PARTICULATE MATTER ACCUMULATION TO URBAN ROCK PIGEON (*Columba livia*) FEATHERS

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This research investigates particulate matter (PM) deposition to rock pigeons (*Columba livia*) in urban environments within Denton County, Texas. Pigeon habitat was characterized within a 2-km radius at eight locations using the 2016 National Land Cover Database (NLCD). In summer 2020, feathers were sampled from 10 rock pigeons at two locations (n = 20) differing in degree of urban development. Birds were captured using walk-in funnel traps baited with bird seed. Based on molt pattern and appearance, four old flight feathers were identified and sampled from each bird. New primary feathers were obtained from each population as reference samples. Feathers were washed three times with double deionized water and acetone, and the solution vacuum filtered through a glass microfiber filter to collect all particles >1.5 µm in diameter. Particulate matter mass was determined by gravimetric analysis and calculated per unit feather surface area. Relative PM accumulation rates were significantly different between the populations. Characteristics of urban land cover, proximity to and types of emissions sources, wind exposure, and building density were drivers of variability in PM deposition to feather surfaces. The results from this study should be useful for subsequent research to help identify best practices for using feathers collected from pigeons or other urban restricted bird species to monitor PM levels across multiple spatial scales.

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CHAPTER 1

PARTICULATE MATTER, URBAN ENVIRONMENTS AND HEALTH: AVIAN BIOMONITORS AS INDICATORS OF CHANGE

Introduction

Particulate matter (PM) is a component of air pollution and a topic of global concern due to its adverse effects on air quality and human health (Bourdrel et al., 2017; Du et al., 2016; Falcon-Rodriguez et al., 2016). This mixture of suspended particles and liquid droplets may be composed of numerous and diverse chemicals dependent upon the emission source (Kim et al., 2015). Particulate matter pollution is regulated according to its size fraction with stricter standards imposed for the finest particles as these present the greatest health risk (US EPA, 2018b). Although PM_{10} (particles between 2.5 and 10 μm in diameter) can contribute to adverse effects in humans, finer particles less than 2.5 μm in diameter ($PM_{2.5}$) are especially dangerous as they can be inhaled deeper into lungs and pass into other tissues (Losacco & Perillo, 2018). In humans, increased rates of cardiovascular and respiratory disease, cancer, impaired neurological development, and increased mortality rates all have been associated with long- and short-term exposure to PM pollution, especially in urban areas (Lu et al., 2015; WHO, 2013). The main sources of atmospheric PM emissions in urban areas are industrial processes, power generation, and the burning of fossil fuels (US EPA, 2018a), especially that of vehicle emissions (WHO, 2013).

Particulate matter is removed from the atmosphere via deposition to receptor surfaces. This occurs via wet deposition, the rainout or washout of particles in precipitation, or dry deposition, the settling of particles directly onto surfaces (Ponette-González et al., 2016;

Weathers & Ponette-González, 2011). Wet deposition is influenced by atmospheric emissions, proximity to sources, precipitation, and orographic effects (Weathers & Ponette-González, 2011). Dry deposition of PM varies by temperature, relative humidity (RH), precipitation, wind speed, and the properties of the particle and deposition surface (Chen et al., 2012; Lou et al., 2017; Weathers & Ponette-González, 2011). Thus, great variability in atmospheric deposition can occur over small spatial scales (i.e., meters) (Griffith et al., 2015).

Elevated atmospheric concentrations of PM are associated with increasing levels of urbanization, defined as the percentage of the total population living in an urban area, as well as urban area size, and population and traffic density (Clougherty et al., 2013; Wang et al., 2019). Increases in vehicle emissions and industrial processes are the main contributors to higher levels of urban PM (Lee et al., 2007; Rodríguez et al., 2004), especially the finer fractions in developing countries (Han et al., 2016). However, direct measures of population migration into urban areas, traffic densities and number, type, and distribution of emissions sources do not always account for the variations in concentrations seen among cities around the world. Causal factors can be attributed to differing levels of socio-economic development; this determines access to more fuel-efficient vehicles and better environmental control technologies, as well as the development of regulatory policies (Wang et al., 2019).

As of 2018, 55% of the world's population occupied urban areas. Projections for the year 2050 indicate that this number will increase to 68% with Asia and Africa contributing to almost 90% of that growth (United Nations, 2019). Included within these urban areas are 33 megacities, whose populations exceed 10 million and collectively house one eighth of the total number of urban inhabitants (United Nations, 2019). The number of megacities is expected to

increase to 43 by 2030, located mostly in developing areas (United Nations, 2019). Given this explosive growth, exposure to PM and other harmful air pollutants can only increase, along with the potential for adverse health outcomes. Thus, for municipalities to mitigate and reduce exposure, the ability to monitor changes in PM levels is important. In such diverse environments, however, monitoring atmospheric PM concentrations is challenging.

Over the last several decades, multiple studies have examined the accumulation of anthropogenic pollutants within various species and the resulting adverse physiological effects to individuals and populations (AL-Alam et al., 2019; Irwin, 2005; Wolterbeek, 2002), a method known as biomonitoring. This field has proven essential to tracking changes in the environment by monitoring the cumulative effects of pollutants in a species. Birds have proven to be apt subjects for such research due to their global distribution, well-known flight ranges, and use of multiple habitat types (Sanderfoot & Holloway, 2017). Methods and techniques using bird feathers in biomonitoring studies have been refined since the 1960s to quantify an increasing number of harmful compounds persistent in the physical environment and to elucidate contamination sources and patterns of redistribution in ecosystems (Rutkowska et al., 2018).

An increasing body of research shows that the adverse health effects from air pollution extend beyond the human animal (Sanderfoot & Holloway, 2017). Birds, who spend their lives in the atmosphere, are also exposed to harmful pollutants. Birds that inhale polluted air suffer respiratory distress, illness, immunosuppression, and reduced reproductivity (North et al., 2017; Sanderfoot & Holloway, 2017). Although inhalation studies are relatively few, biomonitoring studies on avian species have provided a wealth of information on the fate of anthropogenic pollutants in the environment (Brait & Antoniosi Filho, 2011; Cai & Calisi, 2016;

Jaspers et al., 2011; Roque et al., 2016). Even then, most studies are concerned with the internal accumulation of contaminants (Jaspers et al., 2009). External contaminants deposited on feather surfaces are rarely quantified and their contribution to adverse outcomes is not well understood (DuBay & Fuldner, 2017).

This chapter defines PM, discusses sources of PM and the factors that influence its removal from the atmosphere, and summarizes the effects of atmospheric PM exposure on human and avian health. Biomonitoring is introduced as a way to use the feathers of common urban bird species to understand the spatial distribution of PM pollution in urban habitats.

Atmospheric Particulate Matter

A component of air pollution, PM is a mixture of solid particles and liquid droplets suspended in the atmosphere (US EPA, 2018a). Particulate matter is emitted into the atmosphere from natural sources such as volcanoes, dust storms, wildfires and sea spray. Anthropogenic sources include the incomplete burning of fossil fuels, dust from unpaved roads, power plants and industrial processes. It may be composed of many hundreds of components including nitrates and sulfates, organic chemicals, metals, and dust particles (US EPA, 2018b). Particulates can be emitted directly into the atmosphere (primary) or can form in the atmosphere by chemical reactions (secondary) (US EPA, 2018a).

Particulate matter is differentiated by size fraction according to its aerodynamic diameter. Coarse particles (PM_{10}) measure between 2.5 and 10 µm in diameter, and fine particles ($PM_{2.5}$) are those that measure less than 2.5 µm. Generally, natural sources of PM are dominated by the coarse fraction while anthropogenic sources occur mainly in the fine fraction (Clements et al., 2016; Mamun et al., 2020). The smaller, lighter particles of the finer fraction

travel farther than the large particles and remain suspended in the atmosphere longer (Kim et al., 2015). Therefore, fine particles may be transported over long distances and redistribute at local, regional, and even global scales, influencing atmospheric concentrations far from the original emissions source (NRC, 2010). In rural environments, agricultural practices contribute to atmospheric PM through land clearing and cropping, application of fertilizers and pesticides, biomass burning, and the operation of diesel-powered farm equipment (Sun et al., 2017). Concentrated animal feeding operations also contribute to PM emissions (Kundu & Stone, 2014; US EPA, 2020). In an urban environment, industrial processes, energy production, and vehicle-related emissions (fuel combustion, road dust, brake- and tire-wear) are the main sources of PM (Clements et al., 2016; NRC, 2010).

Particulates emitted into the atmosphere are eventually removed via wet and dry deposition. Wet deposition occurs when PM is dissolved in rain, snow, sleet, or hail and returns to the surface via precipitation or when insoluble particles are washed out of the atmosphere. Dry deposition occurs when PM settles directly on surfaces (Ponette-González et al., 2016). While wet deposition is mainly influenced by changes in precipitation, many factors affect dry deposition rates. These include meteorological conditions, the properties of the particle and its atmospheric concentration, as well as properties of the deposition surface (Chen et al., 2012; Lou et al., 2017; Weathers & Ponette-González, 2011). High wind speeds and rough surfaces produce greater turbulence and therefore greater dry deposition (Chen et al., 2012; Weathers & Ponette-González, 2011). Relative humidity, temperature, and particle size are also important factors (Lou et al., 2017). As distance from the emission source increases, atmospheric concentrations of PM decrease, and therefore, both dry and wet deposition rates

decrease as well (Weathers & Ponette-González, 2011).

In exploring ways to mitigate atmospheric PM, many studies have looked at PM deposition onto vegetation surfaces in urban environments. Rindy et al. (2019) found that urban oak trees in the City of Denton, Texas, could accumulate 3.5 tons of soot, a component of fine PM, per year. Other studies consider how vegetation structure (Dzierżanowski et al., 2011) and placement of vegetation within the urban area (Hofman et al., 2014a) influence deposition rates. Mitigation measures to reduce particle pollution at the source and to improve scavenging rates from the atmosphere are of increasing importance due to the negative health and environmental effects associated with exposure to PM (Bourdrel et al., 2017; Du et al., 2016; Falcon-Rodriguez et al., 2016).

Physiological Responses: Particulate Matter and Health

Particulate matter is detrimental to health when inhaled, the finest fraction posing greater risk as it travels deep into the lungs where it can be absorbed into tissues and pass into the circulatory system (Losacco & Perillo, 2018). This can lead to heart and lung disease, which results in premature death in humans (Anenberg et al., 2019). The World Health Organization (WHO) estimates that globally 4.2 million premature deaths can be attributed to ambient air pollution. This number does not account for the adverse health effects to the 3 billion people who burn biomass or use fossil fuels for cooking and heating within their homes (WHO, 2018). Atmospheric PM also provides a surface for adsorption and transmittal of other harmful airborne substances such as heavy metals, polyaromatic hydrocarbons (PAHs) (Ball & Truskewycz, 2013), and pathogens (Kalisa et al., 2019; Zhao et al., 2019). PM-bound substances may have greater adverse health effects because they remain in the body longer than unbound

substances (Ball & Truskewycz, 2013).

In addition to contributing to heart and lung conditions and increased risks of mortality (Lu et al., 2015), exposure to PM may also result in low birth weight and pre-term deliveries in infants, adversely affect lung development in children, and has been correlated with adult diabetes (Kim et al., 2015). Over the next 30 years, the United Nations (2019) estimates that 2.5 billion people will move into urban areas. This increase in population density and associated increases in traffic density and vehicle emissions means an increase in exposure to PM and its harmful effects.

Birds also suffer adverse effects due to exposure to atmospheric air pollution. To meet the high oxygen demands of active flight, the avian respiratory system utilizes a unique combination of lungs and auxiliary air sacs to accomplish a quick and continuous exchange of gases. Inhaled air from the trachea moves first to air sacs posterior to the lungs. On exhalation, this air is returned forward to the lungs where gas exchange occurs. A second inhalation moves the air from the lungs, now depleted of oxygen and rich in carbon dioxide, to further anterior air sacs from which the stale air is finally expelled back out through the trachea in a second exhalation (Proctor & Lynch, 1993). This continuous, unidirectional airflow results in a highly efficient respiratory system, making birds more susceptible to health-related effects from atmospheric pollutants. Consequently, inhalation and bioaccumulation of these pollutants result in altered blood pathogen loads (Gasparini et al., 2014), as well as respiratory distress, illness, immunosuppression, and impaired reproductivity (Sanderfoot & Holloway, 2017). For example, European starlings nesting in areas of Calgary showed a reduction in clutch size with greater exposure to atmospheric contaminants as well as increased oxidative stress (North et

al., 2017), an effect also seen in house sparrows occupying urbanized areas in Spain (Herrera-Dueñas et al., 2014).

External reactions with air pollutants may also affect bird populations. For example, Kylin et al. (2011) hypothesized that sulfonates formed from chemical reactions in the atmosphere between natural volatile organic compounds and urban air pollution act as detergents in dense fog. This may reduce the nesting success of the South African Blue Swallow (*Hirundo atrocaerulea*) by leading to degradation of the water repellant layer on feathers, which allow the swallow to forage in its native habitat, mist-belt grasslands. In a population of Great Tits (*Parus major*), air pollution indirectly affected plumage brightness by reducing the number of carotenoid-rich prey insects in their forest habitat, which provide the necessary pigmentation that influences the color hues in feathers (Eeva et al., 1998). Direct external deposition of PM can alter plumage brightness and color as well. Using museum specimens of various bird species collected within the U.S. manufacturing belt over a 135-year period, DuBay & Fuldner (2017) measured the photometric reflectance of the feathers to show changes in feather brightness caused by accumulations of black carbon, a component of fine PM. The least reflective feathers revealed a peak in black carbon deposition to feather surfaces from 1900 – 1910, declining after the 1920s with the introduction of cleaner fuels and new environmental policies. Chicken feathers exposed to vehicle-related emissions at two urban microhabitats 1.5 km apart collected significant amounts of elemental carbon (EC) over a five-day period, showing an eight-fold difference in median accumulation between the two sites (Pitre et al., 2021). These studies illustrate how feathers can be used to show temporal and spatial changes in atmospheric pollution. Accumulation of atmospheric contaminants darkens feathers, and as a

result may affect mate selection, camouflage, signaling and communication as pigmentation is a key component in these behaviors (Griggio et al., 2011).

Biomonitoring

As many pollutants have persistent footprints in the physical environment, much research focuses on biological monitoring. Biological monitors, or biomonitors, provide quantitative information on the quality of the environment around them (Wolterbeek, 2002). Rather than depending on direct measurements of individual physical parameters such as temperature or pollutant concentrations, biomonitors enable the tracking of changes in the environment by monitoring the cumulative effects in a species. From Holt and Miller (2010), good biomonitors are characterized by: (1) the ability to "provide a measurable response…in proportion to the degree of contamination or degradation" across the entire population, community, or ecosystem; (2) an abundant, common, and stable population; (3) an organism well-studied through simple and affordable surveys so that its life history and ecology are thoroughly understood and documented; and (4) an organism that has the interest of the public.

Birds have proven to be apt subjects for such research due to their global distribution, well-known flight ranges, and use of multiple habitat types (Sanderfoot & Holloway, 2017). Birds common to the human urban environment are continuously exposed to anthropogenic atmospheric pollution in their aerial habitat. Birds have a higher metabolism and as discussed above, a more efficient respiratory system than humans. These unique traits result in greater sensitivity to atmospheric contaminants, and therefore, an opportunity to utilize urban birds, especially, as indicators of changing conditions (Brown et al., 1997).

Internal and External Contamination

In birds, pollution impacts may occur through external deposition to feather surfaces, or through internal accumulation via ingestion or inhalation. Several studies have explored bioaccumulation, or internal ingestion, absorption, and storage of contaminants in bone, tissue, and feathers of birds (Bortolotti, 2010; Jaspers et al., 2009). Bioaccumulation of contaminants in tissue and bone reflect successively longer processes and are indicators of long-term environmental exposure. Contaminant levels in the blood stream are the result of recent ingestion while that in feathers reflects accumulation during feather growth. Heavy metals and organic pollutants are the most widely monitored and may be a component of PM through agglomeration via adhesion or absorption.

By comparing contaminant levels among sample types, regional patterns of contamination can be revealed. Nam et al. (2004) analyzed concentrations of Pb and Cd in the eggs, organ tissue, bone, and crop and gizzard contents of young and adult feral pigeons (*Columba livia*) collected from one urban and one industrial site. Concentrations increased with bird age and were found to be higher in the bone and tissue of the urban birds.

It is also possible to identify the mode of contamination, whether internal or external. For example, to accurately identify lead poisoning from lead ammunition ingestion in two scavenging bird species, Cardiel et al. (2011) compared Pb and Al levels among bone, feather barbs, and rachises. The rachis, or central shaft of the feather is smooth with a small surface area which discourages external PM deposition. Therefore, contaminant levels in the rachis accurately reflect exposure due to ingestion. The complicated surface structure of the feather vanes attracts external deposits and is best suited for quantifying external contaminants. As

aluminum is known to be minimally bioaccumulated due to its low intestinal absorption rate, this metal was used as a marker of external contamination. Aluminum and Pb levels on the feather vanes were positively correlated indicating external contamination. However, Pb levels in the rachis were found in absence of Al and correlated to levels in the bone, indicating internal accumulation.

Common urban species such as sparrows, starlings, and pigeons are providing valuable information on the effects of urbanization on people and other wildlife. Urban birds can be used to detect and monitor anthropogenic pollutants common to urban environments to determine regional and local variations in accumulation and persistence. For instance, in neighborhoods of Manhattan, pigeons were used as bioindicators of lead in the environment. Researchers discovered a positive correlation between the blood levels of pigeons and that of children living in the same neighborhoods (Cai & Calisi, 2016). Furthermore, avian physiological and behavioral responses to toxicant exposure can be studied to understand potential adverse health effects in people. As potential vectors and reservoirs of pathogens, birds are also valuable as monitors of zoonotic diseases which spread more easily in densely populated areas (reviewed in Pollack et al., 2017).

Feathers

Feathers are a widely used sample type for biomonitoring studies (Espín et al., 2016; Janiga et al., 2019; Malik & Zeb, 2009; Pitre et al., 2021; Roque et al., 2016) and have been utilized to track multiple pollutants in diverse environments (Bianchi et al., 2008; Borghesi et al., 2016; Hahn et al., 1993; Hughes et al., 1997). Hughes et al. (1997) found differences in mercury contamination in feathers collected in urban and rural areas, and among breeding

grounds, wintering grounds, and migration routes of nesting Osprey in the Great Lakes region of North America. Hahn et al. (1993) made comparisons of mercury levels between the feathers of urban and rural resident bird species, and between species with different foraging habits.

Feral pigeons have also proven useful for monitoring public and wildlife health concerns in urban areas (Brait & Antoniosi Filho, 2011; Cai & Calisi, 2016; Frantz et al., 2012; Liu et al., 2010; Schilderman et al., 1997). Their small home ranges have allowed researchers to show variation in contaminants within urban habitats. Feathers collected from pigeons in three sites in Brazil helped distinguish between internal and external pathways for accumulation of certain heavy metals, showing greater external accumulation of Cd, Pb and Cr as population and traffic density increased (Brait & Antoniosi Filho, 2011). Metal concentrations in feathers were also used to confirm the restricted home ranges of feral pigeons in areas of Paris with differing degrees of urbanization. Concentrations differences were apparent between populations occupying habitats less than 800 m apart (Frantz et al., 2012). Urban pigeons have also been used to track polycyclic aromatic hydrocarbons (PAHs), a potential component of PM considered especially dangerous because of its mutagenic and carcinogenic effects (Liu et al., 2010, Schilderman et al., 1997).

Feathers are widely used in biomonitoring studies because they are easy to collect, store and transport, and provide a non-destructive sampling method. Different feather types collected from different locations on the bird can reflect the duration and magnitude of exposure. For instance, the outer primaries of wing feathers (remiges) and outer tail feathers (rectrices) experience greater wear due to their constant exposure to the elements (Dauwe et

al., 2003).

Due to their unique structure, certain feather parts are useful for distinguishing between external and internal (i.e., bioaccumulation) accumulation of contaminants. The complex shape of the barbs (vane) of the feather physically trap and retain external contaminants, leaving residues even after multiple washings (Jaspers et al., 2008). Although vanes store contaminants internally during growth of the feather, using vanes to measure internal accumulation becomes difficult as results are confounded by the presence of external deposits. This makes the vane more suitable for examining external deposition.

Internal contaminants within feathers are deposited by the blood stream and accumulate as the feathers grow. When feather growth is complete, the blood vessels atrophy and hence the feather is physically separated from the bird (Dauwe et al., 2003). Resulting contamination levels in the feather reflect levels that were available in the blood stream during the growth period. Contaminants are lost with the feathers during molt, the periodic replacement of worn and damaged feathers. Subsequently, contamination on and within the feathers is representative of a limited timescale of exposure (Cardiel et al., 2011; Niecke et al., 1999). The rachis of the feather is best for obtaining internal contaminant concentrations as its smooth structure does not capture external contaminants as efficiently as the vanes (Borghesi et al., 2016; Cardiel et al., 2011).

For effective use of feathers in biomonitoring studies, consideration of how the feather grows and which elements are essential to the structure of the feather are important (Bortolotti, 2020). For example, sulfur and zinc are essential to the formation of keratin and melanin, respectively, within feathers (Niecke et al., 1999). These elements may be expressed

accurately as mass-dependent and are not suitable for biomonitoring. However, bioaccumulation of non-structural elements (e.g., contaminants) is better expressed in terms of accumulation per length of feather, or time-dependent deposition. This avoids problems associated with variations in mass between different types of feathers and feather parts, as mass varies from rachis to vane, proximally to distally along the feather, and asymmetrically about the rachis. Aerial deposition of external contaminants is also better expressed in terms of length of feather exposed (Bortolotti, 2010).

