HY	39	18	6	6	9
Total	82	33	30	8	11

Table 2.5. Estimates of serostatus transition probability (Ψ_i) and capture probability ($\hat{p}$) from the top approximating model for adult (AHY) and juvenile (HY) mallards captured in Anchorage, Alaska from September through April (2014/15). Seroconversion (-)→(+) and seroreversion (+)→(-) estimates are representative of each age class.

Age	Ψ_i = (-)→(+)	SE	Ψ_i = (+)→(-)	SE	Month	$\hat{p}$	SE
AHY	0.05	0.03	0.05	0.03	Oct	0.46	0.06
					Nov	0.39	0.04
					Dec	0.32	0.03
					Jan	0.26	0.02
					Feb	0.21	0.02
					Mar	0.16	0.02
					Apr	0.12	0.02
HY	0.19	0.07	0.54	0.14	Oct	0.46	0.06
					Nov	0.39	0.04
					Dec	0.32	0.03
					Jan	0.26	0.02
					Feb	0.21	0.02
					Mar	0.16	0.02
					Apr	0.12	0.02

2.8 Appendix

Appendix 2. A. Model selection results and output from models evaluating variation in influenza A virus prevalence and seroprevalence of mallards wintering in Fairbanks and Anchorage, Alaska (2012 – 2015).

Table 2.A-1. Model-averaged class-specific estimates and (SE) of influenza A virus prevalence for mallards sampled in Fairbanks and Anchorage, Alaska between August – April of 2012/15. Adult (AHY) and juvenile (HY) mallards were captured in autumn (Aug – Oct; AUT), early winter (Nov – Jan; E-W) and late winter (Feb – Apr; L-W).

Year	Age	Season	%	SE
2012/13	AHY	AUT	0.11	0.03
		E-W	0.14	0.06
		L-W	0.24	0.10
	HY	AUT	0.21	0.04
		E-W	0.20	0.05
		L-W	0.30	0.11
2013/14	AHY	AUT	0.11	0.05
		E-W	0.00	0.00
		L-W	0.11	0.04
	HY	AUT	0.28	0.09
		E-W	0.00	0.00
		L-W	0.23	0.07
2014/15	AHY	AUT	0.22	0.03
		E-W	0.07	0.02
		L-W	0.10	0.03
	HY	AUT	0.39	0.04
		E-W	0.13	0.03
		L-W	0.18	0.06

Table 2. A-2. Class-specific estimates and (SE) of influenza A virus seroprevalence for mallards sampled in Fairbanks and Anchorage, Alaska between August – April of 2012/15. Adult (AHY) and juvenile (HY) mallards were captured in autumn (Aug – Oct; AUT), early winter (Nov – Jan; E-W) and late winter (Feb – Apr; L-W).

Year	Age	Sex	Season	%	SE
2012/13	AHY	M	AUT	0.72	0.04
			E-W	0.49	0.06
			L-W	0.36	0.09
	AHY	F	AUT	0.64	0.05
			E-W	0.40	0.07
			L-W	0.27	0.08
	HY	M	AUT	0.45	0.05
			E-W	0.23	0.05
			L-W	0.15	0.05
	HY	F	AUT	0.36	0.05
			E-W	0.17	0.04
			L-W	0.11	0.04
2013/14	AHY	M	AUT	0.44	0.07
			E-W	0.14	0.07
			L-W	0.66	0.06
	AHY	F	AUT	0.35	0.07
			E-W	0.10	0.06
			L-W	0.57	0.07
	HY	M	AUT	0.20	0.05
			E-W	0.05	0.03
			L-W	0.38	0.07
	HY	F	AUT	0.14	0.04
			E-W	0.03	0.02
			L-W	0.29	0.06
2014/15	AHY	M	AUT	0.63	0.04
			E-W	0.69	0.03

Table A-2 (continued)

Year	Age	Sex	Season	%	SE
			L-W	0.57	0.05
	AHY	F	AUT	0.53	0.05
			E-W	0.60	0.05
			L-W	0.47	0.06
	HY	M	AUT	0.35	0.04
			E-W	0.41	0.04
			L-W	0.29	0.05
	HY	F	AUT	0.27	0.04
			E-W	0.32	0.04
			L-W	0.22	0.04

Appendix 2. B. Model selection results and output from models evaluating serostatus transition probabilities of mallards wintering in Fairbanks and Anchorage, Alaska (2012 – 2015).

