ABSTRACT

HARTLEY, SUZANNE MARIE. Sparrow Swap: Testing Management Strategies for House Sparrows and Exploring the Use of their Eggshells for Monitoring Heavy Metal Pollution. (Under the direction of Dr. Caren Cooper).

Human movement across the globe, particularly through colonialism throughout the last 500 years, has led to the introduction of species into novel environments where they threaten the biodiversity and ecosystem functioning of those novel environments. In the Anthropocene where other threats such as climate change, pollution, and habitat destruction already occur, invasive species are just one more threat facing ecosystems. But what if we can find a way to use an invasive species to help monitor those other threats while at the same time managing them?

In the following thesis I explore the strategies by which volunteers manage House Sparrows to minimize their negative impact as an invasive species, but also the potential to use their eggs as indicators of heavy metals in the environment. House Sparrows compete with native birds for nesting spaces. They are also commensal with humans, utilizing buildings as nesting spaces and split grains and forgotten French fries as food sources. In order to 1) find effective management strategies for House Sparrows and 2) evaluate their use as indicators of environmental contaminants, a citizen science project Sparrow Swap was created. Sparrow Swaps takes advantage of the ubiquity of House Sparrows and the expertise of volunteer nestbox monitors to gather data about House Sparrow nesting behaviors and eggs across the United States. In Chapter 1, I address the first research goal of Sparrow Swap by comparing the outcomes of two different strategies by which volunteers manage House Sparrows. In Chapter 2, I explore potential links between heavy metal concentrations and morphological characteristics in House Sparrow eggs.

Sparrow Swap: Testing Management Strategies for House Sparrows and Exploring the Use of their Eggshells for Monitoring Heavy Metal Pollution

by
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DEDICATION

To the citizen scientists of Sparrow Swap that made this project possible.

BIOGRAPHY

Suzanne Hartley grew up on a farm in Lancaster County, Pennsylvania. Her love of nature manifested in a love for conservation as a teenager when she had the opportunity to be part of a month-long educational canoe trip with the Chesapeake Bay Foundation. Inspired to learn more about ecology, she completed a Bachelor of Science in environmental biology at Millersville University of Pennsylvania in 2013. During her time as an undergraduate she got her first exposure to research through the Cornell University’s Entomology Summer Scholar program at the New York Agriculture Experimental Station where she studied an invasive cranefly and parasitic nematodes. Following graduation, she spent two field seasons in Alaska as a research technician at the University of Alaska Fairbanks working on a project exploring the food webs in a large glacial river. In addition to her studies, she is passionate about by fostering wonder and love of nature in people of all ages.

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I would also like to thank the Public Science Cluster at NC State for creating an academic environment valuing scholarship and practice in public engagement, science communication and citizen science. The project also wouldn't have been possible without my committee members, Dr. Daniel Hanley, Dr. Catherine LePrevost, and Dr. Robert Dunn. I would also like to thank my committee chair and advisor Dr. Caren Cooper for taking a chance on a graduate student who knew little about birds, citizen science, or contaminants when starting this project. Finally, I would like to thank my fellow graduate students, friends, and family whose support made this project possible; in particular Nate Hartley, Carson, Maria Sharova, and Eli Fry.

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CHAPTER 1

FOOLING THE BIRDS: SWAPPING HOUSE SPARROW EGGS AS A MANAGEMENT STRATEGY

ABSTRACT

House Sparrows (*Passer domesticus* L.) are a non-native, invasive species in the United States that compete with native cavity nesting birds for nestboxes. In addition, House Sparrows are extremely aggressive and have been known to kill and injure eggs, young, and adult native birds. Volunteer nestbox monitors employ diverse management strategies to limit any competitive advantage of House Sparrows over native birds. However, little is known on the effectiveness of these management strategy. One common strategy is removal of the nest and eggs upon discovery. However this may lead to aggression and harassment by House Sparrows towards native species. Instead, swapping real House Sparrow eggs with fake egg replicas may protect native species. The purpose of this study is to determine who uses a nestbox and how long it takes for a nesting activity to occur in a nestbox following a swap or removal. We used a citizen science approach, through a project called Sparrow Swap to engage songbird nestbox monitors across the United States in swapping and/or removing clutches of House Sparrow eggs and reporting their observations on subsequent visits to those nest sites. Our results indicate that removing is associated with high-intensity monitoring with frequent removals to keep nest boxes open for native species. Comparatively, swapping is associated with low-intensity monitoring with occasional swaps to placate house sparrows and keep neighboring boxes open, potentially lower House Sparrow recruitment and with potentially lower artificial selection pressure on House Sparrows to nest faster.

INTRODUCTION

When considering invasive non-native species and their impact on biodiversity and ecosystems, attention is often focused on plant, invertebrate, fish, and mammalian taxa (Martin-Albarracin et al. 2015). However, non-native avian species can also be considered pests with serious impacts on native taxa. Most invasive avian species were intentionally introduced as pets, game, and biocontrol as part of colonialism (Cassey et al. 2004). For example, House Sparrows (*Passer domesticus* L.) have been closely associated with humans for over 6,000 years (Ravinet et al 2018) but were introduced in misguided attempts for biocontrol and by nostalgic Europeans to the continents and surrounding islands of North America, South America, and Australia in the last 200 years (Anderson 2006, Robbins 1973). House Sparrows are an aggressive, non-migratory bird species. In many parts of the world, House Sparrows are considered a pest for damaging agricultural crops (Anderson 2006), transmitting parasites and diseases to livestock (Hoyle 1938), serving as reservoirs for human diseases (Marra et al. 2004), blocking ventilation (Fitzwater 1994), and competing with other native birds species (Gowaty 1984).

In their native range throughout Europe, the Mediterranean coast, and parts of Asia, House Sparrows effectively use aggression to compete for nesting cavities against other species, such as Great Tits (*Parus major* L.) and Eurasian Tree Sparrows (*Passer montanus* L.; Barba and Gil-Delgado 1990). In Israel, House Sparrows outcompete Great Tits by occupying cavities that could have been used by Great Tits and by usurping Great Tit nests (Goldshtein et al. 2018; Charter et al. 2013). In Spain, Cordero and Senar (1994) found that House Sparrows had a clear advantage during encounters with Eurasian Tree Sparrows. House Sparrows won every encounter they initiated, with 9% of encounters leading to fights that had a high risk of leaving the Eurasian Tree Sparrows injured. Eurasian Tree Sparrows can mitigate the aggression by nesting later, after House Sparrows have finished their broods (Cordero and Senar 1994). Outside of their native range, House Sparrow aggression can impact the reproductive success of locally native species (Zeleny 1978).

House Sparrows were introduced to the United States in the early 1850's and have since spread across the country and throughout North America. As an invasive non-native species, House Sparrows have a competitive advantage against Barn Swallows (*Hirundo rustica* L.; Weisheit and Creighton 1989), Tree Swallows (*Tachycineta bicolor* Vielliot; Ghilain and Bélisle 2008), and Eastern Bluebirds (*Sialia sialis* L.; Gowaty 1984), Mountain Bluebirds (*Sialia currucoides* Bechsteinand; Frye and Rogers 2004) and Western Bluebirds (*Sialia mexicana* Swainson; Fiehler et al. 2006). The impressive diversity of songbird species that House Sparrows have been documented to outcompete has contributed to their notoriety as an invasive pest species by native songbird conservationists. Unlike most native species which produce 1-2 broods per year, House Sparrows typically have double that, with 3-4 broods per year (Anderson 2006). Zeleny (1978) detailed four reasons that make House Sparrows problematic for small cavity nesting songbirds. First, while most other avian competitors, such as European Starlings (*Sturnus vulgaris* L.), can be excluded from nest boxes by reducing the size of the entrance hole, House Sparrows are smaller than each species of bluebird and therefore cannot be restricted from nestboxes intended for bluebirds by the reducing entrance hole diameter (Zeleny 1978). Second, House Sparrows are not obligate cavity nesters and therefore can occur at higher densities than obligate cavity nesters (von Post and Smith 2015, Zeleny 1978). Third, as a non-migratory species, House Sparrows have a temporal advantage over native migratory songbirds in finding and defending nesting sites (Zeleny 1978). Fourth, House Sparrows are extremely aggressive, particularly the males (Cordero and Senar 1994). Nestbox monitors have observed House

Sparrows usurping the nests of native species by removing, injuring, and killing nestlings; injuring and killing adults; and pecking or removing eggs (Gowaty 1984, Larson et al. 2015).

In response to House Sparrow competition with native birds, nestbox monitors have tested a wide range of management strategies for preventing House Sparrows from occupying and reproducing in nestboxes (Table 1.1). While most avian species in the United States are protected by the Migratory Bird Treaty Act of 1918, House Sparrows' invasive non-native status make them exempt from this law. Current sources of recommendations for deterring House Sparrows can be found on websites operated by bluebird interest groups (e.g., North American Bluebird Society website, www.sialis.org) and books on monitoring bluebird nestboxes (Berger et al. 2001, Stokes 2007). The North American Bluebird Society (NABS) classifies management strategies as either passive or active (NABS 2012). Passive strategies refer to various non-lethal actions that create undesirable conditions for House Sparrows, such as "spookers" on nestboxes or in the case of two-hole nestboxes, allow for native birds to escape through an alternative entrance (Berger 2001). Active strategies refer to lethal disposal of eggs, young, and adult sparrows by a variety of methods, with the exception that nest removal is considered a non-lethal but active form of House Sparrow management (NABS 2012). However, there is limited research on the effectiveness of both the passive and active management strategies.

The gap in research on effective management strategies for House Sparrows may contribute to volunteer management decisions driven by emotions and desires for retaliation. When volunteers have first-hand encounters with the consequences of House Sparrow aggression towards bluebirds, the experience has been an important determinant in their choice for lethal management (Larson et al. 2015). In addition, emotional dispositions such as feelings of anger, hate, and disgust, were strong drivers of lethal House Sparrow management by volunteer nestbox monitors. Overall, most volunteer bluebird monitors agree that monitors are obligated to undertake some sort of management (passive or active) rather than no management at all, but there is debate over which management strategy is best (Larson et al. 2015). Given that nestbox monitors across the country will continue to engage in wildlife management for this nuisance species, research is needed to determine which management strategies are effective in minimizing House Sparrow reproduction and damage to native birds.

Research addressing House Sparrow management may benefit from taking a citizen science approach. Management strategies also need to work within the current context that management is done mostly by volunteer nestbox monitors. House Sparrow management is not done by government agencies or large organizations, but instead by thousands of individuals or small groups working on disjunct bluebird trails. A citizen science approach, engaging the nestbox monitors in research on management, is a way to ensure that management strategies work in applied field contexts vs a traditional scientific study that does not adequately incorporate the complexities of both biological systems and human behavior in management. For a management strategy to be effective and sustainable, it needs to be adopted by the nestbox monitors and songbird advocacy groups that are currently doing the management. A citizen science approach engages those groups at the beginning of the research and provides essential information on the practical and applied challenges and solutions a management strategy can provide. A citizen science approach may also provide a volume and breadth of data that can address trends in management better than individual and isolated sites.

The lack of scientific studies on the efficacy of avian pest management is particularly problematic given that past research on nuisance wildlife management has shown that management strategies can often be ineffective or have unintended consequences (Warburton and Norton 2009). For example, egg removal is a common management strategy used to control many avian pest species. However, Jacquin et al. (2010) found Rock Pigeons (*Columba livia* Gmelin) increased their reproductive efforts in responses to repeated egg removals. One unintended consequence reported with House Sparrow management has been the death of native birds. Songbird nestbox monitors have reported that following egg removals, House Sparrows, particularly males, will attack and kill native songbirds nesting nearby (Gowaty 1984). There may be a difference in unintended consequences between removing both the egg and nest versus removing only the eggs. When removing eggs and nest, there may be a higher rate of abandonment of the nestbox, leading pairs of House Sparrows to look for another nesting cavity potentially creating conflicts with native species. In comparison, with egg only removal, the pair may continue to occupy and renest the same nestbox without disturbing native birds nesting nearby.

An alternative to removing House Sparrow eggs is to addle them: rendering the House Sparrow eggs nonviable and returning them to the nest (Baker et al. 1993). The objective of this method is to occupy the pair's resources in defending and incubating a clutch of eggs that will never hatch. To accomplish this, nestbox monitors first addle the eggs by boiling, cooling, piercing, shaking, or oiling eggs. Similar methods have been done for the management of Canada Geese (*Branta canadensis* L.; Baker et al. 1993), Australian white ibis (*Threskiornis molucca* Cuvier; Martin et al. 2007), Herring Gulls (*Larus argentatus* Pontoppidan; Blackwell et al. 2000), and Double-crested Cormorants (*Phalacrocorax auratus* Lesson; Shonk et al. 2004), but this strategy has not been studied in House Sparrows. Compared to egg removal, leaving nonviable eggs in the nest may prevent a House Sparrow from immediately attempting another brood and/or disturbing native birds nesting in the vicinity.

While addling eggs can be effective, there are instances where shaken, pierced, or boiled eggs have hatched (Baker et al 1993, Martin et al. 2007). An alternative to addling House Sparrow eggs is to replace the eggs with artificial egg replicas (referred to as swapping). House Sparrows have a poor ability to distinguish their own eggs from that of another bird and are likely to accept the replicas as their own clutch (Manna et al. 2017). By replacing real eggs with replicas, females may spend vital resources incubating a clutch of replicas that will never hatch, thus lowering the frequency with which they reproduce and/or disturb native birds.

The effectiveness of swapping House Sparrow eggs with replicas has not been previously explored in scientific literature. In order for swapping to be effective at reducing the reproductive output of House Sparrows it is necessary for the female to incubate the egg replicas for at least the length of a typical incubation period, and ideally the time span it would have taken for the young to fledge (Blackwell et al. 2000). House Sparrows generally hatch after about 11 to 12 days of incubation starting after the last egg in the clutch is laid (Anderson 2006). After hatching, young House Sparrows fledge at around 14 days (Anderson 2006). If the egg replicas can keep the adult House Sparrows attending the nest for more than 25 days, then the pair would have lost the potential for an entire brood for the season.

We created a citizen science project called Sparrow Swap (Appendix 1.1) to answer the following questions:

1. What species uses the nestbox following egg swaps and removals?
2. Does swapping delay a House Sparrow from nesting in the same nestbox?

METHODS

Study System

Sparrow Swap is a citizen science project that engages nestbox monitors from across the United States to participate in scientific research surrounding House Sparrow management. After a pilot year (2015) with Master Naturalists in Virginia, Sparrow Swap was expanded nation-wide from 2016-2018. In February 2016, 2017, and 2018 the Sparrow Swap Team sent an informational email to bluebird societies, ornithological societies, and Audubon chapters inviting interested nestbox monitors to participate in Sparrow Swap. In order to participate, volunteers signed up for the project via scistarter.org, a website designed to aggregate and run citizen science projects. Once joining and agreeing to the informed consent (Appendix 1.2), volunteers were able to download field protocols and datasheets for both a Swap (Appendix 1.3) and Removal (Appendix 1.4). We, the Sparrow Swap Team, then mailed hand-painted wooden House Sparrow replicas to volunteers interested in swapping eggs (Figure 1.1). We purchased 7/8-inch wooden eggs from American Woodcrafters Supply Company. To make the wooden replicas resemble House Sparrow eggs, a base coat of white acrylic paint was spray painted onto the wooden replicas followed by a wash of blue-grey acrylic paint. To create the speckling, reddish-brown acrylic paint splattered onto the eggs with a toothbrush.