In a review of various studies using feathers for biomonitoring purposes, Jaspers et al. (2019) emphasizes that feathers are not appropriate biomonitoring samples for all environmental pollutants. Therefore, careful research design is imperative for obtaining accurate data, and thus determining appropriate explanations and conclusions. This should include (1) characteristics of the bird species, including its migratory/resident status, foraging strategies, range, and life-history traits; (2) the environment and pollutants to be monitored; (3) the types of feathers and/or feather parts; (4) collection and storage of feathers; (5) preparation and extraction; and (6) quality control and assurance measures across all aspects of a study (Jaspers et al., 2019).

Though biomonitoring has proven effective at tracking internal accumulation of environmental pollutants, less is known about the extent and magnitude of external contamination and how it may affect individuals or populations of birds (Pitre et al., 2021). Many studies which quantify external accumulation do so only to correct for its presence and thus determine more accurate measurements of internal accumulation (Borghesi et al., 2017; Dauwe et al., 2003; Jaspers et al., 2004).

Conclusion

As urbanized areas grow, atmospheric PM associated with concentrated human activity will continue to pose a threat to human health and to the health of species that share the urban environment. Common urban birds have been utilized in multiple studies to understand the source and fate of anthropogenic pollutants (Brait & Antoniosi Filho, 2011; Frantz et al., 2012, 2016; Scheifler et al., 2006) and highlight potential risks to human (Cai & Calisi, 2016; Kim et al., 2015; Lu et al., 2015) and avian (Bauerova et al., 2017; North et al., 2017) health. While many studies address the adverse outcomes from inhalation of atmospheric PM, research concerning the accumulation of external PM is sparse. This thesis explores the use of feral pigeons as indicators of the spatial distribution of atmospheric PM in urban areas by quantifying external deposition to feather surfaces.

CHAPTER 2

PARTICULATE MATTER DEPOSITION TO URBAN ROCK PIGEON (*Columba livia*) FEATHERS

Introduction

As the majority of the world's population concentrates in urban centers, the dense traffic and industry associated with human activities leads to ever-increasing amounts of air pollution (Clougherty et al., 2013; Han et al., 2016; Lee et al., 2007; Rodríguez et al., 2004; Wang et al., 2019). Particulate matter, a component of urban air pollution, is emitted into the air from natural and anthropogenic sources. Differentiated by its aerodynamic diameter, PM is very diverse and may be composed of any number of chemical compounds. Particles 10 µm and smaller are regulated because they can be inhaled and cause multiple adverse health effects (WHO 2013). Coarse, PM_{10} (2.5 – 10 µm) and large particles (> 10 µm) are generally dominant in natural emissions, such as dust and pollen. The fine fraction, $PM_{2.5}$ (< 2.5 µm), is mostly dominant in anthropogenic emissions such as vehicle exhaust, industrial processes, and power generation (US EPA, 2018a).

Because of the health burden associated with PM pollution, many studies quantify PM deposition on vegetation surfaces to determine the effectiveness of diverse plant species in monitoring and mitigating PM pollution in urban environments (reviewed in Cai et al., 2017). In this same vein, the feathers of urban birds have been used extensively in biomonitoring studies to track bioaccumulation, or internal storage of ingested contaminants, such as heavy metals which are deposited within a feather during its growth (Bianchi et al., 2008; Cardiel et al., 2011; Espin et al., 2016; Hahn et al., 1993; Jaspers et al., 2009, 2011). Many of these previous studies quantify external accumulation to feathers, but do so to control for its presence, excluding the

measure to obtain more accurate calculations of internal accumulation (Borghesi et al., 2017; Dauwe et al., 2003; Jaspers et al., 2004).

However, the external accumulation of contaminants on feathers can provide valuable information as well (Borghesi et al., 2016; DuBay & Fuldner, 2017; Petri et al., 2021). External contaminants are deposited to the feather surface, trapped in the complex structure of the vanes. These trapped contaminants are observable in micrographs produced via scanning electron microscopy (SEM). Feathers collected from Greater Flamingo (*Phoenicopterus roseus*) chicks revealed densely packed and diverse particulates (Borghesi et al., 2016), and feather vanes of museum specimens showed accumulation of black carbon particles (DuBay & Fuldner, 2017) under SEM. Capture can vary over fine spatial scales as well. Experimentally exposed molted chicken feathers showed an eight-fold increase in black carbon deposits between two urban microhabitats 1.5 km apart (Pitre et al., 2021). These small-scale variations in PM deposition matter, suggesting that birds experience different levels of exposure that could affect feather function. Bright, colorful feathers are signals of individual health and important in sexual selection, and social dominance (Eeva et al., 1998). As such, birds spend much effort to maintain these signals through preening, an effort which may necessarily increase if inhabiting environments with heavy air pollution (Griggio et al., 2011).

Pigeons are ubiquitous year-round residents of the human urban environment. Their sizeable feathers are most likely capable of capturing measurable amounts of PM. Their small home ranges allow for distinct populations to occupy unique microhabitats with minimal overlap, and thus, individuals from different populations may experience different degrees of exposure to atmospheric PM. Previous research using molted feathers that were cleaned prior

to exposure to vehicular traffic over a five-day period showed a significant increase in black carbon deposits that correlated positively with proximity to a highway (Pitre et al., 2021). What is not known is if black carbon or PM deposition varies on feathers remaining on the bird over time, and if so, whether sufficient differences exist that would allow the use of feathers from wild populations for biomonitoring purposes.

In this study, we used feathers collected from wild rock pigeons at multiple urban locations to investigate whether external PM deposition on their feathers differed relative to exposure to vehicular traffic and other land use differences. The results from this study should be useful for subsequent research to help identify best practices for using feathers collected from pigeons or other urban restricted bird species to monitor PM levels across multiple spatial scales.

Research Objectives

Specifically, the objectives of this study were to: (1) observe pigeon behavior and characterize land use/land cover among urban pigeon habitats, and (2) quantify external PM accumulation on pigeon feathers at two sites contrasting in land use/land cover in a rapidly growing urban area. We expected to find greater PM accumulation in more densely urbanized areas, as well as in areas downwind of densely developed areas and high-traffic roads.

Study Area

Location and Climate

This study was conducted in Denton County, Texas, about 30 km north of the Dallas/Fort Worth metroplex. The City of Denton is the county seat, located at 33.1991˚N and 97.1049˚W.

Other principal urban areas are located to the south of the City of Denton, concentrated along the I-35 corridor, State Highway 121, and the region bound to the north by U.S. Highway 380, to the east by the county line, and to the west by Lewisville Lake. Principal rural areas are located north of U.S. 380 and west of I-35W.

The climate is humid subtropical, characterized by hot and humid summers (NWS, 2021b). Though winters are generally mild, northers drop temperatures abruptly and occur approximately three times per month. The 30-year average (1981 – 2010) minimum temperature (0.56˚C) for the year occurs in January and the maximum occurs in August (35.4˚C) (NWS, 2021a). Precipitation occurs throughout the year, mostly in the form of rain, averaging about 97 cm per year with a range between 45 and 144 cm. The month of May receives the most rainfall on average, and August is the driest month (NWS, 2021a). Average annual wind speed is 15 km/hr, with average maximum wind speeds of ~20 km/hr. Throughout most of the year, prevailing winds are from the south (Iowa Environmental Mesonet, 2021).

Urbanization and Particulate Matter Sources

In 2018, the population for the county was estimated at 859,064 and for the City of Denton at 138,541 (U.S. Census Bureau, 2020). This is a 29.7% and 19.1% increase in population for the county and city, respectively, since the 2010 census. This rapid population growth, urbanization, and increase in traffic density contribute to increases in ambient PM concentrations from mobile and point source emissions.

The Interstate 35 corridor travels north to south through the county, splitting in the City of Denton into I-35W towards Fort Worth and I-35E towards Dallas. U.S. Highway 380 is another major transport corridor, traveling east to west. Other major roads include Loop 288,

and State Highways 114, 170, and 121 (Figure 1). Per the Texas Department of Transportation (TxDOT), these roads experience high traffic volumes, especially in commercial vehicles along I-35 (TxDOT, 2018b). Maximum annual average daily traffic (AADT) counts monitored by TxDOT for 2018 are summarized in Table 2.1 (TxDOT, 2018a). In 2018, these roads totaled about 233 centerline kilometers, and experienced around 10.6 million daily vehicle kilometers travelled (DVKT), of which 1.1 million were by commercial trucks (TxDOT, 2018b).

Table 2.1: Annual average daily traffic counts (AADT) for major roads within Denton County, Texas (TxDOT, 2018a).

Road	2018 AADT Maxima
State Hwy 121	186,071
Interstate 35E	174,314
Interstate 35	103,210
State Hwy 114	96,709
Interstate 35W	71,374
State Hwy 170	52,124
U.S. Hwy 380	42,277
Loop 288	29,915

In addition to these local sources of PM emissions, Dallas and Tarrant counties located to the south contribute to air pollution in Denton County. Prevailing winds transport air pollution from this highly urbanized area with a combined population of over 4.7 million people to Denton County (U.S. Census Bureau, 2020). Within these counties are 1,204 centerline kilometers of major roads which experienced 62.7 million DVKT of which 5.25 million were by commercial trucks in 2018 (TxDOT, 2018b).

Figure 2.1: Roads with greater than 48,280 daily vehicle kilometers (DVKT) traveled in the county shown in relation to identified pigeon populations and prevailing winds.

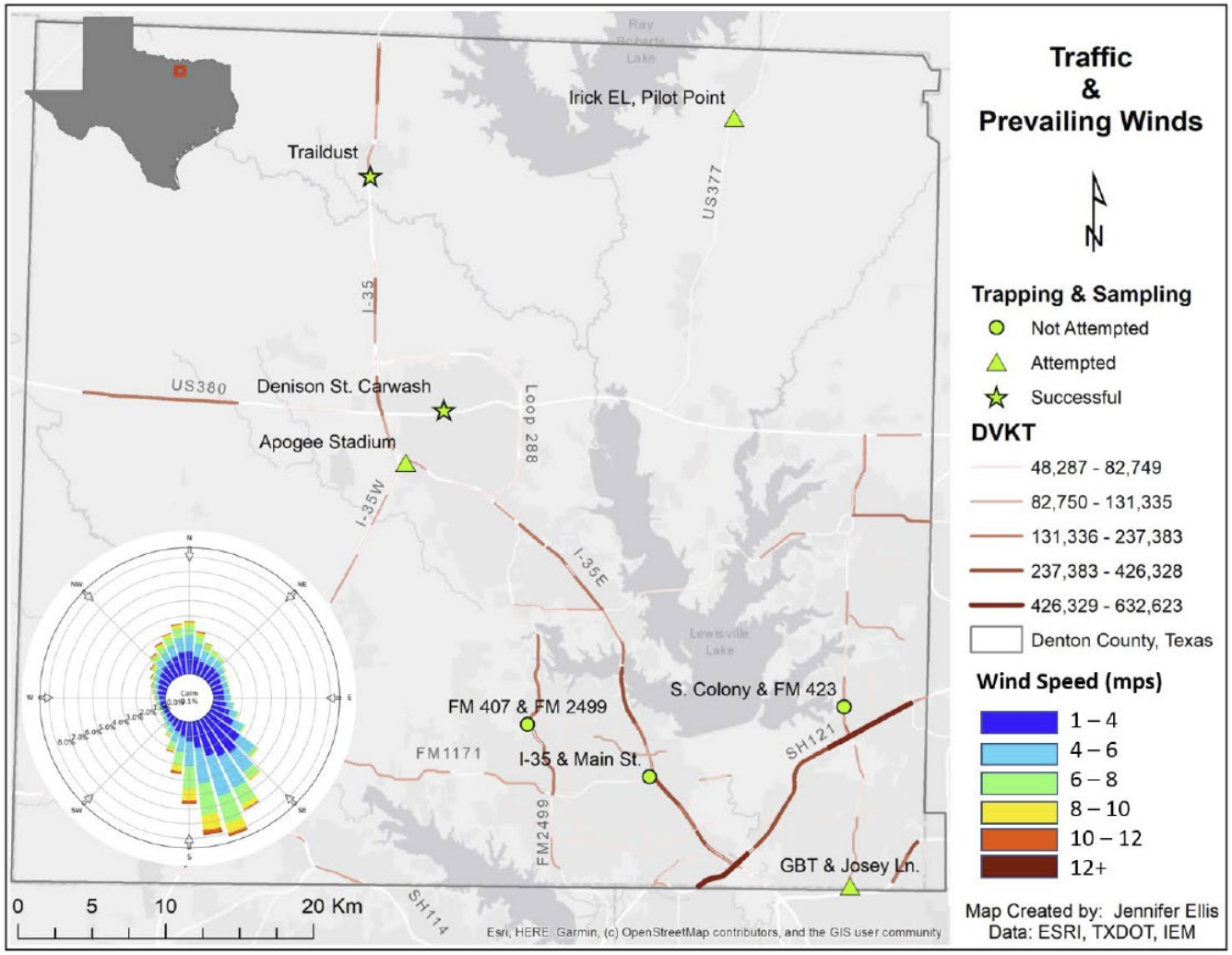


Figure 2.2: Map of land cover types within the Denton County, Texas, as determined by the 2016 National Land Cover Database (NLCD) (Homer et al., 2020; Jin et al., 2019; Yang et al., 2018).

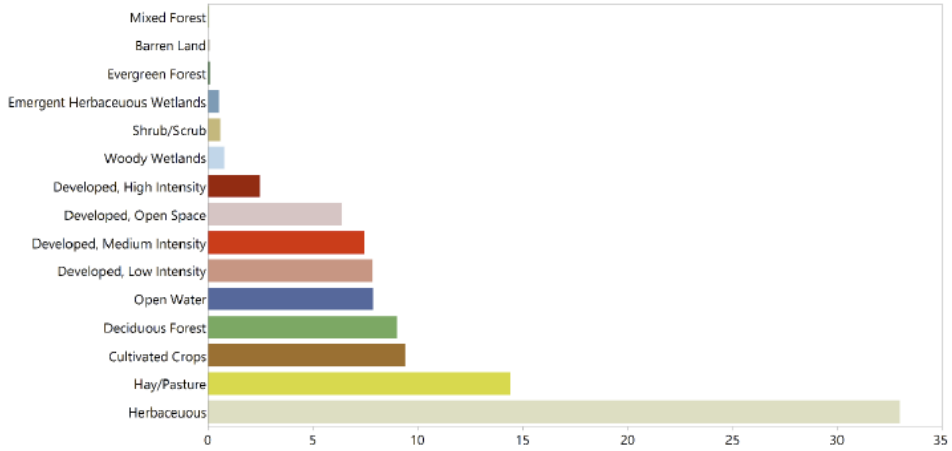
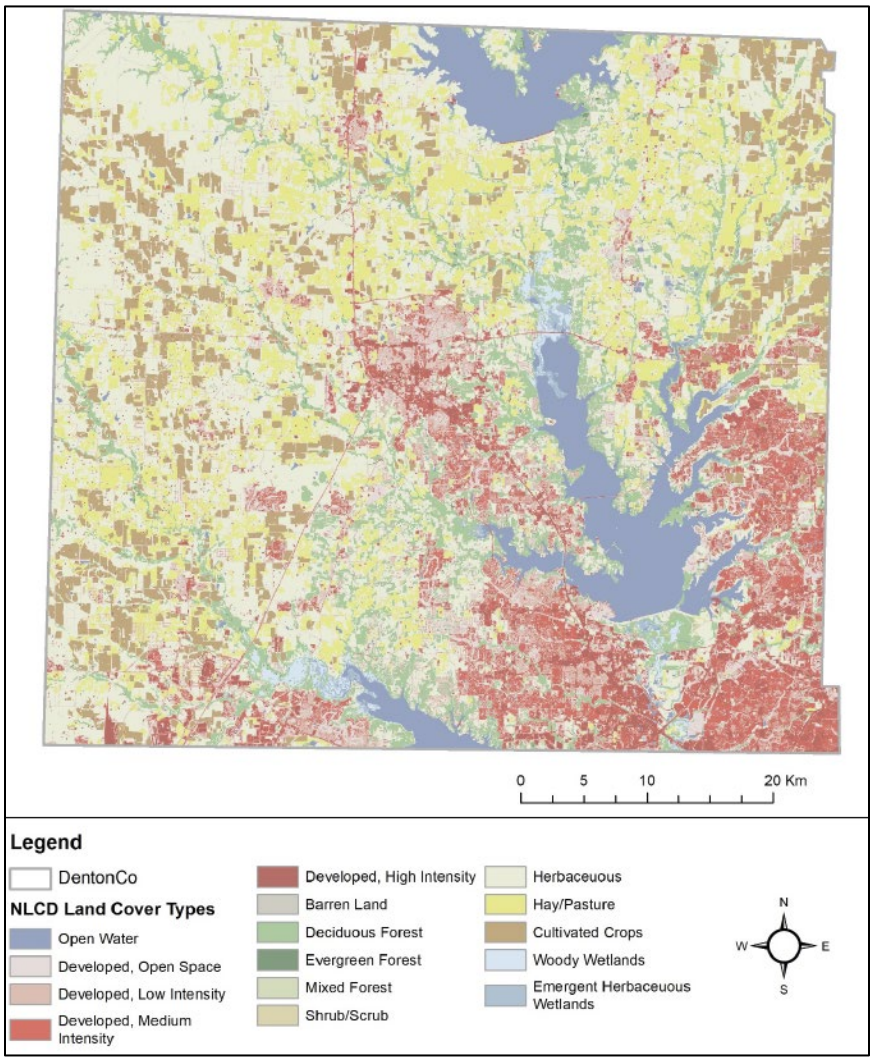


Figure 2.3: Percent land cover within Denton County as determined by the 2016 National Land Cover Database (NLCD) (Homer et al., 2020; Jin et al., 2019; Yang et al., 2018).



Land Cover

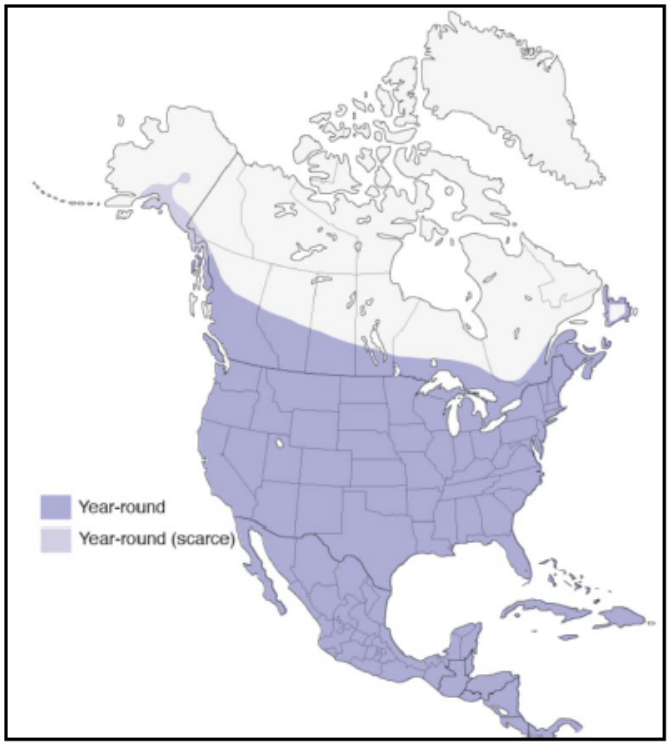
The county covers 227,664 hectares of land. An additional 19,461 hectares is open water comprised mainly of Lewisville Lake and portions of Ray Roberts Lake and Grapevine Lake. The majority of the county is in herbaceous cover (33%). Developed areas account for 23% of the county's land cover. Pasture, cultivated crops, and deciduous forest cover 14, 9, and 9%, respectively (Figures 2.2 and 2.3).

Focal Species: Rock Pigeon (*Columba livia*)

Life History

In North America, the common Rock Pigeon (*Columba livia*) can be found throughout the United States with populations extending into southern portions of Canada and south throughout Central America (Figure 2.4).

Figure 2.4: Range of the feral rock pigeon (*Columba livia*) within North America (Lowther & Johnston, 2020).



This species has a long history of domestication. Wild populations native to Europe, North Africa, and parts of Asia were captured and domesticated beginning as far back as 5,000 years ago (Stringham et al., 2012). Prolific and fast-growing with remarkable homing abilities, pigeons have been and still are raised for food, sport, and companionship. Over the years, escaped domestics known as feral pigeons, have used these human-selected traits to adapt successfully in the wild and are now prevalent the world over.

Originally introduced to the United States around the early 1600s, feral pigeons followed the dispersal of human populations and their distribution and abundance remain closely associated with human activities (Lowther & Johnston, 2020). Rather than employing cliff-side crevices for nesting areas like their wild counterparts, feral pigeons became year-round residents in dense urban communities as well as rural areas. They utilize the nooks and crannies of man-made structures and take advantage of the reliable food sources provided by urban excess and agricultural crops (Lowther & Johnston, 2020).

On average, feral pigeons measure 32 cm (+/- 5% or more) from bill tip to tail tip, have a wingspan of 71 cm (+/- 5% or more), and weigh around 270 g (+/- 30% or more) (Sibley, 2000). Males are larger than females. Breeding birds significantly outweigh non-breeding birds, male and female. In North America, females increase in size with increasing latitude, a trend not found in males (Johnston, 1990). Due to years of selective breeding among their domestic ancestors, modern-day feral pigeons can have many different phenotypes. The blue-bar variant most closely resembles wild populations, but blue-black, reddish, brown, white, pied, and checker variants are common as well and may occur simultaneously in a population (Lowther & Johnston, 2020).

Seeds, particularly from agricultural crops like corn, wheat, and barley, make up the greatest portion of the feral pigeon diet. Fruits are also eaten along with the rare invertebrate. Pigeons are opportunistic foragers and in the presence of humans will eat bread and other common human foodstuffs (Goodwin, 1983). Studies have shown that pigeons are able to live on a single food item for long periods, and that individual birds will show preferences for specific food items. Pigeons forage early in the morning and before nightfall and in urban areas will time foraging to match human activity, typically at mid-day (Lowther & Johnston, 2020). Travel distance from the home roost to a foraging site may be less than 1 km or rarely as far as 25 km, but most birds stay within 2 km (Rose et al., 2006).