Table 2. B-1. Top AICc approximating models predicting variation in capture probability (p) and transition probability (Ψ) of antibodies after an influenza A virus infection for recaptured mallards sampled in Anchorage, Alaska (2014/15), following two stages of model selection.

Model Name	K[a]	AICc[b]	Δ AICc	w_i[b]
Stage 2 Models				
S(.) p(T) Ψ_i(-)→(+)(Age) Ψ_i(+)→(-)(Age)	7	562.98	0.00	0.87
S(.) p(T) Ψ_i(-)→(+)(Age+T) Ψ_i(+)→(-)(Age+T)	9	567.23	4.25	0.10
S(.) p(T) Ψ_i(-)→(+)(Age∗T) Ψ_i(+)→(-)(Age∗T)	11	570.07	7.09	0.03
S(.) p(T) Ψ_i(-)→(+)(T) Ψ_i(+)→(-)(T)	7	577.87	14.89	0.00
Stage 1 Models				
S(.) p(T) Ψ_i(-)→(+)(Age∗T) Ψ_i(+)→(-)(Age∗T)	11	570.07	0.00	0.99
S(.) p(.) Ψ_i(-)→(+)(Age∗T) Ψ_i(+)→(-)(Age∗T)	10	587.98	17.92	0.01
S(.) p(Age) Ψ_i(-)→(+)(Age∗T) Ψ_i(+)→(-)(Age∗T)	11	589.51	19.44	0.00
S(.) p(Sex) Ψ_i(-)→(+)(T) Ψ_i(+)→(-)(T)	11	589.77	19.71	0.00

[a] Number of parameters in the model.
[b] Akaike's Information Criterion corrected for sample size (AIC_c) and model weight (w_i) relative to others in the candidate model set.

Table 2.B-2. Regression coefficient estimates (β) and standard errors (SE) from the top model predicting variation in the production (Seroconversion; Ψ_i = (-)→(+)) or waning (Seroreversion; Ψ_i = (+)→(-)) of antibodies after an influenza A virus infection for recaptured mallards sampled in Anchorage, Alaska (2014/15).

Parameter	Covariate	β	SE
S	(.)	21.86	0.00
p	Intercept (HY)	0.13	0.29
	Age	-0.30	0.06
Ψ_i (+)→(-)	Intercept (HY)	0.17	0.57
	HY	-3.04	0.83
Ψ_i (-)→(+)	Intercept (HY)	-1.42	0.42
	HY	-1.56	0.84

General Conclusions

This study provides an examination of the seasonal health of mallards (*Anas platyrhynchos*) wintering at the northern extent of their winter distribution. I characterized the diet of adult mallards wintering in both Fairbanks and Anchorage, Alaska to assess the importance of natural and anthropogenic food subsidies and associated changes in body condition throughout the non-breeding season. Additionally, I examined the dynamics of influenza A viruses (IAVs) within these two urban wintering mallard populations, assessing the potential maintenance of IAVs in high-latitude wintering waterfowl populations.

Most species occupying urban habitats are abundant in nature (Chace and Walsh 2006, McKinney 2006), and the dietary plasticity of mallards may be the most significant factor allowing them to make use of habitats considered to be unfavorable during winter (Brodsky and Weatherhead 1984, Heussman 1988). Using SIAR (Parnell et al. 2008), a Bayesian mixing model to estimate dietary proportions for Anchorage mallards, I found evidence of a shift in diet from ~60% anthropogenic food items (e.g. corn, bread) in autumn, to a diet consisting of ~86% anthropogenic subsidies by late winter (Feb – Apr) based on serum isotope values. Comparing estimates from serum, which provides a diet perspective of about a week, to estimates from whole blood, which provides a diet perspective of about a month (Schmutz et al. 2006, Hahn et al. 2012), revealed similar patterns. However, as invertebrate and fish contributions declined through late winter, the seeds & berries group remained between 8-13% of the diet based on serum and whole blood. While mallards may have consumed natural seeds and a few berries in Anchorage, the similarity between the seeds & berries and bread group may have inflated those estimates.

