

# **Managing Urban Pigeons: Understanding Welfare in the Balance of Control**

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## **Abstract**

Pigeons and humans have been in commensal relationship for thousands of years. Pigeons have benefitted from living with humans who provided a rich source of food. Humans have used pigeons as a food source, too, but also considered them as pets. Owning pigeons was considered a status symbol. Another benefit to humans was pigeons' nitrogen-rich feces used to make gunpowder, saltpeter, and fertilizer. When humans migrated to all parts of the globe, pigeons went with them. Approximately 100 years ago, human attitudes towards pigeons became negative because of bird droppings and the realization that pigeons carried diseases transmissible to humans. As urban centers provided abundant food and nesting sites, pigeon populations increased, becoming a point of conflict between pigeons and humans. To ease this conflict, several methods of population control have been tried. Many of these methods produce short-term success and long-term failure, but a method using chemical contraception appears to work, albeit slowly, requiring years to see reductions in number of pigeons. Because this method of population control shows promise, it makes sense to consider as many consequences as possible of administering a chemical contraceptive from the point of view of all stakeholders, including the pigeons. The work reported in this dissertation addresses some of the challenges inherent in adapting a strategy of population control from the lab where it was developed to the application of the treatment to pigeons in their natural environment. Research was done to assess less invasive methods of sample collection, and to explore how prolonged exposure to chemical contraception would impact the physiology and behavior of pigeons. A satellite research facility was built to provide an intermediary step between the control of a lab, and the lack of control in the field. The smaller scale of this facility allowed closer observations of how the pigeons interfaced with the contraceptive. Our approach of balancing pigeons' social and reproductive

behaviors and physiology with the benefits and side effects of NCZ exposure can improve the effectiveness of chemical contraception, an important method to ease pigeon-human conflicts in urban areas.



*When you touched Flint, you reached me*

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## **Chapter 1. Introduction**

Human beings are a part of the ecosystem on Earth—not above it or below it. Yet while we make up only 0.01% of life on Earth (Ritchie, 2019), how we congregate and sustain ourselves alters how other species live and die. According to the 2024 Living Planet Index report, there has been a 73% average decline in wildlife populations since 1970 largely due to extensive farming causing fragmentation of wildlife habitats (Ritchie, 2019), even though more than half of the world's human population currently lives in urban areas (Ritchie et al., 2024). Wild animal welfare researchers are studying ways to systematically understand how resources can be better allocated to find a balance between the interests of humans and wildlife to maximize survivability for humans *and* wildlife and thereby reduce human-wildlife conflicts (Dubois et al., 2017). My perspective on this complex issue is that wildlife can benefit from scientists adapting our current technology to create less invasive methods of understanding what is happening to wildlife populations in their natural environments. We can then create strategies of co-existence that will support both humans and existing wildlife populations by working with the characteristics and needs of all stakeholders. It is with this purpose in mind that I seek to ease the conflict between humans and feral pigeon populations coexisting in urban habitats by focusing on how pigeons interact with current methods of population control.

### **Feral Pigeons**

Pigeons live in urban habitats worldwide. They nest in niches in cliffs in mountainous habitats, allowing for easy transitioning to nesting in crevices in buildings. They are non-migratory and tend to have one nest site for several years. Pigeons have a foraging radius of up to 26 km, with a typical range of 3-10 km. Males are likely to stay in their colonies for the duration of their lives,

and females will leave if they lose their mates, attaching themselves to their new mate's territory. They are monogamous and pair for life. Both males and females will aggressively defend their nest. Pigeon predators include owls, peregrine falcons, crows, racoons and opossum (Lowther & Johnston, 2020).

“DNA evidence suggests that the earliest pigeons may have evolved roughly 60 million years ago...after a catastrophic asteroid strike...” (Mosco, 2021). Evidence of pigeon - human coexistence dates back as far as 10,000 years (Shapiro & Domyan, 2013). Pigeons were used as a food source, and as such were brought along by migrating people. Colonists introduced pigeons to North America in 1606, with feral pigeons, or domesticated pigeons returning to the wild, appearing soon after (R. F. Johnston & Janiga, 1995; Lowther & Johnston, 2020). Pigeons' nitrogen-rich feces was valued for fertilizer, saltpeter, and help with tanning animal hides. Laws and culture in France and England regarding pigeon ownership gradually changed to allow commonfolk to keep pigeons. Previously, ownership was restricted to landed people as a status symbol (Mosco, 2021). Pigeons served in the armed forces and were recognized as heroes. One example is a homing pigeon named Cher Ami. When the Lost Battalion came under friendly fire in the Argonne Forest during World War I, Cher Ami flew through hostile fire with a note attached to him advising commanders to attack elsewhere (Larsen, 2021). Pigeons have been well-studied across several scientific disciplines ranging from evolution (Darwin, 1875) to behavior (Morse & Skinner, 1958) to genetics (Bruders et al., 2020). Oscar Riddle discovered prolactin using pigeons as his model (Riddle, 1938; Riddle et al., 1932). Yet, for all of this research, there is much to learn about their breeding biology and population ecology (Lowther and Johnston 2020).

In spite of all these positive pigeon-human interactions, the prevailing current human attitudes about pigeons are negative. This paradigm shift had its start around the time of World War II when complaints about nesting and defecation on landmarks and sidewalks increased. In the 1950s and 1960s, the negative attitudes took hold on a larger stage when it was learned that pigeons carried disease (Jerolmack, 2008; Soniak, 2016). With the release of an article in 1966 quoting Parks Commissioner Thomas P. F. Hoving as saying that pigeons are “rats with wings”, the shift from humans valuing pigeons to humans reviling pigeons was firmly established (Duffy, 2007; Jerolmack, 2008; Mosco, 2021).

An attitude shift towards a more positive, cooperative view of pigeons may be re-emerging. There are currently 700 racing pigeon clubs in the United States (American Racing Pigeon Union | Homing Pigeons | ARPU Sport Flyers, 2025). Pigeon fancier clubs dedicated to particular breeds of pigeons exist around the world (Pigeon Cote Pigeon Club Locator, 2025; Seed, 2025). Pigeon advocacy groups like Palomacy have over 40K members whose mission it is to foster, rehabilitate, and find homes for feral, abandoned, and wounded pigeons (Young, 2025).

Wildlife animal welfare researchers, urban managers, and this body of work seek to build on this upswing of positive attention. The field of wild animal welfare is a relatively new scientific discipline that focuses on how an individual wild animal interfaces with its habitat, conspecifics, non-conspecifics, humans, climate, and itself. That list is not complete as wild animal researchers are in the process of crafting definitions and mission statements that are accurately descriptive of the work. For example, the Centre for Effective Altruism (CEA) says, “Wild animal welfare is the welfare of non-human animals under natural conditions, and the study of interventions aimed at improving the welfare of these animals (2020). International Fund for Animal Welfare (IFAW) offers this definition: “We define animal welfare as the physical and mental wellbeing of

animals; physical, physiological, and behavioural measurements are used to assess animal welfare” (2024). The broad range of projects of what these and other global non-profit organizations are supporting include Wolf Monitoring and Integration in Germany, maintaining a water supply system for people and animals in Malawi, and Big Cats in Captivity: Rescue and Advocacy - United States (International Fund for Animal Welfare, 2024). Primarily, these organizations provide like-minded scientists, wildlife managers, and funders a platform from which to connect and collaborate on research to achieve such broad goals. It is under this umbrella that the studies reported in this thesis fit. Wild Animal Initiative, whose stated mission is, “ ... to accelerate science that helps wild animals”(2025), funded this work.

During my time as a graduate student, I was able to honor my driving interest in research focused on improving the living experiences of vertebrates in general, and feral pigeons in particular, in a manner beneficial to the pigeons \*and\* the humans they live with. I was able to employ and improve my skills as an avid bench-scientist and physiologist to better understand what information can be gleaned from less invasive field techniques. Further, I report how I not only designed an experiment to explore possible impacts of the physiology and behavior of feral pigeons exposed to chemical contraception, but also how I designed and built a satellite research facility from which to run my experiment. One of the following chapters has been published (Wright-Lichter et al., 2022); two chapters will be submitted for publication.

Here are brief summaries of each chapter:

Chapter 2--Exploring less invasive methods of assessing wild animal welfare in their natural environment

An investigation into whether levels of corticosterone (cort) deposited in sparrow feathers, which grew and were plucked several years before this study was done, would degrade over time. The question of whether deposited cort was more concentrated at the edges of the feathers as previous studies suggested they might be was tested.

Chapter 3—Creating a low-impact, environmentally friendly satellite research facility to conduct non-lethal research with feral pigeons (*Columba livia*) in a suburban setting

This is a methodology paper describing what went into designing and running the satellite research facility. Included are suggestions for getting IACUC approval, as well as diagrams and photos which may help future researchers in their work.

Chapter 4—Physiological and Behavioral effects of chemical contraception in feral urban pigeons

This study explores what impact prolonged exposure to chemical contraception in combination with a rapid rise in ambient temperature had on feral pigeons' physiology and behavior.

## **Chapter 2. Average measurable corticosterone in house sparrow (*Passer domesticus*) primary feathers varies little across life-history stages**

Jessica X. Wright-Lichter<sup>1\*</sup>, Brenna M. G. Gormally<sup>1,2</sup>, Christine R. Lattin<sup>3</sup>, and L. Michael Romero<sup>1</sup>

**ABSTRACT**—The relatively new technique of measuring corticosterone (cort) levels extracted from feathers provides a less invasive, more integrated method of assessing a bird's stress physiology. Current understanding is that cort is deposited into the feather via blood when the feather is replaced during molt. The blood supply in the feather is cut off when the new feather completes growth, thereby ending the influx of cort. Previous studies assumed that cort deposited during feather growth remained constant throughout the feather's attachment to the bird, allowing an accurate retrospective index of circulating cort at the time of feather growth. We tested this assumption by measuring cort levels in feathers collected from different wild house sparrows (*Passer domesticus*) across six important life history stages spanning a single year, thus representing feathers of different ages but grown during similar environmental conditions. If the common assumption is correct, we predicted that feather cort would not vary across the year. We further simulated substantial feather wear by removing the top 20% length of duplicate samples from the same birds across the same time span. We found significant differences in cort levels across life history stages, but no effect of sex or 20% feather removal. After excluding feather wear and sex and re-running the analysis, the effect of life history stage was no longer significant. Furthermore, despite uncontrolled individual and environmental variation, there was not a sustained decrease in feather cort over time. These data support the assumption that feather cort levels are stable while feathers are on the bird, regardless of feather age or typical wear.

*Keywords:* Feather age, feather corticosterone, feather growth, plasma corticosterone, stress mediator.

Measurements of stress mediators, like corticosterone (cort), can be useful in assessing an animal's probability of survival (Romero, 2012). For many decades, avian researchers have focused on measuring birds' corticosterone levels in plasma (Nagra et al., 1963; Wingfield et al., 1992). As useful as this type of measurement is, it has the drawback of requiring handling of the bird in order to obtain a sample. The development of a technique to measure cort deposited in feathers (Bortolotti et al. 2008), and subsequent explorations of the practical and theoretical underpinnings of feather cort and its measurement (Bortolotti et al. 2009; (Bortolotti, 2010); Lattin et al. 2011; (Fairhurst et al., 2013); Jenni-Eiermann et al. 2015) have provided a less invasive method of measuring cort levels in birds. This technique appears to yield an integrated profile of cort circulating in the bird's blood throughout the time of molt (genesis) of the feather during which time the feather is vascularized (Maderson et al., 2009; Proctor & Lynch, 1993). Once it completes its growth, the blood supply then dries up, leaving behind detectable levels of cort deposited in the feather (Bortolotti et al., 2008a; Jenni-Eiermann et al., 2015). The fully-grown feather can then be harvested either by plucking the feather or by picking up feathers naturally dropped by the bird. The feather cort assay is proving to be a powerful tool providing the basis for a wide range of studies of birds both in the field and in captivity, and both alive or dead (Romero & Fairhurst, 2016).

One assumption of this technique is that the concentration of measurable cort remains stable for the life of the feather. In other words, the amount of measurable cort would be equivalent, and reflect the amount originally deposited in the feather, regardless of what time of year the feather was plucked. This is a reasonable assumption considering a number of species

only undergo one molt per year, and limited work has generally supported this assumption (Romero and Fairhurst 2016). However, because feathers are exposed to year-long conditions that can potentially deteriorate feather structure, the goal of this study was to test this assumption. We analyzed primary feathers from house sparrows (*Passer domesticus*) collected across six life history stages, with each individual sampled only once. However, all birds were captured from the same suburban site in the same year, and because house sparrows have a limited adult range (Lowther & Cink, 1992), we assumed that all feathers were molted under similar environmental conditions. We determined whether feather cort levels changed over the period of a feather's functional lifespan, i.e. from genesis to shed, regardless of the bird's life history stage at sampling. We then compared the feather cort levels to the significant differences in plasma cort that were previously reported from house sparrows in general (Romero et al. 2005), and these birds in particular (Lattin et al., 2012; Romero et al., 2005). Because the feathers were grown under roughly equivalent natural conditions (during fall molt 2010), we did not expect to find differences in feather cort across life history stages. Furthermore, the ends of primary feathers often abrade over time; specifically, the distal 20% of the length can often wear away the longer the primary feather stays on the bird (Willoughby et al., 2002). Therefore, we also tested whether feather cort could change as a result of abrasion by removing the distal 20% of feathers prior to assay, thereby mimicking the effect of abrasion, and comparing cort concentrations to adjacent primaries that did not have the distal portion removed. In previous studies using European starling (Lattin et al., 2011) and pigeon feathers (Jenni-Eiermann et al., 2015), higher cort concentrations were found in the distal portion of the feather. Consequently, we predicted lower levels of cort in the cut feathers (Fig. 1)

## Methods

### Study Subjects

Note that this was not a repeated measures design – all birds were sampled only once during the study. However, all birds were captured at the same field site in Medford, MA, USA. During each life history stage, approximately equal numbers of males and females were sampled.

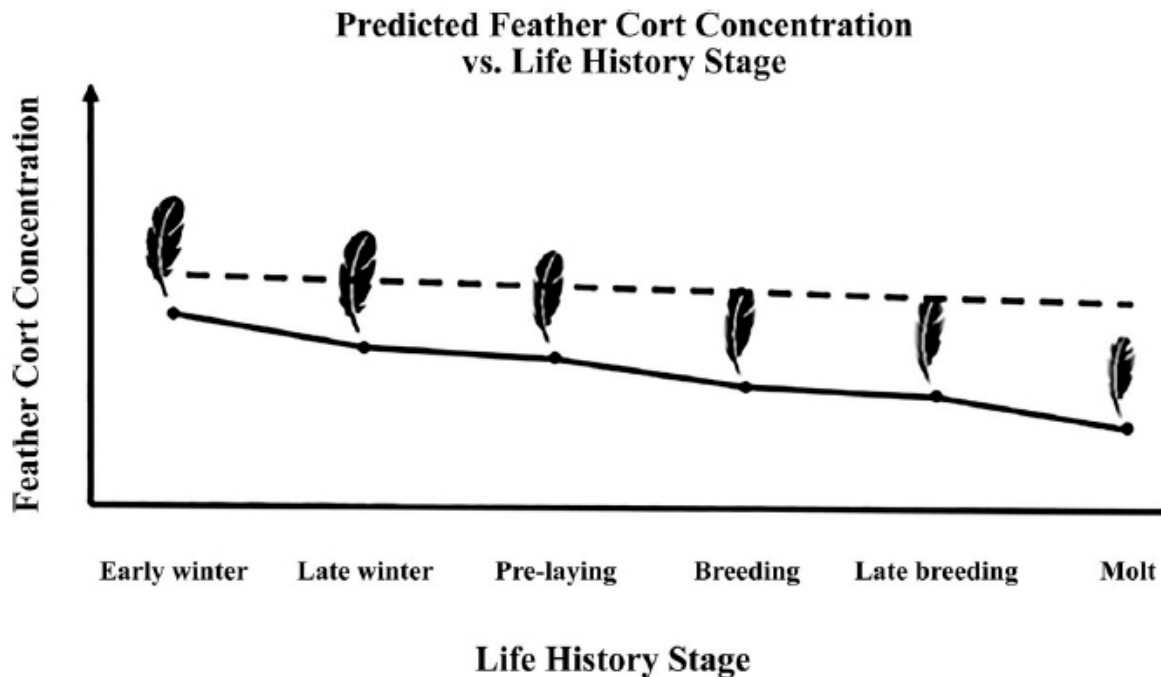


Figure 2.1. Predicted relationship of cort concentrations of whole feathers (dotted line) and naturally or artificially eroded feathers (solid line and shrinking, fading feather symbols) across one year of life history stages. Previous studies measuring cort levels in whole feathers assume that, once grown, feather cort levels will not change. In naturally worn feathers, studies suggest that any decrease in feather cort concentrations may result from loss of distal feather mass.