Management of House Sparrows

When a volunteer found a nestbox with a House Sparrow (HOSP) nest, the volunteer could choose to remove or swap the House Sparrow eggs. For both swapping and removing, we instructed volunteers to wait until the female had finished laying the entire clutch. During egg-laying, female House Sparrows lay one egg per day ioto attain a clutch size of 3–6 eggs (Anderson 2006). Once the clutch was completed, we asked volunteers to perform the chosen management strategy (swap or removal) and record the date, nestbox location, and if they saw the adult birds(s). For a removal, volunteers indicated whether they removed both the nest and eggs, or only the eggs. If a volunteer swapped, they were instructed to warm the replicas in their hands before swapping them into the nest. The volunteers then replaced the real House Sparrow eggs with an equal number of wooden egg replicas.

Follow-up Visits

In 2016, the Sparrow Swap Team asked volunteers to return to the nest for two follow-up observations approximately seven days apart to determine the outcome of the management strategy. In 2017 and 2018, the Sparrow Swap Team asked volunteers to return to the nestbox for an additional follow-up visit. At each follow-up, volunteers recorded whether they thought the nest was attended or abandoned, if they saw House Sparrows during the visit (flushed or nearby), and what was in a nestbox. For a removal, they were asked whether the nestbox contained nest material, eggs, or nestlings. For swaps, volunteers were asked to count the number of replicas and whether there were any real eggs. To assess whether the eggs were being incubated, participants were also asked three questions. First, volunteers were asked whether the replicas felt warm to the touch. Second, they were asked to take a photo of the replicas on each visit to determine whether the replicas had been rotated since the prior visit, indicating that an adult had been present since the last visit. Third, volunteers were asked to place a small object (e.g. a piece string) across the replicas to determine on the following visit if an adult bird had visited the nest. For both swaps and removals, if another clutch (native species or House Sparrow), was laid, the observations for that initial clutch were considered completed. The outcomes of the swaps can be summarized into the categories listed in Table 1.2. For all three management strategies we classified the outcome of each House Sparrow clutch as Native Nest, House Sparrow Nest, House Sparrow Attended, Abandoned, or Inconclusive.

Statistical Analysis

We removed the 2015 Sparrow Swap data from our analysis due to incompleteness. We conducted our analyses in R (R Core Team 2019) using a two-way ANOVA to confirm that there was no bias in number of days between visit between swaps and removals or visit number (Figure 1.2). Egg only removals were removed from the remaining analyses due to small sample size. We conducted a Chi-square test to compare the frequencies of each outcome for each of the remaining management types: Swap and Removal (nest and egg). Due to non-normality, we conducted a Mann-Whitney U test to determine differences between management strategies in time until a House Sparrow laid a clutch.

RESULTS

General Summary

Over the course of 3 years (2016-2018) approximately 200 participants joined the project Sparrow Swap, with 84 individuals sending in datasheets on the outcomes of management. Collectively, these volunteers managed a total of 536 House Sparrow clutches (2182 eggs) from 326 nestboxes. Most volunteers were in the eastern United States (Figure 1.3). The average (median) volunteer sent observations from 2 nestboxes and collected 3 clutches totaling 13 eggs. Volunteer participation was skewed, with some volunteers contributing more than others with max contribution being 22 nestboxes, 58 clutches equaling 231 eggs. Most volunteers only participated in Sparrow Swap for one year (69.0%). Few volunteers participated in Sparrow Swap for two years (21.5%), and even fewer for three years (9.5%).

Of the total House Sparrow clutches managed, 248 were swaps and 130 were removal. For both swaps and removals there were instances (n= 158) where no follow up visits were recorded. These were excluded from analysis along with 30 clutches from the pilot year in 2015. Of removals, 89 included nest and egg while 36 were eggs only. Partly due to the name of the project and the novelty of hand-painted egg replicas, swapping was a more popular option than removing (Table 1.3). While instructions indicated to visit the nestbox every seven days, the amount of time between visits varied from 1 to 52 days, with a mean visit day of 7.68 ± 11.03, and a median of 7.00. Our ANOVA test revealed that management type (swap versus removal) or visit number did not significantly ($p > 0.05$) impact the days since last management.

Management Outcomes

A Chi-square test of independence was performed to examine the relation between management strategy and outcome (Table 1.4). Data with an inconclusive outcome were removed from analyses. The relation between these variables was significant, (chi-square test of independence, X2 = 143.06, df = 3, $p < 0.0011$) with both House Sparrows and natives nesting in boxes more than expected in Removals and natives nesting less than expected in Swaps (Figure 1.4). Based on our Mann Whitney test (U = 403, p-value < 0.001), House Sparrows took twice as long to nest again following a Swap (18.2 ± 6.39 days) compared to Removals (9.8 ± 4.21) (Figure 1.5).

DISCUSSION

We found that swapping real eggs with replicas delayed House Sparrows from starting a new nest attempt in the nestbox compared to removing. However, without management it would likely be more than 25 days until another breeding attempt would be made. Thus, even though swapping delays another House Sparrow nest attempt, it still might encourage more frequent nesting if compared to no management at all. Jacquin et al. (2010) found replacing eggs with decoys did shorten the nest attempt cycle in urban feral pigeons (*Columba livia*) from an average of 11 weeks to 4 weeks. Jacquin et al. (2010) also found females on a shorter egg-laying cycle had poorer quality eggs. Thus, both swap and removals may increase reproductive attempts compared to if the clutch was not altered at all. However, the delay caused by swapping still has the potential to reduce the number of broods a pair can attempt to raise within a breeding season compared to removing.

Given the complexity of our results, there is no obvious choice of which management strategy is better (Table 1.5). The preferred management strategy, swap or removal, may depend on the goal of those managing House Sparrows. If the motivation of a nestbox monitor is to have a native bird in their nestbox, then nest and egg removals are the preferred strategy. Nest and egg removals clear the way for a native to nest in the nestbox. In boxes with swaps, the presence of the replicas occupying the House Sparrows means that natives are less likely to utilize the nestbox. Alternatively, if the goal is to prevent House Sparrow reproduction in hopes of impacting local population of House Sparrows, then swapping is the more ideal strategy. Swap delays renesting and is less likely to increase brood attempts than removals. Timing may also be another consideration. Because House Sparrows lay another clutch more quickly after a removal than a swap (Figure 1.5), a nestbox monitor who chooses to remove will have to visit nestboxes more frequently to ensure that a House Sparrow does not re-nest.

One downside of swapping is that the nestbox tended to remain occupied by a House Sparrow or was abandoned all together. Very few native species nested in a nestbox after a swap. On the other hand, removing the nest and eggs allows for both House Sparrows and native species to compete for the open nestbox. However, this competition may lead to native – House Sparrow conflicts that could lead to injured or dead native species (adults, chicks and eggs). Out of the 365 swaps and removals there was one reported incident of a native bird being found dead in a nestbox following a management strategy. In this case, it followed an egg only removal. Since participants did not always return data on all House Sparrow nests, it is impossible to calculate the frequency at which House Sparrow aggression occurred. In addition, several participants corresponded with the Sparrow Swap Team that after seeing a dead native bird they no longer participated in the project and chose to use a lethal management strategy instead. This is consistent with the finding by Larson et al (2015) that firsthand encounters are a driver of choosing a lethal management strategy. Therefore, our results might be biased towards incidents where aggression did not happen.

There are also some limitations of our study. Since the birds were not banded, we cannot be certain that the House Sparrow that attended or subsequently nested in the nestbox is the same pair that laid the initial clutch. However, House Sparrows are known to have site and nest fidelity within a season, so it is likely that at least one of the adults is the same from the previous clutch (Anderson 2006). Another limitation of this study is that we treated each management event (swap or removal) as independent. Since some of the management events happened within in the same nestbox within the same season it is very likely that these management events are not truly independent. In addition, trails with multiple boxes managed simultaneously also adds to the lack of independence.

While swapping may reduce competition between House Sparrows and native species by reducing events where native- House Sparrow conflicts occur, it still does not remove competition for nesting cavities. In order to determine which management strategy is more effective, it would be necessary to know whether competition via usurpation and conflict is more of a deterrent to native birds than the loss of a potential nesting location. In order to further assess which management strategy is more effective at reducing competition and destruction to native birds, information on the success or failure of native birds in neighboring nestboxes is crucial. Future studies that provide a more holistic picture on the success of native birds on trails before and after a swap are needed.

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Table 1.1 Management strategies used by nestbox monitors

Type	Management	Description	Life Stage	Source
Active	Trapping	Using in-box traps or bait traps to capture, wing clip, euthanize, and/or relocate birds.	Adult	Zeleny 1976, Berger et al. 2001
Active	Nest removal	Removing of any combination of nest material, eggs, and nestlings.	Eggs Nestling	Davis and Blankenship 1995
Active	Non-viable eggs	Shaking, piercing, boiling, coating in oil, or chilling eggs and returning non-viable eggs to the nest.	Eggs	Berger et al. 2001
Active	Swapping eggs	Removing and replacing real HOSP eggs with fake replica eggs.	Eggs	Berger et al. 2001
Passive	Food availability	Avoiding birdseed with millet or placing novel objects on bird feeders to deter HOSP.	Adult	Dennis 1978, Kessler et al. 1994
Passive	Nestbox availability	Plugging entrances to nestboxes, removing nestboxes from the vicinity, or relocating nestboxes that have attracted HOSP.	Adult	Zeleny 1976, Berger et al. 2001
Passive	Nestbox timing	Waiting to install nestboxes or open nestbox entrances to coincide with the arrival of migrating birds.	Adult	Zeleny 1976
Passive	Nestbox deterrents	Dangling a monofilament or a novel object from nestboxes to deter HOSP.	Adult	Pochop et al. 1993 Agüero et al. 1991
Passive	Hole restrictor	Restricting the entrance size when nestlings of a native species are ~10 days old to prevent HOSP from getting inside.	Adult	Berger et al. 2001
Passive	Nestbox design	Altering nestbox designs to deter HOSP, includes nestboxes made of PVC and two-holed nestboxes.	Adult	Berger et al. 2001
Passive	Nestbox Placement	Installing more nestboxes to provide nesting locations for native birds.	Adult	Berger et al. 2001

Table 1.2 Possible management outcomes. A list of possible outcomes following a swap or removal event occurs in a nestbox (HOSP = House Sparrow).

Outcomes	**Description**
Attended	HOSP activity for 3 weeks after the management
Abandoned	HOSP activity not reported
Native Nest	Native bird started nest building in the nestbox (with or without eggs)
HOSP Nest	HOSP started a new clutch of eggs.
Inconclusive	Participant did not provide enough information to determine outcome

Table 1.3 Participation in Sparrow Swap. Participation separated by management strategy.

Management Strategy	**n**	**%**
Swap	238	65.2
Removal- Nest & Egg	91	24.9
Removal - Egg Only	36	9.9
Total	365	100

Table 1.4 Observed and expected counts for management outcomes. Expected values in parentheses for each management strategy. Inconclusive outcomes were removed for analysis.

	Abandoned	Attended	HOSP Nest	Native Nest	Total
Removal	18 (25.86)	9 (23.16)	42 (26.94)	11 (4.04)	80
Swap	78 (70.14)	77 (62.84)	58 (73.06)	4 (10.96)	217
Total	96	86	100	15	**297**

Table 1.5 Considerations for management implications. The preferred management strategy may depend on both biological considerations and practical limitations by the volunteer nestbox monitor. (HOSP = House Sparrow)

Considerations		**Swaps**	**Removals**
Biological	Native reproduction	Lower use by native species following management	Higher use of by native species following management
	HOSP aggression	May reduce impacts to neighboring native species	May have impacts on neighboring native species
	HOSP reproduction	More likely to have HOSP abandon nestbox	More likely to have HOSP renest
Volunteer	Effort	Requires effort to wait until a clutch is complete	Does not require the clutch being finished*
	Supplies	Requires participant to have access to replica eggs	No materials needed
	Time	Longer intervals until next management	Shorter interval until next management

*for this study we asked volunteers to wait until the clutch is finished to make swaps comparable to removals, however, in practice most monitors remove nests whenever they encounter them whether the nest and/or clutch is completed.

Figure 1.1 Comparison of a real House Sparrow egg (A) to a wooden egg replica (B).

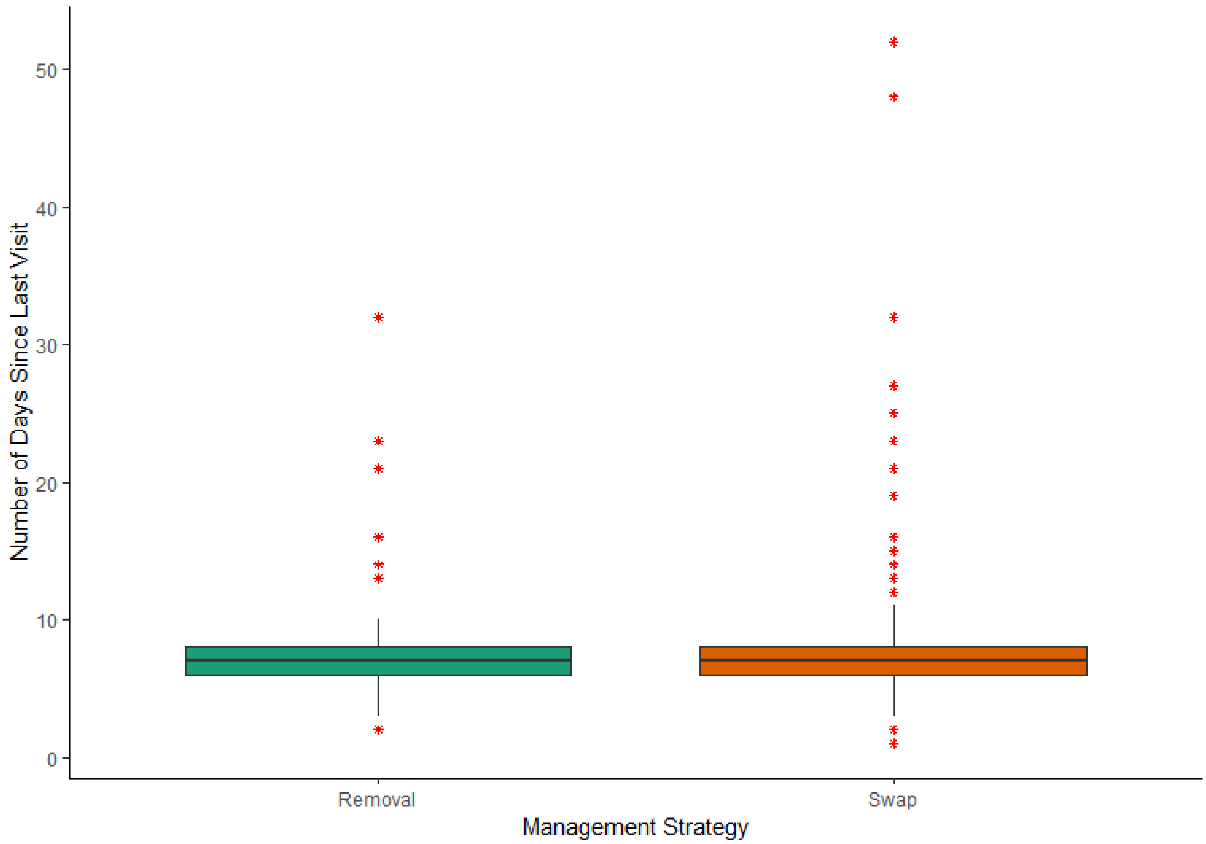

Figure 1.2 Distribution of days between volunteer visits. The number of days between visits to managed nestboxes for each management strategy.