Although we did not have dietary endpoints from the Fairbanks population preventing the application of a dietary mixing model, $\delta^{13}C$ isotope values of mallard serum and whole blood followed a trend similar to mallards wintering in Anchorage. This supports field observations of heavy consumption of anthropogenic foods such as corn during winter in Fairbanks (Spivey, personal observation, UAF). I expected $\delta^{13}C$ isotope values to increase for individuals in both populations, however $\delta^{15}N$ values remained above that of foods such as corn or bread, suggesting natural foods such as invertebrates or natural seeds may provide important nutrients during winter. Alternatively, mallards wintering on the Chena River and mallards frequenting several salmon streams in Anchorage may consume marine-derived nutrients contributing to elevated $\delta^{15}N$ isotope values (Gende et al. 2002). Furthermore, mallards harvested on the Anchorage Coastal Wildlife Refuge (ACWR) consumed fish, an important diet item for mallards wintering in North Dakota (Olsen et al. 2011), and the $\delta^{15}N$ values may be indicative of low-level consumption of this food source. Thus, our observed isotope patterns identify how mallards may opportunistically acquire small amounts of natural diet items to supplement human-provided foods deficient in necessary nutrients.

In addition to seasonal variation in diet, the interaction between season and year covariates was the most influential source of variation relative to body mass of mallards in Fairbanks and Anchorage. I expected trends in body mass for individuals in both populations to be similar due to anthropogenic provisioning, with Fairbanks mallards weighing more than Anchorage mallards due to consistently colder temperatures. Contrary to my expectations, patterns in body mass, which we defined as the relative condition of wintering mallards (Boos et al. 2000, Schamber et al. 2009), differed between the two populations. In Fairbanks, mallard body mass increased from autumn to early winter, then remained relatively high during late

winter, whereas in Anchorage, mallard body mass declined from autumn to late winter. This finding suggests that despite similar isotope values indicative of comparable diets from mallards wintering at these two locations, Fairbanks mallards may consume greater quantities of anthropogenic foods. Moreover, this suggests that the disparity in body mass trends of Fairbanks and Anchorage mallards may reflect both the level of reserves needed to offset local climate conditions and potential differences in the level of subsidy at these two locations. Additional research may be needed to distinctly separate specific mechanisms contributing to body mass patterns of mallards wintering at these urban locations.

In the second chapter of my thesis, I examined IAV dynamics in mallard populations overwintering at urban locations in Alaska. Seasonal variation in putative prevalence of IAV infection occurred during all three years of the study, and we found evidence of IAVs actively circulating during the early winter (Nov – Jan) and late winter (Feb – Apr) seasons. This suggests infections with IAVs still occur after high levels of population immunity are reached. While I expected IAV prevalence and seroprevalence to follow similar patterns, infection and immune responses were somewhat inconsistent. Therefore, these high-latitude wintering mallard populations may experience high temporal variability in IAV infections and associated immune responses. Nonetheless, our higher prevalence rates in juveniles than adults (Hinshaw et al. 1980, Runstadler et al. 2007, Wilcox et al. 2011), and higher seroprevalence in adults than juveniles (Wilson et al. 2013), are similar to results from prior IAV investigations of waterfowl. Thus, in addition to the hypothesis of perpetuation of IAVs in Alaskan wetlands (Ito et al. 1995, Lang et al. 2008, Reeves et al. 2011), our findings suggest that waterfowl populations remaining at high latitudes during winter may facilitate maintenance of IAVs during the non-breeding season.

In addition to population variation in IAV infection and immune response of mallards in both Fairbanks and Anchorage, I estimated the probability of seroconversion or seroreversion after an IAV infection using infection histories of recaptured individuals in Anchorage. I identified higher seroconversion and seroreversion rates in juveniles than adults, yet I expected higher probabilities of seroconversion for both age classes due to the isolation of multiple IAV subtypes throughout the winter in Anchorage. I also examined trends in antibody titers to specific hemagglutinin (HA) subtype viruses from recaptured seropositive individuals. Using virus microneutralization (MN) assays, I found evidence of antibody production to multiple IAV subtypes that were not isolated during discrete capture occasions. I expected trends in subtype-specific antibody titers to exhibit directionality, yet the majority of HA subtype antibody titers showed little indication of overwhelming increases or decreases across the study period. This suggests that specific IAV subtypes may induce a long-lasting immune response following infection (Wille et al. 2016), or that certain IAV subtypes may only occur at unique times in a given non-breeding period (Ramey et al. 2014). Nevertheless, antibody titer trends for several IAV subtypes reflected the timing of circulation of those HA subtype viruses. Therefore, we provide evidence of the utility of combining virus MN assay data with virus isolation techniques to provide a more comprehensive understanding of IAV subtype diversity in host populations (Guinn et al. 2016, Wong et al. 2016).