Feathers used in this study were collected as part of a different study (Lattin et al., 2012) and stored whole in a dark cabinet for ~8 years. Briefly, wild house sparrows were caught at six times of year corresponding to important life history stages in New England and sampled for plasma cort (Lattin et al., 2012). These stages were defined as early winter (11-15 December 2010,  $n_1 = 9$ ,  $n_2 = 20$ ,  $n_3 = 20$ ), late winter (1-14 February 2011,  $n_1 = 8$ ,  $n_2 = 18$ ,  $n_3 = 20$ ), pre-

laying, (31 March -7 April 7 2011,  $n_1 = 8$ ,  $n_2 = 12$ ,  $n_3 = 12$ ), breeding (23-24 May 2011,  $n_1 = 8$ ,  $n_2 = 11$ ,  $n_3 = 12$ ), late breeding (12-22 July 2011,  $n_1 = 8$ ,  $n_2 = 20$ ,  $n_3 = 20$ ), and molt (9-12 September 2011,  $n_1 = 9$ ,  $n_2 = 9$ ,  $n_3 = 8$ ), with  $n_1$  = plasma cort,  $n_2$  = whole feather cort, and  $n_3$  = cut feather cort. Two whole feather samples in late winter, one whole feather sample in breeding, and one cut feather sample in molt were lost due to pipetting errors during the assay. We only measured old feathers from molting birds, so that they were grown during the previous year's molt (late August - September 2010), and all feathers used were primary feathers (P3-P9).

### **Feather Preparation**

Two groups of feathers per bird per life history stage were assayed, with each group consisting of 3 to 5 primary feathers. Combining multiple feathers in each group was necessary to ensure that all samples weighed at least 30 mg to control for effects of small sample mass (Lattin et al., 2011). In addition, storage containers did not label individual primaries, so we could not control for specific feather identity. For group 1, referred to as whole feathers, the calamus of each feather was removed and the rachis length measured from the proximal end of the feather to the distal tip (mm). For group 2, referred to as 80% feathers, after removal of the calamus, the rachis was measured to ascertain 100% length and then the top 20% of the feather was removed with a cut perpendicular to the rachis. The sum total length of all rachises per sample was recorded and the final cort concentration was expressed as cort/mm of feather as per recommendation of (Bortolotti et al., 2008b). Following this, all feathers were cut into pieces for the assay. Sample order (cut and intact feathers from six different life history stages) was randomized within each radioimmunoassay.

### **Feather Cort Assay**

Prepared feathers were assayed following Bortolotti et al. (2008) with some modification. Feathers were mixed with 7 ml of methanol (HPLC grade, Fisher Scientific), placed in a sonicating water bath at room temperature for 30 min, then incubated overnight in a shaking 50°C water bath. Methanol was separated from feather pieces using vacuum filtration with #4 Whatman filter paper in a filtration funnel. Feather pieces, the sample vial, and the filter paper were rinsed twice with ~2.5 ml of additional methanol, with the rinses added to the total methanol extract. Methanol extracts were dried under nitrogen gas in a 50°C water bath. Extracts were reconstituted in Tris-HCl buffer and run through a standard radioimmunoassay as originally described by (Wingfield et al., 1992). The anti-corticosterone antibody was produced in rabbit (Sigma-Aldrich C 8784-100TST, Saint Louis, Missouri, USA, lot 047M4870V). This antibody has been used in many avian species (Musgrove et al., 2017; Studholme et al., 2018). Interassay variation from three assays was 16.3%, and intraassay variation was 3.4%.

### **Plasma Cort Concentrations**

All baseline plasma cort concentrations were collected < 3 min after capture from the same birds as those used for the feather assays. These concentrations were reported in an earlier study (Lattin et al., 2012) and reproduced here for qualitative comparison purposes. Plasma cort values were not included in statistical models of feather cort.

### **Statistical Tests**

Statistical tests were done using R 3.6.0 and SigmaPlot 14.0 (Systat Software Inc.). After using a log transformation to normalize data distributions, a three-way ANOVA was used to analyze differences in cort with life history stages, feather treatment (whole or 80% feathers), and sex as main factors, including all interactions. Because there were no effects of sex or feather treatment on cort (see results below), these factors were removed, and data re-analyzed solely on

life history stage. However, after removing these factors, life history stage data were no longer normally distributed despite the log transformation, so we analyzed data using Kruskal-Wallis One Way Analysis of Variance on Ranks.

## Results

A three-way ANOVA showed no effects of whole and 80% feather treatments ( $F_{1,158} = 1.30$ ,  $P = 0.26$ ) or bird sex ( $F_{1,158} = 0.59$ ,  $P = 0.45$ ) on feather cort levels. However, feather cort levels across life history stages were significantly different ( $F_{5,158} = 3.44$ ,  $P = 0.01$ ). There were no significant two- or three-way interactions ( $P > 0.10$ ) for any tests. Therefore, the data were re-run excluding sex and treatment (in order to determine pair-wise post-hoc differences in life history stages), which failed normalcy. A Kruskal-Wallis One Way ANOVA on ranks showed

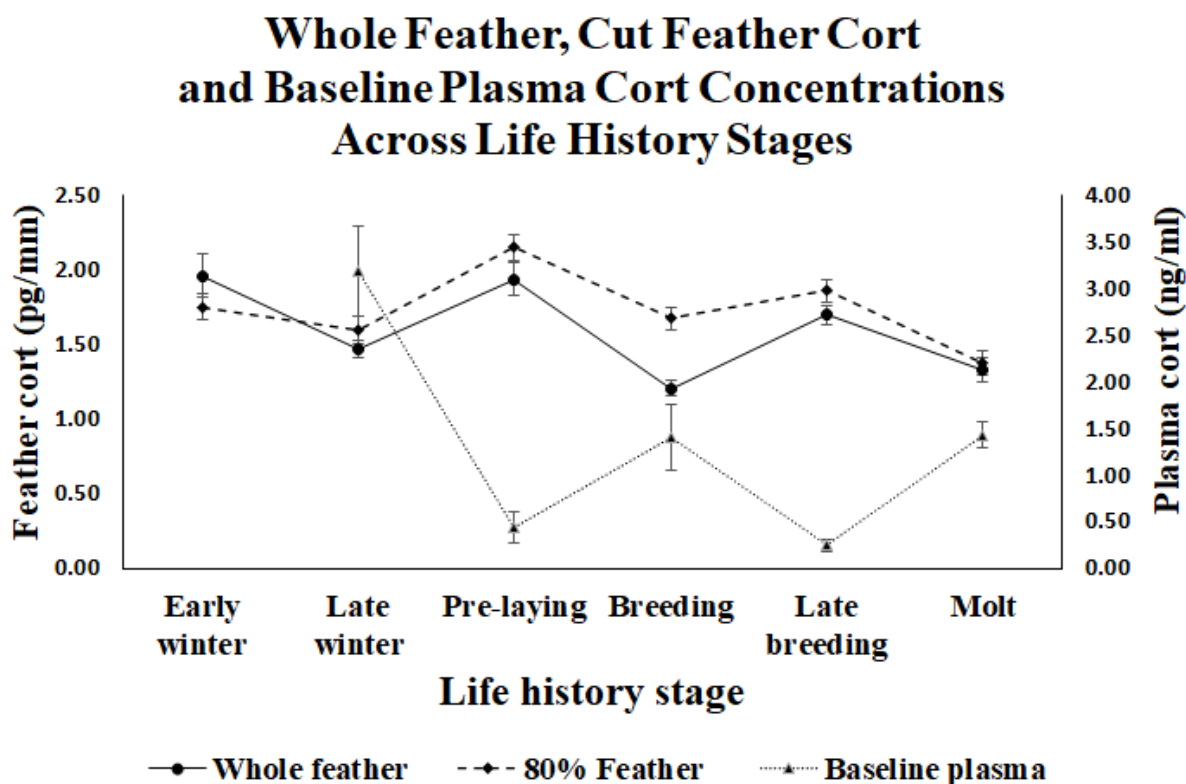


Figure 2.2. Whole and 80% (cut) feather cort concentrations and baseline plasma cort concentrations across life history stages. Plasma cort measurements were taken at the same time the feathers used for this study were collected (previously reported in Lattin et al. 2012). Bars indicate SE.

no significant differences across life history stages ( $H_5 = 10.88, P = 0.054$ ). Although approaching significance, the data do not fit the predicted pattern in Fig. 1 with the highest cort being in the youngest feathers and the lowest being in the oldest (i.e., no systematic decrease over time). When the pattern of feather cort was compared to baseline plasma cort, the patterns did not align (Fig. 2).

## Discussion

In this study, we examined the assumption that corticosterone deposited into feathers at the time of their growth remains stable across one year of life history stages as they remain attached to the bird. By necessity, this study was a factorial, not repeated measures, design. Cort measurements thus reflect the age of the specific feathers as they are maintained naturally by the bird, without confounding effects of repeated sampling required for a repeated measures design. It would be difficult, if not impossible, to repeatedly capture wild house sparrows in order to test our hypothesis using repeated measures. However, this design does present some limitations. We could not control for individual or environmental variation within each life-history stage, meaning that we only compared broad patterns. On the other hand, all birds were caught at the same site and within 12 months of the previous molt, so that all feathers in this study were grown during the same molting season (Aug - Sep 2010). Because house sparrows have a limited adult range (Lowther & Cink, 1992), it is reasonable to assume that all the feathers in this study were grown during similar environmental conditions, and thus should have started with approximately equivalent cort, notwithstanding individual variation and variation inherent in using multiple primaries from each bird. We consequently expected the differences in feather wear across life-history stages to be substantial enough to demonstrate a trend, especially when comparing winter (relatively new feathers) to molt (oldest feathers).

Our inability to demonstrate a declining trend as feathers aged, despite the substantial variation discussed above, suggests that perhaps feather cort concentrations remain constant across life history stages. The initial analysis showed the highest feather cort during pre-laying, a middle time point during the year, which did not fit the a priori prediction of a gradual decrease in feather cort and may reflect a methodological artifact. Furthermore, feather cort was equivalent in whole feathers and feathers cut to 80% of initial length. The lack of a difference between whole and cut feathers suggests that normal feather wear has no significant effect on measured feather cort levels. Consequently, despite studies showing that there can be a disproportionate amount of cort in the distal end of feathers (Jenni-Eiermann et al., 2015; Lattin et al., 2011), cutting distal ends off feathers in our study did not significantly affect total feather cort.

In contrast, baseline plasma cort varies significantly in these same birds over the year (Fig. 2; Lattin et al. 2012) and the feather cort pattern does not match the plasma cort pattern. This was expected since feather cort levels are believed to be an integrated measure of plasma cort during the 2 – 3-week span when the feather was grown, whereas plasma cort levels reflect cort in the blood at the moment of capture. This study provides further evidence that feather cort is isolated from the annual variability in plasma cort outside of molt, suggesting feather and plasma cort reflect distinct aspects of a bird's physiology.

These results support the assumption of a wide range of research that cort levels are stable across the functional life span of feathers. If feather cort levels remain stable, it allows collection of feathers outside of molt to infer physiological condition of the bird during the prior molt. This provides further support for using feather cort concentrations to assess physiological carry-over effects from the prior molt to subsequent life-history stages such as reproduction (e.g.,

(Bortolotti et al., 2002; Crossin et al., 2013; Grunst et al., 2015; Harms et al., 2015; Monclús et al., 2017), or to relate this to variation in feather pigmentation and mate choice (e.g., (Grunst et al., 2015; Lendvai et al., 2013). It is important to point out, however, that this study only addressed whether feather cort changed during the year it was on the bird's body. Unaddressed is whether feather cort degrades over time once collected, such as in museum specimens.

In addition, the factorial experimental design used in this study did not allow us to conclude that feather cort remains stable despite feather wear in an individual bird, only that it does not appear that feather cort decreases on a population level as feathers age. Further studies could correlate feather cort with actual feather wear, either macroscopically (e.g. light penetration through the feather vanes) or microscopically (e.g. tip abrasion or barbule damage).

In conclusion, these data provide moderate support to the assumption that cort levels deposited in growing feathers remain stable at least through the time when that feather is replaced during the following molt. This should improve confidence that measurements of feather cort allow inferences of physiological condition at the time of feather growth.

### **Acknowledgments**

Funding provided by National Science Foundation (USA) IOS-1048529 and 1655269 to L. M. Romero. Funding for feather collection (Lattin et al. 2012) was provided by the EPA STAR Fellowship program and a Tufts University Graduate Student Research Award to C. R. Lattin. All experiments were performed in accordance with the guidelines for the use of wild birds in research (Fair, J., E. Paul, Jones, 2010) and approved by the Tufts University Institution Animal Care and Use Committee.

**Chapter 3. Creating a Low-Impact, Environmentally Friendly Satellite  
Research Facility to conduct Non-Lethal Heat Trial research with Feral  
Pigeons (*Columba livia*) in a Suburban Setting**

Jessica X. Wright-Lichter, Lori Donohoe, Rebecca Partan, Allen Rutberg

**ABSTRACT**— The goal of minimizing wildlife-human conflict by the least invasive means possible is greatly benefited by using technological and pharmacological advancements to create effective urban pigeon population management strategies. Several studies have examined whether contraception, either chemical or hormonal, can produce the desired reduction of urban populations without culling or poisoning. To date, results are encouraging but mixed. What is clear from these studies is that the challenging lack of control inherent in field studies clouds the researcher's ability to understand the in-situ interface mechanisms between the treatments and the pigeons' social structure and physiology. The lack of control in the field highlights the desirability of a testing method in a facility that provides an environment that is less like a controlled lab environment and more like a partly controlled version of the environment inhabited by the target species. Such an intermediary testing facility will aid in maximizing successful field outcomes while saving resources for the researcher and minimizing social, behavior, and physiological disruptions in feral pigeon communities. Here we describe the actualization of such an intermediary research facility used to better understand the effects of chemical contraception in feral pigeon populations in a non-lethal, semi-controlled manner. We also describe our creation of a non-lethal heat assay where we were able to assess the behavioral impact of the chemical contraception in concert with a rapid rise in ambient temperature. This

paper provides a blueprint for how to establish an effective, relatively low-cost start-up research facility in a non-traditional setting.

## **BACKGROUND**

It is a commonly held view that feral pigeons are “fugitives from captivity” or “outlaws” and therefore not worthy of notice (R. F. Johnston & Janiga, 1995). This is an anthropocentric view likely based on the fact that feral pigeons are defined as coming from, or descended from, pigeons once living under human care and losing that protection, with or without the pigeons’ consent, and having to fend for themselves in whatever habitat was their point of release. Feral pigeons have demonstrated such resilience to this change of habitation to the extent that global population estimates range from 260 – 400 million (Hausheer, 2022; IUCN, 2016). The result of such resilience is that feral pigeons live in urban habitats worldwide. Crevices in urban buildings offer nesting sites similar to the cliffs their ancestors came from (Murton et al., 1972; Shapiro & Domyan, 2013). Of no less importance is that feral pigeons equate presence of humans with presence of food (Giunchi et al., 2012; Stukenholtz et al., 2019). Unfortunately, the excreta liberally shared and the diseases they carry (Haag-Wackernagel & Bircher, 2010) have become more worthy of notice from fellow city-dwelling humans, over-riding thousands of years of positive pigeon-human interactions (Lowther & Johnston, 2020; Mosco, 2021; Shapiro & Domyan, 2013).

An attitude shift towards a more positive, cooperative view of pigeons may be re-emerging. Researchers in South Africa report that survey respondents from employees at a college campus view pigeons in a favorable light (Harris et al., 2016). The Audubon Society is reminding the public of how extraordinary feral pigeons are (Soniak, 2016) as is Animal Control in NYC & New Jersey USA (Adamo, 2021). Building on that energy, wild animal welfare scientists are

researching effective methods of pigeon population control to assist urban managers while minimizing disruptions to the natural social and physiological processes of the pigeons. Methods that have been tried, such as physical deterrents like spikes on building ledges, sonar disruptions, hunting, and poisoning, are short-term solutions. Pigeons repopulate and relocate back to the cleared areas (Harris et al., 2016, 2017; R. F. Johnston & Janiga, 1995). Hormonal and chemical contraception appear to offer promising solutions to pigeon overpopulation. Several lab and field studies have shown this method of population control can work over time (Albonetti et al., 2015; Avery et al., 2008; Cowan et al., 2014; González-Crespo & Lavín, 2022a) while other studies did not come to the same conclusion (Senar et al., 2021; Xenakis et al., 2023). What becomes clear when evaluating the limitations of those studies is that it is difficult to assess what is happening to the pigeons exposed to these treatments in their natural habitats. If a pigeon count decreases after a period of time, it is difficult to ascertain whether the cause is contraception, relocation, or death. Longitudinal studies can reasonably argue that the treatments caused the intended result, but the expense in time and resources can be prohibitive to researchers looking to produce a clearer understanding as to how the treatment impacted pigeons' physiology and daily behavior in situ. It is in this area that an intermediary step between lab and field research can be of great use. A semi-controlled research site in an environment that more closely resembles an urban pigeon's natural territory, in the case of our study an outdoor parking garage, would allow an experimental design that could mindfully examine what the pigeon experiences while undergoing treatment. This facility could also be located in non-traditional research areas, like a suburban backyard, and for a lower cost than a typical start-up research facility which can range from \$500,000 to \$1 million (Powell, 2024). Such a research model could then be accessible to a larger population of researchers who could use their sites to train more students. We have created

and tested such a facility to study the physiological and behavioral effects of chemical contraception in feral pigeons. This paper is a roadmap to how we accomplished this.

## **Methods**

Aims:

1. Create a low-budget suburban research facility from the ground up and from which to conduct non-lethal research to benefit feral pigeons in their natural habitat while maintaining the well-being of the pigeons. This facility was designed to be safely run by minimal staff, easily maintained, and visually unobtrusive to passers-by, essential for a facility in a suburban neighborhood setting. Our facility had to be approved by the Tufts Institutional Animal Care and Use Committee (IACUC). Because of our affiliation with Tufts, we had access to resources such as instrumentation for plasma analysis. We also provide guidance on creating a laboratory space for experiments and sample processing as well as creative ideas for storage of lab, cleaning, and building supplies.
2. Create a non-lethal and only moderately stressful method of exposing subjects to controlled temperatures and humidity in order to assess behavioral and physiological outcomes exhibited by pigeons exposed to experimental treatments. In our experiment, we were looking at the physiological and behavior effects of chemical contraception on feral pigeons. The contraceptive was administered as bait marketed in the US as OvoControl (Innolytics, LLC, La Quinta, CA). The active ingredient is nicarbazin (NCZ).