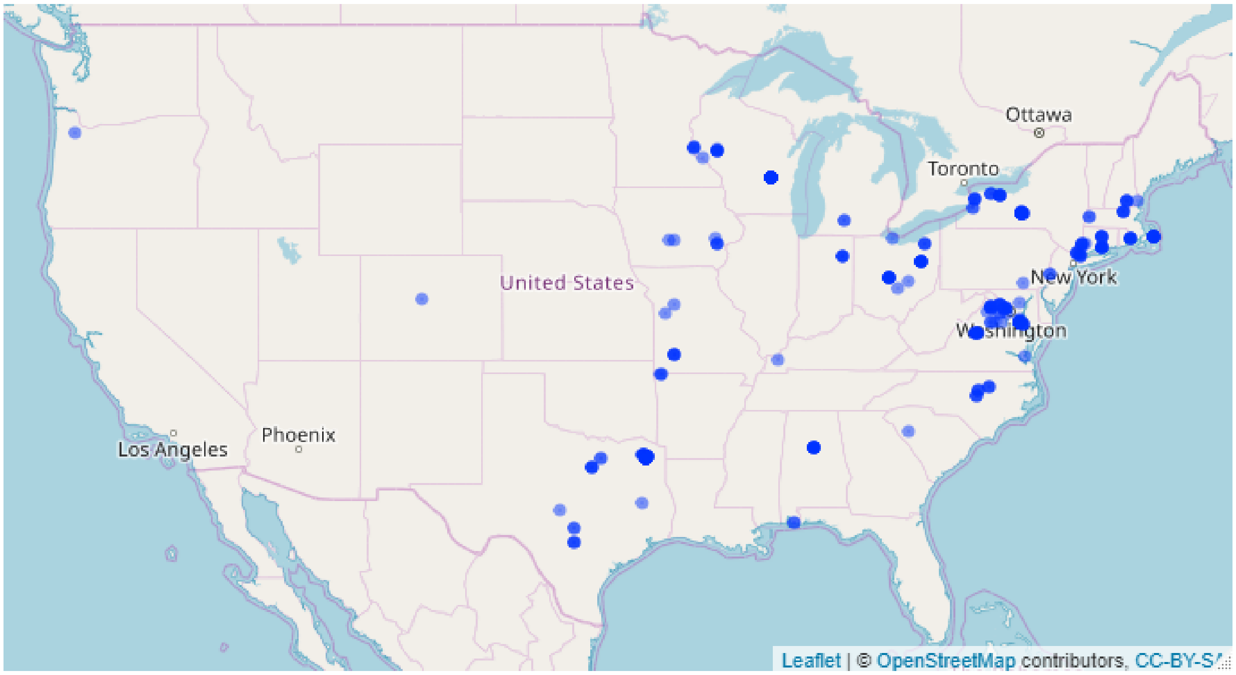

Figure 1.3 Map of Nestbox Locations. House Sparrow clutches were collected from nestboxes across the United States (n= 320). Darker points represent nestboxes that had more than one clutch per season.

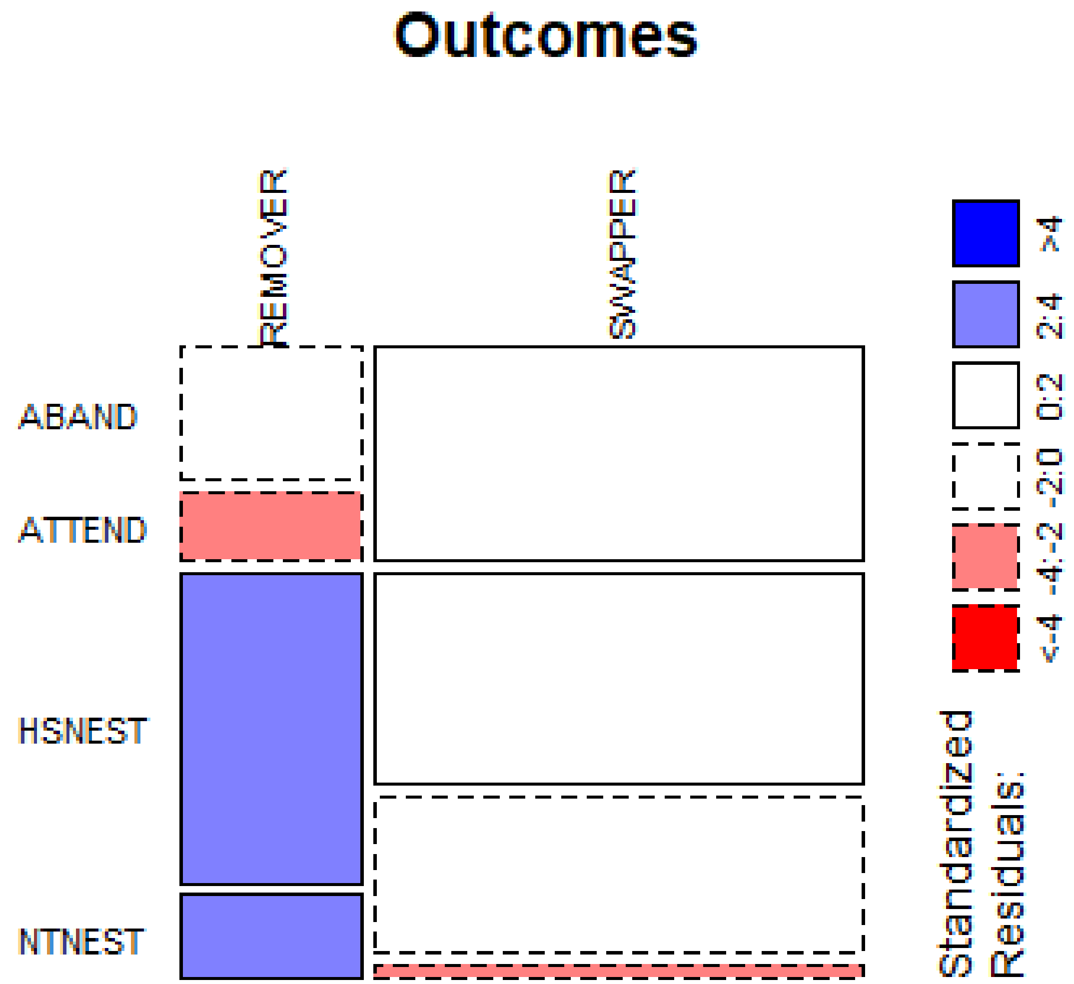

Figure 1.4 Frequency of outcomes by management strategy. (ABAND) Box had no activity (ATTEND) House Sparrow still visiting that nestbox after 3 weeks; (HSNEST) – House Sparrow nest and eggs present; (NTNEST) Native bird nesting in the nestbox. Size of box indicated its relatively proportion compared to other boxes. Boxes shaded blue and/or with a solid line represent outcomes whose observed values are higher than the expected. Boxes that are shaded red and/or have a dashed line represent outcomes where the observed is lower than the expected. The larger width of the Swap (Swapper) boxes is a product of the larger samples size (n = 217) compared to removals (Remover, n= 80).

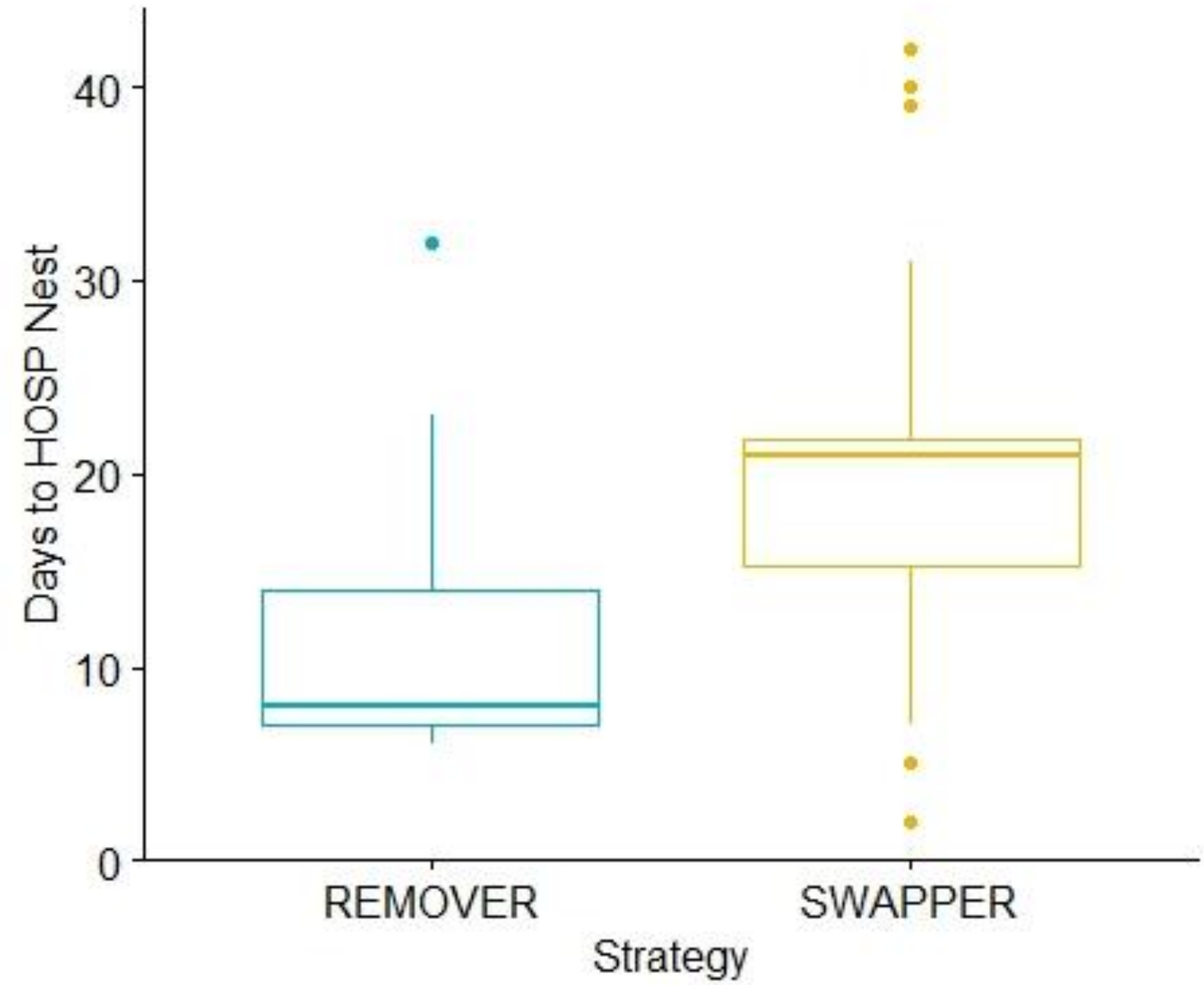

Figure 1.5 Days to House Sparrow nest following management. A comparison of management strategy (Swap vs Removal) on the days after a management until another House Sparrow (HOSP) clutch was discovered in the nestbox.

CHAPTER 2

EXPLORING HOUSE SPARROW EGG CHARACTERISTICS AS INDICATORS OF HEAVY METAL CONCENTRATIONS

ABSTRACT

Persistent environmental contaminants pose serious health risks to humans and wildlife alike. However, the high costs of testing and monitoring for contaminants mean the presence of contaminants in the environment may be unknown. Environmental contaminants can alter the chemical pathways involved in avian eggshell formation and pigmentation. Determining whether these contaminants alter eggshell characteristics (color and speckling) in predictable patterns has important applications for eggs as indicators of environmental contaminants. We used House Sparrows (*Passer domesticus*) as a model organism for determining patterns between eggshell color and speckling and heavy metal concentrations. House sparrows are ubiquitous non-native species, that are commensal with humans, potentially exposing them to similar environmental contaminants as humans. Volunteer songbird nestbox monitors from across the United States collected approximately 536 clutches, totaling 2,182 House Sparrow eggs, as part of the citizen science project, Sparrow Swap. We examined how eggshell characteristics varied across a geographic scale and throughout season. We investigated if metals concentrations were predicted by eggshell color, speckling, thickness, geography, season, and calcium concentrations. We measured metal and calcium concentrations using mass spectrometry with one hundred eggshells representing a hundred clutches and used the software SpotEgg to classify eggshells based on color and speckling. Our results indicated that metals, including As, Se, Cd, Cu, Pb, are present in detectable levels in House Sparrow eggs found across the country. Eggshell characteristics are not strong predictors of metal concentrations, but eggs tend to get darker with

many small speckles as metal concentrations increase. Our work provides a basis for future work exploring using this invasive pest species for environmental monitoring.

INTRODUCTION

As human activity continues to modify the environment, finding organisms to serve as indicators for the health of wildlife and the environment becomes important for monitoring environmental changes and even potential human health hazards. Birds can successfully serve the role as bioindicators because they are easy to observe, widely distributed, well studied, and of interest to the general public (Becker 2003). For example, the decline in Bald Eagle populations because of DDT induced eggshell thinning served as an indicator of the impacts of DDT in the environment that led to political and social change in environmental monitoring (Grier 1982). Since then, studies have examined using blood, feathers, excrement, and eggs as indicators of various pollutants in the environment, including heavy metals, organochlorines, pyrethroids (Becker 2003). But what if we could use the color of eggs as indicators of potential geographical areas that need closer monitoring?

Eggshell coloration between species can vary greatly from the pure white of owl eggs to the red speckled eggs of great tits. Yet this extraordinary diversity in eggshell coloration is derived from only two pigments synthesized by birds: biliverdin (blue) and protoporphyrin (reddish-brown) (Kennedy & Vevers 1976). Several hypotheses for why eggs are colored have been proposed, including crypsis from predators, protection from inter- and intraspecific parasitism, female signaling, and potentially contaminants (Brennan 2010; Davies & Brooke 1989; López de Hierro & Moreno- Rueda 2010; Gosler et al. 2005; Gillis 2012).