In summary, this research will provide wildlife managers and regulatory agencies with empirical data that may be used when making decisions about feeding migratory waterfowl at urban locations in Alaska. Because mallards wintering in Fairbanks and Anchorage, Alaska have the potential to maintain IAVs throughout the non-breeding period, wildlife managers should strongly consider developing regulations to dissuade large-scale supplementary feeding efforts.

As cities in Alaska continue to sprawl, regulations on feeding these wintering waterfowl populations may allow wildlife managers to continue to promote successful wildlife-human interactions in urban environments.

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Appendix

Appendix A. Stable isotope values from diet item samples collected in Anchorage, Alaska during autumn (Sept – Nov) of 2014 – 2015. Anthropogenic foods were purchased from grocery and local feed stores. Samples were analyzed as singular (n = 1), pooled (n < 20), or mash (partially digested) diet items.

Location	Collection Method	Type	Order	(n)	δ15N	δ13C	%N	%C
Cuddy	Collection	Berry	Rosales	Pooled	0.0	-23.3	1.13	41.00
Cuddy	Collection	Berry	Rosales	Pooled	0.4	-23.4	0.99	41.11
Cuddy	Collection	Berry	Rosales	Pooled	-0.6	-23.8	1.54	40.73
Cuddy	Collection	Berry	Rosales	Pooled	-0.6	-23.6	1.34	40.90
Cuddy	Collection	Berry	Rosales	Pooled	-0.1	-23.7	1.50	41.48
Cuddy	Collection	Berry	Rosales	Pooled	-0.6	-23.5	1.50	41.06
Store	Purchased	Bread	Wheat B.	1	2.9	-24.4	2.56	41.43
Store	Purchased	Bread	Wheat B.	1	2.8	-24.4	2.56	42.05
Store	Purchased	Bread	Wheat B.	1	2.9	-25.7	3.05	41.20
Store	Purchased	Bread	Wheat B.	1	3.0	-25.6	3.27	43.26
Store	Purchased	Bread	White Bread	1	2.5	-23.9	2.70	41.51
Store	Purchased	Bread	White Bread	1	2.6	-23.9	2.82	43.25
Store	Purchased	Bread	White Bread	1	2.6	-23.9	2.81	43.49
Store	Purchased	Bread	H. Wheat Bread	1	2.9	-24.8	3.02	44.48
Store	Purchased	Bread	H. Wheat Bread	1	3.0	-24.9	2.92	44.83
Store	Purchased	Bread	H. Wheat Bread	1	3.0	-24.9	2.99	44.95
Store	Purchased	Bread	H. Wheat Bread	Pooled	4.1	-25.0	2.50	44.02
Store	Purchased	Bread	H. Wheat Bread	Pooled	4.1	-25.1	2.48	44.13
Store	Purchased	Bread	White B.	1	3.4	-24.9	2.25	42.67
Store	Purchased	Bread	White B.	1	3.2	-24.9	2.25	43.52
Store	Purchased	Bread	White B.	1	3.0	-24.7	2.33	41.16
Store	Purchased	Bread	White B.	1	2.9	-24.6	2.34	41.96
Cuddy	Purchased	Corn	Poales	Mash	5.7	-12.1	1.30	44.47
Cuddy	Purchased	Corn	Poales	Mash	3.6	-11.9	1.42	44.84

Appendix A (continued)