Facilities

Aviaries: Two aviaries were designed and created based on guidelines from "Minimum Standards for Wildlife Rehabilitation, 4th edition" (Miller, 2012), and conversations with pigeon rehabilitators, bird fanciers and avian farmers. To lessen the stress of captivity, structures were

kept simple so as to minimize the amount of time researchers needed to be in the aviaries for maintenance and husbandry. Environmentally friendly products, low volatile organic chemical (VOC) paints, glues, sealants and varnishes, and human grade items were used for the safety of the pigeons. Maximum capacity of each aviary was 10 pigeons. All wood used for the structures and the nest boxes were sealed with Eco Advance Wood Siloxane Waterproofer (Kensett, AR) prior to construction. Waterproofer was reapplied several times prior to pigeons' arrival providing a smoother surface to facilitate cleaning.

The coops (10' w x 12' d x 8' h) were built using a Berkley Jensen cedar wood pavilion kit (BJs Wholesale Club, Stoneham MA) for framing and constructed on cinder block supports set on gravel substrate to assist with air circulation and water drainage. Flooring was made of wooden pallets placed on top of cinder blocks with low VOC plywood layered on top. A drain was placed in the center-back of the aviary floor. The floors were painted with an exterior grade semi-gloss paint producing a smooth surface to facilitate cleaning. The back walls were solid wood, providing protection from rain and wind. The topmost sides (approximately 1') of the back wall were left open to improve air flow and stability to the structure during high wind events. The two side walls adjoining the back wall had solid wood panels extending three feet from the back wall, providing further shelter from wind and rain. The remaining parts of the side walls and the entire front wall had ½" hardware cloth from roof to floor (Fig 3.1). ¼" hardware cloth ran under the floor. The roof was made of reinforced galvanized steel construction with rust resistant powder coating. Gutters were installed along the eaves for drainage during rain events (Fig. 3.2). The entryway, also used for research and cleaning supplies, consisted of a small room (8' h x 5' w x 5' d) with square aluminum pole framing and enclosed in ½" hardware cloth. Therefore, the coop was a protective enclosure with a double door entry way keeping predators and rodents out while

allowing the birds over 720 cubic feet (referred to as the run) to freely roam in an environment open to ambient outdoor temperature and natural day/night lighting. All interior sharp edges and

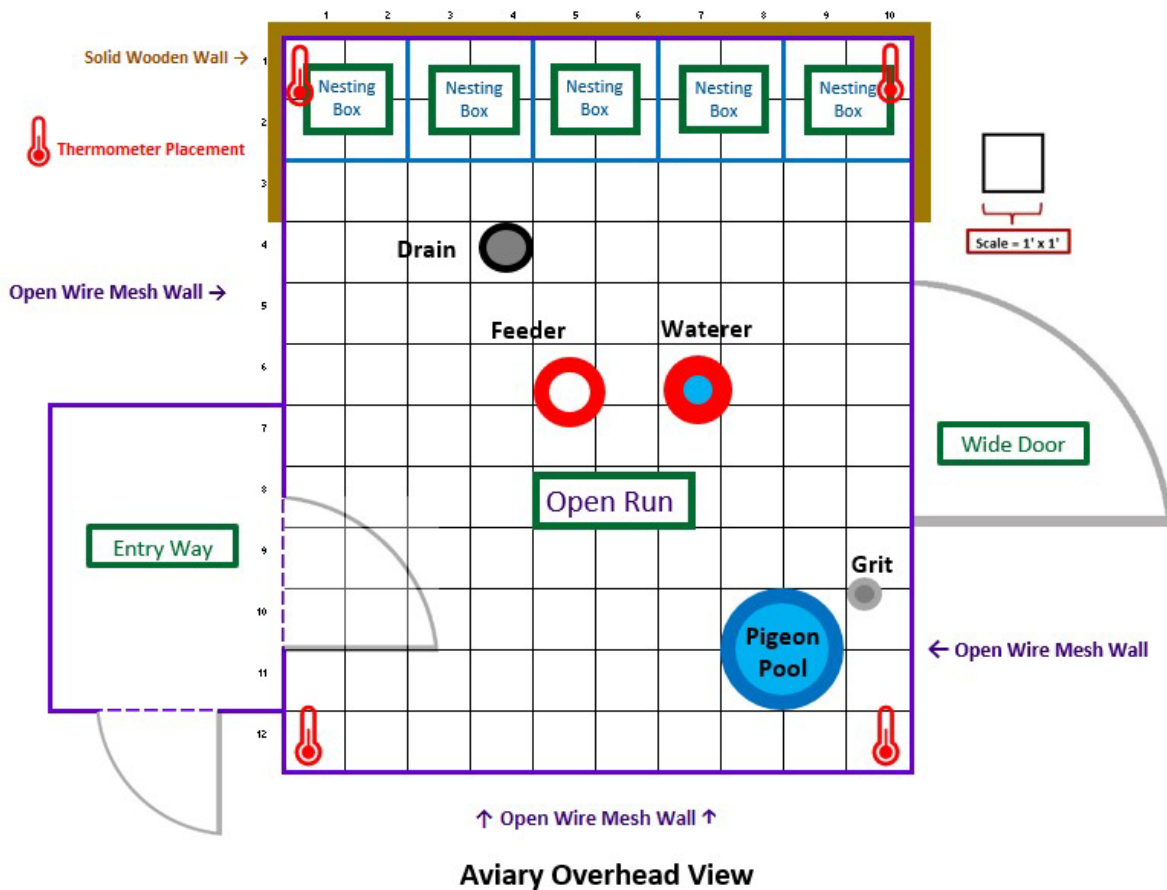


Figure 3.1: Overhead layout of aviary. Wide door (right side of structure) is used only for between-protocol cleaning. It was kept locked and sealed when pigeons were in the aviary.

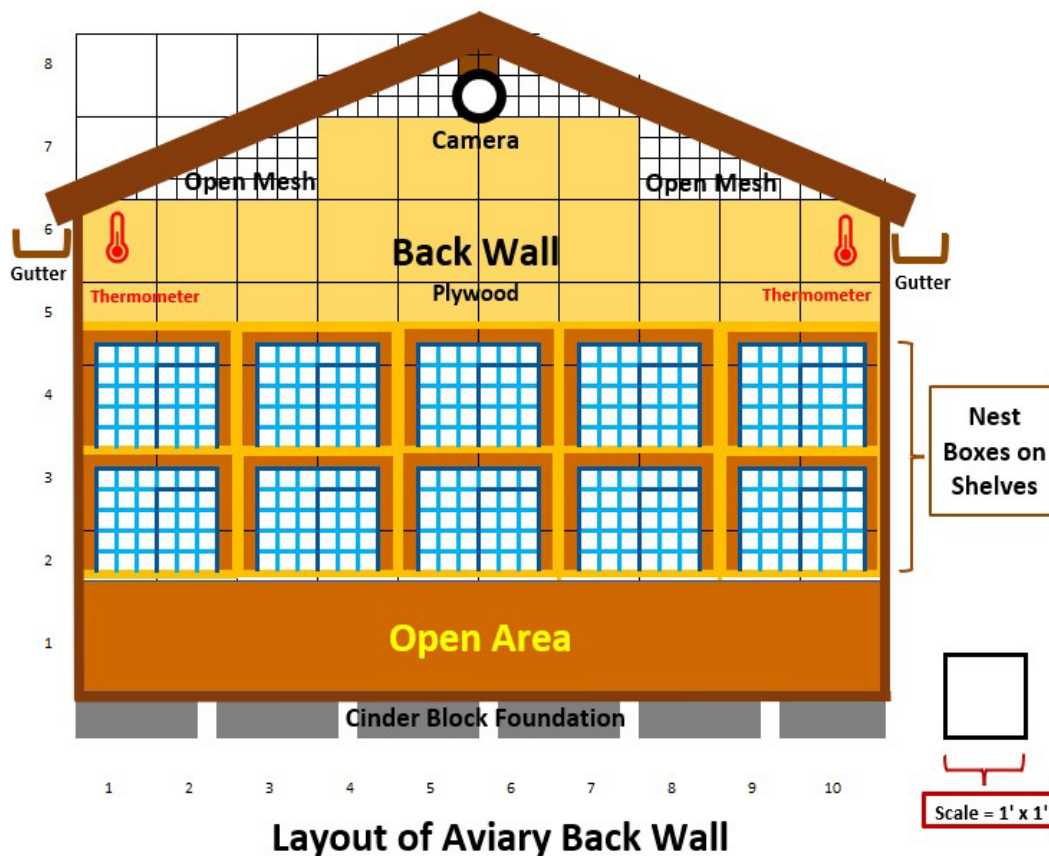


Figure 3.2: Layout view of aviary back wall. Cut-away view from front to back showing placement of shelves, nest boxes, camera, and thermometers.

gaps large enough for the pigeons to get caught in were covered with plastic mesh sheets. All edges were sealed with caulking compound. A wider door made of mesh affixed to wooden framing was put in the wall opposite the entry. This door was used to remove shelves and nest boxes for cleaning and disinfecting the coop after pigeons were released. This door was kept locked with the edges sealed during the experiment. Translucent Greenhouse Heavy Duty poly tarps, (14 mil, UV resistant, waterproof, Glasgow, Greater New York NH7689, UK), were deployed over the hardware cloth sides during rainstorms. These tarps were folded back during dry weather (Fig 3.3). In our location, no building permits were required for these structures.



Figure 3.3: Outdoor aviaries. Aviaries are set back on the property approximately 47' from the main building and at least 30' from property boundaries. Vegetation visibly shielded the aviaries from surrounding properties and also acted as a sound buffer for the pigeons. These aviaries are mirror images of each other.

Aviary furnishings: Chrome wire shelves with two levels were located against the back wall with the lowest level approximately 2' above the floor. Each shelf was covered with acrylic sheeting to provide a solid, removeable surface, facilitating cleaning and protecting the pigeons' feet from the wire mesh shelf surface. The shelves were wide enough to accommodate 5 individual wooden nest boxes (22" w x 18" h x 18" d) for a total of 10 nest boxes/aviary. Nest boxes were made using plywood (4' w x 8' l x .75" d) (See Appendix – 1). After cutting out the nest box pieces and prior to assembly, the plywood was pre-treated with AFM Safecoat Safe Seal (San Diego, CA) to reduce toxic outgassing. Handles were cut into the upper sides for easier handling.



Figure 3.4: Nest box. All materials used to construct these boxes were low VOC and human-grade products.

The fronts of the nest boxes were modified pet carrier doors (Petmate, Arlington TX) (Fig 3.4) with wire mesh that allowed full sight of the birds while they were secured in their boxes. Interiors and exteriors of nest boxes were painted with exterior semi-gloss paint to facilitate cleaning and disinfection during and between experiments. The tops of the nest boxes were covered with brown butcher paper while pigeons were in the aviary. Each nest box, lined with poly coated cage liners (BirdCageLiners, Eagle ID), contained a biodegradable paper pulp nest bowl (Foy's Pet Supplies, New Brighton, PA) and a brick (7.5" l x 3 5/8 " w x 2 1/2" h). These items remained in the nest boxes throughout the protocol. Ten plastic perches (Moduoduo, Amazon.com) were placed, at varying heights, throughout each aviary. Pine Pellet Stall Bedding

(Tractor Supply, Pelham NH) was used as a substrate. One large plastic planter (21.25" top diameter x 17.5" bottom diameter x 3.75" h), placed in a sunny corner of each aviary, was filled with 2 gallons of water and served as a bathing pool. For common use, a plastic feeder (Flock Party, Tractor Supply, Pelham NH), a 1-gallon waterer (Farm-Tuff Chicken Drinker, Tractor Supply, Pelham NH), and a ceramic ramekin filled with grit were placed on the floor in the center of each aviary. Straw was available in the enclosure for nesting purposes. Temperatures in each aviary were monitored by four bluetooth-enabled thermohygrometers (Govee, Springfield IL), one posted about 6' above the floor in each corner. Security video cameras were placed inside the aviaries and powered with Cat6 outdoor, waterproof ethernet cable (amazon.com). The pigeons were videoed for the entire time they occupied the aviaries.

The entryways were used as the only point of entry during the experiment. The doors into the entryways and from the entry into the coops were kept shut at all times for the pigeons' protection. The doors were locked at all times unless researchers were feeding the birds or cleaning the aviaries. There was ample room in each entry for 2 researchers to put on personal protective equipment (PPE) with both doors securely shut. In order to provide protection during rain events, tarps were secured to the outside of the entries. Aviary cleaning supplies, weighing scales, husbandry forms, and PPE supplies were kept in Devoko 30 Gallon Resin Outdoor Waterproof Storage Box (Dallas, GA). Hooks were installed to hang up lab coats. A step ladder and small resin folding table were also stored in the entry way (Fig 3.5). Each aviary had its own complement of cleaning supplies, equipment, and PPE to prevent cross-contamination between treatment exposed and control aviaries.



Figure 3.5: Aviary and entryway interiors. Panel A. Set-up for weighing birds. Panel B. Entryway organization. Panel C. Step ladder set-up for cleaning upper tier nest boxes.

Laboratory shed (Lab Shed): A composite wood outdoor shed (Heartland Sheds, Monroe, MI) (12' w x 8' d x 8' 10" h) was used to conduct heat trials (HTs) and process samples. (3.6). The interior and exterior were painted with semi-gloss exterior paint. All edges were sealed with caulking compound. Foam mats covered the floor. This building was equipped with a steel lab table, a field centrifuge, stools, pigeon traps, and other lab supplies. Local building permits were required for this structure.



Figure 3.6: Laboratory shed interior and exterior. Panel A. View of incubators used for heat trial assays. Note cameras directed at each incubator. Panel B. View from the doorway. Panel C. Exterior view.

Experimental shed: A Keter Manor (Anderson, IN) 4' d x 6' w x 6.5' h Resin Outdoor Storage Shed was used to store the experimental variable supplies. We used this shed to store anything that came in contact with the experimental bait used in our protocol to avoid cross-contamination with the control group supplies. A canopy (ABCCANOPY Outdoor Pop up Tent 10' x 10', Ontario CA) was used to provide shade for researchers and keep temperatures down in the shed. Shed temperature was monitored with a bluetooth-enabled thermo-hygrometer (Fig 3.7). This

was built on top of paving stones and ¼” hardware cloth was placed underneath the shed. All edges were sealed with caulking compound. No permits were required for this structure.



Figure 3.7: Experimental shed: This shed was constructed to store experimental bait to avoid cross-contamination with control feed and supplies. Panel A. Side view showing steel shelves used as a workstation and storage. The black stock tank on the ground to the left of the shed was used for washing and disinfecting lab supplies exposed to the experimental bait. Panel B. Front exterior view. Panel C. Interior view.

Additional workspace: The Stow-Away<sup>®</sup> Horizontal Shed (5.9’ w x 3.7’ d x 4.3’ h; Suncast Chicago, Il) was used for feed processing and storage. This resin shed provided us with a mini-lab and storage which was placed near a dishwashing station. It was placed on top of paving stones and all edges were sealed with caulking compound. No local permits for this structure were required. (Fig. 3.8)



Figure 3.8: Mini workstation/storage shed. Panel A. Exterior view of closed unit. Panel B. Interior view displaying lab supplies containers (left) and work desk used for a food weighing station (right).

Maintenance: All trash produced during experimental protocols were taken to the Tufts University campus and disposed of by the waste management department in compliance with EPA regulations. Fecal matter on aviary and nest box surfaces were scraped at least every other day. This included scooping up feces on the pellet substrate. Aviary and nest box surfaces were disinfected with RES-cue Ready-to-Use (Virox Technologies, Oakville, ON Canada) 2 – 3 times per week. Butcher paper covering the nest boxes were changed once a week. Nest boxes were pulled off the shelves at that time and all interior and exterior surfaces were disinfected. Also, the acrylic sheets on the shelves were thoroughly disinfected. Common use feeders and waterers were cleaned daily and disinfected every other day. Water in the pool was changed every other day and the saucer containing the water was disinfected at the same time. Individual feeding cups were cleaned daily by rinsing with water, soaking in dilute SaniCoop (Schiller Park, Illinois) concentrate as per manufacturer's instructions, drying, then, immediately before feeding the pigeons, run through a sanitizing cycle using a Wabi baby bottle sanitizer (W9900N-PT, Frisco, Texas). All cleaning supplies were wiped with 70% isopropyl alcohol immediately after each use. Between experimental protocols, everything not permanently affixed to the aviary structure

was removed, washed and sanitized. Pellet substrate was removed from the aviaries and double bagged with garbage can liners. The entire interior of the aviaries and entryways were hosed down and disinfected with RES-cue concentrate, dispensed using a garden sprayer, hosed down again, and left to dry.

#### Non-Lethal Heat Study Assay:

This experiment is described in more detail in Chapter 4. For the purpose of this paper, we list data pertinent to this methodology.