A large part of the successful global dispersal of feral pigeons can be attributed to their prolific breeding ability (Lowther & Johnston, 2020). Individuals may attain sexual maturity and breed in their first year, however they are not as successful as adult birds and are limited by the availability of nesting sites. A level covered ledge is all that is needed for a feral pigeon nest. The male brings nest material to the female who tucks it around her. Subsequent nestings occur in the same site, the pair building a new nest on top of the old. Older nests are robust, as accumulation of fecal pellets glues the materials together. In North America, nesting may occur year-round, but most commonly from February through September. Each brood consists of two eggs, laid about 40 hours apart, after which incubation begins, lasting about 18.5 days. Incubation duties are divided equally among daylight hours by both sexes (Lowther & Johnston, 2020).

Hatchlings are born altricial, or underdeveloped and helpless, but grow quickly, flying around day 30-32 and leaving the nest between 25 – 45 days depending on the season. Fast

growth and thermoregulation of the young are possible because of the crop milk fed to the young by both parents. A product of the crop epithelium, crop milk is high in fat and protein. Crop milk is fed exclusively until day 4, after which seeds slowly replace it, transitioning completely to an adult diet after day 9. In summer, breeding pairs may have overlapping clutches, hatching two squabs *after* laying eggs of the subsequent clutch. Intervals between clutches may be as short as 29 days in the summer, extending up to 60 days in the fall. The life expectancy of feral pigeons averages 2.4 years. Adult breeding pairs average 6.0 young per year (Lowther & Johnston, 2020).

Feathers and Molt

Birds follow a general pattern of feather replacement, known as a molting sequence. A complete molt replaces body feathers and flight feathers. An incomplete molt may replace only body feathers, or some of the flight feathers. Flight feathers include the feathers of the wings (remiges) and those of the tail (rectrices). Rock pigeons have 10 primaries and 15 secondaries on each wing, and a total of 12 tail feathers. These feathers are generally numbered according to the order in which they molt (see Materials and Methods section). Primaries molt proximally to distally (P1 – P10). Secondaries molt outwardly from S10 and distally to proximally from S1. Molt of the tail feathers begins with T5 and most commonly ends with T2, the sequence varying for the remaining feathers (Johnston & Janiga, 1995).

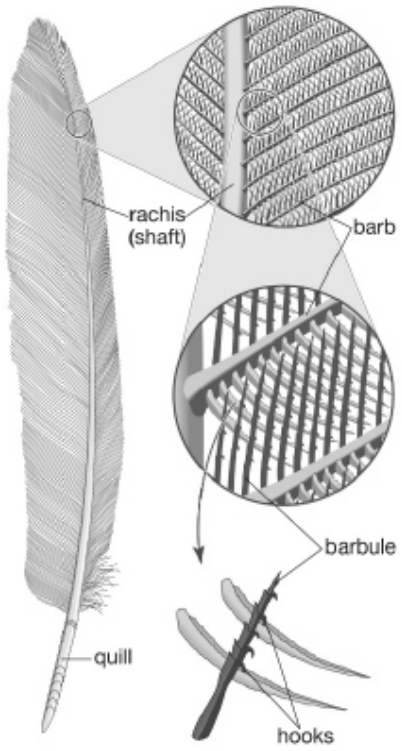
Rock pigeons follow what is called a “Complex Basic Strategy”. Unlike many other species of birds, these common birds do not develop elaborate alternate breeding plumage for attracting mates. Though there are periods during the year which are common to see certain plumages or the beginning of molts, rock pigeons may replace feathers year-round. A complete

molt is usually accomplished over 6 months from June through November and overlaps with the breeding season. Various factors including photoperiod, temperature, location, breeding status, dietary protein intake, and illness are known to affect the timing of molt in populations and individuals (Johnston & Janiga, 1995).

Feather Structure and Function

The molting process ensures that the feather coat remains in good condition. Healthy feathers are required to protect the skin from injury, to provide insulation, efficient flight, waterproofing, communication, and sensory reception. Six major types of feathers have been identified among birds, each differing in shape, structure, and function. Rock pigeons possess five of these types: contour feathers, down feathers, semiplumes, filoplumes, and powder feathers (Proctor & Lynch, 1993).

Figure 2.5: The structure of a typical contour feather (Lewis, 2021).



Contour feathers are the vaned (pennaceous) feathers of the body, wings and tail. Their structure includes a central shaft (rachis), with a vane on each side. These vanes are formed by parallel barbs that branch off from the rachis. Each barb has smaller branches called barbules, and these have even smaller projections called barbicels. Hamuli on the barbicels act as tiny hooks (Figure 2.5). The combination of these structures secures the feather surface, preventing the vanes from separating. Body contour feathers have symmetrical vanes, whereas flight contour feathers are asymmetrical; anterior vanes are narrower than posterior, resulting in the airfoil cross-section necessary for flight (Proctor & Lynch, 1993).

Down feathers do not have a rachis. The barbs are connected at the base of the feather (calamus) and do not have the finer barbicel-hamuli structures to fasten them together, hence their "fluffy" (plumulaceous) appearance. Semiplumes are plumulaceous as well but have a central rachis. Down and semiplumes lie under the contour feathers and are vital for insulation (Proctor & Lynch, 1993). Filoplumes are long feathers, hair-like, with a mostly bare rachis containing only a few unattached barbs. These feathers are positioned between and may extend beyond the contour feathers. Conditions outside the protective feather coat are transmitted to the bird via sensory corpuscles located at the base of the feather. This tactile feedback may include information about wind, airspeed, and position of the flight feathers (Proctor & Lynch, 1993). Powder feathers are unique. The barbs of this feather break down into a fine powder which is used for grooming and possibly waterproofing the feathers. Powder feathers never molt and are constantly grown. These feathers are scattered throughout the down feathers (Proctor & Lynch, 1993).

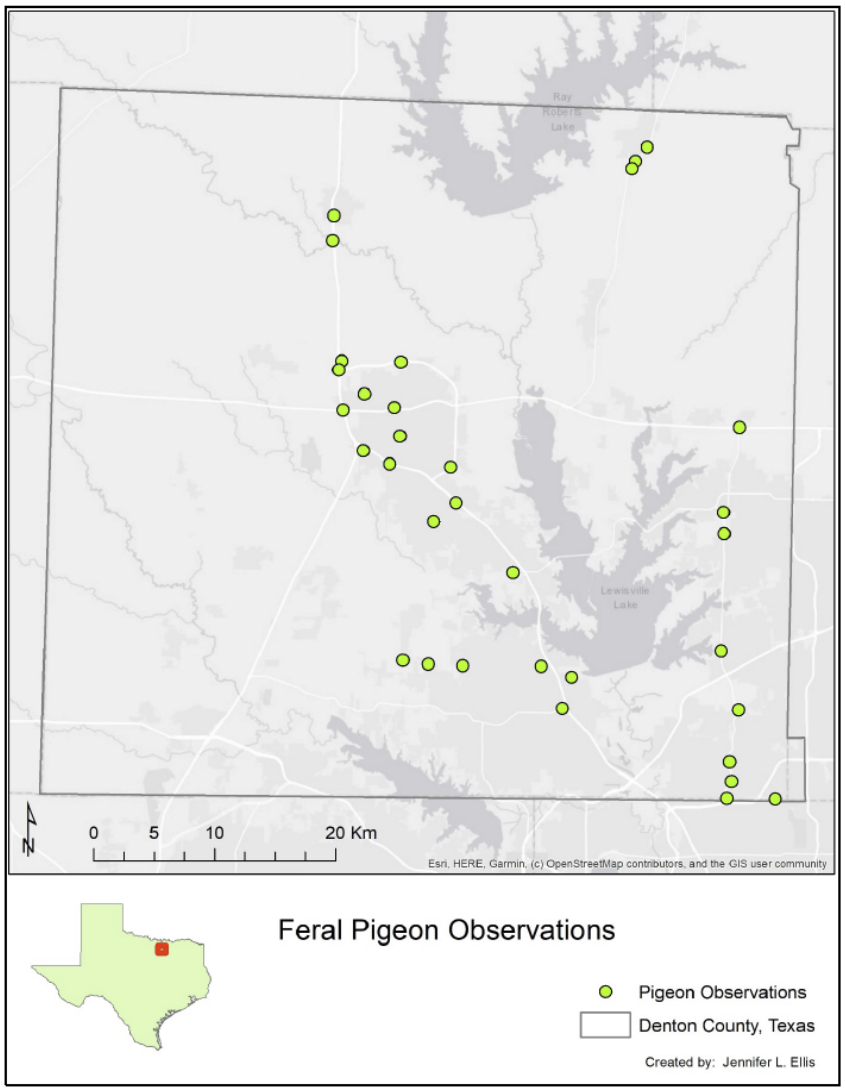
Most birds, including the pigeon, possess a uropygial gland. Located at the base of the tail, the gland secretes preen oil through the pores, which birds access via their beaks or heads and spread across the feathers. This oil conditions the feathers and varies in morphology and excretions across species. It may provide varying degrees of waterproofing or act as an antiparasitic (Salibian & Montalti, 2009). The use and function of the uropygial gland in the rock pigeon is still unclear. Removal of the gland in one study resulted in degradation of pigeon feathers over a four-month period. This was attributed to breakage of the barbules during preening in absence of preen oil (Moyer et al., 2003). Despite this study, it is generally accepted that rock pigeons do not utilize their uropygial gland for preening, but instead depend upon their powder feathers for maintenance and waterproofing of feathers (Goodwin, 1983; Delhey et al., 2007).

Materials and Methods

Habitat Characterization and Observations

Pigeon populations in Denton County were identified by observation of nesting or roosting locations during the spring of 2020. A total of 33 populations were identified (Figure 2.6). Land cover within a 2 km radius buffer of eight sites with identified rock pigeon populations was characterized using the 2016 National Land Cover Database (NLCD) classification (Homer et al., 2020; Jin et al., 2019; Yang et al., 2018). Pigeon behavior was observed closely at the five locations where traps were placed. Observations occurred at least once per week for 1 - 3 hours throughout the summer of 2020. I was interested in the birds' foraging patterns (timing, distance, and duration), use of water sources for drinking and bathing, location and use of roosting sites, and interaction with people.

Figure 2.6: Locations of feral urban rock pigeons observed in Denton County, spring 2020.



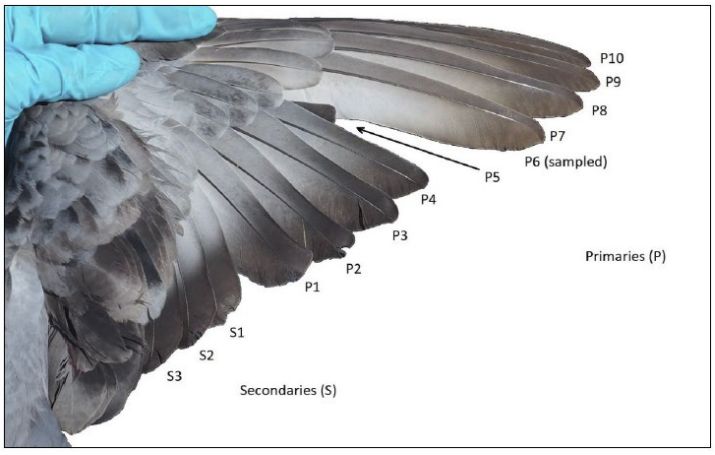
Feather Sampling

Pigeon trapping was attempted at five locations chosen based on variations in degree of urbanized habitat. These areas were pre-baited using a wild bird seed mix of milo, millet, and wheat for at least two weeks. When the birds became accustomed to the provided seed, an open (funnel removed) walk-in funnel trap made of wire mesh measuring 24”W x 24”D x 8”H (Figure 2.7) was placed on the ground and baited for an additional two weeks. When birds started arriving in numbers, the trap was set by inserting the funnel.

Figure 2.7: Left, a baited open trap with the funnel removed and the top access door open. Right, a similar trap with the funnel attached and top access door secured.

Trapping was successful at two locations contrasting in urban land use/land cover. Ten individual pigeons were caught at each location. From each bird caught at the two sampling locations, one primary feather from each wing and two tail feathers were plucked. Plucking is painless and assures the bird will receive the proper stimulus to regrow the feathers. "Older" feathers were chosen, their age determined by molting sequence, general wear, frayed edges, and fading color. From each population sampled, an additional two "newer" primary feathers were chosen. These feathers were identifiable by crisp colors, smooth edges, and lack of wear (Figure 2.8).

Figure 2.8: This picture shows a clear molting pattern in the right wing of a rock pigeon. Primaries 1-4 were recently replaced, as evident by their richer colors and crisp edges. Primary 5 is still growing. Primary 6 was plucked as a sample feather as it was next in line in the molting sequence. Primaries 7-10 are older, faded in color, and have worn edges.



Feral pigeons accomplish a complete molt once per year, replacing all feathers of their coat. Molting most commonly occurs from June through November, though worn flight feathers of the wings and tail can be replaced throughout the year (Lowther & Johnston, 2020). The first samples were collected July 24 – 27 at a restaurant located in the town of Sanger, just off I-35 in the northern portion of the county. The second set of samples were collected August 24 – September 8 at a self-service carwash in the city of Denton. Hereafter, these are referred to as the Traildust and Carwash populations, respectively. At both locations, the birds were actively molting flight feathers and the feather sampled was identified as next in sequence to molt (Table 2.1). As such, though the exact age of the feathers sampled is unknown, I estimate the feathers to be roughly one year old. This estimation is supported by one study of captive rock pigeons in which primaries 4 -10 had mean lifetimes ranging from 10.5 – 12.2 months with outer primaries P6 – P10 replaced less often than inner primaries P1 – P5 (Mallet-Rodrigues, 2012).

Table 2.2: Identification of feathers sampled from each population. Feathers are identified as primary (P) or tail (T) and the number indicates their position on the wing or tail, corresponding to the molt sequence.

Population & Sampling Date	Primaries (P)							Tail (T)						
	P4	P5	P6	P7	P8	P9	P10	T1	T2	T3	T4	T5	T6	Total
Carwash Aug. 24 – Sep. 8	2	2	3	3	8	0	2	2	8	5	2	1	2	40
Traildust Jul. 24 - 27	0	3	4	7	2[a]	2	0	1	1	7	5[a]	0	4	36[b]
Total	2	5	7	10	10	2	2	3	9	12	7	1	6	76

[a] Two tail feathers (T4) and two primary feathers (P8) sampled from one bird of the Traildust population were excluded from analysis but are included in this table. [b] Two secondary flight feathers (S2), and two tail feathers of unknown position are excluded from this table.

The new primary feathers sampled are estimated to be roughly 45 days old. This is

based on the growth rate and pattern of replacement reported in Mallet-Rodrigues (2012), in which an old feather would drop once the feather prior to the sequence in the molt had grown ¾ of its length or about 2 weeks. Complete growth is accomplished between 19 and 39 days depending on the size of the feather. These ages are a rough estimation as many factors can influence molt in feral pigeons, such as breeding status or nutritional deficiencies (Johnston & Janiga, 1995).

Each feather was placed in a paper envelope and the envelopes sealed in a plastic bag labeled with the site name and date. The samples were transported to the lab and stored at room temperature. Each set of four feathers collected from a bird were photographed on a solid background with a ruler for scale. The calamus, or base of the feather shaft, was clipped with acetone-cleaned scissors just below the base of the vanes. This was done so that the longer primary feathers would fit inside the capped amber bottles for the extraction process. For consistency among the samples, the calamus was clipped this way for all feathers.

Extractions and Filtration

Several preliminary trials were run to determine the most suitable methods for extraction, filtration, and gravimetric analysis (Appendix A). Glassware used for extraction and filtration was soaked overnight in double deionized (DDI) water, washed in a 10% nitric acid bath a minimum of 4 hours, and finally baked in a muffle furnace at 450˚C for 5 hours to remove all PM.

The four feathers plucked from each bird were pooled into one sample in order to collect measurable amounts of PM. The feathers were sonicated tip-down in a capped 500 mL amber bottle filled with 300 mL DDI water for 5 minutes, the feathers flipped on end, and

sonicated an additional 5 minutes. The feathers were then removed with tweezers and each side rinsed three times using an acetone squirt bottle over a graduated cylinder fitted with a large funnel until a total of 10 mL acetone was collected. The acetone rinse was added to the amber bottle. To improve the recovery of elemental carbon particles from the solution, 4.65 g of $NH_4H_2PO_4$ salt (1.5 g per 100 mL solution) was added to the amber bottle and the contents sonicated an additional 10 minutes (Torres et al., 2014). This process was repeated twice more for a total of three extractions per sample (930 mL). The bottles were stored in the refrigerator overnight. After each sample, used glassware was soaked for 24 hours in DDI water, washed in a 10% nitric acid bath a minimum of 4 hours, and baked in a muffle furnace at 450˚C for 5 hours. This procedure was repeated for all feathers, for a total of 20 samples, 10 per population.

Feathers were laid out on a clean lab bench, wrapped lightly in a KimWipe, and allowed to air dry. Once dry, each side of each feather was scanned to a digital image in the open-source software ImageJ (Ferreira & Rasband, 2012) to calculate the surface area, determined by averaging the areas of the dorsal and ventral surfaces. One method blank was run after every five samples, following the same extraction process in absence of feathers to determine if the glassware cleaning process was effective or if it was a source of contamination. One lab blank was also run after every five samples. Lab blanks duplicated all treatments (fume hood, desiccator, oven) except for filtration to determine if any contamination was occurring in the lab environment. Four method blanks (two for each population) and four lab blanks (two for each population) were run, totaling eight blanks for all samples.

Whatman 934-AH Ready-to-use (RTU) 47-mm glass microfiber filters (GMF) come in

labeled aluminum pans and are pre-conditioned and pre-weighed according to U.S. EPA Standard Method 2540 Part D (American Public Health Association, 2018): (1) pre-rinsed under vacuum with three successive lots of 20mL reagent grade water; (2) dried at 103 – 105˚C for 1 hour; (3) cooled in desiccator to ambient temperature and weighed; and (4) drying and weighing repeated until weight measurements differ by less than 4%. Labeled foil covers were created for each filter tray and the filter number and pre-weight recorded in the filtration log. Filters were stored in the desiccator until ready for filtration.

The extraction solution was filtered through a filter funnel assembly composed of a base beaker fitted via a silicone stopper to a fritted glass support base, and borosilicate glass funnel, connected to a manual vacuum pump. The solution was filtered through the glass microfiber filter to collect all PM greater than 1.5 µm in diameter. Following filtration, filters were air-dried on wire mesh in the desiccator for 2 hours.

Gravimetric Analysis

To establish a constant weight after filtering, sample filters were returned to their aluminum pans and dried for 1 hour in a Thermo Scientific Heratherm General Protocol OGS 180 oven at 103˚C, cooled for 1 hour in a desiccator to room temperature, and then weighed on an internally calibrated Mettler Toledo MS105DU Semi-Micro balance to the nearest 0.01 mg.

To obtain reliable filter weights, pre- and post-weights of filters should be recorded in similar conditions of temperature and relative humidity to control for hygroscopic growth of the filters. The ideal conditions are 20 -23˚C and 30 – 40% relative humidity which do not vary by more than ±2˚C and ±5% relative humidity over a 24-hour period (Papp & U.S., 2016). Samples were processed during the spring of 2021, from March 11 – 30 for the Traildust

samples and from April 6 – May 11 for the Carwash samples. Using a Kestrel 4500 Pocket Weather Tracker, temperature and relative humidity readings were recorded every hour, 24 hours per day, from March 17 – May 18, 2021. For each filter weight measurement, temperature and relative humidity were matched to the nearest recorded time stamp to determine any correlation between these parameters and the final filter mass.

The drying-weighing process was repeated until the weight difference between the measurements did not exceed 0.07 mg. Most samples were weighed twice while some samples required a third weight. The two weights with the minimum difference in post-weights were averaged to determine the final post-weight of the filter. The difference in filter media weights before and after filtering determined the mass of PM collected. Particulate matter accumulation is reported in micrograms per square centimeter ($\mu g/cm^2$) of feather surface area.

The same processes of extraction, filtration, and gravimetric analysis were completed for the three single "new" feathers, two from the Carwash population and one from the Traildust population. One method blank was processed along with the new samples. In total, 23 feathers were processed, 20 old and three new. All dried filters were transferred to covered plastic petri dishes and photographed for comparison of the filter color and then transferred to the freezer for storage.

Statistical Analysis

Absolute PM mass (mg) was determined as the difference between the filter weights before and after filtration. The final weight of the method blanks was averaged for each population and this value subtracted from each sample to obtain the corrected total PM

accumulation. The PM mass was converted to micrograms (µg) and divided by the total surface area (cm^2) of the 4-feather sample to calculate PM accumulation per unit feather surface area for each sample bird. Mean feather surface area values are reported in cm^2 ± SE and median PM accumulation values are reported in $\mu g/cm^2$. The relative accumulation factor (RAF) was calculated to compare differences in time-dependent deposition (1) between the populations which were sampled one month apart, and (2) within a population by relating PM accumulation on older feathers exposed for a full year to a new feather exposed for approximately 45 days (Martínez-Carrillo et al., 2010). This factor was calculated using the formula

$$RAF = \frac{PM_{Old} - PM_{New}}{PM_{New}}$$

where PM_{Old} is the PM accumulated ($\mu g/cm^2$) on the four-feather sample; and PM_{New} is the PM accumulated ($\mu g/cm^2$) on the single new feather, sampled from the same population as the older feathers.

A Shapiro-Wilk test was used to determine if total feather surface area, absolute PM, the relative accumulation factor, temperature, and relative humidity data were normally distributed. A two-sample t test was used to test for mean differences in feather surface area and a Wilcoxon rank-sum test was used to test for median differences in absolute PM accumulation and the relative accumulation factor between the two sampling locations as these were not normally distributed.

The glass microfiber filters used in this study can be affected by changes in temperature and relative humidity (Widziewicz-Rzońca and Tytła, 2020). The non-parametric Spearman's rank correlation coefficient (r_s) was calculated to determine any correlation between the final filter weight differences (total PM accumulation in mg), relative humidity, and temperature.