Location	Collection Method	Type	Order	(n)	δ15N	δ13C	%N	%C
Cuddy	Purchased	Corn	Poales	Mash	2.1	-12.3	1.53	41.59
Cuddy	Purchased	Corn	Poales	Mash	1.9	-12.3	1.53	41.98
Cuddy	Purchased	Corn	Poales	Mash	2.8	-12.7	1.66	42.58
Cuddy	Purchased	Corn	Poales	Mash	2.4	-12.3	1.71	42.06
Cuddy	Purchased	Corn	Poales	Mash	2.0	-12.4	1.77	42.92
ACWR	Harvest	Fish	Gasterosteiformes	1	9.9	-27.6	8.24	57.03
ACWR	D-net	Fish	Gasterosteiformes	1	8.3	-26.6	8.22	40.02
ACWR	D-net	Fish	Gasterosteiformes	1	7.4	-27.6	8.31	42.35
ACWR	Harvest	Fish	Gasterosteiformes	1	13.5	-34.2	10.11	42.65
20mi/p	Harvest	Invert	Amphipoda	Pooled	1.6	-31.1	10.84	48.29
20mi/p	Harvest	Invert	Annelida	Pooled	11.3	-32.8	6.34	32.37
20mi/p	Harvest	Invert	Odonata	1	4.5	-29.6	4.98	28.82
20mi/p	Harvest	Invert	Odonata	1	2.9	-29.6	5.43	40.36
ACWR	Harvest	Invert	Odonata	1	4.5	-34.5	3.82	40.19
ACWR	Harvest	Invert	Invert Mash	Mash	6.3	-23.1	6.91	50.98
ACWR	Harvest	Invert	Invert Mash	Mash	7.9	-24.4	4.84	38.02
ACWR	Harvest	Invert	Amphipoda	Pooled	4.1	-28.5	3.28	54.45
ACWR	Harvest	Invert	Amphipoda	Pooled	2.8	-29.9	1.41	13.21
ACWR	Harvest	Invert	Amphipoda	Pooled	2.8	-30.6	3.00	30.76
ACWR	Harvest	Invert	Amphipoda	Pooled	6.2	-30.1	4.45	38.49
ACWR	Harvest	Invert	Odonata	Pooled	2.5	-40.6	7.59	32.65
ACWR	Harvest	Invert	Odonata	Pooled	4.2	-33.8	8.66	52.28
ACWR	Harvest	Invert	Odonata	Pooled	5.3	-34.8	9.43	53.25
ACWR	D-net	Invert	Amphipoda	1	10.1	-20.6	11.14	47.23
ACWR	D-net	Invert	Odonata	1	6.5	-27.8	2.80	51.59
ACWR	Harvest	Invert	Diptera	1	4.9	-22.1	6.01	42.88
ACWR	Harvest	Invert	Amphipoda	Pooled	6.2	-22.7	4.92	36.07
ACWR	Harvest	Invert	Diptera	Pooled	4.9	-20.7	8.84	45.07

Appendix A (continued)

Location	Collection Method	Type	Order	(n)	δ15N	δ13C	%N	%C
ACWR	Harvest	Invert	Annelida	Pooled	11.0	-31.2	8.88	45.93
ACWR	Harvest	Invert	Polychaeta	Mash	6.3	-25.1	5.47	37.43
Cheney L.	D-net	Invert	Hemiptera	1	7.6	-27.5	2.85	17.40
C. Gate	D-net	Invert	Diptera	Pooled	4.0	-24.8	2.46	44.01
Cuddy	D-net	Invert	Invert Spp.	Pooled	5.8	-28.8	8.67	47.36
Cuddy	D-net	Invert	Gastropoda	Pooled	6.5	-31.2	9.02	57.21
Cuddy	D-net	Invert	Invert Spp.	Pooled	2.7	-26.6	10.21	46.00
Cuddy	D-net	Invert	Invert Spp.	Pooled	4.4	-28.3	2.46	15.42
E. Chester	D-net	Invert	Diptera	Pooled	8.4	-26.2	9.41	56.91
Ship Cr	D-net	Invert	Invert Spp.	Pooled	5.0	-30.4	9.86	49.02
Ship Cr	D-net	Invert	Invert Spp.	Pooled	6.4	-22.0	7.36	39.73
Ship Cr	Hatchery	Fish	Coho Salmon	Pooled	14.7	-23.0	11.34	55.74
Ship Cr	Hatchery	Fish	Coho Salmon	Pooled	14.9	-22.3	11.45	53.99
Ship Cr	Hatchery	Fish	Coho Salmon	Pooled	13.5	-24.1	12.28	59.86
Ship Cr	Hatchery	Fish	Coho Salmon	Pooled	14.3	-23.3	11.43	54.87
Ship Cr	Hatchery	Fish	Coho Salmon	Pooled	14.0	-24.2	11.28	55.46
Ship Cr	Hatchery	Fish	Coho Salmon	Pooled	13.9	-24.1	11.31	56.08
Ship Cr	Hatchery	Fish	Coho Salmon	Pooled	14.1	-22.8	11.50	53.84
Ship Cr	Hatchery	Fish	Coho Salmon	Pooled	14.0	-22.8	11.28	53.22
Ship Cr	Hatchery	Fish	Coho Salmon	Pooled	13.9	-23.2	11.46	54.10
Ship Cr	Hatchery	Fish	Coho Salmon	Pooled	14.1	-22.8	11.08	53.21
Ship Cr	Hatchery	Seed	Asterales	Pooled	1.3	-27.9	2.10	42.09
20mi/p	Harvest	Seed	Sedge spp.	Pooled	2.5	-26.2	2.01	42.64
20mi/p	Harvest	Seed	Sedge spp.	Pooled	2.3	-26.0	1.64	41.80
20mi/p	Harvest	Seed	Poales	Pooled	2.1	-27.7	1.96	41.94
20mi/p	Harvest	Seed	Sedge spp.	Pooled	1.2	-28.8	2.28	47.29
20mi/p	Harvest	Seed	Sedge spp.	Pooled	1.8	-26.4	2.02	52.70
20mi/p	Harvest	Seed	Poales	Pooled	2.4	-28.6	1.77	45.26