Experiment: Sixteen pigeons were trapped at an urban site in Cambridge, MA. The pigeons were randomly assigned to either the experimental (NCZ) group (N = 8; 4 males, 4 females) or the control group (N = 8; 6 males, 2 females). Birds in both groups had access to the open runs in their respective aviaries (Fig. 3.1) during the day and were secured in individual nest boxes overnight. Both aviaries were videoed 24/7 to study behavior post-hoc. The pigeons' daily diet of seed (Hagen Pigeon & Dove Seed (Rigaud, Quebec Canada) was supplemented as follows: The experimental group were given a full dose of NCZ bait (5g/bird/day) for two weeks; the control group were given cracked corn (5g/bird/day). These supplements were provided to the bird in their nest boxes overnight; the amount of bait ingested was measured to assess bait consumption for each bird. To maximize the opportunity for the birds to ingest a full dose of NCZ, birds received an additional dose in the common feeder for an average of 4.75 hrs after they were released from their nest boxes into the open run. Bait consumption from this method of distribution was not assessed. Every pigeon was subjected to a HT.

Materials: Two Chick Chalet 3 Parrot Brooder incubators (Avey Incubator, LLC, Hugo CO; 32" w x 16" d x 18" h) were used to expose the birds to a rapid increase in ambient temperature. To validate unit temperature and humidity readings, a Govee thermohygrometer (model H5101,

Springfield IL) was attached to the interior back wall of each incubator, fully visible to the recording camera through the clear polycarbonate doors. Each incubator had a brown cage liner



Figure 3.9: Still shot from video taken during a HT. The plastic planter saucer (left) contained tap water; the ceramic ramekin (center) contained feed. The brick the pigeon is perched on comes from its nest box. Brick was placed in the incubator immediately before HT started and was returned to the bird's nest box at the end of the HT.

(to mimic the color of the substrate in the aviary), a small planter saucer (8" diameter) filled with water, and a small ramekin containing regular feed. When a bird was put into the incubator, we also put in the brick taken from its nest box. There was sufficient room in the incubator for the bird to freely walk around. Each incubator had a dedicated camera (Axis models M1138 or M1135) recording behavior data exhibited during the HT (Fig. 3.9).

Heat Trials: The pigeons were exposed to a rapid increase in ambient temperatures that went from 25° C to 40° C over a period of 200 minutes. The number of heat-mediating behaviors (HMBs) exhibited were recorded. The mean number of HMBs for each temperature interval per

bird were analyzed by a repeated measures multivariate analysis of variance (MANOVA) test run on JMP 17.2.0. The fixed factors analyzed were temperature, experimental group, and sex. To determine which HMBs to assess, we did a trial HT with two birds (not included in this study) and observed behaviors they exhibited as the incubator temperature increased. Along with gular fluttering (a rapid movement of the neck muscles drawing air around internal air sacs to create an evaporative cooling effect), we saw a slow blinking movement with eyelids appearing thickened and white. We also saw feather fluffing, wing spreading, leg stretching, yawning, gulping, gaping, panting, and lying down, where the bird's ventral surface was in full contact with the substrate. To simplify the scoring process yet leave room for variation in HMBs between birds, we limited the behaviors to five different behaviors exhibited at each observation point. The five we scored were lying down, thick eyelid blinking, open beak, wing spreading, and gular fluttering.

## **Results**

**Satellite Research Site:** The site was designed and built by the lead researcher under the supervision of the research advisor. One master's student and three undergraduate students provided support during construction of the facility as well as running the experimental protocol for which the facility was created. Of this team, two researchers did most of the maintenance and husbandry of the facility while also conducting the experiment. Five additional researchers assisted with husbandry and sample collection during the experiment.

**Constructing and maintaining the facility:** The satellite research facility was approved by the Tufts Institutional Animal Care & Use Committee (IACUC). The site passed several on-site inspections concurrent with the construction of the site as well as during our experiment. We successfully ran two experimental protocols as part of a pilot study reported in Chapter 4

producing publishable results. Our total expenses for construction, conducting the study (including running 16 HTs), and payment for student researchers and behavior scorers were approximately \$40,000. The facility withstood high temperatures and wind gusts of up to 50 mph during the summer of 2024 (Boston July 2024 Historical Weather Data (Massachusetts, United States) - Weather Spark, 2025). As the aviaries were open to these conditions, the pigeons were exposed to the weather as they would have been in their home territory. The pigeons had access to shade, their nesting areas, food, and water, as they would have been in their home territory

Heat Trial Assay:

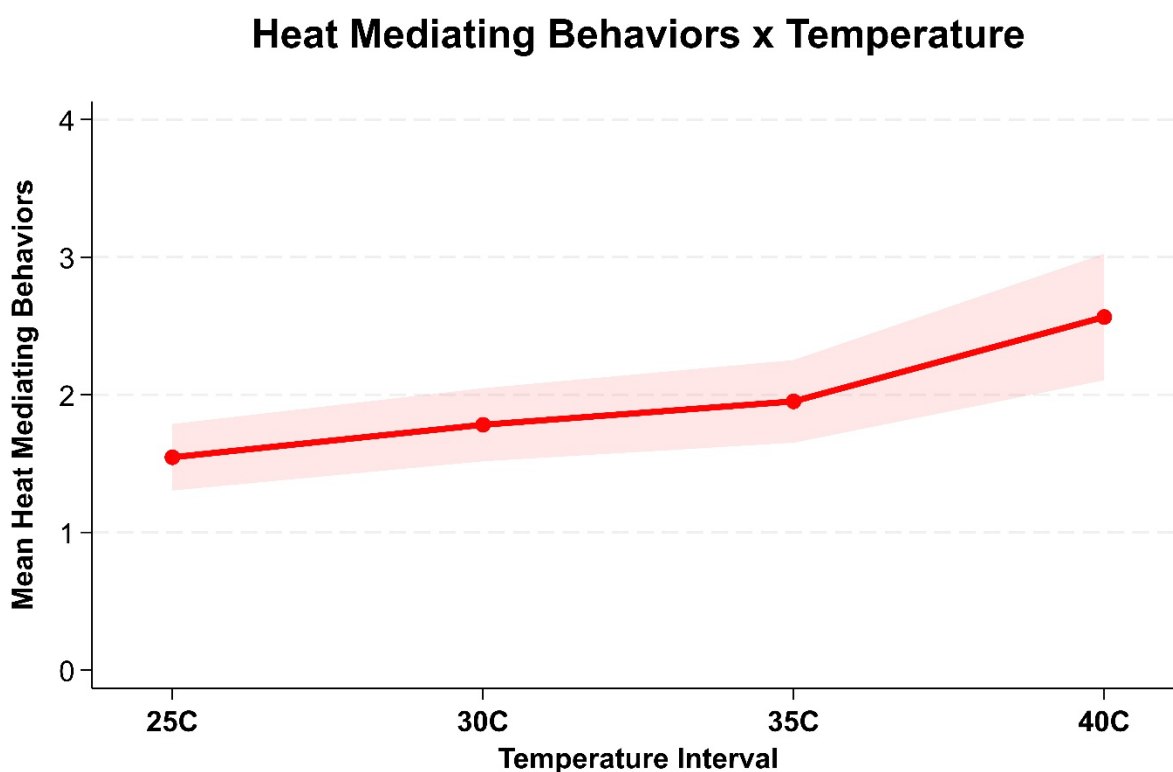


Figure 3.10: Number of mean HMBs for all birds (N = 16) across four temperature intervals. The number of HMBs displayed by all birds increased significantly as ambient temperature increased. Shaded area = 95% confidence interval.

The mean number of heat-mediating behaviors exhibited by all birds across both experimental and control groups increased significantly as the ambient temperature in the incubators rose ( $F(3, 10) = 8.0660, P = 0.005$ ) (Fig. 3.10). There was no significant difference of mean HMBs exhibited between the Control and NCZ-exposed groups across temperature intervals ( $F(3, 10) = 0.4004, P = 0.7558$ ) (Fig 3.11).

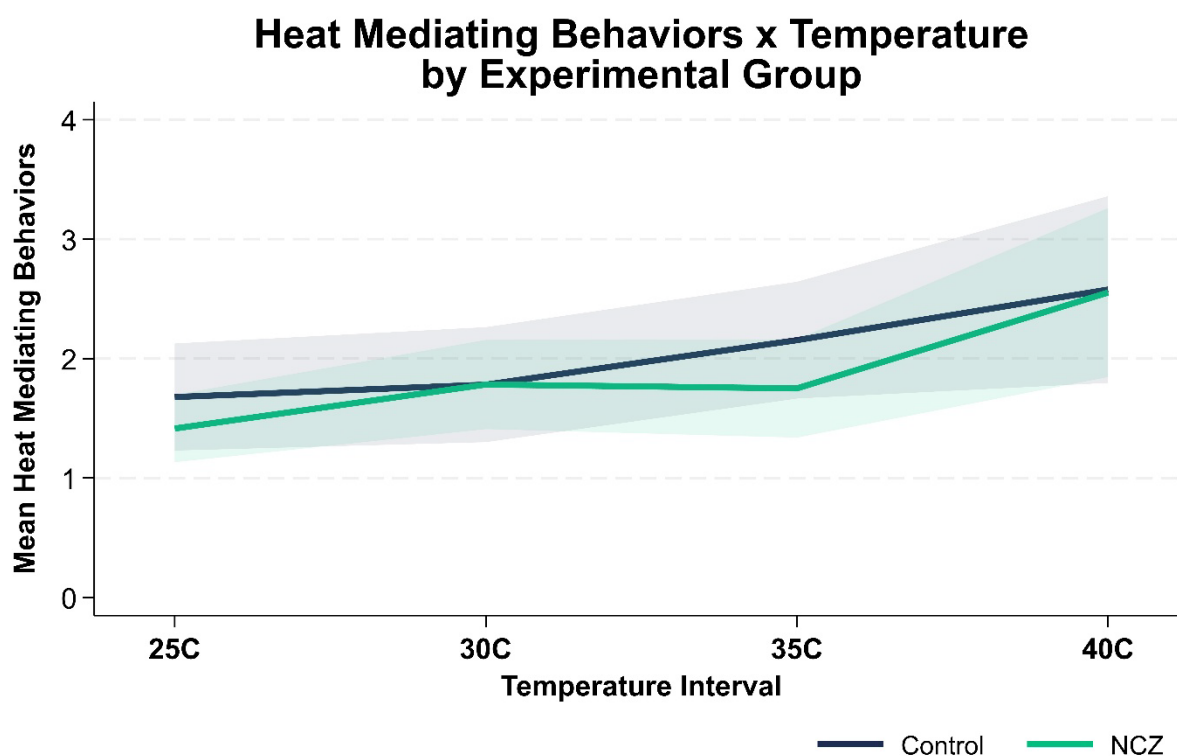
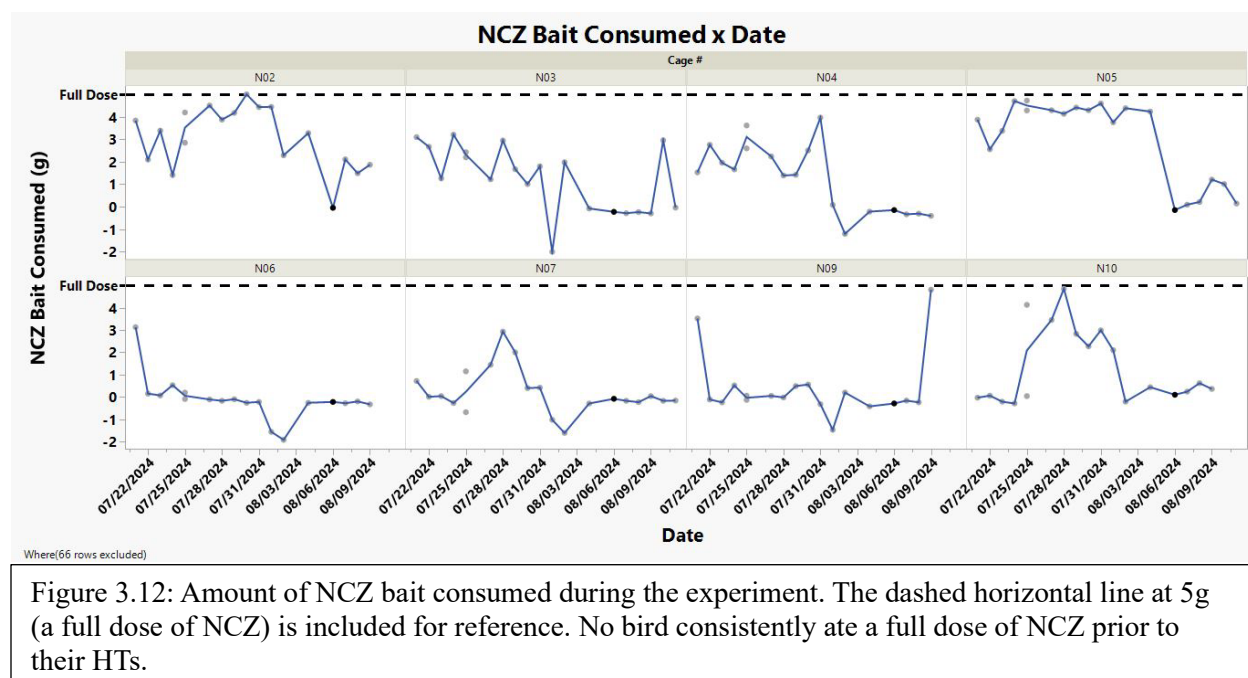


Figure 3.11: Mean HMBs by experimental treatment. There was no difference between NCZ birds and control birds in HMBs exhibited across all temperature intervals. Shaded areas = 95% confidence intervals.

NCZ Bait consumption: According to measured bait intake during the experiment, no pigeon in the experimental group consistently ingested a full daily dose of bait (Fig. 3.12).



## Discussion

We created this satellite research facility to conduct non-lethal research mindful of how urban pigeon subjects might interact with the facility. The facility was designed to balance experimental control with field-realistic conditions. We used this facility to study possible physiological and behavioral impacts of rapid heat increases on pigeons exposed to chemical contraception, a method of population control generally shown to be successfully administered to pigeons in urban centers. This project was necessitated by the lack of an existing facility on or near the Tufts University campuses. Our background research did not produce any one source providing clear instructions or measurements on how to design such a facility, thus creating a gap we sought to fill with this roadmap. We created a low-cost, viable, easily managed and maintained facility, approved by Tufts IACUC. This was not an insignificant outcome as the committee had no experience with a facility that wasn't completely controlled. Complete transparency and goodwill from all parties led to the cooperation necessary to figure out an

effective method of oversight, for the benefit of all. Because of our affiliation with Tufts, we had access to department-wide shared resources, such as instrumentation for plasma analysis and waste disposal, which kept costs down.

Our research produced an important finding in the area of chemical contraception in feral urban pigeons. We found that there were palatability issues with the contraceptive bait. We came to this conclusion because we were able to monitor our pigeons as they were being exposed to the bait while they were interacting with each other as a community, something that is difficult, if not impossible, to do in field studies or in a lab. Previous studies have mentioned that lack of palatability may have caused pigeons to reject the bait in favor of other available food (Giunchi et al., 2007; Senar et al., 2021; Xenakis et al., 2023). González-Crespo & Lavín, (2022) conducted a palatability study as part of their research and found no difference in the number of pigeons eating at a feeder distributing cracked corn or one distributing Ovistop bait (an NCZ bait marketed in Europe). However, due to the number of birds at the feeders, they could not identify if the birds eating at one feeder were also eating at another. Although this was not the primary objective of our original study design, our facility and observational set-up allowed us to observe how the NCZ-exposed birds were interacting with the bait on a day-to-day basis. Our pigeons would ignore the bait in anticipation of delivery of standard seed rations, even if provision of those rations was delayed by several hours. Our observations strongly suggest that making the bait more palatable to pigeons will be critical to getting them to eat enough bait to make a difference. When that obstacle is cleared, we can better assess how long it could take to see a reduction in urban populations and costs in administering the bait. We would also be able to see what effect NCZ may have on pigeons' behavioral and physiological processes.

Assessing HMBs presented a challenge as the goal was to quantify behaviors without invasive or lethal interventions. Pigeons exhibit several thermoregulating behaviors, from panting to wing-spreading to gular fluttering. Studies have shown that how a pigeon deals with heat depends on whether it's used to high heat and low humidity (Marder & Arieli, 1988; Marder & Gavrieli-Levin, 1987). It was also found that, under those circumstances, gular fluttering is displayed at approximately 38°C - 42°C (Bartholomew et al., 1968; Dawson, 1982). Local temperatures at the study site didn't go over 40°C (*Boston July 2024 Historical Weather Data (Massachusetts, United States) - Weather Spark, 2025; Boston Weather Records, 2025*). Considering these data and based on discussions with IACUC and the manufacturer of the incubators, it was not feasible to subject the birds to temperatures higher than 40°C, so it was not certain that we would see gular fluttering during our HTs, but we included it because it was obvious to see and might suggest the onset of increased discomfort induced by the rapid increase of ambient temperature. We also quantified the following HMBs: Lying down; Open beak; Thick eye-lid blinking; and Wing-spreading. Going forward, we would recommend excluding lying down as there is evidence that pigeons dissipate heat from their legs (Martineau & Laroche, 1988), a finding which our observations could support. We would include leg stretching and perhaps feather fluffing instead.

The intimacy of our research facility brought out other advantages. In having to put each bird back into its nest box every night so that we could measure their food consumption, we got to know these birds as individuals as well as a colony. We learned how agile these birds were and how quickly they learned to avoid our nets. One bird did a full reverse in mid-air the moment his beak touched the back of the net. As the birds' time in captivity increased, a limitation of our study occurred. The 2 aviaries appeared to be forming two separate colonies, with each aviary

developing its own day-to-day rhythms of daily interactions with the researchers, the aviaries, and each other. For example, the NCZ birds took daily baths in their pond but did so in small groups. They often took their dips after the control birds took theirs. When the control birds took their baths, most of the birds went in at the same time. On one particularly hot day, all control birds were in the pond at once. This limitation could be addressed by altering the experimental design.