Values closer to ±1 indicate strong correlations. All analyses were performed using JMP v14.2 software (SAS Institute Inc.) set at a significance of $p < 0.1$.

Results

Habitat Characterization

Figure 2.9: Land cover proportions determined by the 2016 National Land Cover Database (NLCD) (Homer et al., 2020; Jin et al., 2019; Yang et al., 2018) within a 2-km radius buffer around each of the eight identified urban rock pigeon habitats in Denton County, Texas. Values shown in black above the bars represent the total percentage of developed land within each habitat. Total developed land includes high, medium, and low intensity development as well as open space.

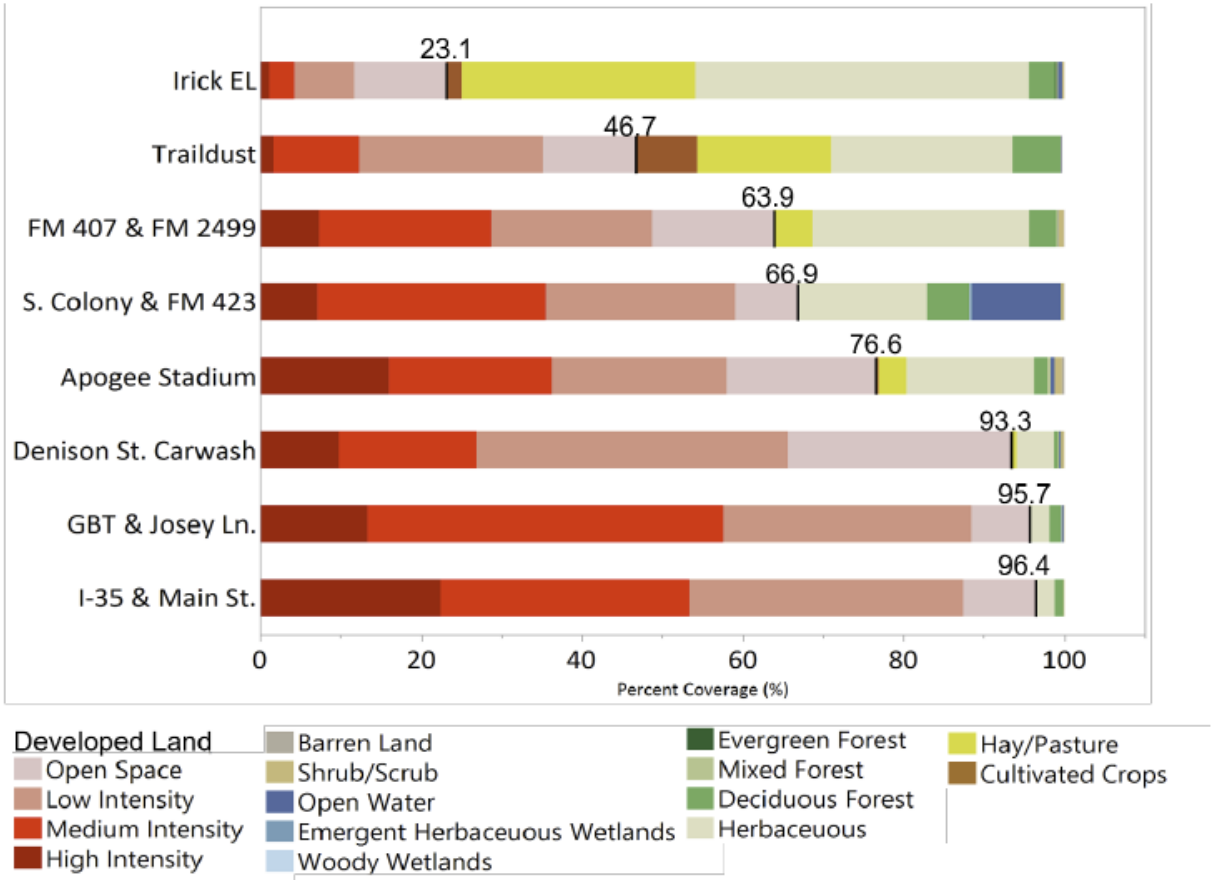
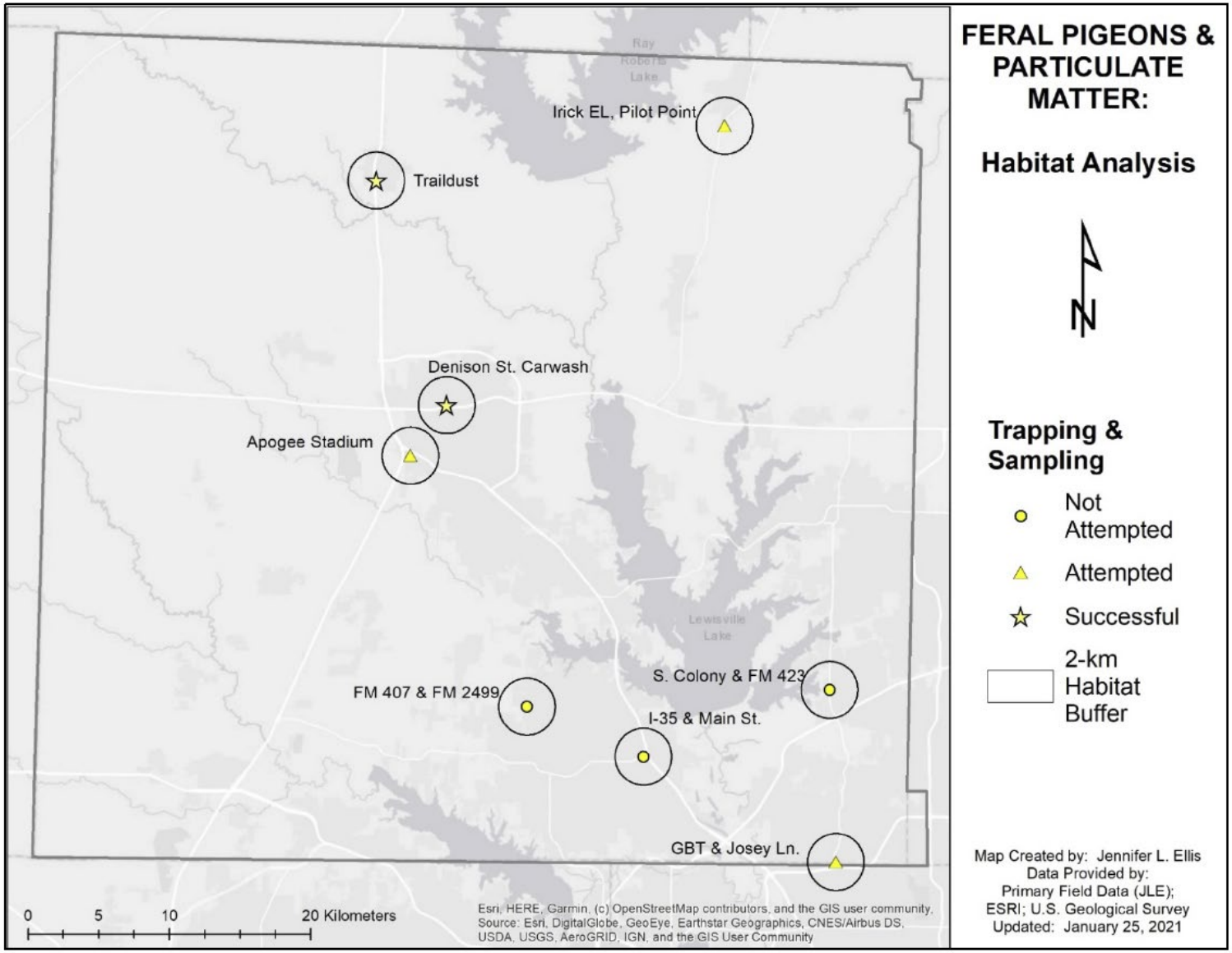


Figure 2.10: Locations characterized by land cover types within 2 km of observed urban rock pigeon populations in Denton County.



Irick Electric Line, Pilot Point

The Irick Electric Line (EL) population is the most rural site and experiences the least traffic of the eight locations. Only 23% of the land is developed, about half of which is open space (Figure 2.11). Here, birds roost on an electric line about 200 meters east of a farm-to-market road, above a hay field on private property and regularly number 50 – 70 individuals. Birds frequently visit a stock pond for water. Birds also travel every afternoon to a large horse ranch 1.3 km to the west. Pigeons were observed roosting on the barn roof and foraging in the dirt of the paddocks.

Traildust Steakhouse, Sanger

Traildust has 47% total developed land, most of which is low intensity or open space. The area is also surrounded by agricultural fields, pasture, and herbaceous cover (Figure 2.11). This location has a restaurant with few structures nearby. It is less than 100 meters east of I-35 and is exposed to high winds. The parking lot is a mix of paved and gravel surfaces which are heavily trafficked during peak dining hours. About 24 individuals were observed regularly at this site. Pigeons nest under the eaves of the restaurant building, roost on the roof and other tall structures nearby, such as billboards and highway overpasses. These birds flock with a larger group (~30 or more pigeons) that pass through the region and were observed to forage in the parking lot and nearby vacant grassy lots. Pigeons access bathing and drinking water from parking lot potholes filled with rainwater, or the ditch next to the frontage road which, at the time of observation, was supplied with water via a leaking water pipe.

FM 407 and FM 2499, Highland Village

This area is a densely developed shopping center located at the intersection of two

roads which receive moderate stop-and-go traffic. Developed areas account for 64% of the land cover. It is surrounded by residential areas and herbaceous cover (Figure 2.12). About 20 pigeons were observed roosting on electric lines paralleling roadways and on business roofs and foraging in parking lots and grassy medians. The roof of the McDonald's was an especially popular perch.

South Colony and FM 423, The Colony

This site is north and downwind of SH121 which receives extremely heavy traffic. Developed land covers 67% of the area. An inlet of Lake Lewisville is less than 1 km to the west and accounts for 11% of the land cover. This shoreline is bounded by vegetative cover, mostly herbaceous with some small amount of shrub/scrub and deciduous forest. Commercial businesses are concentrated at the intersection and to the south along FM 423. The remainder of the area is residential (Figure 2.12). About 30 birds were observed roosting on electric lines in the area, and at least three nesting pairs were seen on the roof of a vacant gas station situated in front of a self-serve carwash.

Apogee Stadium, Denton

This site is surrounded by the Interstate 35 corridor. The University of North Texas campus is located to the east and north, an industrial park to the west, and residential areas to the southeast. Developed areas account for 77% of land cover (Figure 2.11). About 15 – 25 birds were regularly observed in the area, roosting at the edge of the stadium roof line or on the bleacher railings. Four nesting pairs were observed under the bleachers or above concession stands. Small groups of 2-5 individuals would make frequent trips for water to a pond west of the stadium, to culverts draining irrigated practice fields, and to roadside ditches.

Denison St. Carwash, Denton

Developed land surrounding the carwash comprises 93.3% of the total cover, which is also predominantly low intensity and open space. Structures are moderately dense in this area, sheltering this site from high winds. Highway U.S. 380 is < 120 m south of this site (Figure 2.11). There is regular traffic in the area, which generally peaks during afternoon and early evening hours, and especially on weekends. About 10-12 pigeons were observed at this location, nesting under the roof of the carwash and vacuum bays. Water is readily available, and I observed one pigeon drinking water from AC condensation pipes on roofs. Another 15-20 birds were observed in the area, roosting on electric lines, rooftops of nearby buildings, parking lot light poles, and signage. When traffic is quiet, pigeons forage on the ground in parking lots and medians.

George Bush Turnpike and Josey Ln., Carrollton

This site is a strip center at the intersection of two roads which receive heavy traffic. The area is surrounded by residential properties. Developed area accounts for 96% of the total cover with some trees and a riparian greenbelt to the northwest (Figure 2.12). Many pigeon nests were located on the roof of the strip center under AC units, and in signage along the front of the building. Birds roost on rooftops and on light poles. During early to mid-morning hours when traffic is light, pigeons forage on the ground in the parking lots to the east and west of the building.

Figure 2.11: Four northern-most pigeon habitats in Denton County listed from left to right in order of increasing development. Land cover composition as determined by the 2016 National Land Cover Database (Homer et al., 2020; Jin et al., 2019; Yang et al., 2018). Image source: "Denton County, Texas." 33˚10'53.30"N, 97˚04'50.63"W. Google Earth Pro. November 15, 2020. January 25, 2021.

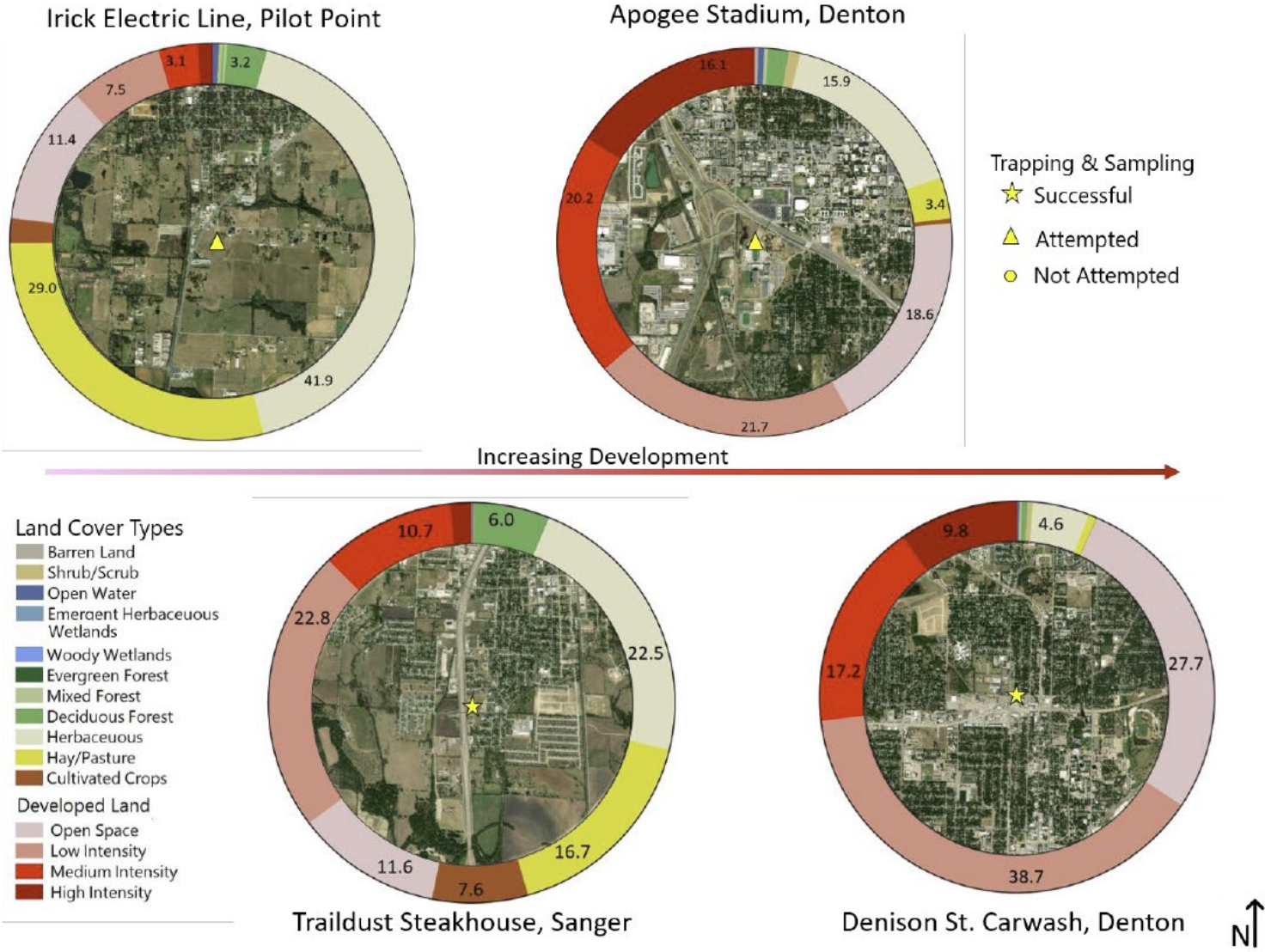
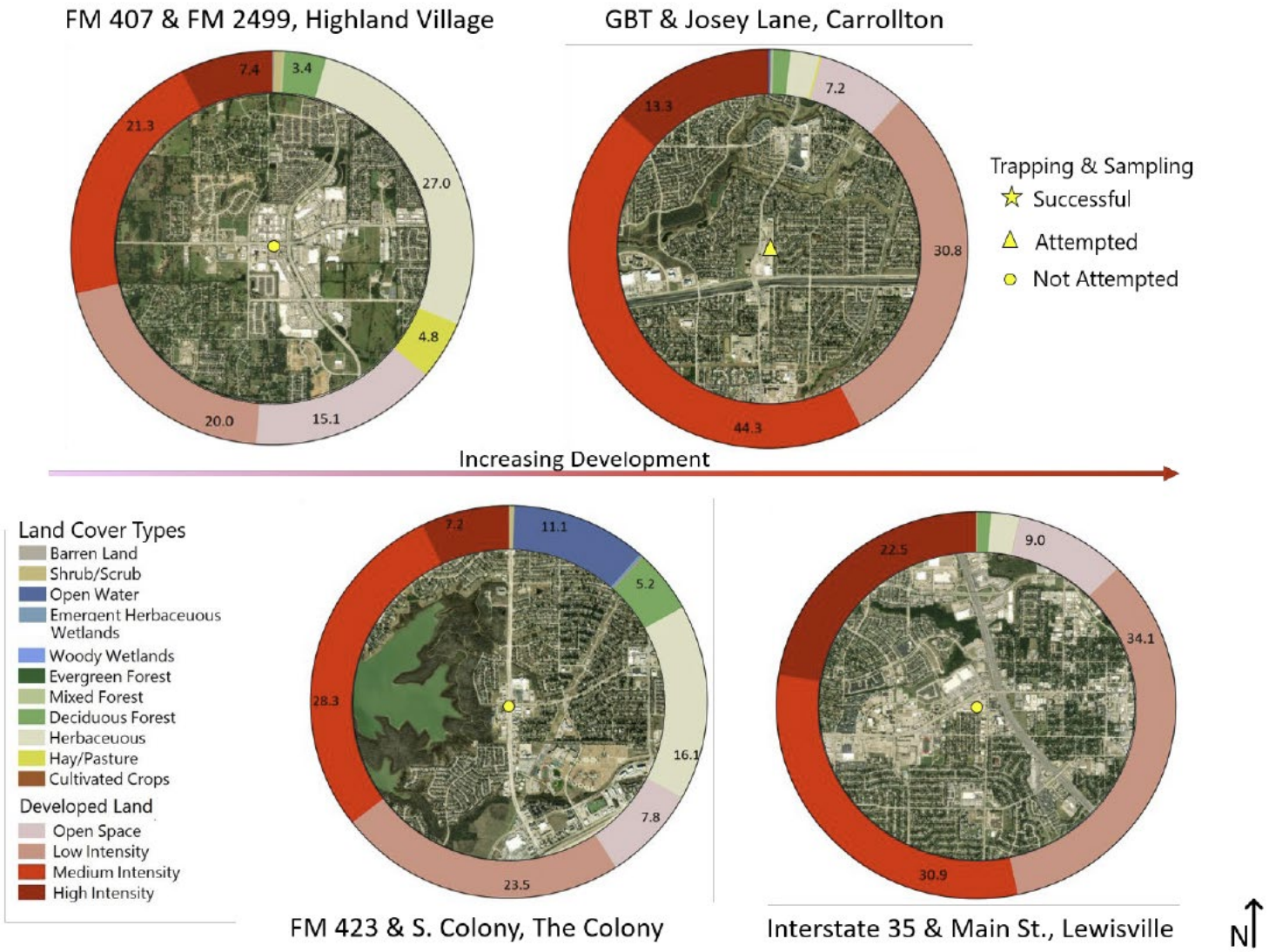


Figure 2.12: Four southern-most pigeon habitats in Denton County listed from left to right in order of increasing development. Land cover composition as determined by the 2016 National Land Cover Database (Homer et al., 2020; Jin et al., 2019; Yang et al., 2018). Image source: "Denton County, Texas." 33˚10'53.30"N, 97˚04'50.63"W. Google Earth Pro. November 15, 2020. January 25, 2021.



I-35 and Main St., Lewisville

Roughly 250 meters west of I-35E, this site is a vacant commercial building with the highest percentage of developed land at 96%. Open space accounts for 34% of this developed area and includes parks, outdoor sports complexes, and lawns. Commercial development is concentrated at the intersection and along Main St. while the remainder of the area is mostly residential (Figure 2.12). Pigeons were observed on the roof of the building and nearby electric lines and regularly numbered 40 - 50 individuals. The birds would forage in the parking lots and fly to various rooftops in the area. Many nests were in signage on the building front.

Table 2.3: Observations of pigeon foraging patterns, and traffic and building density for each location.

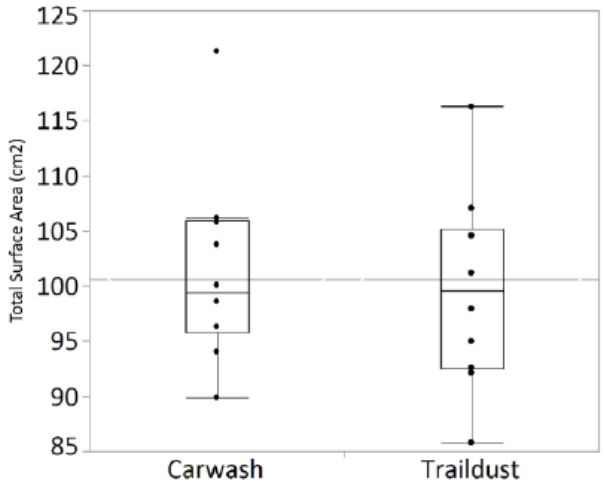
Site	Water Source	Foraging	Traffic	Building density
Irick EL	Pond	Pasture Horse paddocks Paved parking lots	Low	Very Low
Traildust	Roadside ditch Rain puddles	Gravel parking lot Paved parking lot Vacant grassy lots	High	Low
FM 407 & FM 2499	Unknown	Paved parking lots Grassy medians	Medium-High	High
S. Colony & FM 423	Unknown	Paved parking lots	High	Medium-High
Apogee	Pond Irrigation culverts Roadside ditches	Paved parking lots Stadium walkways Maintained fields	Medium-High	Low
Carwash	Puddles AC condensation	Paved parking lots Grassy medians	Low-Medium	Medium
GBT & Josey Ln.	Unknown	Paved parking lots	High	Medium-High
I-35 & Main St.	Unknown	Paved parking lots	High	High

Particulate Matter Accumulation

Mean total feather surface area did not differ significantly between populations.