Appendix A (continued)

Location	Collection Method	Type	Order	(n)	δ15N	δ13C	%N	%C
ACWR	Harvest	Seed	Poales	Pooled	2.1	-28.6	1.31	43.89
ACWR	Harvest	Seed	Poales	Pooled	2.6	-28.8	4.64	46.93
ACWR	Harvest	Seed	Poales	Pooled	2.6	-28.7	3.36	43.36
ACWR	Harvest	Seed	Poales	Pooled	2.8	-26.4	2.68	44.74
ACWR	Harvest	Seed	Alismatales	Pooled	5.1	-25.1	3.81	47.71
ACWR	Harvest	Seed	Alismatales	Pooled	3.8	-23.8	3.37	51.94
ACWR	Harvest	Seed	Alismatales	Pooled	5.3	-26.0	1.57	50.84
ACWR	Harvest	Seed	Alismatales	Pooled	4.8	-25.5	3.81	40.52
ACWR	Harvest	Seed	Alismatales	Pooled	3.8	-24.7	4.29	44.79
ACWR	D-net	Seed	Alismatales	Mash	3.4	-24.8	3.82	39.65
ACWR	D-net	Seed	Alismatales	Mash	4.6	-25.2	3.63	39.89
ACWR	D-net	Seed	Alismatales	Mash	4.5	-25.3	1.31	45.64

Appendix B. Virus microneutralization (MN) assay results reported as influenza A virus (IAV) titer value for mallards captured in Fairbanks (below) during 2012/2013 or Anchorage (next pages) during 2014/2015. Values greater than or equal to 20 represent a positive result and the final dilution at which cytopathic effect (cell death) was observed.

Band #	Month	H1N1	H2N3	H3N8	H4N6	H5N1	H6N1	H7N9	H8N4	H9N2	H10N7	H11N9	H12N5
1927-63151	Sept	<20	<20	20	<20	<20	<20	20	<20	<20	20	80	80
	Oct	<20	<20	40	<20	20	<20	20	<20	<20	20	20	80
1927-63184	Oct	<20	40	<20	40	<20	<20	<20	<20	<20	<20	20	<20
	Dec	<20	40	<20	40	<20	<20	<20	<20	<20	<20	80	<20
1927-63187	Oct	<20	<20	<20	<20	<20	<20	20	40	<20	<20	20	160
	Dec	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	80
1927-63208	Oct	20	<20	20	20	<20	<20	20	<20	<20	<20	<20	80
	Dec	<20	80	20	80	<20	<20	320	<20	<20	<20	<20	160
1927-63263	Oct	<20	<20	<20	<20	<20	<20	160	20	<20	40	<20	160
	Dec	40	<20	<20	40	<20	<20	80	<20	<20	80	<20	640
1927-63299	Oct	<20	40	<20	20	<20	<20	80	80	<20	20	20	80
	Dec	40	<20	<20	20	<20	<20	80	<20	<20	<20	<20	40
1927-63249	Oct	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
	Dec	<20	<20	<20	20	20	<20	<20	<20	<20	<20	<20	40
1927-63360	Dec	<20	<20	<20	20	320	<20	<20	<20	<20	<20	<20	<20
	Apr	<20	<20	<20	20	80	<20	<20	<20	<20	<20	<20	<20

Appendix B (continued)