Our experiment provided a valuable opportunity to the students, faculty, and the IACUC committee members to engage with wildlife in a meaningful way. In addition to experiencing firsthand what it takes to build and run a research facility, the undergraduate students learned how to handle the pigeons, take samples from them, and most importantly, observe the pigeons' behaviors to see how to work *\*with\** them to achieve good outcomes, such as taking accurate weights and gentle application of leg bands. Our master's student received valuable insight into what makes a well-designed scientific experiment, excellent preparation for future graduate and professional research. All student research assistants had the chance to see if wild animal welfare was a field they wished to pursue thus promoting the best-hands on outcome for them.

## **Conclusions**

We report this methodology to provide a blueprint for creating a satellite research facility in a suburban setting in which to conduct non-lethal, semi-controlled studies on feral pigeons. In realizing this goal, we also developed a non-lethal heat assay which we used to study possible impacts NCZ and heat may have on behavioral and physiological processes in feral urban pigeons. Our experiment provided evidence that this form of chemical contraception may not be as effective as it could be due to low palatability. This result highlights the importance of implementing an experimental phase in a facility that has more of the control of a field study and

less of the control of a laboratory study. It allows researchers to closely observe how small populations of a model species might interact with a technique or product such as a chemical contraceptive that it will encounter in its natural habitat. Observations at this scale will provide clarification as to whether a product or technique is ready to be tested in a wider population or if there are aspects that need improvement before expending more resources in efforts that may not succeed on a larger scale. The relatively low cost of setting up such a facility makes this level of research accessible to more researchers outside of a large research facility. The size of this facility allows for more hands-on learning opportunities for students and dedicated wildlife researchers. In the promising field of chemical contraception as a method of pigeon population control, these resources can direct stakeholders to a successful, user-friendly outcome, one that minimizes disruption to a remarkably resilient pigeon population.

### **Acknowledgements**

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## Chapter 4. Physiological and behavioral effects of chemical contraception in feral urban pigeons (*Columba livia*)

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**ABSTRACT**—Nicarbazin (NCZ), the active ingredient in OvoControl bait in the US and OviStop bait in Europe, has been shown to be an effective and reversible oral contraceptive in feral urban pigeons (*Columba livia*) in both lab and free-ranging birds. Although NCZ is EPA-approved and widely marketed, no published studies examine the impact of prolonged exposure to NCZ on the pigeons' internal environment or behavior beyond the contraceptive effect. . This is of particular concern because NCZ exposure has also been linked to an impaired ability to cope with high heat in other avian species, a condition called heat stress. The increased incidence of rapid changes in temperature associated with global warming elevates the importance of determining whether NCZ-dosed and heat-exposed pigeons can maintain social, behavioral, and physiological processes similar to those of non-dosed birds during challenging weather events. In this study, we subjected NCZ-treated pigeons to a short-term, rapid increase in ambient temperature over a range that is consistent with thermal variation in the local climate near the study site. We measured the presence of five heat-mediating behaviors as well as plasma NCZ levels in both control and treatment groups. Previous laboratory studies found that pigeons acclimated to extreme temperatures are highly thermally resilient with a high upper critical temperature. Our data demonstrate the onset of heat-mediating behaviors at temperatures far below this threshold in pigeons living in moderate climates. Our data also demonstrate wide variation in blood plasma NCZ levels amongst birds in the NCZ treatment group, likely due to

individual variation in consumption rates of NCZ-laced bait. Our data do not support a difference in HMB between control and treatment birds, though our study was underpowered to test this specific outcome because of low blood plasma levels in most of the treatment birds. These findings suggest that heat stress causes behavioral changes in pigeons at temperatures lower than previously appreciated, and that individual variation in consumption rate of NCZ-laced bait may lessen its impact as a population control method when other food sources are readily available. Our protocols could be extended to test for the effects of NCZ on heat stress in pigeons in larger samples, an important research direction for assessing the joint impacts of population control and climate change on avian populations.

## **Introduction**

Feral pigeons (*Columba livia*) have demonstrated not only an opportunistic ability to thrive in urban centers, but also remarkable persistence in the face of humans' aggressive and often short-term attempts to control their population (R. F. Johnston & Janiga, 1995; Murton et al., 1972). Human impacts, including both direct effects like population management and indirect effects such as urbanization and climate change, are likely to have substantial impact on urban-associated pigeons. However, few (if any) studies have considered the joint effects of population control and climate change on pigeon behavior and physiology. Chemical contraception is assumed to be a humane method of pigeon population control that can substantially decrease urban pigeon populations (e.g., by 65% in 8 years; Albonetti et al., 2015). At the individual level, chemical contraceptives can also exacerbate the effects of heat stress in other avian species (Keshavarz & McDougald, 1982; Onagbesan et al., 2023). Though pigeons are thought to be highly thermally resilient, it is not clear how the joint effects of chemical contraception and

climate change will affect pigeon population control and individual thermal physiology in pigeons, especially within rapidly warming urban microenvironments.

Nicarbazin (NCZ) is the most commonly administered drug for population control of pigeons. It is the active ingredient in OvoControl bait in the US and OviStop bait in Europe, has been shown to be an effective and reversible oral contraceptive in feral urban pigeons in both lab and free ranging birds (Avery et al., 2008; González-Crespo & Lavín, 2022b). NCZ, which was developed as a coccidiostat for the poultry industry (Cuckler et al., 1955), causes exposed birds to lay unhatchable eggs which is the basis for its contraceptive effect (Avery et al., 2006, 2008; Baker et al., 1957; Bynum et al., 2007; Polin et al., 1957). At a more basic level, it alters the structure of the vitelline membrane in eggs, a thin tissue encasing the yolk, and the zona pellucida, layers of sperm-binding proteins attached to the vitelline membrane, thereby preventing fertilization (Cunningham & Ylander, 1980; Reinoso, 2008). The exact molecular mechanism is not known but is suspected to interfere with cholesterol metabolism in the formation of the membrane. Because NCZ is excreted from the birds' bodies within 2 days, its effects regarding egg hatchability have been shown to be reversible (Fagerstone et al., 2010; Polin et al., 1957). The reversibility of NCZ's contraceptive effect makes it an attractive option for use as a humane option for population control of invasive avian species, such as pigeons. However, its reversible quality requires that, in order to achieve and maintain its contraceptive effect, pigeons must be exposed to NCZ during the entirety of their breeding season. As pigeons can breed from 5 – 12 months per year, it is wise to consider other possible consequences resulting from prolonged exposure.

Though the effects of NCZ on population control are well-known, its effects on individual physiology are not well characterized in pigeons. Early work on the effects of NCZ in chickens

revealed that it has a substantial effect on thermal performance. Beers et al., (1989) found that broiler chicks exposed to NCZ for 4 - 6 weeks had significantly higher internal body temperatures, heart rates, and mortality than non-exposed chicks when subjected to an increase of ambient temperature from about 25° C to 38° C over 4 hours. Studies have demonstrated pigeons' ability to thermoregulate in extreme high temperatures (Calder & Schmidt-Nielsen, 1956, 1967; Marder & Arieli, 1988; Marder & Gavrieli-Levin, 1987), but these were performed on birds bred in captivity and acclimated to 35° - 60°C. To our knowledge, no studies exist about the effect to their thermoregulation that prolonged exposure to NCZ may have on their thermoregulation during rapidly elevating temperatures, such as high-heat events commonly occurring due to climate change. Climate change is characterized by increased day-to-day weather variability as well as increases in prolonged high heat events (Dodman et al., 2022; Medvigy & Beaulieu, 2012; US EPA, 2016, 2016), and hence the response to both sustained and rapid heat exposure are of interest in natural populations

NCZ is a dimer composed of two molecules: 4,4-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethyl-pyrimidine (HDP). DNC is the active part of the dimer that produces the contraceptive effect. However, its low solubility in water (< 0.02 mg/L) impedes it from crossing cell membranes. Therefore HDP, which is hydrophilic, is necessary to increase DNC absorption. (Bacila et al 2017). HDP is excreted via urine within 24 hours while DNC is excreted in 4 days via feces (J. J. Johnston et al., 2002). The dimer dissociates in contact with fluid and does not recombine, leaving the inactive DNC adsorbed to surfaces via fecal matter. However, NCZ bait and residue from pigeon excreta have low impact on the environment and pigeon predators due to the rapid dissociation of the dimer (Greenlees, 2018; Intorre et al., 2023; PubChem, 2021)

In this study, we sought to assess how pigeons modified their behavior in response to both thermal challenges and treatment with NCZ. Feral pigeons were randomly assigned to control and treatment groups. Birds in the treatment group were dosed with NCZ for two weeks, then they and their control counterparts experienced a 200-minute heat trial (HT). We video-recorded their behaviors and scored individuals for five heat-mediating behaviors (HMBs) and obtained corresponding blood samples. The frequency of HMBs was tallied across each heat trial and blood was analyzed to assess whether plasma levels contained an effective dose of NCZ.

## METHODS

### Subjects

Twenty feral pigeons (*Columbia livia*) were trapped in June 2024 from an urban site in Cambridge, MA, USA. An initial physical intake examination was done to assess the health and estimate the age of the birds. The pigeons were transported to a suburban satellite research facility twenty-two miles away where they were randomly assigned to either the control or experimental (NCZ) group (10 birds/group). Each bird was assigned an identifying leg band and individually videoed to record physical appearance and plumage coloration to assist with identification. All birds were treated for 5 days with All-in-One powder (Belle Plaine, MN) mixed into the birds' drinking water, a prophylactic treatment for common intestinal parasites and also containing pro-biotics to support beneficial gut bacteria. Upon completion of treatment, birds were given tap water for the remainder of the protocol. The pigeons were given a full week post-treatment to allow the medication to fully pass out of their bodies because the coccidiostat was a possible confounding variable. Hagen Pigeon & Dove Seed (Rigaud, Quebec Canada) (30g/bird/day) was provided to meet the birds' daily nutritional requirements for the duration of this study. Purina® Oyster Shell Grit was available ad libitum. Diet for the NCZ treatment group

was supplemented with the manufacturer-recommended effective dose (5g/bird/day) of OvoControl contraceptive bait (Innolytics, LLC, LaQuinta, CA). Pigeons' weights were monitored to assess overall health. With regards to age determination, the pigeons were classified as either adult or juvenile based on keel score, weight, beak shape, and eye color. Sex of the birds was determined by DNA analysis of blood samples by IQ Bird Testing (Miami, FL) at the end of the heat trials. Of the ten birds in the NCZ group, eight birds completed the entire protocol. Two birds died of unknown causes prior to the beginning of the heat trials. Of the surviving birds, there were four females (2 juveniles, 2 adults) and four males (3 juveniles, 1 adult). The control group had three females (2 juveniles, 1 adult) and six males (2 juveniles, 4 adults), one bird having died of accident at the beginning of the protocol. One adult female was removed from the protocol when she laid two eggs, a potentially confounding variable due to the energy cost of producing and laying eggs (Zhang et al., 2023), leaving N=8 in the control group. During the day (10 – 12 hrs/day), birds had free access to over 729 cubic feet within their respective aviaries (referred to as the open run). Each bird was secured in its individual nest box within its respective aviary during the night (12 – 14 hrs/night). Birds were provided feed, bait, or cracked corn (Kaytee Cracked Corn, Chilton WI) and water while they were in their nest boxes; they had feed or bait, water, and grit provided in a common feeder, waterer, and ceramic ramekin when they were in the open run. All birds except for the mother and father and their squabs, hatched July 31, 2024, were released at the place of capture upon the completion of the heat trials (Aug. 12, 2024). Mother, father, and both squabs were released at place of parents' capture 1 week after squabs fledged (Sep. 6, 2024)

## **Facilities**

Aviaries: Two aviaries were designed and created based on guidelines from "Minimum Standards for Wildlife Rehabilitation, 4th edition" (Miller, 2012), and conversations with bird fanciers and avian farmers. Each structure measured 10' w x 12' d x 8' h and was constructed on cinder block



Figure 4.1: Outdoor aviaries. Aviaries are set back on the property approximately 47' from the main building and at least 30' from property boundaries. Vegetation visibly shielded the aviaries from surrounding properties and also acted as a sound buffer for the pigeons. These aviaries are mirror images of each other.

supports to assist with air circulation and water drainage. The back walls were solid wood, and the remaining 3 walls were ½" hardware cloth (wire mesh) secured to wooden support beams. ¼" hardware cloth ran under the floor. The entryway, also used for research and cleaning supplies, consisted of a small room (8' h x 5' w x 5' d) made of square aluminum pole framing and enclosed in ½" hardware cloth. Therefore, the coop was a protective enclosure with a double door entry way keeping predators and rodents out while allowing the birds an environment open to ambient outdoor temperature. Translucent Greenhouse Heavy Duty poly tarps (14 mil, UV resistant, and waterproof, Glasgow, Greater New York NH7689, UK) were deployed over the

hardware cloth sides during rainstorms. These tarps were folded back during dry weather (Fig 4.1)

Aviary furnishings: Chrome wire shelves with two levels were located against the back wall with the lowest level approximately 2' above the floor. Each shelf accommodated 5 individual wooden nest boxes (22" w x 18" h x 18" d) for a total of 10 nest boxes/aviary. The fronts of the nest boxes were modified pet carrier doors (Petmate, Arlington TX) (Fig. 4.2) with wire mesh that allowed full sight of the birds while they were secured in their boxes. Each nest box, lined with poly coated cage liners (BirdCageLiners, Eagle ID), contained a biodegradable paper pulp nest bowl (Foy's Pet Supplies, New Brighton, PA) and a brick (7.5" l x 3 5/8" w x 2 1/2" h).



Figure 4.2. Nest box. All materials used to construct these boxes were low VOC and human-grade products.

These items remained in the nest boxes throughout the protocol. Ten plastic perches (Moduoduo, Amazon.com) were placed, at varying heights, throughout each aviary. Pine Pellet Stall Bedding (Tractor Supply, Pelham NH) was used as a substrate. One large plastic planter saucer (21.25" top diameter x 17.5" bottom diameter x 3.75" h), placed in a sunny corner of each aviary, was filled with 2 gallons of water and served as a bathing pool. For common use, a plastic feeder (Flock Party, Tractor Supply, Pelham NH), a 1-gallon waterer (Farm-Tuff Chicken Drinker, Tractor Supply, Pelham NH), and a ceramic ramekin filled with grit were placed on the floor in the center of each aviary. Straw was available in the enclosure for nesting purposes.

Temperatures in each aviary were monitored by four Govee thermohygrometers (model H5104, Springfield, IL), one posted about 6' above the floor in each corner. Coops were cleaned regularly with Rescue™ Ready to Use disinfectant (Oakville, ON, Canada). Feeding cups were cleaned daily with Sani-Coop Germicide (Binghamton, NY) then run through a bottle sterilizer



Figure 4.3: Facility buildings. Panel A. Lab shed used to run HTs, process samples and store research supplies. Panel B. NCZ shed used to process NCZ bait and to store anything that was exposed to NCZ bait.

cycle. Two additional sheds, a Lab Shed (12' w x 8' d x 8' h), and a shed (6' w x 4' d x 8' h) for processing NCZ-exposed materials and bait were used for the study (Fig. 4.3)

Cameras: Six exterior security cameras were donated by Axis Communications (Lund Sweden). Each aviary had two cameras (model M3058). One camera was located approximately 6' above the floor and secured to the interior front of the aviary and the other camera was placed in the back of the aviary approximately 7' above the floor. The other two cameras, models M1138 and M1135, were used to record bird behaviors during the heat trials. When two of the aviary cameras failed during the course of the protocol, they were replaced as follows: model P3818-PVE was placed in the back of the NCZ aviary; model P3268-LVE was placed in front of the control aviary. Video data was managed by an Axis S3008 Mk II Recorder.