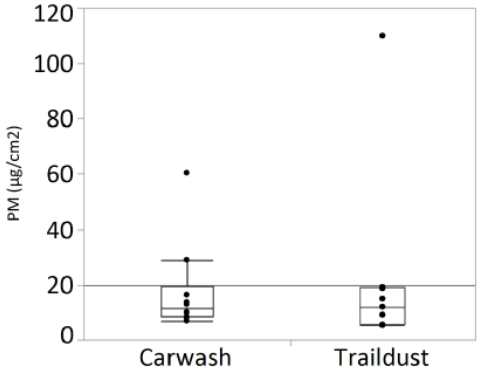
Feathers sampled from the carwash population (*n* = 10) ranged from 89.90 – 121.32 cm^2 with a mean of 101.48 ± 2.74 cm^2. Feathers sampled from the Traildust population (*n* = 10) ranged from 85.79 – 116.28 cm^2 with a mean of 99.71 ± 2.80 cm^2 (Figure 2.13).

Figure 2.13: Boxplots of total feather surface area (cm^2) for urban rock pigeon populations sampled in 2020 in Denton County, Texas.



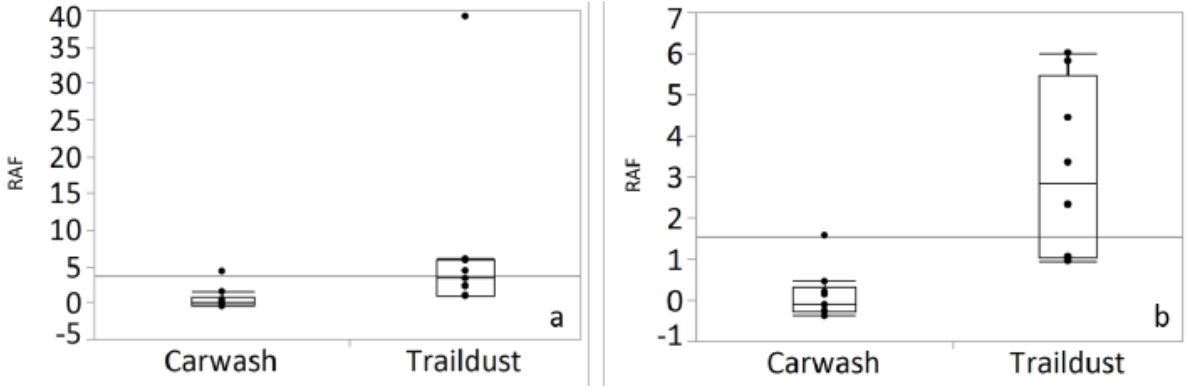
All 20 samples accumulated measurable amounts of PM per unit feather surface area. However, one sample from the Traildust population was excluded from analysis as bits of filter media stuck to the weighing pan after drying. Median absolute PM accumulation did not differ significantly between populations. Amounts on feathers from the Carwash population (*n* = 10) ranged from 6.96 – 60.44 µg/cm^2 with a median of 11.44 µg/cm^2. Amounts on feathers from the Traildust population (*n* = 9) ranged from 5.34 – 110.07 µg/cm^2 with a median of 11.92 µg/cm^2 (Figure 2.14). The interquartile range was 11.14 and 13.33 µg/cm^2 for the Carwash and Traildust locations, respectively. One sample in each population accumulated very high amounts of PM. When these outliers were excluded, the results did not change. Absolute PM accumulation values for the new feather samples were 2.74 µg/cm^2 for the Traildust population and 8.46 and 14.06 (mean = 11.26) µg/cm^2 for the Carwash population.

Figure 2.14: Boxplot of absolute PM accumulation (µg/cm^2) on pooled four-feather samples collected in 2020 from urban rock pigeon populations in Denton County, Texas.



Median relative accumulation factor values were significantly different between populations ($p < 0.1$). Pigeons in the Traildust population accumulated PM on feather surfaces at a median rate of 3.35 with a range of 0.95 – 39.17. At the Carwash, rates of accumulation ranged from -0.38 – 4.37 with a median of 0.02. The Traildust population had a much greater range of rates and accumulated PM much faster than the Carwash population. When outliers were excluded, the relative PM accumulation between populations remained significantly different ($p < 0.1$) at a median rate of -0.11, ranging from -0.38 – 1.58 for the Carwash population and a median of 2.84, ranging from 0.95 – 6.01 for the Traildust population (Figure 2.15).

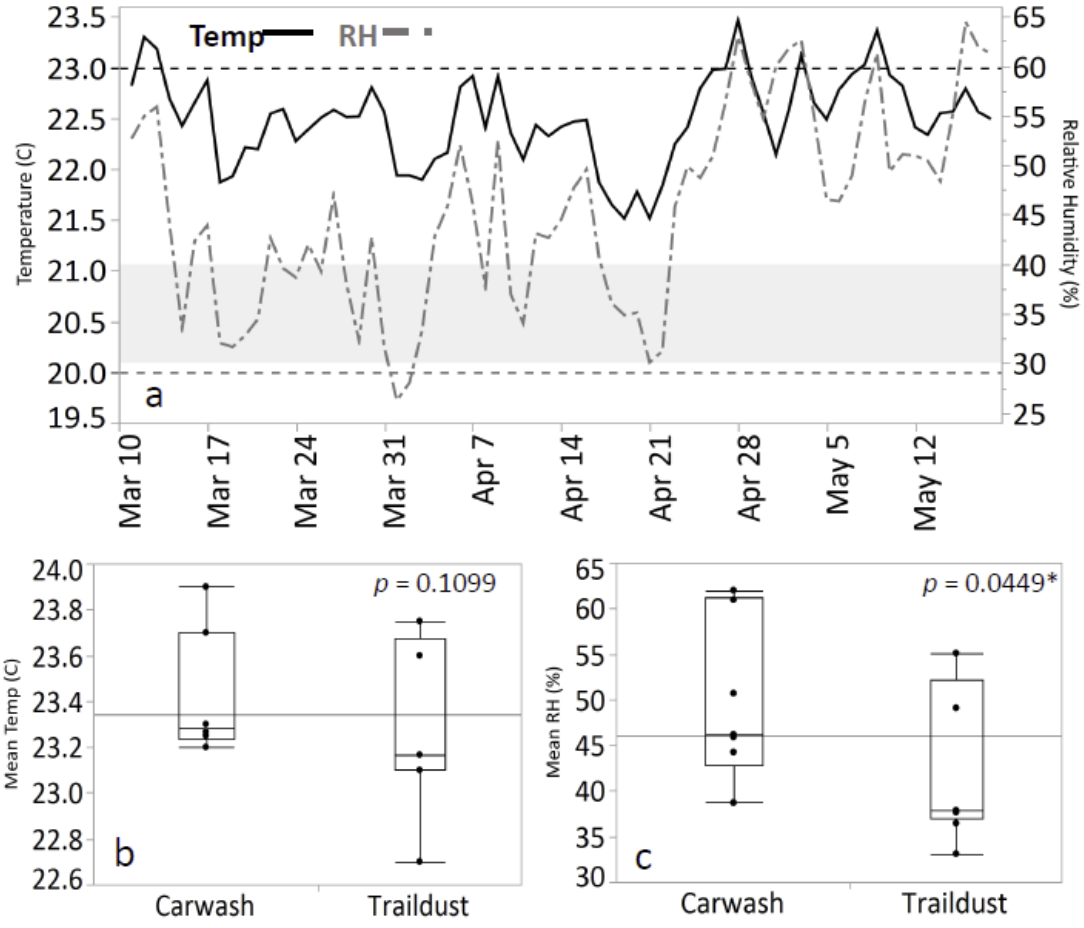
Figure 2.15: Boxplots of relative accumulation factors (a) with outliers and (b) excluding outliers for urban rock pigeon population sampled in 2020 in Denton County, Texas.



Temperature and Relative Humidity

During the weighing periods, relative humidity ($p < 0.0449$) differed significantly while processing each population of samples.

Figure 2.16: Using a Kestrel 4500 Pocket Weather Tracker, temperature and RH were recorded every hour, 24 hours per day, from March 17 – May 18, 2021, and data aggregated to chart (a) changes in mean daily RH and temperature. To obtain reliable filter weights, ideal conditions of RH, indicated by the shaded region, and temperature, values between the dashed lines, are recommended. The Traildust samples were processed from March 11 – 30 and the Carwash samples from April 6 – May 11. Temperature and RH readings were averaged for each sample post-weight and the median values compared between populations for each parameter (b) temperature and (c) RH. Asterisks indicate a significant difference.



Median values were 23.3˚C and 46.2% relative humidity while processing the samples from the Carwash population and 23.2˚C and 37.9% relative humidity for the Traildust population samples (Figure 2.16 b and c). A Spearman's ρ correlation determined that there was a

moderate but significant correlation between absolute PM accumulations, relative humidity, and temperature for the Traildust samples when outliers were excluded. This correlation weakened and was insignificant when outliers were included in the analysis (Table 2.4). No correlation was found for the Carwash samples.

Table 2.4: Spearman's ρ correlation (r_s) and significance values (*p*) for the correlations between total PM mass and RH, and total PM mass and temperature for each population with and without outliers. Asterisks indicate a significant correlation.

	With Outliers			Excluding Outliers		
	n	**r_s**	**p**	**n**	**r_s**	**p**
Traildust						
Temp & PM	9	0.4104	0.2726	8	0.7274	0.0409*
RH & PM	9	0.4051	0.2794	8	0.6794	0.0664*
Carwash						
Temp & PM	10	-0.2080	0.5643	9	-0.0420	0.9145
RH & PM	10	-0.2569	0.4737	9	-0.3109	0.4154

Discussion

Feral Pigeons Occupy Diverse Habitats in Urban Environments

From my observations of urban rock pigeons in Denton County, these birds occupy diverse habitats. Key requirements are nesting areas which are flat and sheltered, a nearby reliable source of water, and perches away from tree lines to avoid predators. Even the most rural of the eight populations observed remained close to human activity, foraging in parking lots and medians near restaurants, or around barns and livestock. Nests were observed on support columns below rooflines, under air conditioning units on rooftops, in the dark gaps between concrete support beams below highway overpasses, under the eaves of roofs, at the intersection of metal support beams, and within billboards and other highway signage. Water

sources included condensation from AC units, stock ponds, roadside ditches, rain puddles, and runoff from carwash hoses, leaking pipes, and irrigated fields.

Generally, traffic density and urbanization at the observed pigeon habitats decreased from the southern boundary of the county to the north. However, northern locations are downwind of urban pollution sources due to the prevailing southerly winds in the study area. Although I was not successful at trapping at other locations, I would expect proximity to major roads and land cover composition to affect ambient concentrations of PM available for deposition. For instance, the southern-most locations in Carrollton, The Colony, Lewisville, and Highland Village are all located downwind of major roads in highly urbanized areas. Even though The Colony and Highland Village have similar profiles of developed land, The Colony population has more tree cover, which contributes to ambient PM removal (Ferrini et al., 2020; Rindy et al., 2019).

How individual birds use these diverse habitats may also influence PM deposition to their feathers and explain some of the variability within populations. Male pigeons spend more time preening, important for maintaining healthy, louse-free feathers, and subsequently, for attracting mates (Johnston & Janiga, 1995). Female pigeons living in urban areas of Basel, Switzerland flew further distances from the home loft than males to reach more dependable food sources. Males were active in the morning and females in the afternoon and early evening (Rose et al., 2006). These patterns of activity may influence PM accumulation to the feathers if birds are exposed to greater concentrations of ambient PM during specific times of day, such as rush hour when traffic is at its heaviest. Sex-related differences were found in the accumulation of external trace metals on the feathers of Parisian pigeons. Females showed high

external accumulations of certain trace metals while males' feathers were clean of these same contaminants, a result attributed to the increased time spent preening by males (Frantz et al., 2016).

Relative Accumulation Factors Differ Between Contrasting Urban Rock Pigeon Habitats

Our results show that urban rock pigeon populations occupying two sites with contrasting land cover within a 2-km radius buffer accumulated measurable amounts of PM and differed in their relative accumulation factors. Although few studies investigate external PM on feathers (DuBay & Fuldner, 2017; Griggio et al., 2011; Pitre et al., 2021), numerous studies have quantified PM accumulation on plant surfaces.

Some of these studies show values of PM accumulation similar to those measured on urban rock pigeon feathers. For example, our feathers collected between 6.96 – 60.44 and 5.34 – 110.07 $\mu g/cm^2$ of PM after one year, for the Carwash and Traildust populations, respectively, while leaves from eight broadleaf tree species near a busy urban road in Warsaw, Poland, accumulated approximately 8 – 16.5 $\mu g/cm^2$ of surface PM and 12.5 – 25.8 $\mu g/cm^2$ of total (surface + wax deposited) PM after one growing season (Dzierżanowski et al., 2011). Thirteen different woody species grown under identical conditions in a nursery collected 4.5 – 17.1 $\mu g/cm^2$ of surface PM and 7.5 – 32.1 $\mu g/cm^2$ of total PM. Hofman et al. (2014b) measured the average surface PM of all particles greater than 0.2 µm on leaves collected from a single species of tree growing along a busy street canyon in Antwerp, Belgium. These leaves were exposed for four months and not protected from precipitation. This study seems most comparable to amounts found on the pigeon feathers as there was no upper limit on particle size collected and only one species was considered. The mean total PM accumulation (74.66 $\mu g/cm^2$) was much

higher with a greater range (8.38 – 282.07 μg/cm^2) than that found on the feathers.

Contrary to expectation, we found that the Traildust population had a median relative accumulation factor that was significantly higher than that of the Carwash population, where total developed land was approximately two-fold higher (Figure 2.9). There was a 38-fold difference in the range of relative accumulation factors for the Traildust birds and the accumulation rate was always positive. The range of relative accumulation factors for the Carwash birds was much lower. A caveat of this finding is that we detected a positive correlation between PM mass and relative humidity for the Traildust samples when one outlier was *excluded* from the analysis, suggesting that the total PM accumulated for the Traildust feathers could be overestimated. When we compared the relative accumulation factor between populations without the samples weighed at the highest relative humidities, the results remained the same. Thus, we consider that differences between populations were greater than the potential error introduced during the weighing process.

What could be driving the differences in PM accumulation between habitats? Particulate matter accumulated onto a surface is the result of deposition (wet and dry), retention, and removal processes (Van Stan et al., 2021). The same rain that deposits particles can wash them off, and wind can drive resuspension into the atmosphere. These processes of particle input and output are further affected by the duration of exposure, structural and morphological characteristics of the deposition surface and, in the case of feral pigeons, the natural behaviors of the bird.

As has been shown in vegetation studies, exposure to rain and wind influences PM accumulation on feather surfaces. As no weather stations are located near our sites, we cannot

confirm if there were differences in cumulative precipitation between the sites during the study period. However, we suspect that precipitation amount was similar as the locations are only 14 km apart. The same feather types, primaries and tail feathers, were collected from both populations. Therefore, surface structure and shape likely did not differ between populations, affecting deposition rates. In addition, all samples were estimated to be roughly one year old, and thus the duration of exposure was similar.

Natural activities such as bathing and preening are shared behaviors within a species. Bathing occurs in shallow water and most often in rain, the bird alternately raising each wing to allow the rain to runoff (Goodwin, 1983), thus cleaning the feathers and potentially removing particulates. At both locations, bathing behavior was similar. Birds utilized puddles formed in the parking lots. Preening maintains feather structure and function, which may be degraded in birds who are ill or have high parasite loads, leading to "dirtier" feathers and higher PM accumulation. Although these bird behaviors may explain some of the variability among individuals within the sampled populations, we do not consider behavior to be a major factor affecting between population differences in relative accumulation factors.

We speculate that differences in PM accumulation rates at the two locations were most likely influenced by building density and types of and proximity to emissions sources. The Traildust location is subject to vehicle emissions from the heavily traveled interstate highway, dust from agricultural fields, and air pollution from upwind urban areas. With its low building density, the open nature of the location leaves it more exposed to the wind, which could enhance dispersion but also increase deposition. This atmosphere of potentially abundant PM

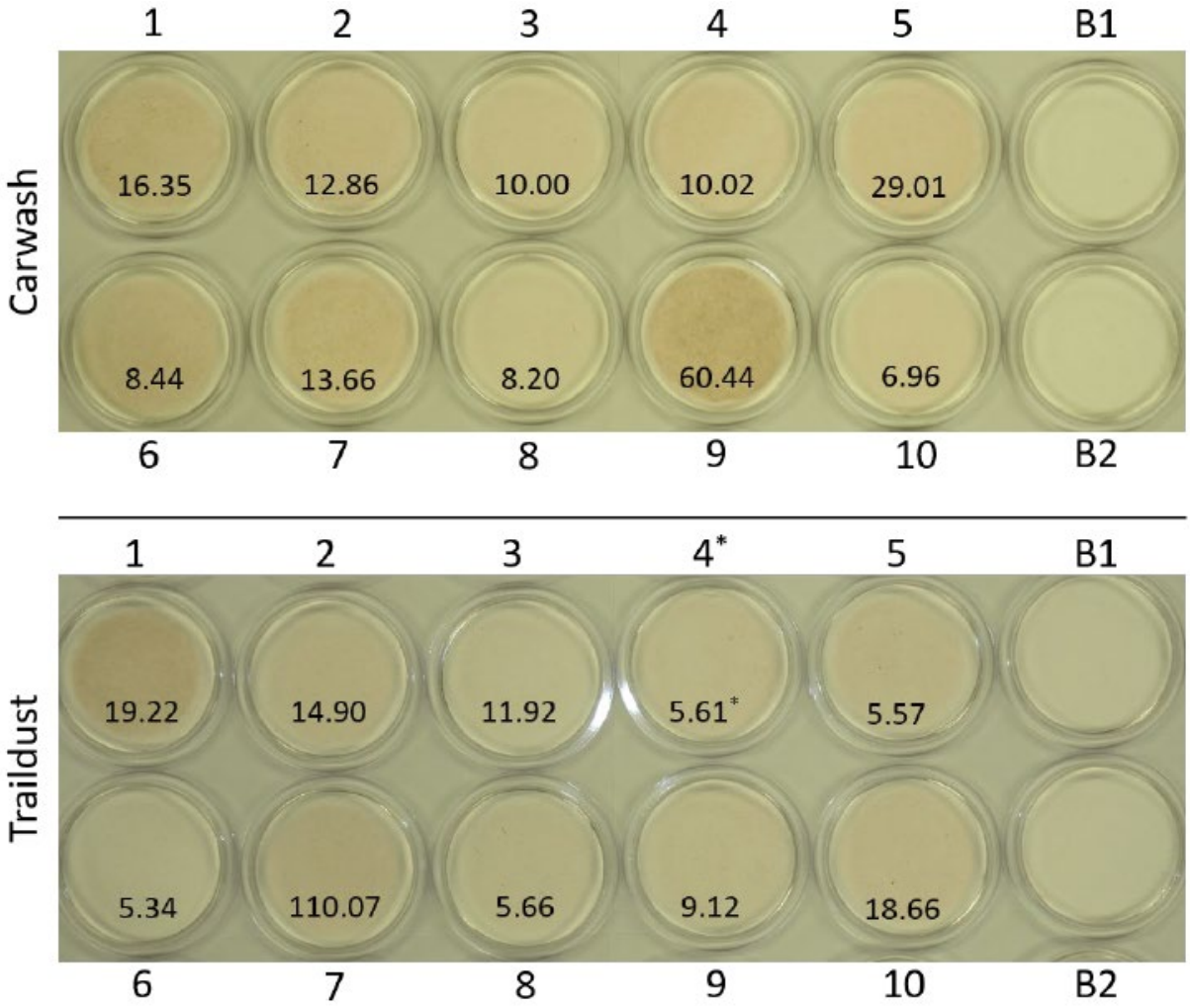
sources and wind exposure could increase PM deposition rates and result in the much higher relative accumulation factors at the Traildust.

The Carwash population is situated in a densely urbanized area surrounded by roads which experience moderate traffic (in comparison to the interstate highway). Sources of PM affecting this population are most likely from passing traffic and idling vehicles. Due to the greater building density, this site is somewhat sheltered from the wind. Lighter traffic and less PM emissions from vehicles coupled with protection from the wind may have reduced deposition rates at this site. In addition, the Carwash birds nested above the vacuum and wash bays and foraged on the ground in the paved lot. When the wash bays are in use, a fine mist of water is constantly present which may serve to remove PM from the air or wash it off the pigeon feathers. This self-service carwash also receives regular maintenance. Each Monday, the pavement of the wash bays was cleaned with a high-pressure hose. This, and the runoff from regular use, would reduce the pigeons' exposure to dust as they forage on the ground. These factors may explain the much lower relative accumulation factors found for the Carwash population.

We note that in addition to the relative accumulation factors, the size fraction and composition of particulates might also differ between populations. PM_{10} (2.5 -10 µm) is generally dominant in dust and other natural sources while $PM_{2.5}$ (≤ 2.5 µm) is indicative of traffic-related emissions. Though fine particulates contribute to a smaller percentage of the total mass, this size fraction can capture a much greater number of particles (Dzierżanowski et al., 2011; Popek et al., 2013). Some of this possible variation is noticeable in the color variation of the dried filters. As can be seen from photographs of the filters, filter color and "dirtiness"

do not necessarily correspond with the PM accumulation (μg/cm^2) measured per sample.