Band #	Month	H1N1	H2N3	H3N8	H4N6	H5N1	H6N1	H7N9	H8N4	H9N2	H10N7	H11N9	H12N5
2047-55423	Sept	160	<20	<20	<20	80	<20	<20	<20	20	<20	320	<20
	Mar	160	<20	<20	<20	<20	<20	<20	<20	<20	<20	160	<20
2047-55487	Sept	320	<20	<20	<20	40	20	40	160	20	20	160	<20
	Oct	160	<20	<20	<20	<20	80	<20	80	<20	<20	160	<20
	Nov	80	<20	<20	<20	20	<20	<20	160	<20	<20	160	<20
2047-55311	Sept	<20	160	<20	<20	160	80	40	<20	160	20	80	80
	Dec	<20	160	<20	<20	160	160	20	<20	160	20	160	80
	Jan	<20	80	<20	<20	80	160	<20	20	160	40	160	160
	Mar	<20	<20	<20	<20	80	80	20	40	160	40	80	40
2047-55454	Oct	80	<20	80	<20	<20	80	<20	80	80	<20	320	<20
	Nov	80	<20	20	<20	<20	160	<20	320	<20	<20	160	20
	Feb	160	<20	20	80	<20	<20	<20	40	<20	<20	160	<20
2047-55263	Sept	<20	<20	<20	<20	<20	40	<20	<20	20	<20	80	80
	Oct	160	<20	<20	<20	<20	40	<20	80	320	<20	320	40
2047-55089	Nov	160	<20	<20	<20	<20	20	<20	40	160	40	160	20
	Dec	160	<20	<20	<20	<20	20	<20	20	160	20	160	320
	Mar	320	<20	<20	<20	20	20	<20	20	160	20	40	160
2047-55079	Oct	<20	<20	<20	<20	<20	<20	<20	80	160	<20	160	<20
	Nov	<20	<20	<20	<20	<20	<20	<20	20	40	<20	80	20
2047-55312	Nov	<20	160	80	<20	<20	<20	320	20	<20	<20	160	80
	Jan	<20	160	160	160	<20	<20	160	40	<20	<20	160	20
2047-55036	Oct	<20	<20	<20	<20	<20	<20	40	40	<20	<20	160	20
	Nov	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	80	<20
2047-55155	Sept	<20	<20	<20	<20	<20	<20	<20	<20	20	<20	80	<20
	Nov	<20	<20	<20	<20	<20	<20	<20	<20	80	<20	80	<20
2047-55394	Dec	<20	20	20	40	<20	80	<20	20	80	<20	80	<20
	Jan	<20	20	20	<20	<20	80	<20	80	80	<20	80	80
2047-56022	Nov	<20	<20	<20	160	<20	40	<20	160	320	<20	320	160
	Dec	<20	<20	<20	320	<20	<20	<20	<20	160	<20	320	80

Appendix B (continued)

Band #	Month	H1N1	H2N3	H3N8	H4N6	H5N1	H6N1	H7N9	H8N4	H9N2	H10N7	H11N9	H12N5
2047-56026	Nov	80	20	<20	<20	<20	80	<20	80	160	<20	80	20
	Dec	40	<20	<20	<20	<20	40	<20	80	80	<20	80	<20
	Jan	80	40	<20	<20	<20	320	<20	40	160	<20	80	20
2047-55020	Nov	<20	40	<20	160	<20	<20	80	40	<20	20	160	<20
	Dec	<20	40	<20	320	<20	<20	40	80	<20	<20	80	<20
	Jan	<20	40	<20	320	<20	<20	80	20	<20	20	320	<20
	Mar	<20	40	<20	320	<20	<20	160	<20	<20	<20	40	<20
2047-55458	Dec	80	<20	<20	80	160	<20	<20	40	160	<20	80	<20
	Jan	320	<20	40	80	320	80	<20	<20	80	<20	160	<20
2047-55028	Oct	<20	<20	<20	20	<20	<20	<20	40	40	20	80	160
	Dec	<20	<20	<20	<20	<20	<20	<20	<20	<20	40	40	40
2047-56057	Nov	<20	<20	<20	<20	<20	<20	<20	20	<20	<20	80	40
	Dec	<20	<20	<20	<20	<20	<20	<20	20	<20	<20	80	320
2047-56058	Nov	320	20	80	80	<20	40	20	40	<20	<20	80	320
	Dec	320	20	80	20	<20	160	<20	40	40	<20	160	320
	Jan	160	20	80	<20	<20	80	<20	80	<20	<20	80	160
	Feb	160	40	80	<20	<20	40	20	<20	40	<20	80	160
2047-55238	Dec	80	<20	<20	<20	<20	80	<20	<20	<20	<20	80	<20
	Jan	<20	<20	<20	<20	<20	40	<20	<20	<20	<20	80	<20
2047-55058	Oct	<20	<20	<20	<20	<20	80	20	80	<20	20	160	<20
	Jan	<20	<20	<20	<20	<20	<20	<20	40	<20	<20	320	<20
2047-55029	Oct	<20	<20	<20	<20	<20	80	<20	80	160	20	320	160
	Jan	<20	<20	<20	<20	<20	<20	<20	<20	160	<20	320	320
2047-56084	Dec	<20	<20	<20	<20	<20	160	<20	80	80	<20	320	<20
	Jan	<20	<20	<20	<20	<20	<20	<20	40	160	<20	160	<20
2047-55253	Dec	40	320	20	<20	80	40	<20	80	320	40	80	320
	Jan	<20	160	20	<20	20	40	<20	80	160	80	160	320
2047-55396	Feb	<20	160	<20	<20	<20	40	20	<20	160	20	160	160
	Mar	<20	80	<20	<20	<20	40	<20	20	40	<20	160	40