### **Experimental Design**

Acclimation: Pigeons in both groups were allowed free access to the open runs in their respective aviaries during the day and put into individual nest boxes overnight. Water and food were provided in the nest boxes. Food consumption was measured by subtracting the weight of the remaining feed in the cup (when the birds were released from their nest boxes in the morning) from the pre-weighed amounts they received the night before as they were put into their nest boxes. As pigeons are dawn feeders (R. F. Johnston & Janiga, 1995), we fed the birds in their individual nest boxes, their location at dawn. They were initially given 20 g/bird/day of feed in their nest boxes and given the remaining 10/g/bird/day of feed to fulfill their daily dietary requirement in the common feeders. Due to weight loss observed at the end of the first week, probably from adjustment to captivity, (Kangas & Branch, 2006) (Fig. 4.9), the amount of feed provided overnight was increased to 30 g/bird/day in addition to the 30g/bird/day in the common feeder. From this point on, birds received 30 g/bird/day in their common feeders regardless of the amounts of feed or bait provided in their nest boxes overnight. When the birds' weights stabilized (approx. 3 weeks), the overnight food allotment was changed to 5g/bird/day cracked

corn in preparation for acclimation to OvoControl bait for the NCZ group. After 1 week, the NCZ birds were gradually introduced to the bait by providing 1 g bait and 4 grams of cracked corn. The amount of bait increased by 1 g/bird/day and the amount of cracked corn decreased by

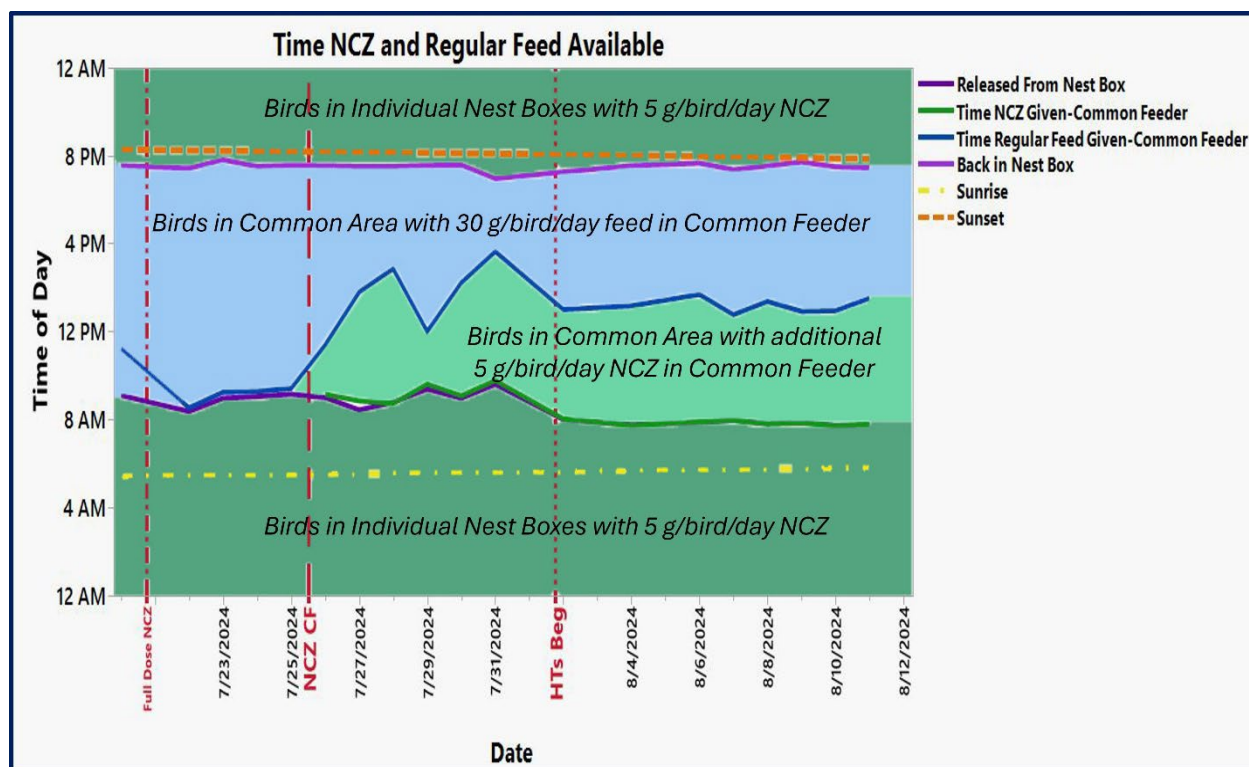


Figure 4.4: Times and methods of distribution of NCZ and regular feed from beginning of acclimation to bait till the end of the study. The three red vertical lines indicate (from left to right): 1. Date when NCZ birds first received a full daily dose of bait in nest boxes overnight; 2. Date when an additional full dose of bait was made available via the common feeder (CF); Date when HTs began. There was no mixing of NCZ bait and regular feed in the common feeder—the feeder had either NCZ or regular feed.

1g/bird/day until the effective dose of NCZ (5g/bird/day) was reached. The control birds received 5g/bird/day of cracked corn overnight from the beginning of the acclimation period until the heat trials were completed (Fig 4.4).

#### Heat Trials (HT)

Materials: Two Chick Chalet 3 Parrot Brooder incubators (Avey Incubator, LLC, Hugo CO; 32" w x 16" d x 18" h) were used to expose the birds to a rapid increase in ambient temperature. To validate unit temperature and humidity readings, a Govee thermohygrometer (model H5101,

Springfield IL) was attached to the interior back wall of each incubator, fully visible to the recording camera through the clear polycarbonate doors. Each incubator had a brown cage liner (to mimic the color of the substrate in the aviary), a small planter saucer (8" diameter) filled with water, and a small ramekin containing regular feed. When a bird was put into the incubator, we



Figure 4.5: Still shot from video taken during a HT. The plastic planter saucer (left) contained tap water; the ceramic ramekin (center) contained feed. The brick the pigeon is perched on comes from its nest box. Brick was placed in the incubator immediately before HT started and was returned to the bird's nest box at the end of the HT.

also put in the brick taken from its nest box. There was sufficient room in the incubator for the bird to freely walk around. Each incubator had a dedicated camera recording behavior data exhibited during the HT (Fig 4.5).

Heat trials (HT) began after NCZ birds had been exposed to a full dose of NCZ for two weeks. A total of 9 days of heat trials were run over an eleven-day period. Trials were not run on two of the days included in that period due to extremely high outdoor temperatures. The trials began at 7 am when outdoor temperatures were approximately 25C, matching the initial temperature setting

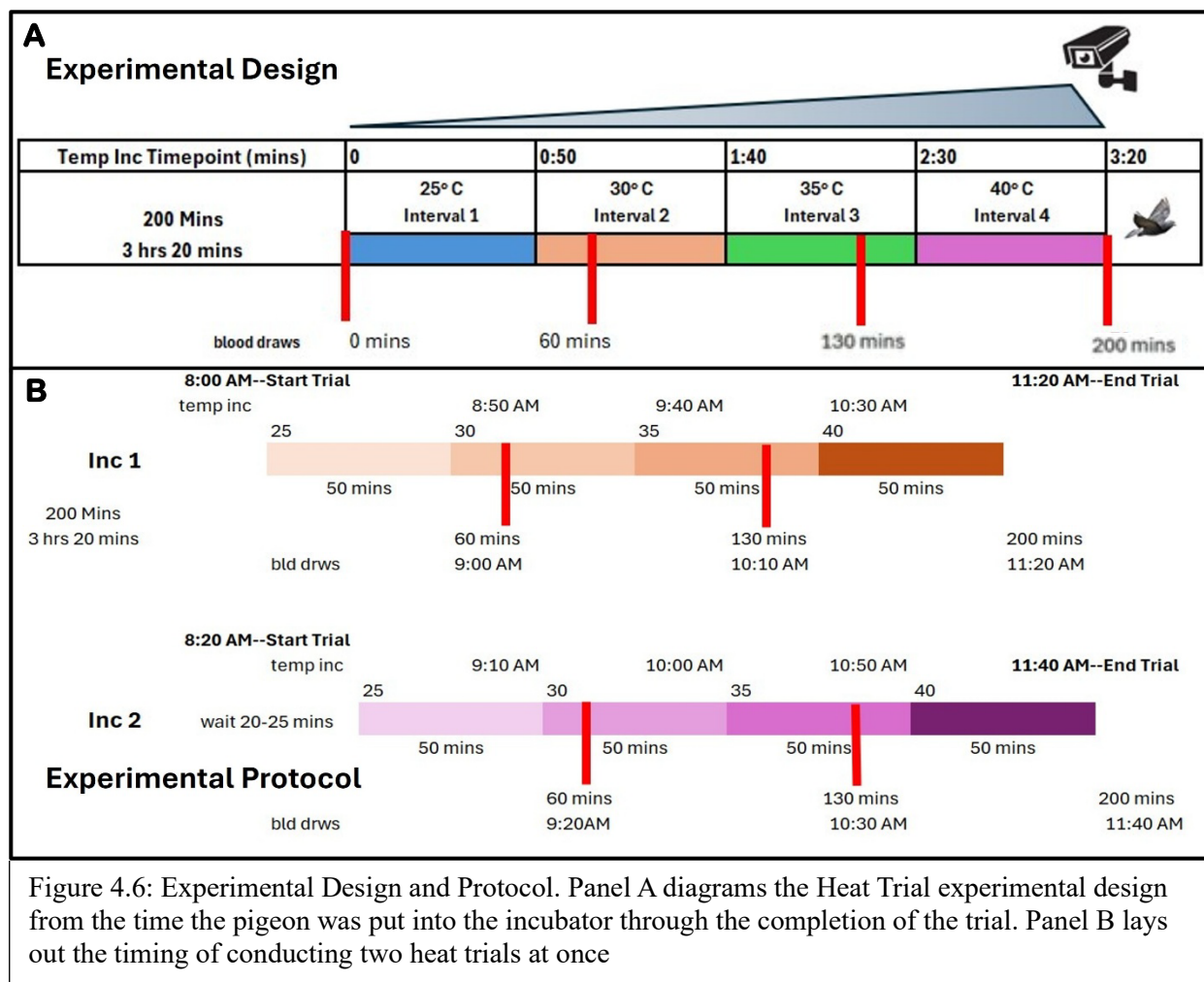
of the incubators. Two heat trials were run at a time. The order in which the birds were tested was randomly chosen. We alternated HTs between NCZ birds and Control birds so as to give the birds in their respective aviaries a day with minimal human interaction between trials.

Lab Shed Set-Up: The interior of the Lab Shed (LS) was kept as dark as possible to maximize the quality of the videos produced. The windows in the shed were covered in light-blocking black paper. A black double curtain was hung across the entry way to keep the LS dark when a researcher had to exit and/or enter during a heat trial. The only light sources in the LS during a HT came from the internal lighting of the incubators and two hurricane lanterns, providing sufficient lighting for note taking and sample processing, which was done on a steel lab table, using a ThermoScientific My Spin 12 centrifuge (Vernon Hills, Illinois). Two stools, placed 8' – 10' away from the incubators, were available for researchers. Researchers minimized movements and maintained quiet during the HTs.

Plasma sample collection: Two researchers were required to draw blood samples, one to hold the bird and one to draw blood. Blood was drawn from the medial metatarsal (caudal tibial) vein or from closely clipped toenails and collected in heparinized microtubes. Samples were centrifuged at 3000 rpm for 10 minutes. The separated plasma was pipetted into microtubes and placed in an ice chest containing crushed ice. Upon conclusion of each HT, samples were transferred to and stored in a -80C freezer located off-site until analyzed.

Procedure: On the day of their HTs, two pigeons in the selected aviary were removed from their nest box, had blood drawn, and placed in a small, covered holding cage. They were taken to the LS where their cages were placed on the floor in a quiet dark area until their HT began. Two researchers were required to oversee the HTs. Each bird had a dedicated researcher taking notes on observed behaviors and ensuring the bird's wellbeing throughout the trial. The two heat trials

were run concurrently, but starting times were staggered to allow for researchers to assist with sample collection as needed (Fig. 4.6B). A total of 16 heat trials, one per bird, were conducted.



At the start of the trial, the bird was placed into an incubator set at 25C. The trial ran for a total of 200 mins (3hrs 20 mins) (Fig 4.6A). The trial was divided into 4 temperature intervals lasting 50 mins each with an increase of temperature at the rate of 5C/50 min interval. Temperatures went from 25C to 40C. Blood samples were taken at 60 mins, 130 mins, and 200 mins into the trial. When the final blood sample was drawn, the HT was complete. Pigeons were put back in their individual, covered holding cage containing food and water. They were closely monitored

by the researchers to ensure that they were sufficiently recovered from the stress of the trial.

Every bird tested was fit to be released into its assigned aviary within about an hour. Tested birds were monitored for the rest of the day. All birds maintained good health and social behavior post-trials based on observations taken during feeding times.

Scoring Heat Mediating Behaviors: Heat-Mediating Behaviors (HMB): Five behaviors that pigeons use to thermoregulate were chosen because they are used by pigeons in the wild and they could be easily seen on video. They are:

1. **Sitting:** Feet and legs are NOT visible; body is in full contact with substrate. Requires less energy than standing
2. **Open Beak:** Upper and lower beak are not in contact. This is one of the earliest signs of heat stress (St-Laurent & Larochelle, 1994). It indicates panting behavior which increases evaporative cooling by increasing respiration rate (Teeter & Belay, 1996).
3. **Thick Eyelid Blinking:** Slow blinking with eyelids that are whitish which were easily seen. Closed eyelids better maintain moisture
4. **Wing Drooping:** Wing joints (connecting the humerus with the radius and ulna) are held apart from the body. The white feathers on the pigeon's back are easily visible when wings are drooping. There is a gap between the wing tips thus increasing surface area. This elevates blood flow and heat transfer, which then reduces peripheral to blood flow and increases resistance to the viscera (Teeter & Belay, 1996). White coloration absorbs less heat (Angelier, 2020).
5. **Gular Fluttering:** Defined as rapid movement of the floor of the buccal cavity and the upper throat region (Calder & Schmidt-Nielsen, 1967). It promotes internal evaporative cooling. It is identified as a rapid movement of the neck feathers.

Three scorers each scored all 16 videos taken during the HTs resulting in three sets of scores for every bird. Videos ran for the full 200 mins of the HTs with the temperature display on the incubators and the thermohygrometers in the incubators blurred out. Videos started when birds were put in the incubators (timepoint = 0:00). Scorers were directed to advance the video to a specified data point and observe pigeon behavior for 15 seconds/data point. Behaviors were scored as 1 for being present or 0 if not present at any time during the 15 second viewing interval. Scorers recorded the presence of 5 different HMBs at 5-minute intervals with the first data point occurring at 5 mins from the beginning of the video. The number of HMBs exhibited were totaled for each 15-second data point. HMB scores for each 15 second interval were averaged across each temperature interval. The resulting mean number of HMBs per interval were used for analysis. To reduce the impacts of researchers' interactions with the pigeon (blood draw) or the incubator (temperature change) on the pigeon's behavior assessment, the first data point that happened after either event was not included in the final scoring.

NCZ plasma analysis: Plasma samples taken during HTs were analyzed for DNC levels using a PerkinElmer QSight 110, with LX-50 solvent delivery module Liquid Chromatograph-Mass Spectrometer (LCMS) tandem using the AOAC Official Method 2013.07 for Determination and Identification of Nicarbazine in Chicken Tissues. Because the DNC molecule stays in the pigeon's system for 2-4 days, plasma DNC is used as a proxy for plasma NCZ levels. Going forward, DNC levels will be labeled as parts per million (ppm) of NCZ. Plasma was analyzed to determine whether an effective level of NCZ of approximately 3.5 ppm was present in the birds' bodies (Avery 2006). Samples were prepared using a modified version of the protocol created at the USDA for pigeon plasma analysis. We used 60 ul plasma/sample but maintained the 1:2 volume ratio of plasma to acetonitrile. Samples were then vortexed and centrifuged at 3000 rpm

for 10 minutes. The resulting supernatant was transferred to low volume LCMS tubes, then analyzed by LCMS. Data processing to determine plasma DNC levels was performed in Simplicity 3Q software from PerkinElmer. (See Appendix – 2 for LCMS specifications)

### **Statistical Tests**

The mean number of HMBs for each bird for each temperature interval were analyzed by a repeated measures multivariate analysis of variance (MANOVA) test run on JMP 17.2.0. The fixed factors analyzed were temperature, experimental group, and sex.

## **Results**

**Heat Mediating Behaviors:** The mean number of heat-mediating behaviors exhibited by all birds across both groups increased significantly as the ambient temperature in the incubators rose ( $F(3, 10) = 8.0660, P = 0.005$ ). There was no significant difference of mean HMBs exhibited between the Control and NCZ-exposed groups across temperature intervals ( $F(3,10) = 0.4004, P = 0.7558$ ) (Fig 4.7). Neither was there any detectable relationship between temperature and sex ( $F(3,10) = 0.9300; P = 0.4616$ ) (Fig. 4.8).

To see if a trend was present within temperature intervals between the NCZ birds and the control group, we ran an Independent-Samples Mann-Whitney U test comparing the mean HMBs shown by the experimental compared to the control group within each temperature interval in IBM SPSS Statistics (Version 29). There was no evidence for a relationship between NCZ exposure and number of HMBs within any individual temperature interval (Table 4.1).

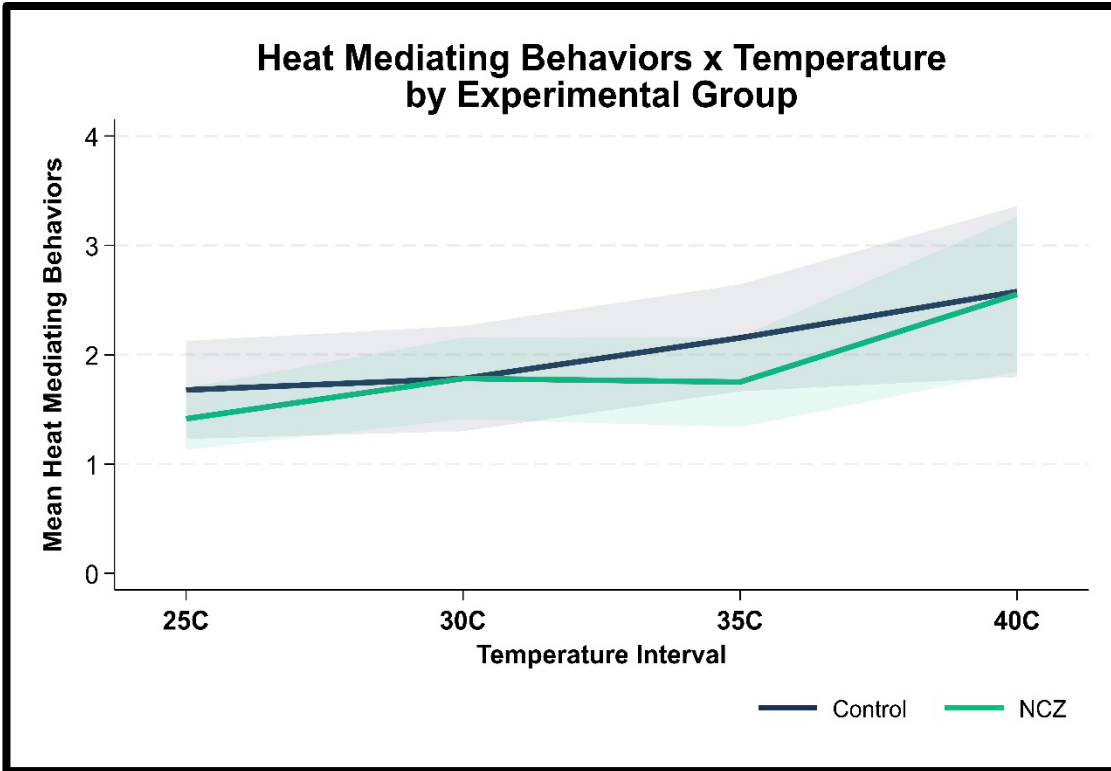


Figure 4.7: The overall mean of heat-mediating behaviors exhibited by all birds in this study is significantly correlated with an increase in ambient temperature. There is no statistical difference of mean HMBs displayed between the Control and the NCZ-exposed pigeons across temperature intervals.