Figure 2.17: Comparison of filter color. Filters are labeled with the sample number (1 – 10) and method blanks (B) are shown for comparison. Numbers on each filter show the mass of accumulated PM per unit feather surface area (μg/cm^2) after method blank correction. *Sample 4 from the Traildust population was excluded from statistical analysis as filter media was lost to the pan during the drying process, affecting the filter weight.



Conclusions

To the author's knowledge, this is the first study to directly quantify external PM accumulation on feathers of a wild urban bird. We found significant differences in PM accumulation rates to pigeon feathers sampled from two urban populations with habitats contrasting in land use/land cover. Types of and proximity to emission sources, building

density, wind exposure, and characteristics distinct to each location likely explain the differences in relative PM accumulation. This illustrates that the external accumulation of atmospheric PM pollution can vary greatly among urban microhabitats. Bright, colorful feathers are important for signaling and communication in birds, indicating fitness and health. Feathers darkened by atmospheric pollutants may obscure these signals. Future studies should explore how this may impact bird health.

CHAPTER 3

CONTRIBUTIONS TO THE FIELD

This study characterized urban rock pigeon habitat within Denton County, Texas, and quantified PM accumulation on the feather surfaces of two urban rock pigeon populations to explore the effects of urban land cover on variations in PM deposition at fine spatial scales. This research found that urban rock pigeons occupy diverse habitats. Absolute PM accumulation did not differ between habitats, but the rate of PM accumulation did.

As more people concentrate in urban centers, increases in traffic density and industry lead to increased air pollution. As a component of air pollution, PM has been well-studied as it is especially dangerous to human health (Bourdrel et al., 2017; Du et al., 2016; Falcon-Rodriguez et al., 2016). Birds, particularly those living in urban environments, are exposed to this same particulate pollution and suffer the same ill effects (North et al., 2017; Sanderfoot & Holloway, 2017). Within the fields of environmental science and conservation biology, a wealth of research examines bioaccumulated pollutants in wild bird populations, associating elevated levels of contaminants with proximity to anthropogenic activities (Adout et al., 2007; Brait & Antonioso Filho, 2011; Jaspers et al., 2009; Nam et al., 2004). However, few examine external contaminants (DuBay & Fuldner, 2017; Griggio et al., 2011; Pitre et al., 2021). This thesis focused on directly quantifying external PM accumulation on feather surfaces of feral pigeons to better understand the spatial distribution of atmospheric PM pollution in diverse urban microhabitats.

Feral Pigeons as Biomonitors of Atmospheric PM Pollution, or Not

This study contributes to a rich body of literature in which birds have proven valuable as

biological indicators of anthropogenic pollution. Although PM as a whole has not been quantified in such studies, many contaminants that may be components of PM, such as heavy metals and organic pollutants, have been found to accumulate within and on the feathers of birds. Variations in urban land cover and human activity influence the type and size of PM contributed to the atmosphere while wind, rain, duration of exposure, surface texture, and proximity to emission sources affect deposition of PM to surfaces. A feather's unique structure makes it very efficient at capturing contaminants, while preening and bathing behaviors remove them. This study found a significant difference in the relative accumulation factors between populations. The most likely drivers of this variability were differences in land use/land cover, types of and proximity to emissions sources, building density, and exposure to wind.

While previous studies used molted feathers (Pitre et al., 2021) and museum specimens (DuBay & Fuldner, 2017) to reveal temporal and spatial changes in PM deposition to feathers, this is the first study to directly quantify external PM accumulation on feathers of a wild urban bird. In exploring the usefulness of feathers collected from wild bird populations as monitors of atmospheric pollution, this research contributes to the field of environmental science. However, to successfully build upon the results of this novel research, we propose that future studies should incorporate several recommendations to address the challenges involved.

Recommendations for the Field

The funnel traps used in the study are designed to be placed on rooftops. Trapping on the ground takes considerable time and effort. For efficient trapping resulting in more

sampling sites and a greater sample number, roof access is imperative and should be secured well ahead of planned trapping activities.

To better assess the drivers of PM accumulation, air monitors should be placed at each sampling site to record ambient atmospheric PM concentrations and meteorological data. Information on individual birds such as sex and age might also prove useful in determining potential routes of exposure as these factors affect how the habitat is utilized (Frantz et al., 2016; Nam et al., 2004). Geotagging individuals would also prove beneficial in determining foraging patterns when lack of time, personnel, or access renders field observations inadequate.

For determining the relative accumulation factor, a new feather should be sampled from *every* bird in addition to the four older feathers. Variation in PM accumulation among individuals ranged widely and this would provide a more accurate measure of the rate of accumulation, per bird and per population, than a single feather per population.

Recommendations for the Lab

Filter media utilized in gravimetric analysis are sensitive to changes in temperature and relative humidity which affect the filter weight due to hygroscopic growth. For accurate filter weights regardless of the type of filter media used, the lab environment must be controlled for temperature and relative humidity. The ideal conditions are 20 -23˚C and 30 – 40% relative humidity and must not vary by more than ±2˚C and ±5% relative humidity over a 24-hour period (Papp & U.S., 2016). Quantification by size fraction would be possible under these controlled conditions, taking advantage of the multiple pore sizes available in cellulose filter papers.

In measuring PM deposition to vegetation, Dzierżanowski et al. (2011) recommends a total leaf surface area of 300 – 400 cm^2 to obtain measurable amounts of fine PM. The surface area of the individual feathers collected from each population in our study ranged from 19.11 – 33.20 cm^2. Therefore, feather samples were pooled to ensure a sufficiently sized surface area from which PM could be extracted. However, during the extraction, the four feathers would stick to each other during sonication which might affect the efficient recovery of PM from the samples. Additionally, as measurable amounts of PM were found on the new, single-feather samples, extracting individual feathers would provide a greater number of samples per population and make comparisons between feather types (tail and primary) possible.

Particulate Matter and Bird Health

By determining how external PM accumulation may affect urban birds, this study also contributes to the field of conservation biology. Feathers soiled by pollutants affect the feather color, which is important as a signal of fitness and mate choice in birds (Griggio et al., 2011). However, to our knowledge, there are no studies that directly measure a dose-response relationship between external PM accumulation and potential adverse health effects. Future research should examine the consequences of external contaminants to bird feathers, and furthermore, consider the cumulative effects of internal and external contamination on avian behavior and health.

Conclusion

The relative rate of PM accumulation to feathers was significantly different between two populations of pigeons occupying diverse urban habitats while absolute PM accumulations

did not differ. Characteristics of urban land cover, proximity to and types of emissions sources, wind exposure, and building density were drivers of variability in PM deposition to feather surfaces. This study contributes to the fields of environmental science and conservation biology by quantifying the relationship between an atmospheric pollutant and a synanthropic species of the human urban environment.

APPENDIX A

PROTOCOL TRIALS

Purpose

Three trials were performed to determine the most effective lab protocols for extracting, filtering, and quantifying external PM accumulation on feathers.

Research Questions and Objectives

Question 1: What number of feathers within a sample is required to obtain detectable amounts of PM?

Objectives:

- Quantify PM accumulation on samples of one, two, and four feathers by size fraction
- Quantify PM accumulation by using various extraction methods.

Question 2: What are the best practices for quality assurance and control?

Objectives:

- Determine sources of contamination by utilizing method blanks, lab blanks, and different glassware cleaning procedures.
- Verify the best method for determining constant weights of various filter media.

Methods

These trials were performed using molted pigeon primaries (remiges) and tail (rectrices) feathers collected from the ground at various locations throughout Denton County. Feathers from each sampling day and location were stored together in a plastic bag; labeled with the date, time, and location of collection; and stored in the freezer. Before processing the feather samples, all glassware used in extraction and filtration was soaked for 24 hours in DDI water, washed in a 10% nitric acid bath for a minimum of 4 hours, and finally baked in a muffle furnace at 450˚C for 5 hours.

Generally, feathers were placed tip-down in double deionized (DDI) water and sonicated

for 5 – 7.5 minutes, flipped on end, and sonicated again for the same amount of time. Feathers were removed with tweezers and each side rinsed three times with acetone. The DDI and acetone rinse water were combined. To improve the recovery of elemental carbon particles, $NH_4H_2PO_4$ salt (1.5 g per 100 mL solution) was added to the solution (Torres et al., 2014) and contents sonicated for 10 minutes. The bottles were labeled and stored in the refrigerator overnight. Feathers were placed in a clean glovebox to dry. Once dry, each side of each feather was scanned to a digital image in the open-source software ImageJ (Ferreira & Rasband, 2012) to calculate the surface area, determined by averaging the areas of the ventral and dorsal surfaces.

The extraction solution was filtered through a filter funnel assembly consisting of a base beaker fitted with a fritted glass support base, borosilicate glass funnel and base, and a silicone stopper. This assembly was connected to a manual vacuum pump. In the first two trials, the solution was filtered sequentially through three filters of decreasing pore sizes to separate PM by size fractions. Based on results from the first two trials, the third trial used a single filter collect all PM greater than 1.5 μm in diameter.

The difference in filter paper weights before and after filtering determines the mass of PM collected. To obtain reliable weights, cellulose and glass microfiber filters were dried to a constant weight by identical methods before and after filtration. The filters were stored in labeled foil trays or aluminum pans with foil covers. The filters were dried in an oven, cooled in a desiccator, and acclimated to lab conditions before weighing. Among the three trials, the drying temperature and time spent at each step varied. Once weighed, the filters were stored temporarily in a glove box and then transferred to covered plastic petri dishes and stored in the

freezer when the trials were completed. Quartz fiber filters were stored in a plastic petri dish within a small piece of foil. The petri dishes were covered, individually labeled, and stored in the freezer until ready for use. Before filtration, filters were acclimated in the desiccator for 24 hours, acclimated to lab ambient conditions for 3-5 minutes, and then weighed. After filtration, these filters were dried in the desiccator for 12 hours, acclimated for 3-5 minutes, weighed, and stored in the freezer. Quartz fiber filters were treated identically for all three trials. All filters were weighed to the nearest 0.01 mg on a Mettler Toledo MS105DU Semi-Micro balance which was internally calibrated at the beginning of each weighing session.

The trials differed in the number of feathers and extractions per sample, amount of extraction solution, total sonication time, constant weight determination, and the method used to clean the glassware between samples and blanks. A summary of the methods used is in Table A.1.

Trial 1

Trial 1 used relatively clean feathers, on the basis that these might better represent the amount of PM accumulation you would find on feathers plucked directly from a pigeon. Before processing the feather samples, all glassware used in extraction and filtration was soaked for 24 hours in DDI water, washed in a 10% nitric acid bath for a minimum of 4 hours, and baked in a muffle furnace at 450˚C for 5 hours.

Filter Preparation

To determine constant weights before and after the filtration process, cellulose filter papers were placed in individually labeled foil trays and dried uncovered in a gravity convection oven for 24 hours at 60˚C.

Table A.1: Summary of methods for protocol trials 1, 2, and 3 and the finalized protocol.

	Extraction Method	Glassware Rinse Between Samples	Constant Weight Method	Results & Conclusions
Trial 1	Molted "Clean" feathers		Filter Media: Cellulose	
<u>Replicate 1</u> 1-, 2- and 4-Feather Samples	Single extraction 400 mL DDI water in 500 mL capped amber bottle Sonicate 7.5-7.5-10 minutes One method blank per sample Filter 3 size fractions (Dzierżanowski et al., 2011)	5x DDI water only	Covered foil trays 24-hour oven-dried at 60˚C 30 minutes in desiccator Acclimate 3-5 minutes in lab ambient, weigh (Eaton et al., 1969)	Replicate 1: Least PM accumulation on 4-feather sample; no clear pattern of accumulation Significant PM accumulation on method blanks Replicate 2: No PM accumulation for samples or blanks in large and coarse fractions <u>Conclusions</u>: DDI-acetone-DDI rinse method most effective However, weights of hygroscopic filters suspect due to upward scale drift
<u>Replicate 2</u> 2- and 4-Feather Samples		5x DDI water 1x acetone 1x DDI water		
Trial 2	Molted "Very dirty" feathers		Filter Media: Cellulose	
2- and 4-Feather Samples	Triple extraction 300 ml DDI water in 500 mL capped amber bottle Sonicate 5-5-10 minutes One method blank per sample Filter 3 size fractions	5x DDI water 1x acetone 1x DDI water	Covered foil envelopes 24-hour oven-dried at 60˚C 30 minutes in desiccator Acclimate 24 hours in lab ambient, weigh	Rinse method appears ineffective due to large carryover to blanks However, though filter weights were more stable, post-weights were suspect due to an 11.3% rise in ambient RH between pre- and post-weighing periods <u>Conclusion</u>: Cellulose papers are not suitable for use in situations where ambient RH is not controlled
Trial 3	Molted "Very dirty" feathers		Filter Media: GMF	
4-Feather Sample	Triple extraction 300 ml DDI water in 500 mL capped amber bottle Sonicate 5-5-10 minutes One method blank and two lab blanks per sample Filter once	5x DDI water 1x acetone 1x DDI water	Aluminum pans with foil cover 1-hour oven-dried at 103˚C 30 minutes in desiccator Weigh, repeat (APHA, 2018)	Some filter media stuck to the pan after oven-drying, affecting the filter weights Significant carry over of PM to blanks Conclusions: It is necessary to fully clean glassware between samples (DDI soak, acid bath, oven bake) Filters must be air-dried on breathable surface before transfer to oven to prevent loss of filter media

	Extraction Method	Glassware Rinse Between Samples	Constant Weight Method	Results & Conclusions
Final Protocol	Plucked feather samples		Filter Media: GMF	
4-Feather Samples	Triple extraction 300 ml DDI water in 500 mL capped amber bottle Sonicate 5-5-10 minutes Two method blanks and two lab blanks per population Filter once	5x DDI water 1x acetone 1x DDI water	Aluminum pans with foil cover 2-hour air-dry in desiccator on wire mesh 1-hour oven-dried at 103˚C 1 hour in desiccator Weigh, repeat	Refer to CHAPTER 2 Measurable amounts of PM found for all samples Some carryover of PM to method blanks Positive correlation between RH and filter weight for the Traildust population This may explain some of the increase in weight of the blanks Though GMF filters performed better, filter media is still affected by changes in temperature and RH

The filters were cooled in a desiccator for 15-30 minutes, then removed and acclimated to lab ambient conditions for 3-5 minutes before weighing (Eaton & Likens, 1969). Filters were stored covered in their foil trays in the desiccator until ready for the filtration process. Quartz fiber filters were stored in a plastic petri dish within a small piece of foil. The petri dishes were covered, individually labeled, and stored in the freezer until ready for use. Before filtration, filters were acclimated in the desiccator for 24 hours, acclimated to lab ambient conditions for 3-5 minutes, and then weighed. After filtration, these filters were dried in the desiccator for 12 hours, acclimated for 3-5 minutes, weighed, and stored in the freezer.

Extraction and Filtration

Samples of 1, 2, and 4 molted feathers were photographed on a solid background with a ruler for scale. The calamus, or base of the feather shaft, was clipped with acetone-cleaned scissors just below the base of the vanes. This was done so that the longer primary feathers would fit inside the capped amber bottles for the extraction process. For consistency among the samples, the calamus was clipped this way for all feathers.

For each sample of 1, 2, and 4 feathers, the feathers were placed tip-down in a capped 500 mL amber bottle filled with 400 mL of DDI water and sonicated for 7.5 minutes. The feathers were flipped on end and sonicated an additional 7.5 minutes. After sonication, each side of each feather was rinsed 3 times using an acetone squirt bottle, and the acetone collected in a 200 mL beaker. The acetone was transferred to the amber bottle and 6.0g of $NH_4H_2PO_4$ (1.5 g per 100 mL solution) added. The bottle was sonicated for 10 minutes and then stored in the refrigerator overnight. After each sample extraction, an extraction blank was prepared using the same technique in the absence of feathers. Between each extraction, the

200 mL beaker was rinsed 3-5 times with DDI water. The feathers were air-dried and stored in a clean glove box on top of KimWipes. Once dry, each side of each feather was scanned to a digital image in the open-source software ImageJ (Ferreira & Rasband, 2012) to calculate the surface area, determined by averaging the areas of the dorsal and ventral surfaces.

The same extraction process was replicated for 2-, and 4-feather samples each followed by an extraction blank. For this second replicate, the 200 mL beaker was rinsed five times with DDI water, once with acetone, and once more with DDI water between samples and blanks.

Extraction solutions were filtered through a filter funnel assembly composed of a base beaker fitted via a silicone stopper to a fritted glass support base, and borosilicate glass funnel, connected to a manual vacuum pump. Each extraction solution was filtered twice to separate two fractions of PM: (1) large particles greater than 10 µm; and (2) coarse particles 2.5 – 10 µm. Whatman Grade 91 and Grade 42 cellulose filter papers (47 mm) were used to collect the large and coarse fractions, respectively (Dzierżanowski et al., 2011). The solution was filtered a third time through a quartz fiber filter to separate fine particulates <2.5 µm (Rindy et al., 2019).

Between replicates, the glassware was soaked overnight in DDI water, dried, and baked in a muffle furnace for 3 hours at 450˚C. For the second replicate, the glassware was rinsed five times with DDI water, once with acetone, and once more with DDI water between sample filtrations.

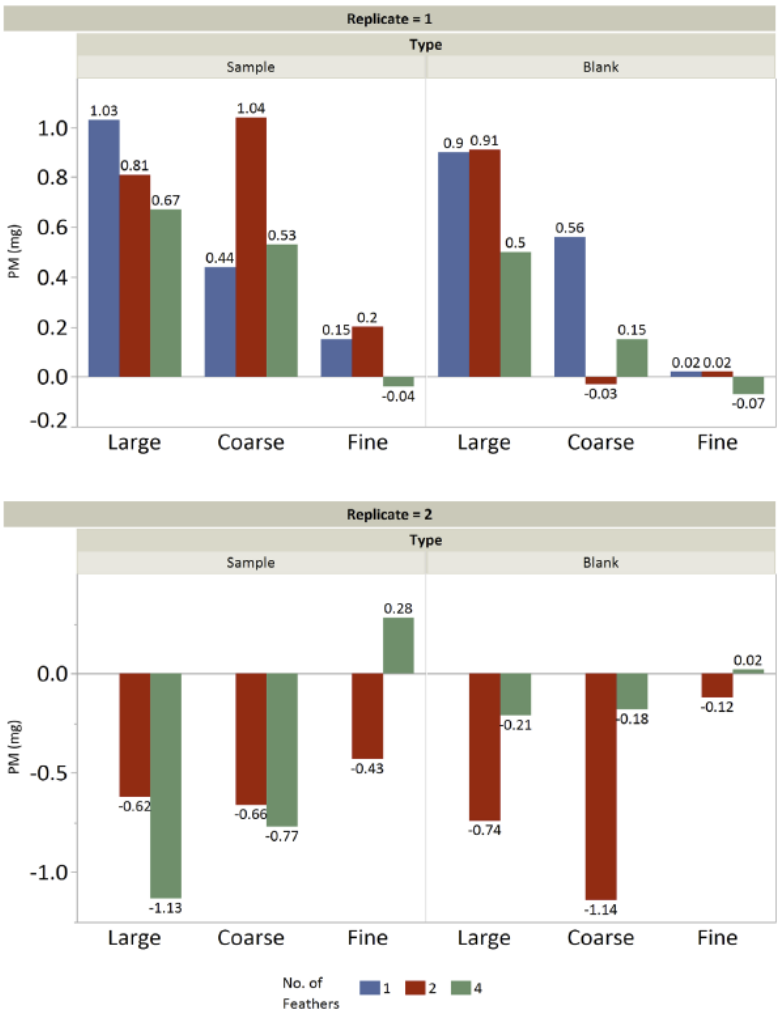
Used cellulose and quartz fiber filters were stored in the desiccator until all samples had been filtered. Cellulose filter papers were transferred to the gravity convection oven and dried for 24 hours at 60˚C. Once dried, they were cooled in the desiccator for 15-30 minutes. Quartz fiber filters were dried in the desiccator for 12 hours. From the desiccator, both cellulose and

quartz fiber filters were acclimated to lab ambient conditions for 3-5 minutes before determining the post-weight.

Results

The results from Trial 1 (Figure A.1) provided no clear pattern of PM accumulation based on the number of feathers included in the sample.

Figure A.1: Total PM measured on samples and blanks in Trial 1. Top: Replicate 1 used a DDI water-only rinse between the sample and blank. Bottom: Replicate 2 used a DDI-acetone-DDI rinse.



In the first replicate, the single feather extraction showed higher accumulation amounts than both 2- and 4-feather samples in the large and fine fractions. In all fractions, the 4-feather

sample showed the least accumulation. The large fraction appeared on the blanks in amounts similar to the samples for all number of feathers extracted. Contamination of the blanks also occurred for the coarse fraction in the single feather samples. This indicates that cleaning the glassware with only DDI water was inadequate as PM was likely carried over to the blanks from the glassware.

The second replicate shows no PM accumulation for the large and coarse fractions for either the samples or blanks. Some fine PM was collected from the sample with minimal carryover to the blanks. However, the negative values indicate that the filter weights are likely inaccurate. A stable post-weight was difficult to obtain for the cellulose filters as micro-balance measurements drifted upward due to changing ambient temperature and relative humidity.

Conclusion

Though results from trial 1 were not clear on how many feathers are needed, it does highlight that the methods are insufficient in preventing contamination of the blanks. Throughout this trial, there was difficulty in timing processes and obtaining accurate weights which likely affected the results.

- Because all extractions for the first trial were performed before filtrations began, the extraction solutions/bottles were refrigerated beyond the recommended 12 -24 hours. Extraction solutions remained in the fridge for a total of 4 days. This could affect the recovery of PM from the solutions.
- Quartz fiber filters from the first replicate were left in the desiccator over the recommended 12 hours. This may have reduced the post-weights of the filters, explaining the negative values.
- Cellulose filter papers from the first replicate were left overlong in the drying oven while completing filtrations. These filters dried for about 47 hours before post-weights were determined. To obtain accurate weights, the drying protocols before and after filtration should be identical.