The crypsis hypothesis (Wallace 1889) states that eggshell speckling helps eggs remain hidden from predators. However, empirical evidence has generated mixed conclusions, with the structure of the nest playing just as an important role as speckling in hiding eggs from predators (Cherry & Gosler 2010). In addition, there are several examples of cavity nesting birds like great tits (*Parus major*) and House Sparrows (*Passer domesticus)* that have speckled eggs despite remaining hidden in cavities (Gosler et al. 2005; Anderson 2006; Cassey et al. 2012).

Another hypothesis for eggshell color variation is that egg speckling and color allow females to identify their own eggs to prevent both inter- and intraspecific brood parasitism (Davies and Brooke 1989; López de Hierro & Moreno-Rueda 2010). However, this fails to explain why high variation in eggshell patterns is found in birds with little to no inter- or intraspecific brood parasitism. Little is known about inter- and intraspecific brood parasitism in House Sparrows (Anderson 2006). Most House Sparrows are unable to visually distinguish their eggs from others, suggesting that the variation seen in House Sparrow eggs is an unlikely result of selection to visually distinguish their eggs from that of another female (Manna et al. 2017).

Another hypothesis for explaining the differences in background color variation is the sexually selected eggshell hypothesis (SSEC). The SSEC states that the blue color (biliverdin) may be a post mating indicator of female genetic quality for species in which the male provides parental care. Biliverdin has antioxidant properties and eggs that are more blue green may indicate that the female is more fit (Moreno & Osorno 2003, Cassey et. Al. 2012). Some studies have indicated that achromatic differences in eggs are more visible to cavity nesters than chromatic differences (Cherry & Gosler 2010). Slight differences in blue-green color are most likely undetectable to the parents once inside the nesting cavity.

Variation in eggshell speckling and color between clutches could be a plasticity in response to environmental gradients associated with latitudinal clines and seasonal trends. Past studies have found geographic and latitudinal trends in clutch size (Greibler 2010; Cooper 2005), egg size (Martin 2008), and egg shape (Duursma 2018). Other research proposed that coloration plays a role in thermoregulation, and therefore could cause overheating in warmer climates

(Gómez et al. 2016, Westmoreland et al. 2010). Research has also shown there may be temporal changes in House Sparrow coloration over the course of a breeding season. López de Hierro & de Neve (2010) found that House Sparrow eggs' speckling got darker and fewer eggs had biliverdin over the course of the breeding season. This may be due to stress due changes in resources or environmental conditions. For example, López de Hierro & de Neve (2010) found that supplemental feeding influences the coloration of eggshells and that the color and speckling patterns changed over the breeding season within individual House Sparrow females.

Both pigments are byproducts of the heme biosynthetic pathway and may be altered by stressors, such as environmental contaminants (Hanley & Doucet 2012; Jagannath et al. 2008). Hanley and Doucet's (2012) studied the coloration of herring gull (*Larus argentatus*) eggs found that the blue-green color was negatively associated with an increasing level of environmental contaminants. In the Eurasian sparrowhawk (*Accipiter nisus*), Jagannath et al. (2008) found that increased concentrations of DDE, a metabolite of DDT, were correlated with an increase in the blue-green color, an increase of speckling of egg, and thinner eggshells. Furthermore, Hargiati et al. (2016) found that higher concentrations of copper were associated with higher concentration of speckling on Great Tit eggs (*Parus major*). To explicitly test the influence of metals on pigmentation, Orlowski et al. (2017b) tested differences between dark and light eggs of Japanese quail (*Coturnix coturnix*) and found that concentrations of copper, iron, manganese, cadmium, lead, calcium were higher in eggs with larger amounts of speckling, while cobalt and magnesium were higher in eggs with fewer speckles. Except for Hanley & Doucet (2012), studies used coarse categorical scales to analyze the differences in eggshell color and speckling.

In addition to the effects mentioned above, heavy metals may disrupt the ionic uptake of calcium, which can alter the creation and composition of eggshells (Rodriguez-Navarro et al.

2002). According to the structural-function hypothesis, protoporphyrin found in eggshell speckling acts as lubricant that provides structural support for thinner areas of the eggshell (Gosler et al. 2005, Solomon 1987). Gosler et al. (2005) found that Great Tit eggshells were thinner in speckled areas. Consistent with this hypothesis, López de Hierro & de Neve (2010) found that female House Sparrows laid eggs with darker speckling later in season, which could be due to a depletion of calcium. In contradiction to this hypothesis, the captive studies by Orlowski et al. (2017b) found that the pigmented segments of Japanese quail eggshells were thicker and contained higher concentrations of calcium. These contradictory results highlight the importance of continued research of relationships between calcium, thickness, and metal concentrations.

The structural-function hypothesis and the hypothesis that stressors, such as environmental contaminants, can alter coloration provide the plausible hypotheses for what is driving variation between clutches within cavity nesting species that experience little brood parasitism (Gosler et al. 2005). House Sparrows (*Passer domsesticus)* are cavity nesting birds that have trouble identifying their eggs from other cavity nesting bird species, making it unlikely that the brood parasitism, sexual selection, or sexual conflict are currently contributing the diversity of eggshell speckling (Manna et al. 2017). House Sparrows also show great variation in eggshell color between and within individuals (Bumpus 1896). On one end of the House Sparrow egg color continuum, eggs can be white with a few pale brown speckles, and at the other extreme, eggs will be a dark blue-gray and mostly covered with dark brown speckles (López de Hierro & de Neve 2010). The reason for this diversity in egg characteristics between House Sparrow remains poorly explored. House Sparrows are also widespread in the Western Hemisphere spanning from Canada to South American. They are also non-migratory meaning their potential exposure to contaminants occurs on a relatively small scale. Thus, House Sparrows provide an exemplary system to test whether the structural-function hypothesis and/or environmental contaminants are influencing variation in egg speckling and background coloration between clutches.

Herein, we leveraged a new visual analysis software to carry out a quantitative analysis of eggshell color and speckling in relation to heavy metal and metalloid concentrations in the invasive House Sparrow. Before assessing the role that contaminants play in egg characteristics, we assessed potential geographic and seasonal patterns. Based on findings of López de Hierro & de Neve (2010), we would expect the base color to decrease over the breeding season in House Sparrows. A decrease in pigment over the season could act as signal of decreased female condition throughout the breeding season. However, because House Sparrows are cavity nesters that are unlikely be able to differentiate slight differences in the background color of the eggs in dim light; we hypothesize that post mating sexual selection plays a minimal role in the variation in eggshell speckling of House Sparrow. We expect to see minor difference in background color. Also based on previous work of López de Hierro & de Neve (2010) on House Sparrows, we expect speckling to get darker over the breeding season which could be due to changes in a heme pathway caused by contaminants or changes in resources. We also plant to explore whether there are geographic trends in eggshell color and speckling characteristics.

To explore the potential role of the structural-function hypothesis and environmental contaminants on eggshell coloration we examined the relationships between eggshell thickness, calcium concentration, color and speckling, and breadth-length ratio on the concentrations of 5 heavy metals and metalloids (copper, lead, selenium, cadmium, arsenic). We chose heavy metals and metalloids, hereafter referred to as metals, as our contaminant of interest because past studies have shown that potential links between eggshell speckling patterns and heavy metals (Hargiati et al. 2016).

Metals and trace elements have been studied in the eggshells of many wetland species, but have been studied less frequently in passerines, and even less frequently in non-migratory passerines associated with humans. They have also not been studied at a great geographic scale, save for Ruuskanen et al. (2014) study on European Pied Flycatchers (*Ficedula hypoleuca*). In addition, previous literature has suggested that eggshells are a way for a female bird to excrete heavy metals from their body (Burger 1994). In general, we selected these metals on the potential to be involved in eggshell speckling and impacts to the health of wildlife and humans. We chose copper to determine if we could find similar trends as Hargitai et al. (2016) with increased aggregation of speckling in House Sparrow eggs with increased copper concentrations. We chose selenium because of its known impacts on fish and bird embryo development (Heinz 1996; Scheuhammer 1987). Lead and arsenic were chosen for their health impacts to both wildlife and humans (Davis et al. 1990; Scheuhammer 1987, Khan et al. 2014). Finally, we chose cadmium because of its potential to increase in toxicity and uptake in organisms with calcium deficient diets (Scheuhammer 1987). Lead, arsenic and cadmium are all non-essential elements for the bird species, while selenium and copper are essential, but toxic at high concentrations (Orlowski et al. 2014; Heinz 1996; Dauwe et al. 1999). Correlative evidence between heavy metal concentrations and eggshell speckling may suggest that environmental exposures could alter chemical pathways. Further research could then investigate if environmental exposures to metals and other persistent environmental contaminants could be driving some of the variation seen between clutches.

Based on the structural-function hypotheses proposed by Gosler et al. (2005), we would expect that as thickness of the eggshell decreases, speckling increases. Specifically, if metals reduce the uptake of calcium, and protoporphyrin adds structural integrity (Gosler 2005), then we would expect eggs with higher metal content to have thinner shells, more speckles, and reduced calcium concentrations. If environmental contaminants act as stressors (Hanley & Doucet 2012; López de Hierro & de Neve 2010), then we would expect the blue-green color of the eggshells to decrease with increasing concentrations of metals. Some research has indicated that eggs become relatively wider as a female birds age (Kendeigh et al. 1956; Brooke 1978). We expect that concentrations of heavy metals increase over the lifetime of a bird. If breadth-length ratio can be used as a proxy for age, we expect stouter eggs to be associated with increasing metal concentrations.

In the following we attempt to examine if seasonal, geographic, and metal concentrations can explain the variation found in House Sparrow eggs found across the United States.

METHODS

Egg Collection

House Sparrow eggs were collected via Sparrow Swap, a citizen science project where volunteers send House Sparrow eggs to scientists. House Sparrows are a non-native species in the United States and exempt for the Migratory Bird Treaty Act of 1918 (Avery and Tillman 2005). The project was open to anyone in the United States from the years 2015-2018. House Sparrow eggs were opportunistically collected by volunteers encountering House Sparrow nests in nestboxes established to attract native songbirds. Participants were instructed to wait until a House Sparrow had completed the clutch before removing the eggs. Once collected, volunteers placed the eggs in a refrigerator for at least 24 hours to stop any further embryo development. Volunteers were instructed to carefully mail eggs to the North Carolina Museum of Natural Sciences in Raleigh, North Carolina by suspending the House Sparrow eggs between two pieces of plastic film inside a plastic egg (Appendix 2.1). Volunteers discarded any eggs that were broken prior to shipment. Upon arrival at the Museum, we unpacked the eggs, gave each clutch a unique identification number, and assigned each egg in a clutch a letter (A-G).

Egg Photography & Photo Processing

We photographed each clutch of eggs with a Nikon 3200 digital SLR camera on the manual setting with aperture set at F16 and a shutter speed of 1/1.6. We saved images as RAW image files (.NEF). In each clutch photograph, we included a scale and six grayscale color patches of known spectral reflectance (Figure 2.1). We used SpotEgg, an image processing tool created by Gómez and Liñán-Cembrano (2017), to quantify the color and speckling of each House Sparrow Eggs. SpotEgg, takes the raw images and linearizes them using DCRAW (Coffin 2015), a tool used for processing raw image files. SpotEgg then employs MatLab software to detect and quantify the area (%) of speckling on each egg, and the color (as RGB) values of each spot. To reduce the highly correlated variables (Appendix 2.2) and their interactions, we conducted a principal component analysis (PCA) with Varimax (orthogonal) rotation using 11 variables relating to eggshell color and speckling, using the psych package in R. Spot size and total area of spots were transformed (1/X) for the PCA. The varimax rotation allowed for easier interpretability in subsequent analyses.

Sample Preparation

One hundred eggs were subsampled from 1426 eggs that were processed in SpotEgg for metal analysis. For this analysis we only selected eggs that were from clutches with complete photo information in order to select a representative egg. In addition, we only selected eggs that were early development stage eggs since calcium and metal concentrations can change in the eggshell over time as the embryo develops (Orlowski et al. 2019). Development stage for each egg was determined when the egg was cut open and categorized as early (yolk still intact, no red blood vessels visible), middle (red blood vessels visible, embryo clearly visible, lacks distinct morphology), and late (beak, eyes, and other morphological traits clearly visible). To capture the variation in clutches over variation in eggs, the most average egg per clutch was determined selecting the egg closest to the average factor loadings for PC1 and PC2. Eggs that deviated the least from the mean were selected as the "average" egg. Average eggs (n=20) that were the extreme in both maximum and minimum for each loading were selected. A remaining 20 eggs were chosen to represent the middle. These 100 eggs also represented a broad geographic area (Figure 2.2). After being photographed, the length and width of each egg was measured to the nearest hundredth of a mm using an UltraTech carbon fiber digital caliper. Some eggs (length; n=4, width; n = 5) that had structural damage (pin hole, crack, piece missing, etc) made either length or width measurement via calipers impossible. Instead, the length and width of these eggs were measured by photograph. Width measurements were consistent between the two methods (calipers vs photograph). However, length measurements were systematically shorter when taken from a photograph versus caliper measurements. We believe that this is due to the angle of the egg when the eggs are photographed. To correct for this discrepancy, we created a regression equation ($y = 0.9412x + 1.6726$, $R^2 = 0.845$, $N = 1312$, $df = 1311$, $p < 0.001$) between caliper and photo length based on 1422 eggs where we had both caliper and photograph measurements.

Egg contents were separated from the eggshell using a Dremel 200 Series rotary tool and eggshells were air dried at room temperature for at least 36 hours. The blunt end of the egg and approximately 8 x 8mm piece from the equator of egg were reserved for thickness measurements. Thickness was measured using a low-force Mitutoyo micrometer (Digimatic Micrometers Series 227) with the force set to 0.05 Newtons and measured to the nearest 0.001 mm. Three measurements were taken from the blunt end of the egg and another three thickness measurements were taken from the piece of egg taken from the egg's equator. The rest of the eggshells were then rinsed with acetone, rinsed with distilled water, and allowed to air dry again. Eggshells were homogenized using a mortar and pestle, rinsing with acetone after each sample.

Calcium and Metal Analysis

Eggshell samples were then sent to the Environmental and Agricultural Testing Service (EATS) at North Carolina State University for sample digestion and elemental testing of Calcium, Copper, Selenium, Arsenic, Lead, and Cadmium. Sample preparation and testing was similar to Hargitai et al. (2016). At the EATS laboratory, HNO3 was added to the dried eggshell samples to begin digestion. Calcium concentrations were analyzed using a Perkin Elmer ICP-Optical Emission Spectrometer Model 8000. Concentrations of metals (arsenic, cadmium, copper, lead, selenium) were diluted 50 times and spiked with 1ML of 2.5 ug/L solution adding 0.5 ug/L elements in the sample. This concentration was subtracted from final reporting. Metals (arsenic, cadmium, copper, lead, selenium) were analyzed using a Perkin Elmer Elan DRCII ICP- Mass spectrometer. Spike solutions were also periodically analyzed with samples to more accurately determine the concentration of the spike solution over sample run times. The practical quantitation limit (PQL), the limit at which the elements can be accurately quantified, and method detection limit (MDL), the threshold where elements can be detected, varied by element (Table 2.1). For eggshells with metal concentrations below the MDL, half the MDL limit was used. For eggshells above the MDL but below the PQL, the mean of the MDL and PQL was used.