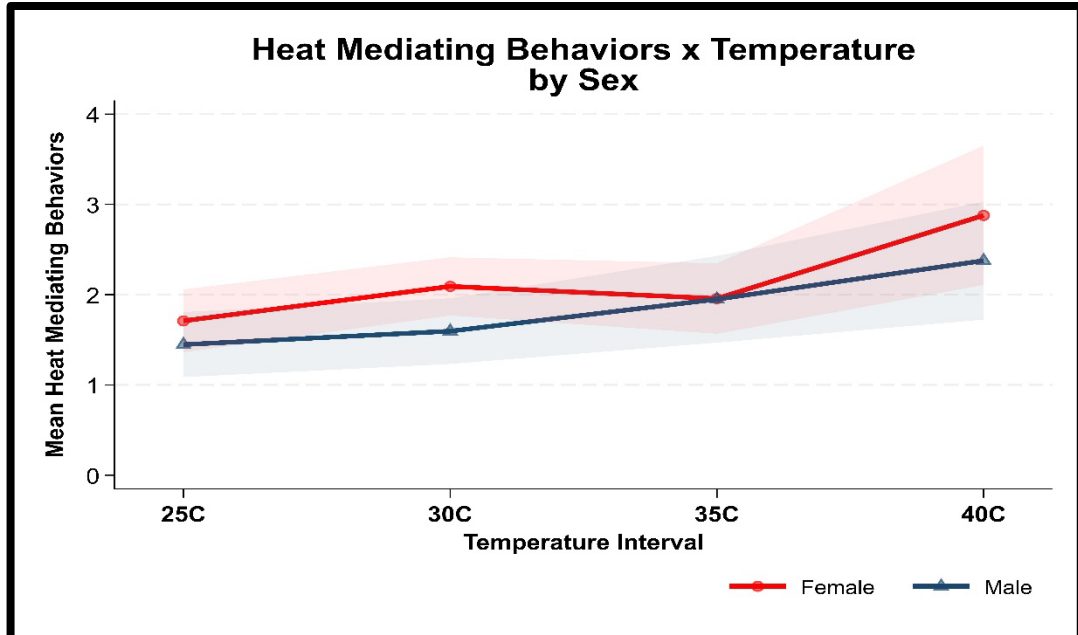


Figure 4.8: Mean HMBs x Sex. There is no correlation between HMBs across temperature intervals by sex regardless of NCZ exposure. N (female) = 6; N (male) = 10

Temperature Interval	Total N	Test statistic (Z)	p
25 C	16	21.000	0.279
30 C	16	35.000	0.798
35 C	16	21.500	0.279
40 C	16	28.500	0.721

Table 4.1: Statistical comparisons of experimental and control groups' mean HMBs within each HT temperature interval using an Independent-Samples Mann-Whitney U test. No significant differences were found.

NCZ bait consumption: The pigeons' weights initially decreased, but recovered to, or continued to increase above, intake (initial) weights (Fig. 4.9). After ten days of providing the bait

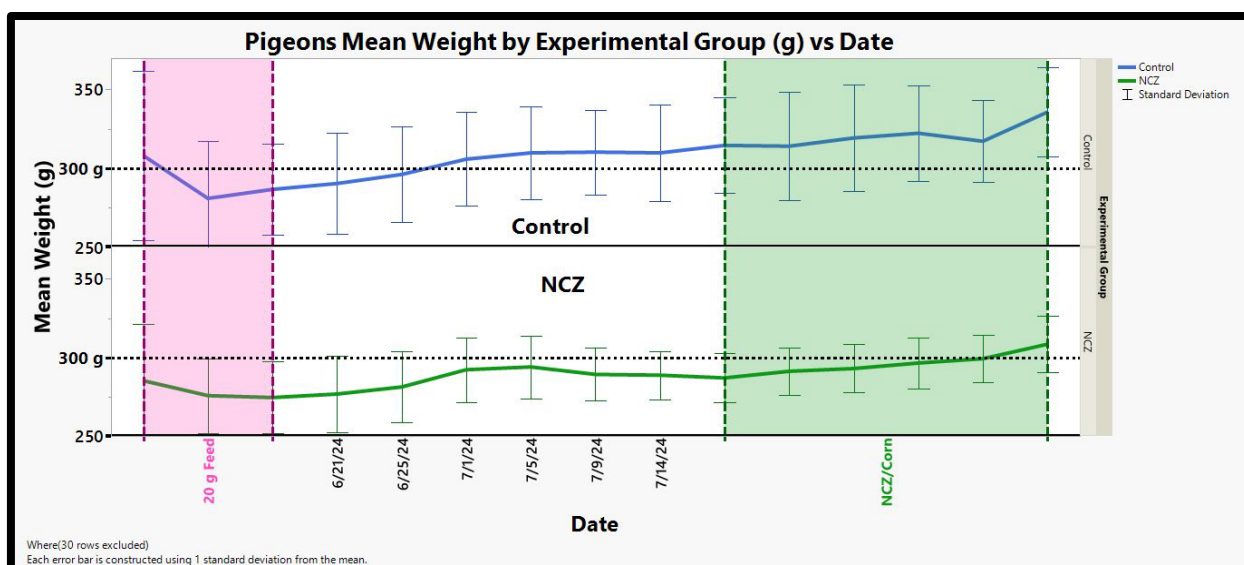


Figure 4.9: Mean weights of pigeons by experimental group. The horizontal dotted line at 300 g inserted for reference. The shaded pink area (left) indicates dates when 20 g/bird regular feed was provided overnight in each nest box. The shaded green area (right) indicates when the NCZ birds were given a full dose of NCZ (5g), while control birds were given an equal amount of cracked corn.

overnight to the birds in their nest boxes, we found that the pigeons were consuming the bait but not completely. When they learned that both types of food would be offered every day, their bait intake dropped or became erratic. To maximize the possibility that birds would consume a daily effective dose, 5g/bird/day of NCZ (i.e., 40g total bait for 8 birds) was provided in the common feeder in addition to the amounts given to the birds in their nest boxes overnight. Therefore, the NCZ birds had free access to an additional full dose of OvoControl bait in the common feeder

for an average of 4  $\frac{3}{4}$  hours after they were released from their nest boxes starting one week before the first heat trial and continuing through the end of the experiment (Fig 4.4). As our study design didn't accommodate this method of administration, we did not quantify the amount of bait each pigeon ate from the feeder. However, based on monitored food intake data, it is likely that most birds did not consume an effective dose of NCZ (Fig 4.10).

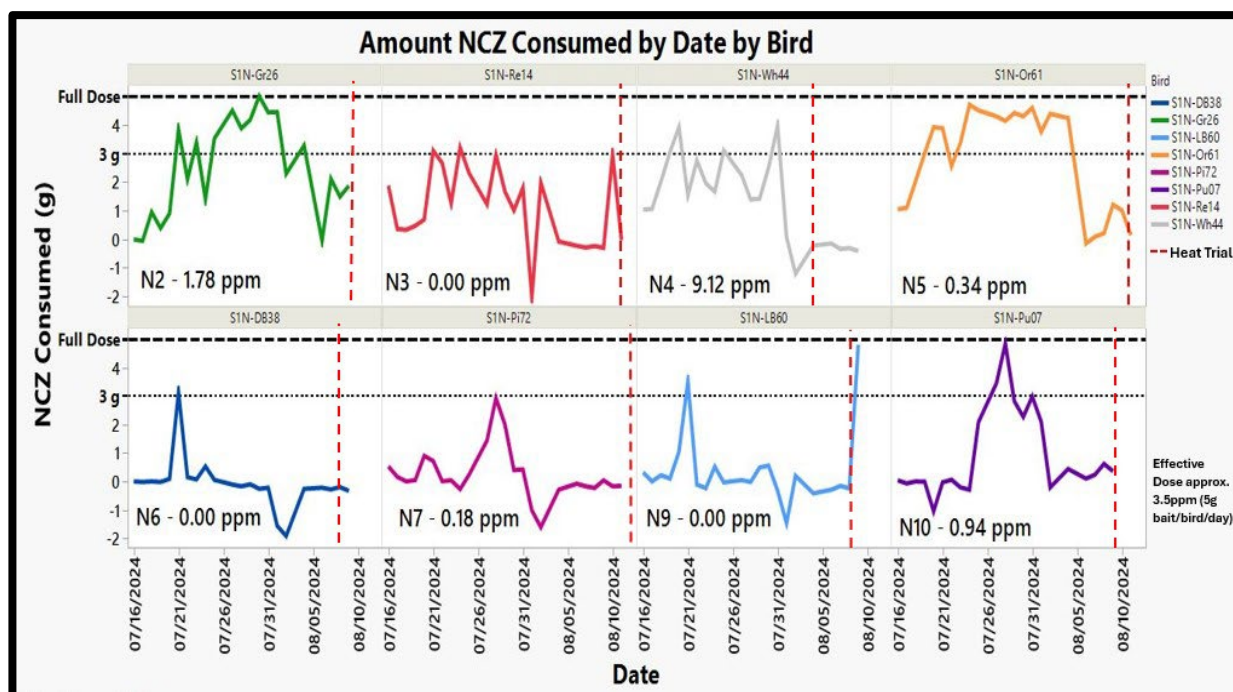


Figure 4.10: Amount of NCZ bait consumed by pigeons in experimental group with plasma NCZ levels/bird shown. The vertical dotted red lines indicate the date when each bird had its HT. The top dashed horizontal line represents 5 g of NCZ required for a full daily dose. The dotted horizontal line at 3 g is included as a reference. Plasma NCZ was analyzed from blood drawn during the HT. The plasma NCZ level required for an effective daily dose is approximately 3.54 ppm.

Plasma NCZ analysis: Of the eight NCZ-exposed birds who completed the protocol, only one bird had an effective dose (9.12 ppm) of NCZ in its system at the time of its heat trial. One bird had half an effective dose (1.78 ppm), and three birds had less than 1 ppm (0.94 ppm, 0.34 ppm, 0.18 ppm). The remaining 3 birds in the NCZ group and all of the control birds had no detectable levels of NCZ in their systems (Fig 4.10).

Video Observations: Video from the aviaries taken from the date NCZ bait was provided via the common feeder (CF) showed the NCZ birds immediately gathering around the CF when they were released from their nest boxes in the morning. When they found the NCZ bait in the CF, only a few birds ate while most, if not all, of the birds began pecking through the substrate around the CF looking for seed. Yet, when the bait in the CF was replaced with regular feed, all



Fig 4.11: NCZ pigeons eating regular feed. Photo taken immediately after researcher loaded CF with regular feed and quickly left the aviary. Every bird in the aviary is at the CF.

eight pigeons ate at once, regardless of the time of day this took place and regardless of their pecking order in their feeding hierarchy (Fig 4.11). This was observed daily throughout the time NCZ was available in the CF. (See Appendix 3 for notes about videos.)

## Discussion

Previous research showed that prolonged NCZ exposure reduced the ability of chicks to tolerate rapid rises in temperature (Beers et al., 1989). Our ultimate goal was to see how pigeons might cope with rapid-onset, high-heat events typical of the climate changes we are currently undergoing. We found that we could not adequately explore the relationship between prolonged exposure to NCZ and heat tolerance in pigeons because they did not choose to consume quantities of bait sufficient for contraception. This outcome was unexpected as there was ample evidence from laboratory and field studies showing that pigeons will readily eat the bait.

Even though feeding behavior of captive pigeons may differ from that of free-ranging pigeons, they had agency in both situations to make choices about what they ate. A feral bird knows where its preferred food stops are and can choose whether it eats at those stops rather than at an automated feeder serving contraceptive bait. Likewise, in our study, the pigeons could refuse to feed on NCZ bait and wait for other feed. In either case, if the most cost effective and least invasive method of administering this sort of drug is for pigeons to eat it voluntarily, it is crucial that the pigeons would want to consume it at least as much as their regular food. In trying to see if pigeons were more likely to consume bait as a community as per published field studies rather than in their individual nest boxes, bait was provided in the CF for several hours after they were released into the open run. Since each bird maintained or gained weight (fig 4.9) and remained in good health, the pigeons appeared to be making a choice: They'd eat the NCZ if they were really hungry, such as after a night's sleep, but they appeared more interested in eating regular seed. A major concern becomes *\*why\** didn't the pigeons consume all of their NCZ allotments. Five grams of bait is only 17% of their daily intake by mass, so consuming it would probably not satisfy the pigeons' hunger enough to keep them from foraging for more food. In our study, if they liked the bait, they would have readily consumed all that was on offer, probably while they were in their nest boxes overnight. Since they didn't eat bait that was available at sunrise, and they weren't going to be let out of their nest boxes for three hours after sunrise, it was reasonable to expect them to readily consume the bait in the CF. Our video data shows they did not, indicating the possibility of an issue with palatability of the bait.

We then focused on what role palatability, or lack thereof, may have played in any of the field-tested contraceptive baits from the studies done in Europe and North America. Studies done in Italy and Spain delivered NCZ via bait pellets mixed with corn kernels (Ovistop), both of which

were coated by a waterproof film. While most of the European studies showed a reduction of urban pigeon populations, questions of palatability were noted as a possible deterrent to consumption, regardless of the outcome in research (Giunchi et al., 2007; González-Crespo & Lavín, 2022b; Senar et al., 2021). Albonetti et al (2015) did not specifically discuss palatability, not surprising due to their strong evidence that Ovistop reduced pigeon populations by 65% - 70% over eight years. The difference between those 4 studies was the method of bait delivery—Ovistop was hand delivered in the Giunchi and Senar studies and mechanically delivered by automated feeders in the Albonetti and Gonzales-Crespo studies. However, a 2-year study done in Vancouver, B.C., Canada, using automated-feeder-distributed OvoControl bait, comprised of pellets without corn, did not find a significant decrease in pigeon populations between NCZ-exposed and non-exposed birds (Xenakis et al., 2023). Based on their observations, these authors raised the concern that low palatability may have impacted their results. This is important because Ovistop and OvoControl, while both having NCZ as the active ingredient, have different formulations. Wolf and MacDonald (2017) posited that Ovistop is less palatable than OvoControl because the amount of NCZ in Ovistop recommended (10 g of bait to deliver 800 ppm) by the manufacturer (ACME Drugs S.r.l.) is higher than the dose recommended by Innolytics, the manufacturers of OvoControl (5 g of bait to deliver 5000 ppm). The differences in dosage mean that pigeons exposed to Ovistop must consume more bait/day than those exposed to OvoControl, making the pigeon consume more of a drug that pigeons and other avian species have been shown to be averse to eating (J. J. Johnston, 2002; VerCauteren & Marks, 2002). As part of a study that showed a 55.26% decline in urban pigeon population over 3 years, González-Crespo & Lavín, (2022) did a 2-year palatability study with Ovistop and found that there was no difference in bait consumption between the OviStop-exposed and non-exposed groups. They

argued that providing the feed by automated feeder from the same locations at the same time on a daily basis was enough for the pigeons to consume an effective dose. Because these are field studies, the authors acknowledge that the reduced number of pigeons in their final census counts cannot definitively prove whether some of the absent pigeons aren't getting their food outside of the observation areas. No solution to the palatability concern is suggested beyond offering either brand of bait from automated feeders at uniform, predictable daily times for longer periods of time (i.e. months or years). In discussion with Innolytics, it was suggested that the user sprinkle finely ground salt on the bait prior to dosing. Clearly, making the bait more palatable to pigeons is critical to getting them to eat enough bait to make a difference. We suggest that we can more accurately assess physiological and behavioral effects of prolonged exposure to NCZ once we overcome this considerable challenge.

In looking to assess the impacts of NCZ and heat, we found that pigeons showed significantly positive correlation between HMBs and rapid increases in ambient temperatures. This is an important finding in that previous studies focused on the mechanisms of how captive-bred laboratory pigeons thermoregulate when acclimated to low-humidity, desert-level heat (Marder & Arieli, 1988; Marder & Gavrieli-Levin, 1987). Our non-lethal study's focus was on how feral pigeons living in cooler urban areas manage high heat weather conditions in their natural habitats—habitats with record maximum temperatures not exceeding 40° C (*Highest Temperatures in Boston History*, n.d.). HTs were run during the third hottest summer on record in Boston, MA (*Assessing the U.S. Climate in 2024, 2025*). Because the pigeons were housed in outdoor aviaries, they could be categorized as heat-acclimated, yet their HMBS increased as the ambient temperatures in the HTs increased. Based on the Marder and Gavrieli-Levin (1987) and the Marder and Arieli (1988) studies, we would have expected to see no significant increase in

HMBs. This is a critical outcome to consider as we continue to use NCZ for pigeon population control.