Appendix B (continued)

Band #	Month	H1N1	H2N3	H3N8	H4N6	H5N1	H6N1	H7N9	H8N4	H9N2	H10N7	H11N9	H12N5
2047-55264	Jan	80	<20	<20	20	40	40	<20	40	<20	<20	160	20
	Feb	80	<20	<20	<20	<20	80	<20	<20	20	<20	160	40
2047-55137	Sept	<20	<20	<20	80	<20	<20	<20	40	<20	<20	320	<20
	Feb	<20	<20	<20	<20	40	20	80	80	<20	<20	320	<20
2047-55211	Dec	320	160	<20	320	80	160	20	<20	<20	<20	160	<20
	Feb	320	40	<20	160	80	160	20	20	40	20	160	<20
2047-55436	Feb	<20	<20	<20	80	<20	80	<20	20	160	20	80	<20
	Mar	<20	<20	<20	80	<20	40	20	40	160	20	160	<20
2047-55505	Feb	20	<20	160	80	20	40	<20	<20	<20	<20	160	<20
	Mar	320	<20	320	80	<20	20	<20	<20	<20	<20	80	<20
2047-55515	Feb	<20	<20	<20	<20	<20	<20	<20	<20	20	<20	160	<20
	Mar	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	80	<20
2047-55141	Sept	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	320	20
	Mar	<20	<20	<20	<20	<20	<20	<20	160	<20	<20	160	<20

Appendix C. IACUC Approval Letter (1)

(907) 474-7800
(907) 474-5993 fax
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www.uaf.edu/iacuc

UNIVERSITY OF ALASKA FAIRBANKS

Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

August 2, 2012

To:	Mark Lindberg, BS, MS, PhD Principal Investigator
From:	University of Alaska Fairbanks IACUC
Re:	[358515-3] Seasonal patterns of low pathogenic avian influenza prevalence and antibody response in an isolated population of high-latitude-wintering mallards

The IACUC reviewed and approved the Amendment/Modification referenced above by Designated Member Review.

Received:	August 1, 2012
Approval Date:	August 2, 2012
Initial Approval Date:	August 2, 2012
Expiration Date:	August 2, 2013

This action is included on the August 24, 2012 IACUC Agenda.

The PI is responsible for acquiring and maintaining all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol, and could result in revocation of IACUC approval.

The PI is responsible for ensuring animal research personnel are aware of the reporting procedures on the following page.

Appendix C (continued)
IACUC Approval Letter (2)

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UNIVERSITY OF ALASKA FAIRBANKS

Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

October 2, 2014

To:	Mark Lindberg Principal Investigator
From:	University of Alaska Fairbanks IACUC
Re:	[662280-2] Temporal and spatial variation in the health and foraging ecology of high- latitude wintering Mallards (Anas Platyrhynchos)

The IACUC reviewed and approved the Amendment/Modification referenced above by Designated Member Review.

Received:	September 29, 2014
Approval Date:	October 2, 2014
Initial Approval Date:	October 2, 2014
Expiration Date:	October 2, 2015

This action is included on the October 9, 2014 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures on the following page.*