Statistical Analysis

To assess potential geographic and temporal trends on eggshell speckling we conducted two generalized additive mixed models (GAMM) using the gamm4 packing in R (R Core Team 2019). In our models, we set Color (PC1) or Speckling (PC2) as the response variable with predictor variables being a linear term for collection date (ordinal date), a combined smooth term of latitude and longitude with clutch as a random effect.

Before conducting any analyzes of metals we determined if there were any correlations between metals. Because distributions of trace elements did not follow a normal distribution, we tested correlations between metals using Kendall's tau (non-parametric). We also conducted a PCA with the metal concentrations to determine if we could create one or two variables that we would consider as "contaminant load". However, we found that the first principal component explained less than 20% of the variation, which is no better than testing the metals individually. We also tested whether eggshell characteristics (Ca, breadth-length, thickness, color, and speckling) were correlated with each other. To test whether eggshell characteristics are a potential indicator of contaminants we used generalized linear model with metal concentrations (As, Cu, Pb, Se) as the response variable and thickness (mm), calcium concentration (%), breadth-length ratio, color (PC1), speckling (PC2), latitude, longitude, and collection date (Julian) as predictor variables with interaction terms for collection date and color (PC1) and an interaction term for latitude and longitude. Cadmium was found in only a small quantity of the eggshells sampled, so we used a binomial generalized linear model with 0 being below the PQL and 1 being above the PQL. The Akaike Information Criterion (adjusted for small sample size)

(AICc), delta AICc, and weight of each model was determined using the dredge function and averaged using model.avg function in the MuMIn package of R. The average model reported here assumes that a variable is included in every model, but in models where the variable is considered less of predicator the corresponding coefficient is set to zero.

RESULTS

General Summary

Between 2015-2018, the Sparrow Swap Team received 536 clutches totaling to 2182 House Sparrow eggs. Of these, 1462 eggs from 431 clutches were received in good enough condition to photographed and used in analysis for seasonal and geographic trends. There was a wide diversity in House Sparrow egg coloration and speckling among the eggs received (Table 2.2). The PCA on the SpotEgg color and speckling variables yielded two principal components (PCs) explaining 90% of the variance between eggs (Table 2.3). The first principal component (PC1) was labeled “color” to the high loadings by the following items: Spots R, Spots G, Spots B, Background R, Background G, Background G. This first PC explained 54% of the variance. Eggs with positive loading values are lighter in color than egg with negative PC1 loadings. The second principal component (PC2) was labeled “speckling” due to the high loadings by the following factors: number of spots, average spot size, and total area of spots, area vs per. The variance explained by this factor was 36%. The spectrum of this factor includes eggs with few large irregular shaped speckled to eggs with many, small more circular speckles (Figure 2.3).

Thickness of eggshells ranged from a minimum of 0.090mm to 0.165mm with a mean and standard deviation of 0.118±0.012. This is thicker than the range of average (0.06-0.09mm) for House Sparrow eggs in India (Dhananjayan et al. 2011). Calcium makeup of the egg ranged from 30.86 % to 37.47 % with an average of 34.69 % (±1.60). This was consistent with other studies on bird eggs. Breadth-length ratios ranged from 0.58 (slender long eggs) to 0.99 (round eggs) with the mean ratio of 0.72±0.04 (Figure 2.4). We did not find any significant correlations between any of the eggshell characteristics of Ca, thickness, breadth-length ratio, color (PC1) or speckling (PC2) (Appendix 2.3).

Seasonal and Geographic Trends

The results of our GAMMs (Table 2.4) showed a negative relationship between collection date (Ordinal) and PC1 (color) indicating that the eggs get darker over the course of the breeding season (Figure 2.5). We found a significant, non-linear relationship between location (latitude: longitude) and PC1(color) suggesting that there is more likely local variation driving color versus larger latitudinal clines (Figure 2.6). Eggs from Texas seem to lighter than other eggs, while eggs in the northeast are generally darker. We found a positive relationship between collection date and speckling, indicating that over the season eggs have fewer, larger, more irregular shaped speckles (Figure 2.7). We found less geographic differences between eggshell speckling (Figure 2.8). The model predicated most to be at the mean, though Texas again differed from the rest of the country and associated with eggs with many small, round speckles.

Metals Concentrations

Only 27 of the 100 eggs had all 5 metals present at detectable levels (Figure 2.9). Most eggs (n = 46) had a combination of 4 of the metals, and 27 eggs had 3 or fewer metals present. Concentrations of metals were in the following order: Cu > Se > As> Pb > Cd (Table 2.5). Burger & Gochfeld (2004) found a similar trend in metal concentrations in the eggs of the Common Tern (*Sterna hirundo*) in New Jersey, except they did not examine copper. These concentrations are within the range of mean concentrations reported in eggshells of House Sparrows and other bird species (Figure 2.10). We found significant correlations between three pairs of metals: arsenic and cadmium (tau = 0.30, $p < 0.001$), arsenic and lead (tau = -0.16, $p = 0.02$), and copper and lead (tau = 0.47, $p < 0.001$) (Table 2.6).

Copper had the highest range of all the metals from no detection to 8.88ppm. We found a mean copper concentration of 1.97 ppm which was higher than the mean concentration of copper (1.00ppm) found in House Sparrow eggs in the West Bank (Swaileh and Sansur 2006). Other studies have found higher mean concentrations of copper with 12.2 ppm being the highest concentration found in an eggshell of a Canada goose in Washington, USA (Rickard and Schuler 1990). In most studies, the mean concentration of copper in the eggshell ranges from 0.5 – 2.0ppm, putting our concentration of House Sparrow eggs among what most other studies have found (Table 2.7). The averaged model from our linear model indicated a positive relationship between collection date and the interaction between collection date and color (PC1) with increasing concentrations on copper. Speckling (PC2), color (PC1), calcium concentration, and latitude were negatively associated with increasing concentrations of copper (Table 2.8).

Our mean cadmium concentration (0.09 ppm) is consistent with other studies that found low levels of cadmium in eggs (Table 2.7). Cadmium concentrations in our House Sparrow eggs ranged from non-detection to 0.99. Only 26% of the eggshells had cadmium concentrations above the PQL, and 48% of eggshells had concentrations below the MDL. Other studies with lower detection limits have found mean concentrations as low as 0.002 ppm in Bridled Terns in Hong Kong (Lam et al. 2005). In general, most studies have reported cadmium concentrations less than 1.0 ppm. However, some recent studies have found concentrations of 21.07 with a mean of 13.28 in American Oystercatchers in Argentina (Simonetti et al. 2015). Other studies have reported high mean concentrations between 1.0-2.4 in Reed Warblers, Tree Swallows, Cattle Egrets, and Little Egrets (Orlowski 2015, Kraus 1989, Hashmi et al. 2013). The averaged model from our linear model indicated a relationship between calcium and latitude and quantifiable concentrations of cadmium. Speckling (PC2), color (PC1), thickness, collection date, and the breadth-length ratio were associated with cadmium concentrations below detection (Table 2.9).

We found the mean concentration of lead in our House Sparrow eggshells to be 0.52 ppm. This was within the range of mean concentration of lead found in other studies. Compared to other studies on House Sparrows eggshells, this concentration is lower than the 3.3ppm mean concentration that Swaileh and Sansur (2006) found in eggshells from the West Bank, but higher than the 0.42ppm concentration found in eggshells from Baghdad, Iraq (Al-Obaidi et al. 2012). The averaged model from our linear model indicated a negative relationship color (PC1), speckling (PC2), and latitude with increasing concentrations on lead (Table 2.10).

Fewer studies have tested the concentrations of metalloids including arsenic and selenium in eggshells. However, arsenic and selenium also have detrimental effects of organisms at high concentrations. As far as we know, this is the first measurement of arsenic in House Sparrow eggshells. We found mean concentration of arsenic to be 0.72ppm. Other studies of Great and Blue tits in Belgium, Willow Flycatchers and Yellow-breasted chats in Arizona, Rooks in Poland, and Brown Boobys in Brazil have been found with much higher concentrations of Arsenic (Dauwe et al. 1999; Mora et al. 2003; Orlowski et al. 2010; Dolci et al. 2017). On the other hand, Ruuskanen et al. (2014) found mean concentrations as low as 0.008 ppm in Pied Flycatchers in Europe. The averaged model from our linear model indicated a positive relationship between color (PC1) and latitude with increasing concentrations on arsenic.

Speckling (PC2), collection date, and breadth-length ratio were negatively associated with increasing concentrations of arsenic (Table 2.11).

We found a mean of 0.87 ppm of selenium in the eggshells of the House Sparrow. Other studies have reported varying means. For example, at the same site in Long Island, Roseate Terns had a mean concentration of less than 0.005 ppm while herring gulls had a mean of 0.40 ppm (Burger 1994). Ikemoto et al. (2005) also reported low concentrations (0.15ppm and 0.08ppm) of Se in Black-footed albatrosses and Short-tailed albatrosses, respectively, on Tarishima Island and Japan. On the other hand, Lam et al. (2005) found relatively high concentrations of Se in eggshells from Black-crowned night herons (8.16), Bridled terns (15.58), Little egrets (7.59) in Hong Kong. In general, correlations between metals were low, but we did find significant correlations between Arsenic and Cadmium ($p < 0.001$), Arsenic and Lead ($p = 0.02$), and Copper and Lead ($p = 0.47$). The averaged model from our linear model indicated a positive relationship between latitude, breadth-length ratio, and calcium concentration with increasing concentrations on arsenic. Color (PC1), longitude, and thickness were negatively associated with increasing concentrations of arsenic (Table 2.12).

DISCUSSSION

Seasonal and Geographic Trends

We found that there were seasonal and geographic trends in House Sparrow egg coloration and speckling. We found that eggs in Texas tended to be lighter than eggs in the rest of the country, and eggs in the northeast tended to be darker in color. In general eggs get darker over the course of the breeding season. This is consistent with another study on House Sparrow eggs. López de Hierro and De Neve (2010) found that that while the background color of the egg got lighter over the course of the season the spots got darker. However, in our present study, both background color and spot color were highly correlated and combined as one variable: color. López de Hierro and De Neve (2010) found that color varied more between females than spread, suggesting that spread is determined by genetics, while color may be influenced by other factors (López de Hierro and De Neve 2010). This is consistent with our results that found a weaker trend between speckling and seasonal and geographic trends. However, we did find that toward the end of the season eggs tended to have few, but larger irregular shaped speckles than compared to the earlier in the breeding season.

Metals

Despite being a terrestrial, mostly granivorous bird, our House Sparrow eggs had concentrations of metals and metalloids comparable to waterfowl and raptors that are generally on a higher trophic level. The concentrations of metals found in our eggs are also relatively similar to the concentrations found in House Sparrow eggs from Iran and the West Bank. The specific toxicity of most of these metals is mostly unknown for eggs and eggshells of birds. However, based on limited other studies on lethal and no effect thresholds, we have no reason to suspect that the metals were found at levels that are lethal to the bird or embryo. However, there still may be sub-lethal effects occurring. In general, heavy metals in high concentrations are known depress the immune response (Bichet et al. 2013), delay development (Pinowski et al. 1994), and increase aggression in birds (Janssens et al. 2003).

Out of the five elements we tested, the two that are essential to life (selenium and copper) were found at higher mean concentrations than the non-essential metals (lead, arsenic, cadmium). Copper is an essential element for blood formation; however, at extreme concentrations copper can be cause liver damage (Chiou et al. 1997). Baselines concentrations of

1.65 ppm have been reported in House Sparrow eggs (Anderson 2006); we found a slightly higher mean concentration of 1.97. Research on chickens found that hens fed a diet of 400 ppm copper produced egg with mean copper concentrations of 4.7 ppm (Chiou et al. 1997). This dietary intake (400ppm) was also considered the maximum tolerable concentration safe to feed chickens (Chiou et al. 1997). Our maximum concentration of copper in House Sparrow eggshells was 8.9 ppm. If a similar maternal transfer from body to egg occurs in House Sparrows, some of our samples (3%) may be at levels that have detrimental effects to the adult birds. We found that as concentrations of copper increased, calcium levels decreased, eggs became darker, with many small speckles. This is consistent with our hypothesis that lower calcium levels would be associated with higher concentration of metals. We also saw in an increase in color with an increase in copper concentrations. This is consistent with the structural function hypothesis, that with a decrease in calcium there would be an increase in color. Our results are also complicated by the interaction between PC1 and collection date as a predicators of copper concentrations.

Like copper, selenium is another essential element that is toxic at high levels (Lemly 1997). At concentrations above 3.0 ppm, selenium reduced hatching success and caused deformities in embryos in several species of aquatic birds (Spallholz and Hoffman 2002). The maximum concentration of selenium reported in our eggs was 2.31ppm. As far as we know, this is the first measurement of selenium in House Sparrow eggshells. While this is still below the 3.0 ppm, we are surprised to find levels this high in a terrestrial bird. A vast majority of the literature on selenium toxicity in birds is focused on waterfowl and other species that feed on mostly aquatic organisms (Heinz 1996). Unlike the other elements we analyzed, speckling was not included as a predicator of selenium concentrations. However, other eggshell characteristics like color, calcium, breadth-length ratio, and thickness were predicators of selenium concentrations.

Based on our averaged model, increasing concentrations of selenium wer eggs with higher selenium concentrations tended to have be darker in color, higher breadth-length ratio, thinner shells and higher calcium concentrations. We are surprised by the relationship between thinner eggs and more calcium. This relationship is not what we expected but we found it for both cadmium and selenium. In selenium, we did see the expected relationship that eggs that were wider and shorter were associated with higher concentration of selenium.