## **Conclusion**

Our study sought to fill the gap of knowledge of what effect prolonged exposure to chemical contraception in combination with rapid ambient temperature increases had on feral urban pigeons. We could not adequately determine what those effects were due to low bait palatability, an unexpected issue based on several field studies. However, we created an assay by which the effects of an experimental variable in conjunction with changes in ambient temperature can be measured. Based on this assay, we were able to show that pigeons exhibit heat-mediating behaviors at lower temperatures than expected. We also modified an existing assay to process lower sample volumes of plasma thereby minimizing the quantity of blood necessary for the quantification of systemic NCZ levels using an internationally validated LC-MS method of analysis. We strongly recommend increasing the palatability of bait-delivered NCZ to not only allow further studies on behavioral and physiological impacts on pigeons, but also to see whether improved palatability could produce faster and therefore more user-friendly population reduction outcomes. Further research into how pigeons in their natural habitats thermoregulate during prolonged high-heat events would contribute to a better understanding of how NCZ might alter pigeons' thermoregulatory processes. This is an important consideration when creating an effective method of pigeon population control in urban centers. Balancing pigeons' natural social and reproductive behaviors and physiology with the benefits and side effects of NCZ exposure can improve the effectiveness of chemical contraception, an important method to ease wildlife-human conflicts in urban areas.

## **Acknowledgements**

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## Chapter 5. Conclusion

In addressing the broad challenge of managing urban pigeon populations, it makes sense to consider as many consequences as possible of administering a chemical contraceptive from the point of view of all stakeholders, including and especially the pigeons'. When taking on such a large, complicated task, it is practical to undertake the work in small, manageable steps. That was the intent of the series of studies in this dissertation. Part of treating pigeons as important stakeholders was to look for ways to conduct studies using the least invasive techniques possible or to minimize the need for invasive methods of sample collection. For example, as part of our study examining possible effects of NCZ and heat, we drew blood samples during the HTs in order to confirm if there were effective levels of NCZ in the pigeons' circulatory system. This presented a confounding variable in our behavior assessment which we addressed by not including data points occurring immediately after a blood draw. Eliminating this disruption in HTs therefore is an important improvement for future studies.

A reasonable way to develop less invasive experimental designs is to explore what the data produced by existing, relevant technology can tell us. The first study described in this dissertation involved using an assay developed to extract cort from sparrow feathers in order to determine whether: A. feather cort levels matched the plasma cort levels when plasma samples were taken at the time feathers were removed from the bird's body; and B. feather cort levels were altered by erosion of the feather edges during the time the feather is on the bird's body. The fact that molting (growing) feathers are vascularized, then are cut off from the blood supply of the bird means that the feather is a storehouse of physiological information gathered during the

approximately 2 weeks a feather is vascularized. If those data can be extracted, life events the bird was experiencing during the time of feather vascularization could be correlated with diet, hormone levels, or any other molecular signal circulating through the bird's body at the time of molt (De Bruijn et al., 2020). However, unless we know which bird the feather came from and when the feather was molted, it is difficult to put the resulting data into proper context.

Therefore, in the case of verifying whether a pigeon has an effective dose of NCZ in its system on a day-to-day basis, a feather assay, a relatively non-invasive method of analysis, is not the best technique to use.

It may be possible, however, to analyze pigeon excreta, providing an opportunity for a method less invasive than blood-sampling of accessing physiological data and more sensitive to short-term biomarker changes. Pigeons excrete 11 g – 26 g of waste per day with voiding occurring approximately 3 – 5 times a day via their cloaca (Spennemann & Watson, 2017). Cloacal fluid, or fluid excreted via the cloaca, is composed of feces, uric acid, urates, water, and mucus (Styles & Phalen, 1998). The cloacal fluid can be assessed via proteomic analysis. A proteome is defined as a complete set of proteins expressed by an organism, or an assortment of proteins produced at a particular time within a particular cell or tissue type. An organism's proteome changes in response to internal and external factors (Nature Education, 2014). A recent study of proteomic analysis of cloacal fluid from European barn swallows (*Hirundo rustica rustica*) revealed differences in protein levels prior to and after egg laying (Míčková et al., 2024). Since this analysis is sensitive to short term physiological changes, it is a promising technique to adapt to perform an in-field test to see if a pigeon exposed to NCZ has an effective dose in its system.

Designing and building a satellite research facility involved a great deal of planning. Having a clear vision of the finished structures is critical to keeping costs down. For example, the cost of

purchasing lumber to use for support beams, framing for hardware cloth, and door construction can be prohibitive. Since we knew exactly what was needed, we used pallets left behind a local hardware store and a vintage-car remodeling warehouse for those purposes. The time investment in pulling the pallets apart was considerable, but the money saved there allowed us to purchase eco-friendly paints and supplies, some of which were quite expensive. Protecting the pigeons from any fumes or off-gassing from the wood we used for nest boxes, walls and flooring was the highest priority. Once they were in the aviaries, the pigeons would not be able to fly away from any noxious odors. Good pre-planning also allowed us to look around for supplies that were not made with pigeon coops in mind. We used empty plastic cat litter containers for cleaning buckets. Inexpensive plastic paint scrapers were the best tools to use in scraping feces off surfaces in the aviaries. All the masonry used for the aviary and shed foundations were found on Craig's list and the only cost was the gas it took to go pick the bricks and pavers up. We found lightly used baby bottle sanitizers on Facebook marketplace which we used to sanitize feeding cups. We continually asked for suggestions and guidance from hardware store employees, pigeon fanciers, avian rehabilitators, veterinarians, or other people skilled in areas in which we were not comfortable. We also took advantage of being affiliated with Tufts University and the Biology department for help with either borrowing, sharing, or purchasing lab equipment. Fellow grad students, faculty, and department administrators generously shared their time and expertise.

Having created the facility and receiving approval from IACUC, we conducted our experiment to determine whether a rapid increase of temperature would have an impact on how an NCZ-exposed pigeon thermoregulates as compared to a non-exposed pigeon. While we were prepared for things to not work out exactly as planned, the pigeons' lack of interest in consuming the NCZ-treated bait was the biggest challenge we encountered. There was sufficient evidence in the

literature to suggest that pigeons would eat the bait once they were acclimated to it (Albonetti et al., 2015; Avery et al., 2006; González-Crespo, 2023). That was not the case with our pigeons in spite of our trying different feeding strategies to address this issue. Even though this was not the finding that the study was designed to investigate, it was an important one. Although previous field studies considered that low palatability may have played a part in the outcomes (Senar et al., 2021; Xenakis et al., 2023), researchers could not be definite about that due to the number of pigeons observed at the feeders and the inability of the researchers to see what pigeons did when they were out of view of the cameras. The size of our facility and our experimental design allowed us to observe how the pigeons interacted with the bait. Increasing the attractiveness of the bait could increase the effectiveness of the treatment and possibly result in faster reductions in pigeon populations. Ultimately, this outcome supports our claim that this intermediary, semi-controlled test can increase the likelihood of a successful application of a treatment in the field. Our efforts to counteract the low palatability of the bait increased the length of the study by at least two weeks. While this was frustrating, it gave us more time with the pigeons. We got to know them as individuals and as two flocks. We gained valuable hands-on experience in how to interact with them and how to interpret their behaviors. We also had the opportunity to observe first-hand a complete breeding cycle. In the control group, two males competed for a female. The males didn't engage with each other as much as they engaged with the female. The successful male placed straw in all the nest bowls in the boxes on the upper shelf. Although we did not want to interfere with this set of behaviors, we decided to remove the straw from three of the five nest bowls. The bowls we left alone were in the nest boxes originally assigned to the bonded pair. Even though this pair had bonded, we continued to secure them in their own nest boxes at night. However, we put their two cages next to each other so that the pair could see and hear each other

through the handle holes cut out of the sides of the nest boxes. When the female laid two eggs, she and the male both spent time brooding during the day, but the female was secured in her nest box with the eggs overnight while the male was secured in his. We removed the female from the protocol at that point. Discovering that the squabs had hatched was a high point for all of us. We then had the experience of watching the squabs mature. One squab developed a blue-bar plumage pattern like the female and the other squab became a check patterned bird like the male (*Pattern, 2025*). As the squabs had not fledged by the end of the last heat trial (August 12, 2024), we decided to keep them as a family unit till they did. All the other birds were taken back to the place of capture and released on August 13, the day after the last HT. In order to maximize the squabs' chances of survival in the wild, we kept them for one full week after the day they were able to fly down from their nest box to the common feeder and fly back up again. Fledging squabs tend to return to their nest rather than to their parents ((R. F. Johnston & Janiga, 1995) which posed a problem in that, when these birds would be released, the squabs would have no nest to return to in their new home. We released the family on September 6, 2024. We scattered generous amounts of seed on the ground to attract the colony. Several pigeons began to eat the seed. The mother and father swiftly walked out of their transfer cage and joined the colony. The squabs walked out of their cage almost immediately when the door was opened but went right back in after several seconds. After a few minutes spent watching the colony feeding together, the squabs walked over and joined the flock. Several minutes after that, something startled the feeding birds and they all launched into the air at the same time. No birds remained on the ground, indicating that the squabs had taken off with the colony. That was confirmation that the squabs were engaged in their new community. We left after that. Even though that was the best moment of the study, going forward it would be best to remove any eggs that are produced by

feral pigeons in a study. As our birds had been trapped in early June, it was likely that one or more of the females would lay eggs during the course of the experiment. It was random chance that only one pigeon produced eggs during our study. It was serendipitous for us that we could observe an entire breeding cycle.

Other alterations to our experimental design were suggested by our study. A major adjustment would be to change which HMBs are included in future scoring assessments for HTs. We recommend removing whether a pigeon is lying down or standing as pigeons may be thermoregulating by releasing heat cutaneously through the skin of their legs and feet. Even though we observed pigeons in our pilot study display thickened eyelids and slower blinking as the HTs progressed, we would seriously consider removing this behavior from the scoring protocol. This behavior indicated that the pigeons were mediating a stressor, but it is not well-documented in previous literature and therefore may not be indicative of just an HMB. Going forward, we would include counting behaviors like feather fluffing (feathers positioned away from the body) and leg stretching. These behaviors have been reported as HMBs in earlier studies. We could also include behaviors like gulping and swallowing, as these may be precursors to panting and gular fluttering.

Our study was impacted by having to handle the pigeons for up to several minutes to get blood samples to determine plasma NCZ levels during our HTs. This metric could be obtained via one blood draw at the end of the HT thus removing the need to handle the bird during the HT. By exploring how pigeons shed waste, or even lose body heat, we could find ways to make more accurate assessments of internal processes without having to draw blood. If an NCZ-exposed bird has a perch it frequents and it drops feces on its perch, a researcher can harvest the feces and run proteomic analyses to find possible differences in certain protein levels from non-exposed

birds. These data could be an urban manager's way of checking to see that pigeons are consuming sufficient doses of NCZ and could then reasonably expect fewer hatchlings. Further, a way to check on the health of urban populations involves using infrared thermography, which is a type of camera that detects heat loss and produces photos using pixel coloration scaled according to pixel temperature ( $^{\circ}\text{C}$ ). Researchers showed that birds dissipate heat through their beaks and that thermography can detect and quantify it. Would application of this technology show differences between NCZ-exposed and non-exposed birds?

Another technique that could be adapted is extraction of DNA from fecal matter (Eriksson et al., 2017). This study is a methods paper showing that kits can be made to extract DNA from the feces of several bird species which can be used to assess bacterial detection. If successfully adapted, would this technique allow determination of sex without having to draw blood?

Harvesting DNA in this manner can also assist in creating computer models based on allele frequencies which can suggest testable hypotheses. For example, I am collaborating on a project with Lawrence Uricchio to create a model predicting how pigeons' plumage coloration may shift over time in a warming environment.

An obvious next step in our NCZ-exposure research is to address low bait palatability. It would be interesting to see if improved palatability would induce pigeons to consume more NCZ-laced bait. In turn, would pigeons' reliably consuming an effective daily dose of bait reduce the time it takes to see decreases in feral pigeon population compared to previous studies? If the pigeons could be shown to have sufficient levels of plasma NCZ, would a difference in thermoregulation between treated and untreated birds exist? Would we uncover differences in HMBs between males and females if we increased our sample size? Would NCZ exposure amplify such a difference? Answers to those questions considered in light of feral pigeons' social structure and

reproductive cycles could assist urban managers create feasible pigeon population management plans. For example, if prolonged NCZ exposure does reduce a pigeon's ability to safely tolerate high heat, and if pigeon counts unique to a specific urban location indicate a reduced egg production in July, an urban manager might consider reducing or ceasing amounts of NCZ bait distributed between the end of June and the end of August.

Another question to be explored is what impact will prolonged exposure to a contraceptive have on pigeons' pairing behaviors? An experienced feral pigeon rehabilitator (in conversation) has seen mating bonds broken after 3 breeding cycles producing no hatchlings. In other words, birds who produce no hatchlings for 3 consecutive breeding cycles leave their mates and re-partner. That brings up questions about the value of pair bonding when there are no parenting demands. Murton et al. (1972) showed a difference in how non-breeding males and females occupy their habitat differently than breeding males and females. These questions present intriguing opportunities for further studies.

In conclusion, this body of work demonstrated the value of designing and running non-lethal research prioritizing the well-being of the subjects of the research—feral urban pigeons. In trying to assess the impact that prolonged exposure to NCZ, a chemical contraceptive, may have on the physiology and behavior of feral pigeons, we found that low palatability of the NCZ-laced bait kept the pigeons from consuming an effective dose, providing practical guidance for future research assessing the effectiveness of this promising method of pigeon population control. This research was conducted in a satellite research facility designed and built to be easily run and maintained by minimal staff. The scale of this facility allowed for close observation of feeding behaviors of the pigeons as they were presented with the bait. We also describe a non-lethal heat assay which was an only moderately stressful method of exposing subjects to controlled

temperatures and humidity in order to assess behavioral and physiological outcomes exhibited by pigeons exposed to experimental treatments. This work will help fellow wildlife welfare researchers continue to ease the pigeon-human conflict and also promote a better understanding of the value of sharing living space with a being of remarkable resilience, and intelligence—feral urban pigeons.

# Appendix - 1: Nest box Cutting Layout



## Appendix – 2: LC-MS specifications

LC-MS/MS Method for determination of nicarbazin active ingredient in pigeon blood.

The LC-MS system used was PerkinElmer QSight 110, with LX-50 solvent delivery module.

LC Method:

Column: PerkinElmer Quasar C8, 3X50 mm, 3  $\mu$ m, Cat. Number N9308888.

Column temperature: 40°C

Mobile Phase A: 1000 mL H<sub>2</sub>O, 1.0 mL FA and 0.38  $\pm$  0.04 g NH<sub>4</sub>OAc.

B: To 1000 mL MeOH, 1.0 mL FA and 0.38  $\pm$  0.04 g NH<sub>4</sub>OAc

Flow rate: 0.2 mL/min

Gradient:

Time, min	Mobile Phase A, %	Mobile Phase B, %
0	100	0
1	100	0
2	100	0
10	0	100
12	0	100
13	100	0
20	100	0

Sample temperature: 20°C

Injection volume: 20  $\mu$ L

Injection mode: Full loop

Flow selector valve: 0 – 2 min: waste, 2 – 10 min: MS, 10 – 20 min: waste

MS Method:

MS Polarity: Negative

Drying gas: 100

HSID Temperature: 200°C

Nebulizer gas: 200

Electrospray voltage: – 5000 V

Source temperature: 60°C

MRM Table:

N	Name	Type	Q1 Mass	Q2 Mass	Dwell time, ms	Resolution (Q1:Q2)	EV	CC L2	CC
1	DNC_Q	Quantitative	301.00	136.70	150	Unit Unit	-34	32	144
2	DNC_R	Qualitative	301.00	106.90	100	Unit Unit	-28	52	144
3	DNC_D8_Q	Quantitative	308.70	140.60	150	Unit Unit	-28	52	144

Scan rate: 0.4 sec/scan

Acquisition window: 2 – 10 min

Data processing: Was performed in Simplicity 3Q software from PerkinElmer.

### **Appendix – 3: Chapter 4 Supplemental data: Feeding behavior videos**

The collection of clips shows behaviors demonstrated by the experimental group (NCZ) pigeons surrounding the administration of NCZ and regular feed. These clips begin on the first day NCZ was offered in the common feeder and run till the penultimate day of the HTs. Each clip is labeled with the date video was taken, and the event of interest are:

- Release of pigeons from their nest boxes,
- Their behavior upon being released
- Their behavior when NCZ is dispensed via the common feeder
- Their behavior when the NCZ in the feeder is replaced with regular seed.

These data are offered in support of our observation that pigeons do not readily consume NCZ bait possibly due to low palatability.

Noteworthy items:

There are no videos for August 3, 2024, and August 5, 2024. For the safety of the birds and the researchers, we did not return the pigeons to their nest boxes on the nights before those dates due to extreme high heat and humidity.

HTs began on August 2, 2024, and with the exception of Aug. 3 and Aug. 5, were run daily until Aug 12, 2024.

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