- For comparison, the cellulose filter papers of the second replicate were dried for 24 hours, weighed, then dried an additional 24 hours and weighed again. The filters' weight increased by an average of 1.08 and 1.24 mg for the large and coarse papers, respectively. Upward drift was observed in the scale readout, suggesting that the filters were still acclimating to ambient conditions of temperature and relative humidity.

To correct for these issues, subsequent methods should:

1. Establish pre- and post-weights under similar if not identical lab ambient conditions of temperature and relative humidity. Temperature should be 20-23 ± 2˚C and relative humidity less than 30-40 percent, constant within ±5 percent over 24 hours (Papp & U.S., 2016). Cellulose filter papers are extremely sensitive to these parameters.

2. To improve recovery of PM:

    a. Increase the number of extractions per sample. Similar protocols to remove PM from feather surfaces perform multiple extractions of the same sample to remove external contamination (Cardiel et al. 2011, Borghesi et al. 2017).

    b. Complete fewer sample extractions (2 samples followed by extraction blanks – total of 4 at most) in a day so that the solutions are refrigerated for the recommended 12 hours, and preparation of filters and glassware are timely.

3. To reduce transfer of PM between samples, wash glassware with DDI water and acetone between each.

4. To reduce contamination of filters, keep filters covered at all times (drying, transport, and storage).

Trial 2

Trial 2 used "very dirty" molted pigeon feathers in order to provide larger amounts of PM for comparison between number of feathers within each sample. Before processing the feather samples, all glassware used in extraction and filtration was soaked for 24 hours in DDI water, washed in a 10% nitric acid bath for a minimum of 4 hours, and finally baked in a muffle furnace at 450˚C for 5 hours.

Filter Preparation

Cellulose filters were placed in individually labeled foil envelopes (covered on 3 sides) and prepared in the same manner – oven-dried for 24 hours at 60˚C and then placed in a desiccator for 15-30 to cool. To determine an appropriate acclimation period before weighing, filters were weighed after 3-5 minutes (Technique 1), and again at 24 hours (Technique 2). For technique 1, the initial weight reading was recorded for all filters, and then the filters were immediately weighed again in the same order. For technique 2, the filters were left out on the lab bench in their foil envelopes, and all envelopes covered lightly with a KimWipe. After 24 hours, the filters were weighed. The initial reading was recorded, and the filter left on the scale until the reading was stable for at least one minute. This second weight was recorded. The range between the two weight values for each sample and each technique is shown in Table A.2.

Table A.2: Range of pre-weight values using different acclimation techniques for cellulose filters.

No. of Feathers	Size Fraction	Type	Pre-Weight Range (mg)	
			Technique 1	Technique 2
2	Coarse	Sample	0.45	0.05
2	Coarse	Blank	0.18	0.03
2	Large	Sample	0.13	0.03
2	Large	Blank	0.14	0.04
4	Coarse	Sample	0.26	0.02
4	Coarse	Blank	0.15	0.03
4	Large	Sample	0.08	0.02
4	Large	Blank	0.14	0.02

Weights were much more stable (less varied) when filters were acclimated for 24 hours after drying and cooling (Technique 2).

Acclimation Experiment with Lab Blanks

To determine if contamination of the filters was occurring in the lab atmosphere, four additional cellulose filters, two each for the large and coarse size fractions, were prepared and treated identically, though not used for filtration. These filters were weighed after acclimation periods of 5 minutes (Technique 1), 24 hours (Technique 2), and 48, 72, and 96 hours to observe any variation in weights.

As can be seen in Table A.3, variation of weights was minimal in each acclimation period, showing no significant improvement with greater drying times. The greatest variation in weights can be seen at 96-hours, where the relative humidity was the highest, leading to greater hygroscopic growth of the filters. Weights for each filter between acclimation periods (Table A.4) varied by less than 1 mg. Again, weight differences were most likely due to changing temperature and relative humidity.

Table A.3: Range in filter weights (mg) of lab blanks, two coarse (C), two large (L), at different periods of acclimation.

Acclimation Period	Temp (˚C)	RH (%)	Weight Range of Filters (mg)			
			C-3	C-4	L-3	L-4
24h	21.90	31.80	0.02	0.04	0.04	0.07
48h	21.80	27.10	0.17	0.04	0.08	0.02
72h	21.94	26.73	0.01	0.03	0.04	0.11
96h	21.60	34.80	0.20	0.08	0.16	0.06

Table A.4: Weights of filters after different periods of acclimation to lab ambient conditions.

Sample	Acclimation Period	Temp (˚C)	RH (%)	Weight (mg)
C-3	24h	21.90	31.80	173.180
	48h	21.80	27.10	172.725
	72h	21.80	27.00	172.523

Sample	Acclimation Period	Temp (˚C)	RH (%)	Weight (mg)
	96h	21.60	34.80	173.006
C-4	24h	21.90	21.90	167.483
	48h	21.80	21.80	166.953
	72h	21.80	21.80	166.960
	96h	21.60	21.60	167.644
L-3	24h	21.90	21.90	106.830
	48h	21.80	21.80	106.530
	72h	21.80	21.80	106.415
	96h	21.60	21.60	106.781
L-4	24h	21.90	21.90	110.254
	48h	21.80	21.80	109.830
	72h	21.80	21.80	109.814
	96h	21.60	21.60	110.400

Therefore, given these results, the pre-weights recorded after the 24-hour acclimation period will be used in the calculations for total PM mass. This same method will be used to determine the post-weights. Prepared filters were stored in the desiccator until ready for filtration.

Extraction and Filtration

Samples of 2 and 4 molted feathers were extracted by placing them in a capped 500 mL amber bottle filled with 300 mL of DDI water. The bottle was sonicated for 5 minutes. The feathers were then flipped on end and sonicated an additional 5 minutes. After sonication, each side of each feather was rinsed 3 times using an acetone squirt bottle. The acetone rinse was collected in a 50 mL graduated cylinder fitted with a large funnel until the total volume of the acetone reached 10 mL. The acetone rinse was added to the amber bottle. This process

was repeated twice more, each time using a clean 500 mL amber bottle filled with 300 mL DDI water. Each sample, therefore, was extracted three times for a total of 900 mL DDI + 30 mL acetone. To each bottle, 4.5g of $NH_4H_2PO_4$ salt was added. The bottles were sonicated for 10 minutes and then refrigerated overnight. The feathers were air-dried and stored in a clean glove box on top of KimWipes. After each sample extraction, an extraction blank was prepared using the same technique in absence of feathers. Between each sample, the graduated cylinder and funnel were rinsed 5 times with DDI water, once with acetone, and again with DDI water.

Extraction solutions were filtered three times using the same assembly in Trial 1 to collect three size fractions of PM. Between each sample, the filtration glassware was disassembled, and each piece washed five times in DDI water, once in acetone, and again in DDI water.

Used cellulose and quartz fiber filters were stored in the desiccator until all samples had been filtered. Cellulose filter papers were transferred to the gravity convection oven and dried for 24 hours at 60˚C. Once dried, they were cooled in the desiccator for 15-30 minutes. Once cool, filters (in foil envelopes) were placed on a bench and covered lightly with a KimWipe. After 24 hours of acclimation, the cellulose filters were weighed. Quartz fiber filters were dried in the desiccator for 12 hours and then acclimated to lab ambient conditions for 3-5 minutes before determining the post-weight. Cellulose filters were stored in the glove box, and quartz fiber filters in the freezer.

Results

The results for Trial 2 (Figure A.2) show considerable carryover of PM from the samples to the blanks. Though glassware was washed with DDI-acetone-DDI method between each

sample, it does not seem to have proven as effective in the second trial as it did in the first. However, there was a 11.3% increase in relative humidity when the pre- and post-weights were recorded (Table A.5) which would increase the filter post-weights, exaggerating the amount of PM accumulated.

Figure A.2: Particulate matter accumulated on sample and blank filters for Trial 2.

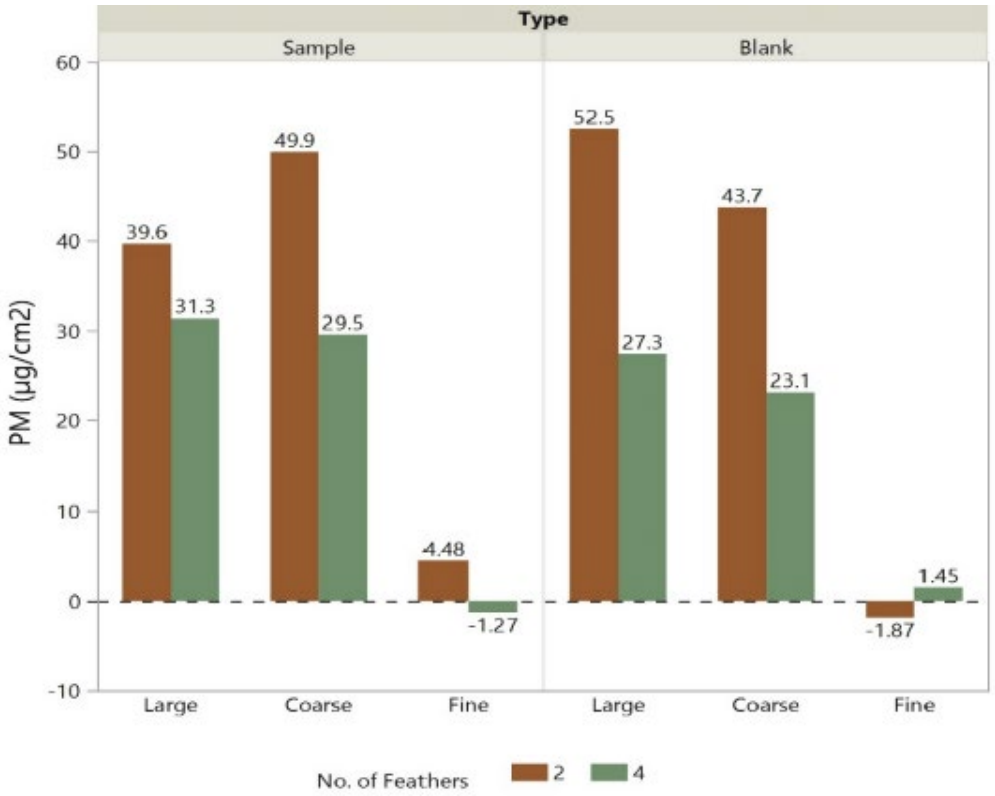


Table A.5: Lab ambient conditions during determination of pre- and post-weights.

Lab Ambient Conditions	Temperature (˚C)	Relative Humidity (%)
Pre-Weights	21.9	32.1
Post-Weights	21.5	43.4

Discussion

Though all lab surfaces, fume hood, glove box, and desiccator were wiped down before all work, other sources of contamination could be affecting the results. The gravity convection

oven was cleaned thoroughly inside and out before the beginning of trials but was not cleaned between drying periods. Lab blanks L-1 and C-1 were prepared identically to sample filters for pre-weights but were not moved back to the oven with the used sample filters for the 24-hour oven drying period, and 24-hour re-acclimation to lab ambient conditions. This would have told us if contamination was occurring in the oven.

Additionally, the lab environment is not controlled for relative humidity. Cellulose filter papers are very sensitive to changes in temperature and relative humidity. To continue this protocol using the actual samples (feathers plucked from birds), pre- and post-weights of filters should be recorded in similar conditions of temperature and relative humidity (Papp & U.S., 2016).

Conclusion

Based on the results of Trials 1 and 2, I determined that cellulose filter papers are not suited to a weighing environment that is not controlled for relative humidity. To save preparation time and avoid complications of changing ambient conditions, I will use a pre-weighed glass microfiber filter media (Whatman 934-AH RTU), also 47 mm in diameter. This filter will collect all particles greater than or equal to 1.5 µm.

To avoid carryover of PM from glassware, it may be necessary to rinse and bake filtration glassware between samples. This will require that only two sample and two blank extractions (four total) can be run at a time. This will ensure that filtration of extraction solutions can be completed within a day (and not remain in the refrigerator over the recommended 12-24 hours). As I am not separating multiple fractions, two samples can be run

at once, followed by the blanks, and the filtration glassware cleaned while performing the next set of four extractions.

Trial 3

This trial used pre-weighed Whatman 934-AH ready-to-use (RTU) glass microfiber filters (47 mm) to collect all PM greater than or equal to 1.5 µm in diameter. These filters come with individual metal pans labeled with ID numbers and the weight (mg) determined by the manufacturer. I created foil covers for each pan and stored them in the desiccator until ready for filtration. No other filter preparation was necessary. Before processing the feather samples, all glassware used in extraction and filtration was soaked for 24 hours in DDI water, washed in a 10% nitric acid bath for a minimum of 4 hours, and finally baked in a muffle furnace at 450˚C for 5 hours.

Extraction and Filtration

One sample of 4 molted feathers was extracted by placing them in a capped 500 mL amber bottle filled with 300 mL of DDI water. The bottle was sonicated for 5 minutes. The feathers were then flipped on end and sonicated an additional 5 minutes. After sonication, each side of each feather was rinsed 3 times using an acetone squirt bottle. The acetone rinse was collected in a 50 mL graduated cylinder fitted with a large funnel until the total volume of the acetone reached 10 mL. The acetone rinse was added to the amber bottle. This process was repeated twice more, each time using a clean 500 mL amber bottle filled with 300 mL DDI water. Each sample, therefore, was extracted three times for a total of 900 mL DDI + 30 mL acetone. To each bottle, 4.65g of $NH_4H_2PO_4$ salt was added. The bottles were sonicated for 10

minutes and then refrigerated overnight. The feathers were air-dried and stored in a clean glove box on top of KimWipes. After the sample extraction, an extraction blank was prepared using the same technique as above, but without any feathers. Between the sample and blank, the graduated cylinder and funnel were rinsed five times with DDI water, once with acetone, and again with DDI water.

Using a similar set up as Trials 1 and 2, the extraction solution was filtered once utilizing the 47 mm frit and funnel. Between the sample and blank, the filtration glassware was disassembled, and each piece washed five times in DDI water, once in acetone, and again in DDI water. Two additional filters were used as lab blanks, duplicating all treatments (fume hood, desiccator, oven) except for filtration to determine if any contamination was occurring in the lab environment.

Once filtration was complete, the filters were returned to their individual pans, covered, transferred to the gravity convection oven and dried for 1 hour at 103˚C. Once dried, they were cooled in the desiccator for 15-30 minutes and then weighed. The drying, cooling, and weighing process was repeated twice more to establish a constant post-weight.

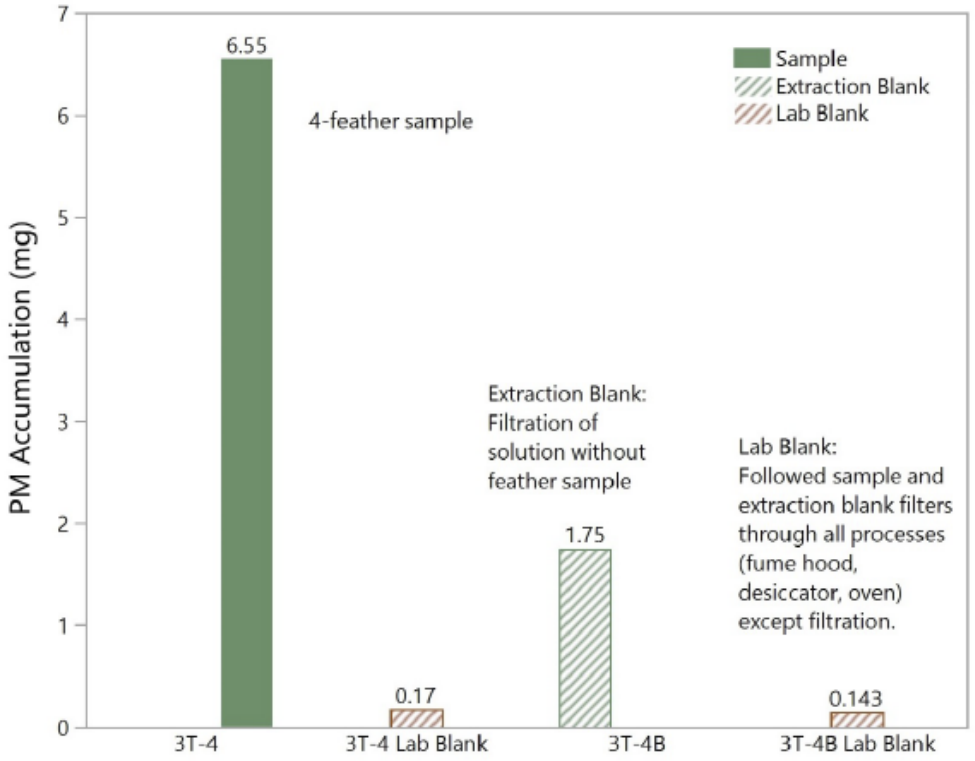
Results

Repeated post-weights showed a maximum variation of 0.10 mg (100 µg), or within 0.08% of each reading (Table A.6). These filters proved to be much less susceptible to humidity than the cellulose paper filters. Particulate matter accumulation was determined by subtracting the pre-weight from the average of the post-weights. There is a significant amount of PM accumulated on the 4-feather sample, a small amount of carryover in the extraction blank, and negligible amounts in the lab blanks (Figure A.3).

Table A.6: Post-weights of glass microfiber filters in Trial 3.

Sample ID	Pre-Weight (mg)	Post-Weight (mg)			Max Weight Change	Max % Change	Average Post-Weight (mg)	PM (mg)
		1	2	3				
3T-4	117.92	124.53	124.44	124.43	0.10	0.08%	124.47	6.55
3T-4 Lab Blank	118.12	118.33	118.28	118.26	0.07	0.06%	118.29	0.17
3T-4B	118.47	120.21	120.27	120.17	0.10	0.08%	120.22	1.75
3T-4B Lab Blank	117.98	118.15	118.10	118.12	0.05	0.04%	118.12	0.14

Figure A.3: Results of total PM accumulation for samples and blanks in Trial 3.



Discussion

Only one issue was encountered. When removed from the oven and cooled, the filters had to be peeled off the metal pan before weighing. Some filter media was left stuck to the pan which affected the final weights.

Conclusion

Trial 3 produced clear results. Though some contamination of the method and lab blanks is present, the repeated drying and weighing process produced much more stable weights and reliable results. The filters will not be acclimated for 24 hours as this is only useful in determining accurate weights when the lab environment is controlled for relative humidity. For the finalized protocol, the used filters will be air-dried for at least 30 minutes before transferring them to the oven. This should prevent them from sticking to the pan. After oven-drying and cooling in the desiccator, the filters will be weighed immediately to minimize exposure to the ambient air. Samples will be corrected to account for PM accumulation to the method blanks. Therefore, the glass microfiber filters and this modified method will be used to process the plucked feather samples.

APPENDIX B

FINALIZED PROTOCOL

This protocol removes external PM from feather surfaces by washing repeatedly in DDI water and acetone. The solution is then filtered, and PM accumulation determined by gravimetric analysis (the weight of the filter before and after filtration) and calculated per unit feather surface area. Method blanks and lab blanks are utilized to determine any sources of contamination.

Preparation of Glassware

The following steps are to be performed on all glassware used in the extraction and filtration processes, including at least one 1L beaker and glass funnel which will be used for transferring the acid wash solution to and from the 4L storage jug and wash bin.

Phase 1: DDI Wash Procedure

1. Remove all labels from the vessel. Sharpie can be removed using ethanol or acetone and a KimWipe
2. Rinse a clean plastic tub 3 times with DDI water
3. Rinse vessel 3 times with DDI water, covering all surfaces inside and out, including any lids or caps
4. Fill reservoirs with DDI water, cap them if they have caps, and soak for 24 hours in the plastic tub. All surfaces should be in contact with DDI water for 24 hours, submerged if possible.
5. After 24 hours, empty reservoirs and rinse again 3 times with DDI water
6. Allow to dry in a clean cabinet lined with KimWipes.
7. Label the shelf with the type of wash and the date (e.g., 'DDI wash 15Oct2020'). Any material that has been drying for more than 2 weeks should be rewashed.

Phase 2: Prepare the Acid Mixture

<u>Never</u> pour water into concentrated acid. ONLY ever pour acid into water

1. Put on PPE: pants, close-toed shoes, lab coat, eye protection, blue nitrile gloves and acid gloves over those

2. In the fume hood, place clean funnel into empty 4 L amber bottle, inside the shallow plastic tub.

3. Using a clean beaker, fill and measure out a total of 3,600mL of DDI water, transferring it to the 4L amber bottle via the funnel, being sure not to overflow funnel (this makes the mixture 90% DDI)

4. In fume hood, measure out 400 mL of nitric acid (HNO_3) into beaker

5. Pour the 400 mL of HNO_3 into funnel, and be sure not to overflow funnel

6. This creates a mixture that is 10% HNO_3 and 90% DDI water (1-part HNO_3 solution to 9 parts DDI water)

7. LABEL the mixture with labeling tape as 10% nitric acid, date it was made, and initials

8. Ensure the label has no other writing on it, or other writing is fully crossed out

9. Ensure the mixture is placed in acid cabinet, which should display proper labeling

Phase 3: Prepare Acid Bath

For glassware that has been washed according the DDI wash procedure (Phase 1) above, and dried.

1. Put on your PPE: pants, close-toed shoes, lab coat, eye protection, blue nitrile gloves and acid gloves over those

2. Make sure fume hood is clean and clear of any extraneous materials. Wipe down the hood with DI water and paper towels.

3. Carefully transfer the nitric acid from the acid cabinet to the fume hood. You may need two or three 4L jugs of diluted acid depending on the volume of glassware you need to clean. Try to use each of the solutions in rotation so that they all reach 25 uses at about the same time.

4. Rinse out one of the large clear Rubbermaid tubs at least 3 times with DI water. Make sure that water touches all internal surfaces during each rinse.