Our low levels of cadmium are consistent with low levels found across species. Furness (1996) argued that cadmium was not transferred into the eggshells, instead that it was always present at low levels. However more recent studies have found relatively highly levels of cadmium, including over 2.0 ppm in Reed Warblers in Poland (Orlowski et al. 2016) and 13.28 ppm in American Oystercatchers in Argentina suggesting that cadmium is excreted into eggshells, but perhaps only when concentrations are high in the female (Simonetti et al. 2015). In addition to being found at low levels, the relationship between cadmium concentration in eggshells to the concentration in body and eggs has not been consistent across studies (Orlowski et al. 2014). Even within studies, differences in site or species have reversed the relationship between eggshells and eggs (Burger 1994; Dauwe et al. 1999). However, this may have been due differences in development stage of the eggshells tested. Orlowski et al. (2019) found that cadmium and copper concentrations in the eggshell decreased with the age of the embryo while they increased in the embryo. This suggests that cadmium is not only likely to be excreted into the eggshells, but it also has the potential transfer to the embryo as the egg develops, potentially causing harm. Cadmium toxicity due to concentrations 75-200ppm in avian organs cause growth retardation, anemia, suppression of egg production, kidney damage, and marrow hyperplasia (Furness 1996, Scheuhammer 1987). Breeding mallards fed a diet of 1.6 ppm of cadmium reported having no reproductive effects (Beyer 2000). Ayas et al. (2007) concluded that concentrations of 0.420 to 1.97 ppm of cadmium in eggshells were unlikely to cause effects since the threshold concentrations for kidneys and liver is much higher. We suspect that our concentrations of House Sparrow eggs from no detection to 0.99 ppm are also unlikely to cause significant health effects to the embryo and adult bird.

We found that quantifiable concentrations of cadmium are associated with dark, slender eggs with many small round spots. Ikemoto et al. (2005) found that concentration of cadmium in egg content increased with breadth-length ratio and suspected that this due to increasing breadth-length ratio as the bird ages, suggested that concentrations of metals in the bird increased over time. We found the opposite trend with increasing cadmium levels associated with a decreasing breadth-length ratio. Also contrary to what we expected; calcium concentrations were higher in eggs with detectable cadmium levels. We would have expected calcium levels to have been negatively associated with increasing concentration of cadmium. However, the relationship of cadmium with thinner, darker eggshells, still supports the structural function hypothesis that protoporphyrin reinforces thinner areas in an eggshell.

Lead is a non-essential element that can rapidly cross biological membranes to accumulate in the yolk of the egg (Forsyth et al. 1985). Lead can compete with calcium for binding sites and be transported to high calcium areas such as bone, or eggshells (Scheuhammer 1987). Lead concentrations of 2 to 15ppm have been found in the bones of adult birds in laboratory settings and from uncontaminated sites, without any known detrimental effects (Scheuhammer 1987). If the transfer of lead into eggs is similar to the transfer of lead to bones, then we suspect the concentrations found in our House Sparrow eggshells (non-detection to 4.66ppm) are likely at concentrations below any noticeable effects. We expected calcium to be included as an important variable for predicating lead concentrations because of its known ability to compete for binding sites, however, calcium was not included as a predictor included in the averaged model. Instead, color, speckling, and latitude were included as predicators in the final averaged model. Eggs generally got darker with many small round speckles with increasing lead concentrations. The darker eggs with increasing metal concentration are consistent with the hypothesis that increased metal concentrations may cause darker speckling in the eggs.

Like cadmium and lead, arsenic is a toxic, nonessential element that can bioconcentrate in organisms (Boncampagni et al. 2003). Eisler (1988) reported the LD50 for sensitive bird species ranged from 17 to 48 ppm body weight in birds. Sublethal effects for arsenic and other metalloids in birds has been poorly studied (Sánchez-Virosta et al. 2015). Other studies suggest that background concentrations in birds are around 3.0 ppm (Lucia et al. 2010). But the impact of arsenic on embryos and the transfer rates from adult to eggshell and eggshell to embryo are unknown. However, we suspect that our metal arsenic concentrations ranging from non-detection to 1.51 ppm are at levels that are unlikely to cause significant health impacts to the adult or embryo. Unlike the rest of the metals and metalloids we looked at, higher concentrations of arsenic were associated with lighter colored eggs. However, like the other metals, higher concentrations of arsenic were predicated by eggs with many small circular spots. Longer thinner eggs (breadth-length ratio) was also a predicator for arsenic concentrations. This trend is opposite of what we would have expected if House Sparrows follow trends in other species where eggs get wider and shorter over time, and if older females have higher bioaccumulation.

We did not find a strong enough relationship between the five metals to evaluate the metals in terms of a general contaminant load. We did however find significant positive correlations between individual pairs of metals (As-Cd and Cu-Pb), and negative correlation between arsenic and lead. Swaileh and Sansur (2006) also found a positive correlation between concentrations of lead and copper in House Sparrow eggshells in the West Bank. We are unaware of papers that shown significant relationship between Cd-As and As-Pb. Instead, previous research has shown a negative correlation between arsenic and selenium. In particular, the presence of arsenic can reduce selenium accumulation in the egg; however, we did not find any significant correlations between arsenic and selenium (Stanley et al. 1994). All five metals we analyzed are elements present in the environment naturally and can't be created or destroyed; however, anthropogenic processes can alter the movement, concentration, and presence of these elements in an area (Wuana & Okieimen 2011). Correlations between metals can provide insight into the potential anthropogenic sources of contamination. For example, arsenic is generally a contaminant associated with pesticide use in agricultural areas whereas lead is found in areas with industrial processes and smelting (Wuana & Okieimen 2011). Hence, the negative correlation we found between arsenic and lead could be a separation between agricultural and industrial areas, though more research into the land use surrounding the eggshell sites would be needed to determine if this correlation is due to geographic difference or not.

Implications and Future Research

The concentrations that we found in the eggshells are likely to be low compared to concentrations in other parts of the body. Burger (1994) suggested that eggshells were a way to excrete contaminants when concentrations in the body are high. Swaileh and Sansur (2006) found out of 10 different areas of the body, eggshells contained the lowest metal richness. However, there has been mixed results when comparing eggs to eggshells. Dauwe (1999) found higher levels of non-essential elements (Pb and As) in the eggshell, while higher levels of essential elements (Cu and Zn) in the egg. However, Agusa et al. (2005) found higher levels of selenium, an essential element, in eggshells than eggs. Relationships between eggshell and egg contents may vary but based on previous research levels of contaminants are most likely higher in the body than in the eggshell. As we mentioned, we do not think that the metal concentrations we found are toxic levels, but little research has been done on the threshold concentrations for contaminants in eggshells to impact embryos. Metal concentrations in vital organs and other parts of the House Sparrows may or may not be at toxic levels.

In order to be effective indicators of environmental contamination, having only a limited number of eggshell characteristics that are reliably associated in one direction with increasing concentrations of metals would be ideal. Our results found different directions of association depending on the metal and the variable. Our models also explain very little of the variance in the data and are unlikely to be reliably predictive of metal concentrations. Examining a larger sample size of eggshells may provide more statistical power to tease out clearer trends. However, we are surprised to find that color was included as a predictor in every single model, and speckling included in all but one. Future research on the mechanics behind potential links between stress via contamination and its impact on color and speckling are worth pursuing.

Our research has shown that these five heavy metal and metalloids are in varying concentrations in House Sparrow eggs. Regardless of the predicative power of the eggshell characteristics, determining if there is a predictable relationship between concentrations in the eggshells and concentrations in the local environment is the next step in determining if House Sparrows can be used as an indicator species for these metals. Previous work suggests that the level of contaminants in eggs is likely to reflect recent diet due to a rapid trophic transfer of nutrients before and during egg laying (Ruuskanen et al. 2014). In the case of House Sparrows, a non-migratory bird, the levels of metals in the egg are likely to reflect an area of a home range of up to 8 hectares, with House Sparrows spending most of their time occupying an area of less than 0.2 hectares (Vangestral et al. 2010). Past studies have shown that various birds species had higher metal concentrations in their body at more contaminated sites when compared to a control (Ruuskanen et al. 2014; Ayas et al. 2007; Lam et al. 2005). Ayas et al. (2007) found high bioaccumulation from sediments to eggshells for copper (19.63 ratio) and lead (22.9 ratio) in herons in Turkey, while bioaccumulation for cadmium was low. Ruuskanen et al. (2014) found that European Pied Flycatcher (*Ficedula* hypolecua) eggshell lead levels were correlated with soil lead levels across Europe, but did not find the same correlation for As or Cu. Lam et al. (2005) found that the concentration of arsenic, lead, an copper in the eggshells of Little Egrets (*Egretta garzetta*) and Black-crowned Night Herons had a consistent correlation to concentrations of those metals in coastal marine sediments. They did not however find this same consistent trend for Bridled Terns. In Bridled Terns, only copper showed a significant correlation between eggshell concentrations and concentrations in marine sediments. The same study did not find any significant correlations between concentrations of selenium and cadmium in eggshell and marine sediments for three different species. Evaluating whether there are similar trends between local soils concentrations and the House Sparrow eggshells would provide insight into the ability to use House Sparrow eggs as indicators.

While difference in local distribution of metals in the environment may be causing the variation, we saw in the metal concentrations, diet differences among House Sparrows can also contribute to difference in metal concentrations. In previous studies concentrations of metals in eggshells concentrations varied between species at the same site (Burger 2002). Burger (2002) found that differences in metal concentrations between five species of marine birds were mainly due to differences in diet. Knowing the diet of House Sparrows may be important in understanding the sources of the contaminants and how concentrations of contaminant reflect the local environment. Studies have shown that humans and House Sparrows have a long history of commensalism (Ravinet et al. 2018, Sætre et al. 2012). House Sparrows' genetics changed in response to human agriculture to adapt to a high starch diet similar to humans and dogs (Ravinet et al. 2018). Future research in determining the diet of House Sparrows, through stable isotopes or other analysis may provide insight on the source of contaminants and their use as indicators of exposure for wildlife and potentially humans.

In conclusion, our research indicated that color and speckling are unlikely to be good indicators of heavy metal concentrations in bird eggshells. However, there may still be future exploration of House Sparrows eggs as heavy metal indicators by exploring links between heavy metal concentrations in eggs to concentrations in House Sparrow diets and the environment.

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Table 2.1 Detection Limits of metals. The limits for PQL and DQL limits with the value used (Value Used) in subsequent analyses.

	Ca (%)	As (ug/g)	Cd (ug/g)	Cu (ug/g)	Pb (ug/g)	Se (ug/g)
PQL limit	0 (<0.0005)	<0.30	<0.10	< 0.20	< 0.20	<0.30
Value Used	NA	0.225	0.075	NA	0.15	0.225
MDL limit	0 (<0.0001)	<0.15	<0.05	<0.10	<0.10	<0.15
Value Used	NA	0.075	0.025	0.05	0.05	0.075

Table 2.2 Summary of SpotEgg Results. The minimum (Min), mean, maximum (Max) and standard deviation (Std. Dev), and definition for each variable computed from SpotEgg,

Variable	Min	Mean	Max	Std. Dev.	Definition*
Spots R	 0.09	 0.23	 0.56	0.07	Mean equivalent reflectance in the camera's Red channel for all the spots in the egg.
Spots G	 0.07	 0.21	 0.53	0.07	Mean equivalent reflectance in the camera's Green channel for all the spots in the egg.
Spots B	 0.06	 0.19	 0.48	0.06	Mean equivalent reflectance in the camera's Blue channel for all the spots in the egg.
Background R	 0.14	 0.33	 0.74	0.08	Mean equivalent reflectance in the camera's Red channel for the background of the egg.
Background G	 0.11	 0.32	 0.78	0.09	Mean equivalent reflectance in the camera's Blue channel for the background of the egg.
Background B	 0.10	 0.30	 0.77	0.08	Mean equivalent reflectance in the camera's Blue channel for the background of the egg.

Table 2.2 Summary of SpotEgg Results (Continued) The minimum (Min), mean, maximum (Max) and standard deviation (Std. Dev), and definition for each variable computed from SpotEgg,

Variable	Min	Mean	Max	Std. Dev.	Definition*
Number of Spots	 113.0	 473.5	 1606.0	346.5	Number (count) of detected Spots in the egg
Tot. Area of Spots	 30.45	 46.26	 75.75	7.26	Total spottiness (%) in this egg
Average Spot Size	 0.02	 0.13	 0.62	0.08	Mean spot size (%) for the spots of this egg
Per Vs Area	 9.05	 20.05	 37.49	0.07	Mean eccentricity for the spots in the egg. Describes how circular (lower) or elliptical (higher) the spots are.

Table 2.3 PCA Results. Correlation for each variable with first (PC1) and second (PC2) principal components.

Variable	PC1	PC2
SpotsR	**0.93**	0.32
SpotsG	**0.92**	0.33
SpotsB	**0.91**	0.34
BackGroundR	**0.93**	0.31
BackGroundG	**0.92**	0.33
BackGroundB	**0.93**	0.32
Number of Spots	0.30	**0.92**
Per_vs_Area	-0.19	**-0.83**
Average Spot Size (1/x)	0.37	**0.96**
Total Area of Spots (1/x)	0.31	**0.74**
Proportion of Variation Explained	0.54	0.36
Cumulative Proportion	0.54	0.90

Table 2.4 Results of a two GAMMs for season and geographic difference in color and speckling.

	PC1 Color	Estimate	Std. Error	p-value
Linear	**Intercept**	0.941	0.191	<0.001
	Collection Date	-0.007	0.001	<0.001
		edf	ref.df	p-value
Smooth	**Longitude + Latitude**	14.33	14.33	<0.001
	R2 (adj.)	0.17		

	PC2 Speckling	Estimate	Std. Error	p-value
Linear	**Intercept**	-0.377	0.163	0.021
	Collection Date	0.003	0.001	0.020
		edf	ref.df	p-value
Smooth	**Longitude + Latitude**	16.43	16.43	0.003
	R2 (adj.)	0.04		

Table 2.5 Summary of eggshell metal concentrations. (ND- Below detection limit)

n=100	**Ca (%)**	**As (µg/g)**	**Cd (µg/g)**	**Cu (µg/g)**	**Pb (µg/g)**	**Se (µg/g)**
Geometric Mean	34.66	0.58	0.05	1.02	0.29	0.63
Arithmetic Mean (SD)	34.7±1.62	0.72±0.37	0.09±0.15	1.97±1.52	0.52±0.62	0.87±0.55
Median	34.95	0.77	0.05	2.06	0.41	0.83
Range (Min.-Max.)	30.90-37.50	ND-1.51	ND-0.99	ND-8.88	ND-4.66	ND-2.31

Table 2.6 Kendall correlation table for metals. Values reported are tau with probably in parentheses.

tau (Kendall)	**Ca**	**As**	**Cd**	**Cu**	**Pb**	**Se**
Ca	********					
As	-0.02 (p=0.73)	********				
Cd	0.06 (p=0.42)	**0.30 (p<0.001)**	********			
Cu	-0.13 (p=0.06)	-0.11 (p=0.12)	-0.09 (p=0.23)	********		
Pb	-0.09 (p=0.21)	**-0.16 (p=0.02)**	-0.08 (p=0.319)	**0.47 (p<0.001)**	********	
Se	0.02 (p=0.73)	-0.01 (p=0.94)	0.02 (p=0.78)	0.10 (p=0.14)	-0.01 (p=0.89)	********

Table 2.7 Mean concentrations of metals found in eggshells in previous studies

Study	Location	Species	Ca (%)	As	Cd	Cu	Pb	Se
House Sparrow								
Present Study	USA	House Sparrow	34.7±1.62	0.72±0.37	0.09±0.15	1.97±1.52	0.52±0.62	0.87±0.55
Swaileh & Sansur 2006	West Bank	House Sparrow	-	-	0.01±0.00	1.00±0.10	**3.3±0.6**	-
Al-Obaidi et al. 2012	Baghdad, Iraq	House Sparrow	97.30±0.85	-	-	-	0.41±0.02	-
		Collared Dove	97.80±0.86	-	-	-	0.42±0.02	-
		Rock Dove	97.80±0.86	-	-	-	0.40±0.03	-
		White-eared Bulbul	97.40±0.84	-	-	-	0.44±0.04	-
Other Passerines and Terrestrial Birds								
Dauwe et al. 1999	Antwerp, Belgium	Great and Blue Tits	-	**1.20±0.60**	0.05±0.01	1.72±0.23	0.37±0.16	-
	Hoboken, Belgium	Great and Blue Tits	-	**4.20±0.80**	**0.80±0.60**	**3.20±0.50**	**15.00±4.00**	-
Hargitai et al. 2016	Budapest, Hungary	Great Tits	-	-	-	1.38±0.37	0.18±0.06	-
	Pilis Mountains, Hungary	Great Tits	-	-	-	1.2±0.22	0.11±0.05	-
Kraus 1989	New Jersey, USA	Tree Swallows	-	-	**1.8**	**2.4±2.5**	**90.9±59.5**	-
Mora et al. 2003	Arizona, USA	Willow Flycatcher	-	**1.30±0.20**	-	**2.50±0.90**	**0.90±0.60**	**1.20±0.70**
		Yellow-breasted Chat	-	**2.10±0.40**	-	**6.20±8.00**	**0.60±0.70**	0.50±0.30
Orlowski et al. 2010	Poland	Rook	-	**32.57**	-	**8.13**	-	-
Orlowski et al. 2014	Poland	Rook	-	-	**0.51**	-	**3.29**	-
Orlowski et al. 2016	Milicz Ponds, Poland	Reed Warbler (no embryo)	25.36	-	**2.1**	**7.91**	**5.65**	-
		Reed Warbler (embryo)	23.13	-	**2.36**	**9.69**	**7.12**	-
Ruuskanen et al. 2014	Europe & Russia	European Pied Flycatcher	27.62-33.58	.008-1.32	-	**2.19-2.83**	0.17-0.45	-
Waterbirds and Raptors								
Agusa et al. 2005	Rishiri Island, Japan	Black-tailed Gull	-	-	0.01±0.01	0.54±0.09	0.06±0.04	0.42±0.15
Ayas et al. 2007	Ankara, Turkey	Black-crowned night heron	-	-	**0.23±0.19***	1.69±0.17*	**1.11±0.87***	-
		Grey Heron	-	-	**0.93±0.49***	**6.76±1.20***	**6.83±2.75***	-
Ayas et al. 2008	Aydinick Island, Turkey	Audouin's gull	-	-	-	1.86±2.57	**0.95±1.01**	-
	Karaburun Island, Turkey	Audouin's gull	-	-	-	**10.2±16.04**	**4.60±5.81**	-

Table 2.7 Mean concentrations of metals found in eggshells in previous studies (Continued)

Study	Location	Species	Ca	As	Cd	Cu	Pb	Se
Burger 1994	Long Island, USA	Herring Gull	-	-	0.05±0.01	-	0.30±0.05	0.40±0.03
		Roseate Tern	-	-	**0.10±0.04**	-	**1.20±0.30**	<0.005
Currie and Valkama 1997	Harjavalta, Finland	Eurasian Curlew	0.24±0.03	-	-	**9.56±0.61**	-	-
	Kauhava, Finland	Eurasian Curlew	0.30±0.05	-	-	**8.03±0.24**	-	-
	Vammala, Finland	Eurasian Curlew	0.30±0.05	-	-	**7.70±0.28**	-	-
Dev et al. 2010	Assam, India	Indian Pond-heron	-	-	0.08	-	**0.91**	-
		Eurasian Bittern	-	-	0.05	-	**0.79**	-
		Cattle Egret	-	-	0.06	-	**0.58**	-
		Little Egret	-	-	0.06	-	**0.81**	-
		Cinnamon Bittern	-	-	0.07	-	**0.84**	-
		Common Little Bittern	-	-	0.07	-	**0.81**	-
Dolci et al. 2017	Currais Island, Brazil	Brown Booby	-	**2.37±1.01**	0.03±0.03	0.99±0.48	-	-
Hashmi et al. 2013	Pakistan	Cattle Egret	-	-	**0.10-1.23**	0.06-0.11	0.13-5.40	-
		Little Egret	-	-	**0.75-1.02**	0.12-0.29	**1.09-1.90**	-
Ikemoto et al. 2005	Tarishima Island, Japan	Black-footed albatross	-	-	**0.10±0.35**	0.78±0.13	0.04±0.03	0.15±0.06
		Short-Tailed albatross	-	-	0.01±0.01	0.77±0.10	0.01±0.006	0.08±0.03
Kim and Oh 2014	Korea	Black Tailed Gull	-	-	**0.45±0.28**	**2.80±0.92**	**3.10±1.35**	-
Lam et al. 2005	Hong Kong	Black-crowned Night Heron	-	-	0.01±0.002	1.12±0.40	0.03±0.01	**8.16±0.20**
		Bridled Tern	-	0.40±0.07	0.002±0.001	1.24±0.41	0.06±0.04	**15.58±1.87**
		Little Egret	-	-	0.006±0.002	1.60±0.73	0.15±0.17	**7.59±0.67**
Metcheva et al. 2011	Antarctica	Gentoo Penguin	17.04±4.75	<0.3	<0.05	1.24±0.40	**0.68±0.30**	<0.05
Morera et al. 1997	Ebro Delta, Spain	Audouin's Gull	-	-	-	**2.14±0.70**	-	**4.12±1.45**
Rickard and Schuler 1990	Washington, USA	Canada Goose	37.8	-	-	**12.2**	-	-
		Bald Eagle	36.4	-	-	**12**	-	-
		Ferruginous Hawk	37.2	-	-	**9.7**	-	-
		Golden Eagle	36	-	-	**10**	-	-
		Great Blue Heron	37.3	-	-	**9**	-	-
		Osprey	35.7	-	-	**9.3**	-	-
		Ring-billed Gull	36.4	-	-	**8.5**	-	-

Table 2.7 Mean concentrations of metals found in eggshells in previous studies (Continued)

Study	Location	Species	Ca	As	Cd	Cu	Pb	Se
Rickard and Schuler 1990	Washington, USA	Swainson's Hawk	32.7	-	-	**8.5**	-	-
Rodriquez-Navarro et al. 2002	Blythe Island, GA, USA	Clapper Rails	-	0.21±0.09	-	1.37±0.64	0.23±0.10	0.71±0.28
	Brunswick, GA, USA	Clapper Rails	-	0.21±0.09	-	1.71±0.58	0.37±0.52	**0.90±0.26**
Simonetti et al. 2015	Bahia Blanca, Argentina	American Oystercatcher	-	-	**13.28**	**2.02**	**7.23**	-

Table 2.8 Model selection table for copper concentrations

	Intercept	Collection Date	PC2	Ca	PC1	Col. Date: PC1	Latitude	df	AICc	Δ AICc	weight	R^2
	1.97	0.40	-0.26	-	-	-	-	4	366.89	0.00	0.035	0.07
	1.97	0.38	-0.28	-0.19	-	-	-	5	367.36	0.47	0.028	0.09
	1.97	0.33	-	-	-	-	-	3	367.70	0.81	0.024	0.05
	1.97	0.34	-0.26	-	-0.18	-	-	5	367.75	0.86	0.023	0.09
	1.97	0.31	-	-0.18	-	-	-	4	368.48	1.59	0.016	0.06
	2.03	0.35	-0.28	-	-0.10	0.18	-	6	368.50	1.61	0.016	0.10
	1.97	0.27	-	-	-0.18	-	-	4	368.58	1.69	0.015	0.06
	1.97	0.40	-0.29	-0.23	-	-	-0.15	6	368.59	1.70	0.015	0.10
	1.97	0.33	-0.28	-0.17	-0.16	-	-	6	368.61	1.72	0.015	0.10
	1.97	0.41	-0.27	-	-	-	-0.10	5	368.64	1.75	0.015	0.08
Avg:	**1.97**	**0.36**	**-0.20**	**-0.07**	**-0.05**	**0.01**	**-0.02**					

Table 2.9 Model selection table for cadmium concentrations

	Intercept	Ca	PC1	PC2	Thickness	Breadth-Length	Collection Date	Latitude	df	AICc	Δ AICc	weight	R^2
	-1.15	0.46	0.51	-0.65	-0.53	-	-	-	5	112.18	0.00	0.028	0.17
	-1.14	0.54	0.51	-0.59	-0.48	-0.37	-	-	6	112.44	0.26	0.025	0.19
	-1.16	0.45	0.41	-0.56	-0.55	-	-0.38	-	6	112.46	0.28	0.024	0.19
	-1.15	0.48	-	-0.57	-0.54	-	-0.51	-	5	112.93	0.75	0.019	0.17
	-1.08	-	0.55	-0.60	-0.60	-	-	-	4	113.07	0.89	0.018	0.15
	-1.10	-	0.44	-0.50	-0.62	-	-0.41	-	5	113.08	0.90	0.018	0.16
	-1.09	0.61	0.51	-0.53	-	-0.40	-	-	5	113.18	1.00	0.017	0.16
	-1.08	0.53	0.50	-0.59	-	-	-	-	4	113.48	1.30	0.015	0.14
	-1.15	0.51	0.44	-0.52	-0.51	-0.29	-0.31	-	7	113.57	1.39	0.014	0.20
	-1.08	0.40	0.41	-	-0.52	-	-0.49	-	5	113.82	1.64	0.012	0.16
	-1.17	0.61	0.55	-0.62	-0.53	-0.41	-	0.25	7	113.88	1.71	0.012	0.20
	-1.18	0.51	0.54	-0.68	-0.56	-	-	0.19	6	113.91	1.73	0.012	0.18
	-1.20	0.51	0.43	-0.59	-0.58	-	-0.43	0.26	7	113.92	1.74	0.012	0.20
	-1.05	-	0.44	-	-0.59	-	-0.52	-	4	113.98	1.80	0.011	0.14
	-1.10	-	-	-0.51	-0.62	-	-0.55	-	4	114.01	1.83	0.011	0.14
	-1.05	0.52	0.55	-	-0.42	-0.45	-	-	5	114.03	1.85	0.011	0.16
	-1.10	0.53	0.41	-0.53	-	-	-0.35	-	5	114.03	1.85	0.011	0.16
	-1.08	0.47	0.44	-	-0.47	-0.35	-0.40	-	6	114.10	1.92	0.011	0.17
Avg:	**-1.12**	**0.40**	**-0.43**	**-0.48**	**-0.46**	**-0.12**	**-0.22**	**0.03**					

Table 2.10 Model selection table for lead concentrations

	Intercept	PC1	Latitude	PC2	df	AICc	Δ AICc	weight	R^2
	0.52	-0.11	-	-	3	189.30	0.00	0.062	0.03
	0.52	-	-	-	2	190.28	0.98	0.038	0.00
	0.52	-0.12	-0.06	-	4	190.64	1.34	0.032	0.04
	0.52	-0.11	-	-0.04	4	191.12	1.82	0.025	0.03
Avg:	**0.52**	**-0.08**	**-0.01**	**-0.01**					

Table 2.11 Model selection table for arsenic concentrations

	Intercept	Breadth/Length	PC1	Latitude	Collection Date	PC2	df	AICc	Δ AICc	weight	R^2
	0.72	-0.06	0.07	-	-	-	4	84.93	0	0.043	0.06
	0.72	-	0.07	-	-	-	3	85.20	0.27	0.038	0.04
	0.72	-0.06	-	-	-	-	3	86.45	1.51	0.020	0.02
	0.72	-0.06	0.07	0.03	-	-	5	86.54	1.61	0.019	0.06
	0.72	-	-	-	-	-	2	86.65	1.71	0.018	0.00
	0.72	-	0.06	-	-0.03	-	4	86.84	1.91	0.017	0.04
	0.72	-	0.07	0.02	-	-	4	86.92	1.98	0.016	0.04
	0.72	-	0.07	-	-	-0.024	4	86.92	1.99	0.016	0.04
Avg:	**0.722**	**-0.25**	**0.054**	**0.005**	**-0.002**	**-0.002**					

Table 2.12 Model selection table for selenium concentrations

	Intercept	Latitude	Ca	Longitude	PC1	Breadth-Length	Thickness	df	AICc	Δ AICc	weight	R^2
	0.87	0.11	-	-	-	-	-	3	167.51	0.00	0.042	0.04
	0.87	0.12	0.06	-	-	-	-	4	168.36	0.85	0.027	0.05
	0.87	0.14	-	-0.06	-	-	-	4	168.69	1.18	0.023	0.05
	0.87	0.10	-	-	-0.05	-	-	4	168.77	1.26	0.022	0.04
	0.87	0.10	-	-	-	0.05	-	4	168.98	1.47	0.020	0.04
	0.87	-	-	-	-	-	-	2	169.04	1.53	0.019	0.00
	0.87	0.11	0.07	-	-0.06	-	-	5	169.31	1.80	0.017	0.06
	0.87	0.10	-	-	-	-	-0.03	4	169.47	1.96	0.016	0.04
Avg:	**0.87**	**0.10**	**0.02**	**-0.01**	**-0.01**	**0.01**	**0.00**					

Figure 2.1 Example of a standardized clutch photograph. Photographs were taken by clutch, with each egg assigned a letter. Photographs included color charts and a ruler.