5. With gloves still on, place one or both plastic bins in fume hood (depending on amount of glassware being washed)

6. Place dry glassware (no plastic) in previously rinsed plastic bin and place the bin inside the fume hood.

7. In the fume hood, SLOWLY pour acid mixture from the 4L bottles into plastic bin until the tops of all glassware are covered, making sure to fill any large reservoirs that cannot be submerged. All surfaces should be in contact with the nitric acid. If the glassware cannot be submerged, rotate it throughout the washing period until all surfaces have been in contact with the nitric acid for at least 4 hours.

8. Replace cap on acid mixture, and place bottle into acid cabinet.

9. Close fume hood completely

10. Place a sign on the fume hood glass, with "10% Nitric Acid (HNO_3) bath" followed by the date you made the bath, and your initials

11. Fill out the acid wash log with the date of use corresponding to the solution IDs on the amber jug that the nitric acid is stored in.

12. Leave bath for a minimum of 4 hours up to 24 hours

Phase 4: Clean Up Acid Bath

1. After the glassware has soaked for the appropriate amount of time, rinse another storage bin with DI water so that you have a bin to transfer your clean glassware to.

2. Carefully remove the glassware from the acid bath draining large objects into the 4L amber storage jug first, then placing the clean, empty glassware into the other rinsed Rubbermaid tub. Always use the funnel and pour slowly so that you don't spill the nitric acid in the fume hood – or on you! Once you have removed all items from the Rubbermaid tub, use the 1L beaker to remove most of the remaining liquid (aka, don't try to pour the tub into the funnel until it is nearly empty of liquid). If you used more than one jug of nitric acid, keep an eye on the level of the acid in the container so that you don't over fill it!

3. Once the Rubbermaid tub is empty of nitric acid, rinse it 5 times with DI water in the sink.

4. Return 4L acid bottle to acid cabinet with cap tightly secured

5. Rinse the glassware with DI water 5 times and place it in the rinsed tub from step 3.

6. Once all your glassware is rinsed, you can set the glass to air dry inverted in a clean, KimWipe-lined cabinet or dry it with KimWipes and bake it in the muffle furnace following that protocol (below, Phase 5).

7. Label the cabinet shelf with the method of cleaning and the date (eg 'acid washed 2/7/17') and let dry.

8. Any material that has been drying for more than 2 weeks should be rewashed.

If A Spill Occurs

Follow the instructions to the right of the fume hood. The drain in the back left of the fume hood can accept acid in the event of a small spill, just turn on the green faucet on the outer left side of the hood to wash the acid down the drain. Allow the faucet to run for at least 5 minutes. Do not dispose of acid in the drain unless directed to do so by RMS staff.

Disposal of Spent Nitric Acid Wash

When the acid bath has been used 25 times for glassware washing and needs to be disposed of, fill out the hazardous waste pick up form at the Risk Management Services website: http://riskmanagement.unt.edu. Make sure you carefully follow the instructions and leave them detailed notes about where to find the waste. Transfer the spent acid solution to a waste container from below the fume hood (DO NOT SEND OUR GOOD AMBER JUGS AWAY TO RMS. WE WILL NOT GET THEM BACK.) Clearly mark the container as 'WASTE FOR RMS DISPOSAL' 'DILUTE 10% NITRIC ACID'. Place the waste container in secondary containment (a Rubbermaid tub) in the fume hood and the waste should be taken away! If it has been more than 24 hours and the waste has not been removed, contact RMS to follow up about the disposal status. Make sure you do not leave any other containers in the fume hood once you

have filed a ticket for RMS to come pick up the waste – you want it to be VERY clear what is to be disposed of.

Phase 5: Muffle Furnace

After the DDI wash and the acid bath, transfer dry glassware to the muffle furnace. Glassware can be air-dried, or you can use KimWipes.

1. Put on PPE: Nitrile gloves, lab coat, closed toed shoes, pants.
2. Cover all openings of glassware in aluminum foil
3. Additional PPE: eye protection (goggles or face shield), high temperature gloves (orange). Fire retardant clothing and apron are also recommended.
4. Transfer glassware to muffle furnace, centering pieces within the chamber. Do not fill chamber more than 2/3 full. Make sure pieces do not touch each other or the thermocouple. WHEN IN USE, THE EXTERIOR OF THE FURNACE IS HOT. DO NOT TOUCH WITHOUT HIGH TEMPERATURE GLOVES.
5. Turn the furnace on. The upper, larger digital readout is the current temperature. The lower, smaller digital readout is the set point (SP1) or desired temperature. Use the up/down arrows to select the settings below. Access to submenus and any changes to settings are confirmed when the new selection flashes once.
    a. Set the temperature (SP1) to 450˚C.
    b. Set the TM.CFG to DWELL for 5 hours
    c. Make sure threshold (THRESH) function is turned OFF
    d. Set the timer end type (END.T) to OFF
    e. To begin the program, set the T.STAT to Run
6. This will heat the furnace from ambient temperatures up to 450˚C, bake the glassware for 5 hours, and then automatically shut the furnace off. [*Specific details on furnace operation can be found in the Lab 258 Manuals folder on the "Margo Ruth Spiegelman" Desktop*.]
7. The oven takes several hours to cool. At < 200˚C , you can crack the door to speed up the process. At < 100˚C, you can open the door completely. Before removing the

glassware, the furnace temperature should be around 50˚C or below to avoid cracking it. Use the metal trays if still warm.

8. Store and label in KimWipe-lined cabinet (e.g., “acid washed and baked, date”) once glassware has completely cooled.

9. Any baked material that has been stored for more than 2 weeks should be re-baked.

NOTE: Furnace should not be powered off until it has cooled to ambient temperatures (~25C).

Extraction: Day 1

- Note all completion and transfer times on extraction and filtration log sheets. Refrigeration must be within 12-24 hours. Drying time must be minimum 1-hour increments.
- Use gloved hands, acid washed and baked glassware, and acetone rinsed tweezers while handling feathers.
- Wipe down all work surfaces with DI water before beginning.
- All procedures should be performed in the fume hood. Wipe clean with DI water.
- The extraction process will be repeated three times for each sample.
- There will be two (2) method blanks and two (2) lab blanks for each population (10 samples), one after every 5 samples.
- All glassware will be soaked 24 hours in DDI water, bathed in a 10% nitric acid solution for a minimum of 4 hours, and baked for 5 hours at 450˚C between samples and between blanks and samples. Glassware cleaned with this method is referred to as “clean” glassware.
- Use the ML scale to weigh salt (to nearest mg).

Supplies

- 6 x 500-mL amber bottle with caps
- 2 x sonic baths
- 2 x 50-mL graduated cylinders
- 1 x scissors
- 1 x small box KimWipes
- 1 x camera

- 2 x large funnels
- 1 x 500-mL beaker
- 1 x acetone squirt bottle
- 1 x DDI squirt bottle
- 2 x tweezers
- Nitrile gloves
- Extraction Log
- Fume hood
- 1 x digital balance (to nearest mg)
- 1 x permanent marker
- ADP salt ($NH_4H_2PO_4$)
- Plastic weigh boats
- Small metal spatula
- Paper towels
- Timer

Method

1. Select two samples (two sets of 4 feathers plucked from two birds). For each sample, on a solid, contrasting background labeled with the sample number and molt ID, photograph both sides of feathers. Record photo numbers in extraction log, along with date, feather origin and molt ID.

2. With acetone-cleaned scissors, clip the calamus, leaving a small portion for grasping with tweezers.

3. Place both sonic baths in the fume hood, filled about half-way with tap water and plug them in.

4. Prepare six 500 mL clean amber bottles with caps, each filled with 300 mL DDI water. Double-check that all bottles have the same amount of water before continuing. You will use three bottles for each sample. Label each bottle with the sample ID and bottle number.

Repeat the following steps (5-12) for each of two samples. <u>Separate sets of clean glassware (graduated cylinder and funnel) should be used for each sample.</u>

5. Using tweezers, place all 4 feathers tip down in the first (#1) of the labeled sample bottles, cap the bottle, and sonicate for 5 minutes.

6. Open the bottle. Using the tweezers, flip the feathers on end, cap the bottle, and sonicate an additional 5 minutes.

7. Remove each feather, one-by-one, and holding it over a 50 mL graduated cylinder fitted with a large funnel, rinse each side of each feather 3 times with the acetone squirt bottle, transferring the feathers to the second bottle (#2) when done. Collect a total volume of 10mL of acetone rinse for all 4 feathers.

8. Transfer the acetone rinse to the first (#1) sample bottle and set aside.

9. Repeat steps 5-8 for a second and third extraction of the same sample. You will have a total of 900 mL DDI water + 30 mL acetone over three bottles for each sample.

10. Once the three extractions are complete, remove the feathers to a clean bench and place them on a KimWipe to dry.

11. Add 4.65 g (0.015 g per 1 mL solution) of ADP salt to each bottle, cap, and sonicate 10 minutes.

12. Move the bottles to the refrigerator for storage, marking the time on the extraction log.

13. Once dry, photograph both sides of feathers and record the photo numbers in the extraction log.

14. Store the clean, dry feathers, labeled and covered, in the glove box.

15. Before continuing with the next set of samples, clean all glassware and wipe down the tweezers with acetone.

Preparation of Method Blanks

After four samples, follow this procedure to prepare a method blank side-by-side with a sample.

1. Select one sample (one set of 4 feathers plucked from one bird). On a solid, contrasting background labeled with the sample number, photograph both sides of feathers. Record photo numbers in extraction log, along with date, feather origin and molt ID.

2. With acetone-cleaned scissors, clip the calamus, leaving a small portion for grasping with tweezers.

3. Place both sonic baths in the fume hood, filled about half-way with tap water and plug them in.

4. Prepare six 500 mL clean amber bottles with caps, each filled with 300 mL DDI water. Double-check that all bottles have the same amount of water before continuing. You will use three bottles for the sample and the other three bottles for the blanks. Label each bottle with the sample/blank ID and bottle number.

5. Open the bottles for the sample and the blank labeled with a #1. Use tweezers to place feathers tip down in one bottle; the other bottle will be your blank and has NO feathers. Cap both bottles and sonicate for 5 minutes.

6. Open the bottles. Using tweezers, flip the feathers on end in the sample bottle while the blank bottle is open. Cap both bottles and sonicate an additional 5 minutes.

7. Open both bottles.

8. Remove each feather, one-by-one, and holding it over a 50-mL graduated cylinder fitted with a large funnel, rinse each side of each feather 3 times with the acetone squirt bottle, transferring the feathers to the second sample bottle (#2) when done. Collect a total volume of 10mL of acetone rinse for all 4 feathers.

9. Transfer the acetone rinse to the first sample bottle and set aside.

10. Using a clean 50-mL graduated cylinder and large funnel, measure and add 10 mL of acetone to the blank bottle and set aside.

11. Beginning with sample bottle #2 now containing the feathers and blank bottle #2, repeat steps 5-10 twice, for a second and third extraction of the same feather sample and blank. The glassware set (graduated cylinder and funnel) used for the sample stays with the sample, and that for the blank with the blank. For each sample and each blank, you will have a total of 930 mL DDI water + acetone in three bottles over the three extractions.

12. Remove feathers to a clean bench on a KimWipe and allow to air-dry.

13. Once all sample and blank extractions are complete, add 4.65 g of ADP salt (0.015 g per 1 mL solution) to each amber bottle (6 total), cap, and sonicate contents for 10 mins.

14. Move bottles to the refrigerator, marking the time in the extraction log. Store overnight.

15. Clean tweezers with acetone from the squirt bottle and wipe dry.

16. Once dry, photograph both sides of feathers. Record photo numbers in extraction log.

17. Store the clean, dry feathers, labeled and covered, in the glove box.

18. Before continuing with the next set of samples, all glassware should be cleaned (DDI soak, 10% nitric acid bath, baked in muffle furnace.

Filter Preparation: Day 2

- Turn on the Kestrel to record ambient lab conditions.
- Put on nitrile gloves before continuing, change gloves after cleaning.
- Wipe down all work surfaces with DI water and a paper towel before beginning and after completion of lab work – lab benches, fume hood, glove box, oven, and muffle furnace.
- Handle all filters carefully, using tweezers wiped clean with acetone.
- Keep filters covered (foil) at all times during drying, transport, and storage.
- Use the MS105DU scale (Semi Microbalance) to weigh the filters (to the nearest 1/100 of a mg).

Supplies

- 2 x Whatman Type 934-AH RTU filters
- MT MS105DU Semi Microbalance (to nearest 1/100 mg)
- 1 x extra metal pan for weighing
- Nitrile gloves
- Foil
- Permanent Marker
- Filtration Log
- Desiccator

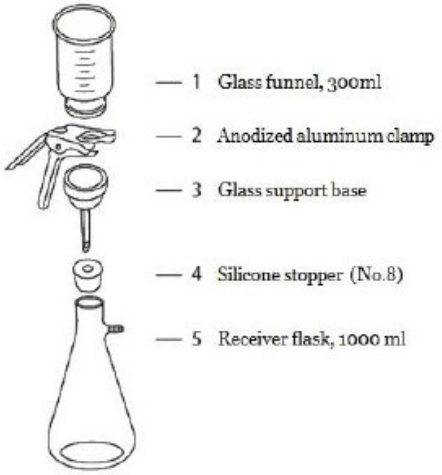
Method

1. Create foil covers for each filter tray, labeled with the sample number.
2. Record pre-weight, filter number, and sample ID in the filtration log.
3. Store filters in desiccator or glove box until ready for filtration.

Note: These filters come in metal trays, pre-conditioned and weighed according to U.S. EPA Standard Method 2540 Part D, described below. No other preparation is necessary.

- Pre-rinsed under vacuum with three successive lots of 20mL reagent grade water.
- Dried at 103 – 105˚C for 1 hour
- Cooled in desiccator to ambient temperature and weighed.
- Repeated drying and weighing until weight measurements differ by less than 4%.

Filtration: Day 2



When filtering a method blank, one lab blank should also be prepared, covered/uncovered, and treated the same way as the sample except for actual filtration. All filtrations should be performed in the fume hood.

Supplies

- 4 x 500 mL receiver flasks w/vacuum attachment
- 2 x 300 mL glass funnels w/47mm base
- 2 x 47 mm glass filter support base (frit)
- 2 x No. 8 silicone stoppers
- 2 x Anodized aluminum clamps, 47mm
- 2 x Manual vacuum pumps
- 1 x 50 mL glass beaker
- 2 x prepared 934-AH RTU filters
- 1 x semi micro-balance (to nearest 1/100 mg)
- Filtration Log
- 2 x tweezers
- 1 x acetone squirt bottle
- 1 x DDI squirt bottle
- Nitrile gloves
- Fume hood
- Desiccator
- Wire mesh drying rack
- Camera
- KimWipes
- Foil

Method

1. Photograph filters before use, recording the photo numbers in the filtration log. Record filter ID, sample ID and picture number in filtration log for the samples and blanks.

2. Attach the stopper to the 47 mm fritted glass support base and place it into the receiver flask

3. Center the filter on the fritted glass support base. If using a lab blank, expose (remove foil cover) the lab blank filter and set it in a safe place in the hood.

4. Center the 300 mL glass funnel with 47 mm base over the filter. Make sure this is centered and that edges of the funnel base are in-line with the frit to avoid leaks. Secure with a 47 mm clamp. Do not put the base of the clamp (bottom of the 'U' bend) flush with the funnel neck, this will push it out of alignment.

5. Attach the hose for the hand pump to the receiver flask.

6. Wet the filter with a small amount of DDI water from a squirt bottle and pump a few times to set the seal.

7. Remove the extraction solution bottles from the fridge. Gently shake/swirl 2 -3 times and then pour it into the funnel slowly, using the hand pump to maintain vacuum pressure within the pressure "box" on the pump gauge (do not go above 5 in-Hg) until all solution has passed through the filter.

8. Rinse the inside of the amber bottle twice with DDI water from the squirt bottle. Pour into funnel until all liquid has passed through the filter.

9. Repeat steps 7 and 8 for the remaining 2 bottles, switching receiver flasks when the maximum volume of 500 mL has been reached. Double-check the funnel alignment after switching.

10. Rinse the funnel sides twice with DDI water and continue pumping until all liquid has passed through the filter.

11. Rinse the filter with 3 aliquots of 10 mL reagent water, measured with a clean 50-mL beaker.

12. Release the vacuum pressure and carefully remove the clamp, funnel, and vacuum hose.

13. Using tweezers, remove the filter, place it in the corresponding pan and cover with the labeled foil.

14. Transfer the filters to the desiccator and place them bare (no pan) on the wire mesh and cover them with the labeled foil. Place the corresponding pan underneath the mesh on the desiccator tray. Allow filters to dry for 2 hours. If using a lab blank, it should follow the other filters – place it out to air-dry as well. Pans can be placed in the oven while warming for about 30 minutes to dry and then return them to the desiccator.

15. In preparation for weighing (next step):

    a. Turn on the gravity convection oven and set the temperature to 103 – 105˚C (takes approximately 30 minutes to heat up).

    b. Perform an internal adjustment.

    c. Place a desiccant tray at the back of the semi-microbalance (NOT ON THE BALANCE PAN).

16. Discard the filtrate.

17. After completing the filtrations, clean the tweezers with acetone and clean all glassware before continuing with the next set of samples.

Drying to Constant Weight: Day 2

Supplies

- 2 x acetone-cleaned tweezers
- Desiccator
- Wire mesh drying rack
- Nitrile gloves
- Box of Small KimWipes
- Filtration Log
- Semi-microbalance
- 3 x Desiccant trays
- Gravity convection oven
- Camera
- Kestrel 4500 Weather Meter

Method

1. Turn on the Kestrel. All weighing should be carried out in similar temperature (20 - 23 ± 2˚C) and relative humidity (30 – 40%, constant within ±5 percent).

2. Place a desiccant tray at the back of the semi-microbalance weigh box at least one hour before weighing (NOT ON THE BALANCE PAN).

3. Wipe down oven with DDI water and a paper towel.

4. Use gloves and acetone-cleaned tweezers to handle the filters and filter pans.

5. After air-drying on the wire mesh for 2 hours, check that the filters easily lift from the mesh (no obvious dampness or sticking). Check to make sure that the labeled metal trays are *completely dry* before returning the filter to the tray. (If not, let

them air dry a little longer or place the empty tray in the oven to remove any moisture. Cool the tray in the desiccator before returning the filter to it.)

6. Transfer the covered filters (including lab blanks) in their labeled trays to the drying oven and dry for at least 1 hour at 103 – 105˚C.

7. Cool for 1 hour in the desiccator to ambient temperature.

8. Remove the entire desiccator tray and take the filters to the scale. Do not handle the filters or metal trays with your hands. This will raise the temperature and affect the weights.

9. Tare the scale. Using tweezers, place the extra metal tray in the center of the balance, and tare again. Place the filter in the center of the tray. After the initial beep, record the weight in the filtration log. Record the temperature and humidity reading from the Kestrel.

10. Repeat drying and weighing until weight measurements differ by ≤0.1 mg (100 µg). Record each post-weight, temperature, and humidity reading in the filtration log.

11. Store filters, covered, in labeled trays in glove box.

12. Upload the temperature and relative humidity data from the Kestrel to your computer; name the file by date series.

Measuring Feather Surface Area

Supplies

- Scanner
- ImageJ Software
- Tweezers
- KimWipes
- Desktop Computer
- Solid color paper background
- Nitrile gloves

Method

After feather samples are rinsed and dried, scan and measure them.

1. Place each sample of feathers to a solid background labeled with the sample ID. Label each feather with its molt ID. Make sure feathers do not overlap and that feather edges do not touch. Label the paper with a feather side (ventral or dorsal).

2. Clean the surface of the scanner with a KimWipe dampened with DI water.

3. Place the feathers on the scanner in the same configuration with the ruler along the top edge and place the labeled sheet on top of them, making sure the labels line up correctly.

4. Open Adobe Acrobat DC. Select File → Create → PDF from scanner. Select button for "Autodetect Color Mode". Click on gear icon to change the scan settings. Set the dpi to 300 minimum, 600 maximum. Select scan.

5. An option menu will pop up when the scan completes. Select "Scan reverse sides (reverse of sheet 1)". DO NOT select OK until you have flipped the feathers over. This will save both pages (ventral and dorsal) to the same PDF file.

6. Flip the feathers over and repeat steps 1 – 4 for the opposite side. Select "complete scan" if needed. Save and label PDF file appropriately. Select File → Close File to begin a new scan.

7. Open each PDF to export it as an image file in JPEG format: File → Export to → Image → JPEG. Select settings, set the quality for color and grayscale to medium or better. Select OK. This will save each page/side as a separate JPEG image, label appropriately.

8. Open ImageJ software and open the first sample JPEG image

9. File → Analyze → set measurements (area only)

10. Select the line tool. Draw a line along the ruler using it to define the length of the line in cm. Select Analyze → Set Scale and enter the known distance in cm and the units (cm). Select OK.

11. Image → Adjust → Color Threshold

12. Uncheck "Dark background" and move top brightness slider to the far left.

13. Drag bottom brightness slighter to the right until feathers are highlighted red with minimal background noise.

Note: White feathers are not recognized on a white background. You might have to use a separate background sheet and scan for white feathers to improve contrast. If using a black/dark background, select Image → Type → 8-bit. Select Process → Binary → Make binary. Select Image → Adjust → Threshold and drag sliders until feathers are highlighted.

14. Open the tool: Analyze → Tools → ROI Manager

15. Use the wand tool to select each feather. If the selection outline needs editing, double-click the oval or rectangle tool, select "enable selection brush" and use it to refine the edges.

16. When you are satisfied with the outline, select "Add[t]" in the ROI Manager. This will add each individual feather selection to a list in the ROI Manager. This will also allow you to save the individual measurements to a file. After you have outlined and added each feather to the ROI Manager, select "Measure".

17. Copy and paste the individual measurements into an Excel worksheet, labeling each measurement with the appropriate molt ID.

18. Repeat steps 1 - 17 for the other side of the feathers in the same sample, recording the measurements to your data sheet as ventral and dorsal.

19. Calculate the sum surface area for the 4 feathers for the ventral side. Do the same for the dorsal side. Then, take the average of the 2 sides to determine the total surface area.

20. Return feathers to storage in the freezer, fridge, or glove box in labeled plastic bag.

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