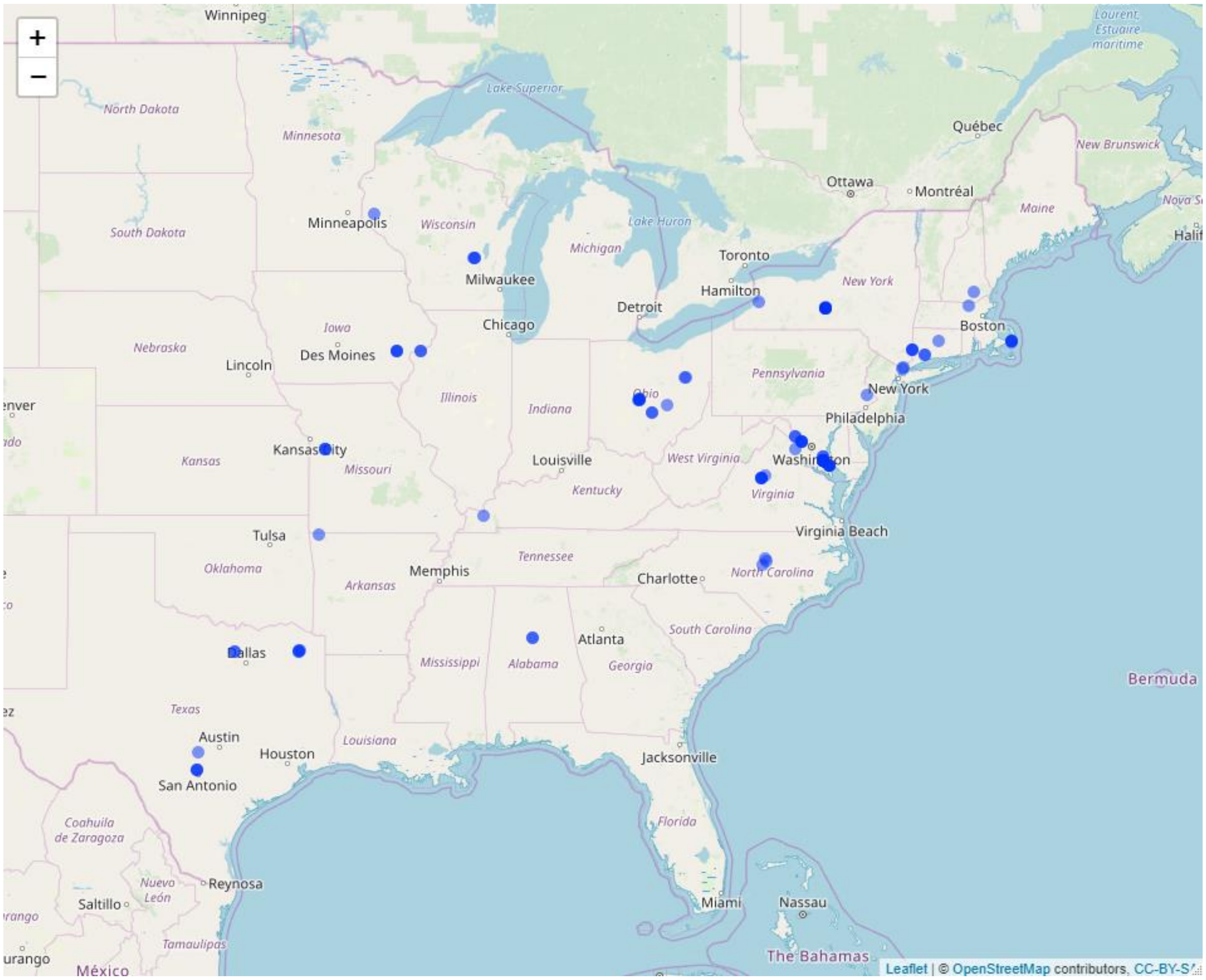


Figure 2.2 Locations of eggs tested for metals. Map of the location of the eggs (n=100) subsampled for metal analysis.

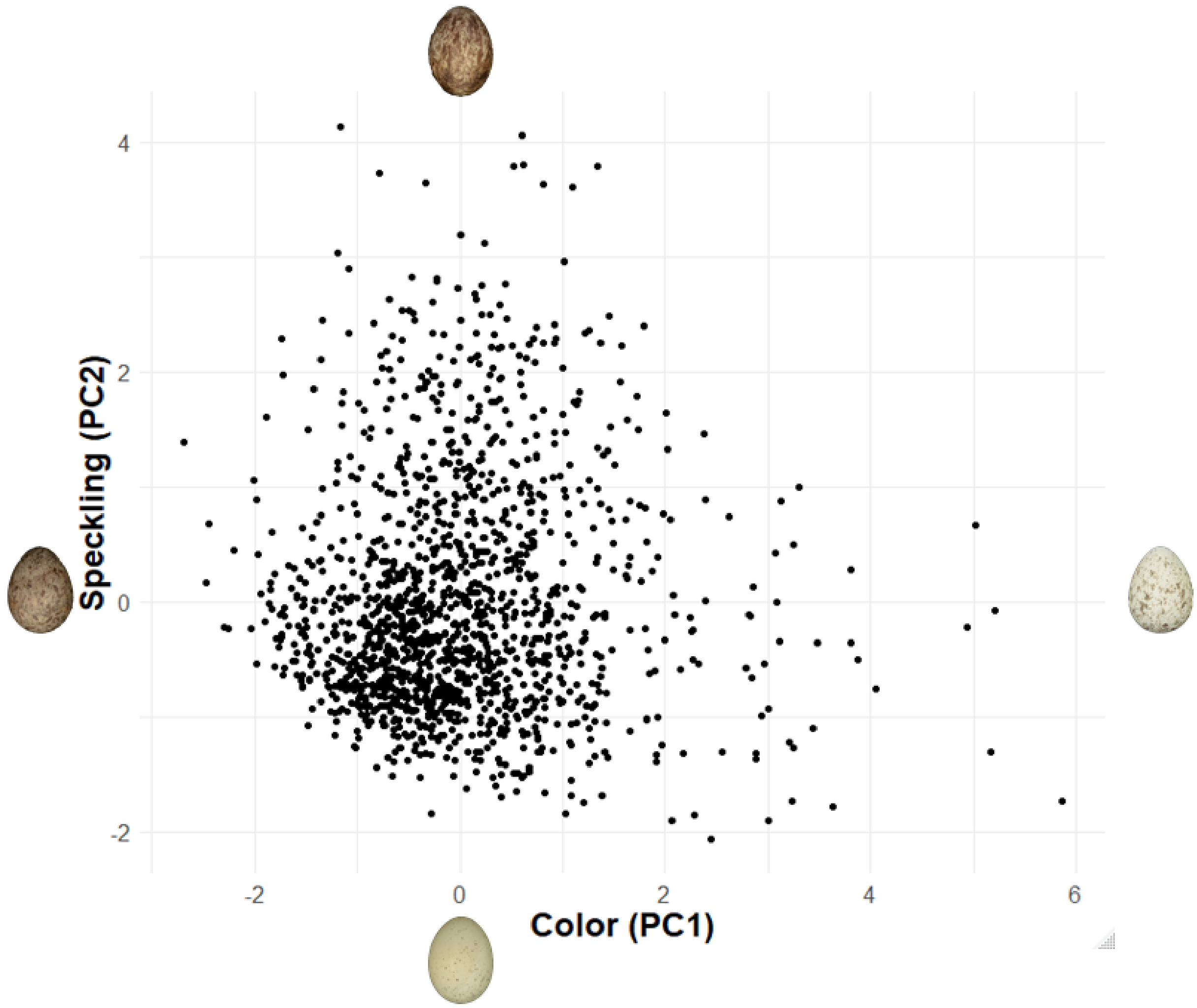


Figure 2.3 PCA Graph. Distribution of eggs (n = 1426) across the two principal components representing color (PC1) and speckling (PC2) of the eggs.

Figure 2.4 Examples of breadth-length ratio. Examples of breadth-length ratios for minimum (1), mean (2), and maximum (3) bread-length width ratios we found in our House Sparrow eggs.

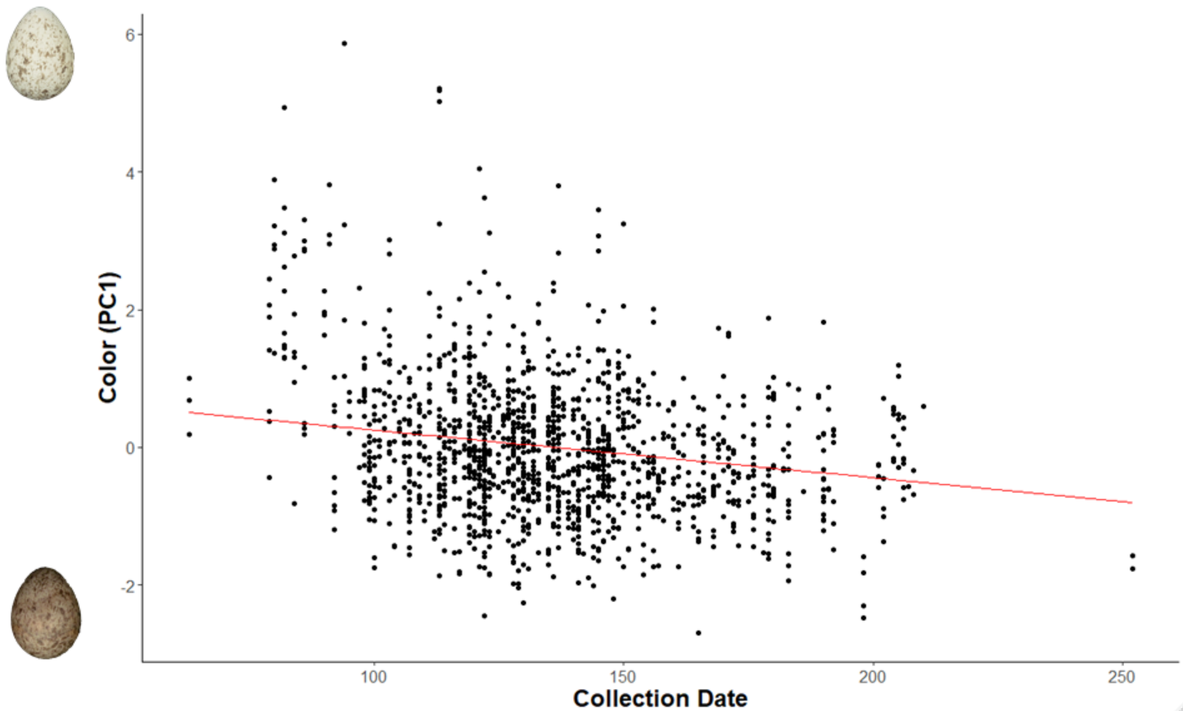


Figure 2.5 Color of eggshells across the breeding season(s). The red predication line from the GAMM results, which included clutch as a random effect.

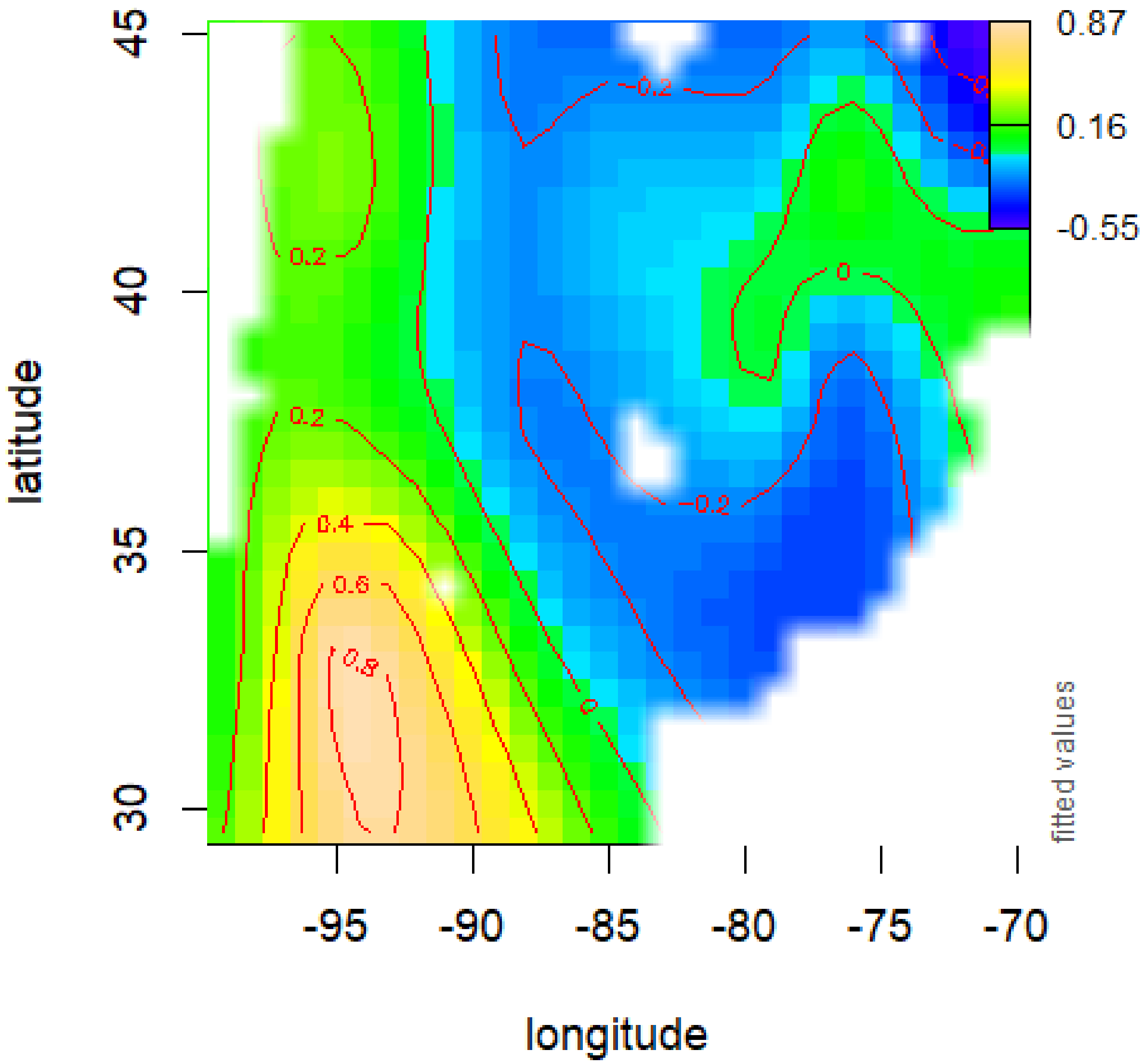


Figure 2.6 Predictions of geographic variation in egg color (PC1) from a GAMM.

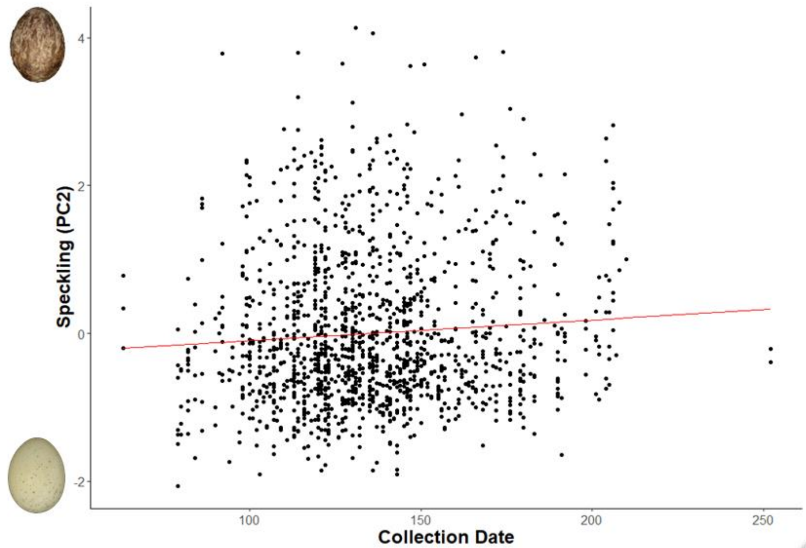


Figure 2.7 Speckling of eggshells across the breeding season(s). The red predication line from the GAMM results, which included clutch as a random effect.

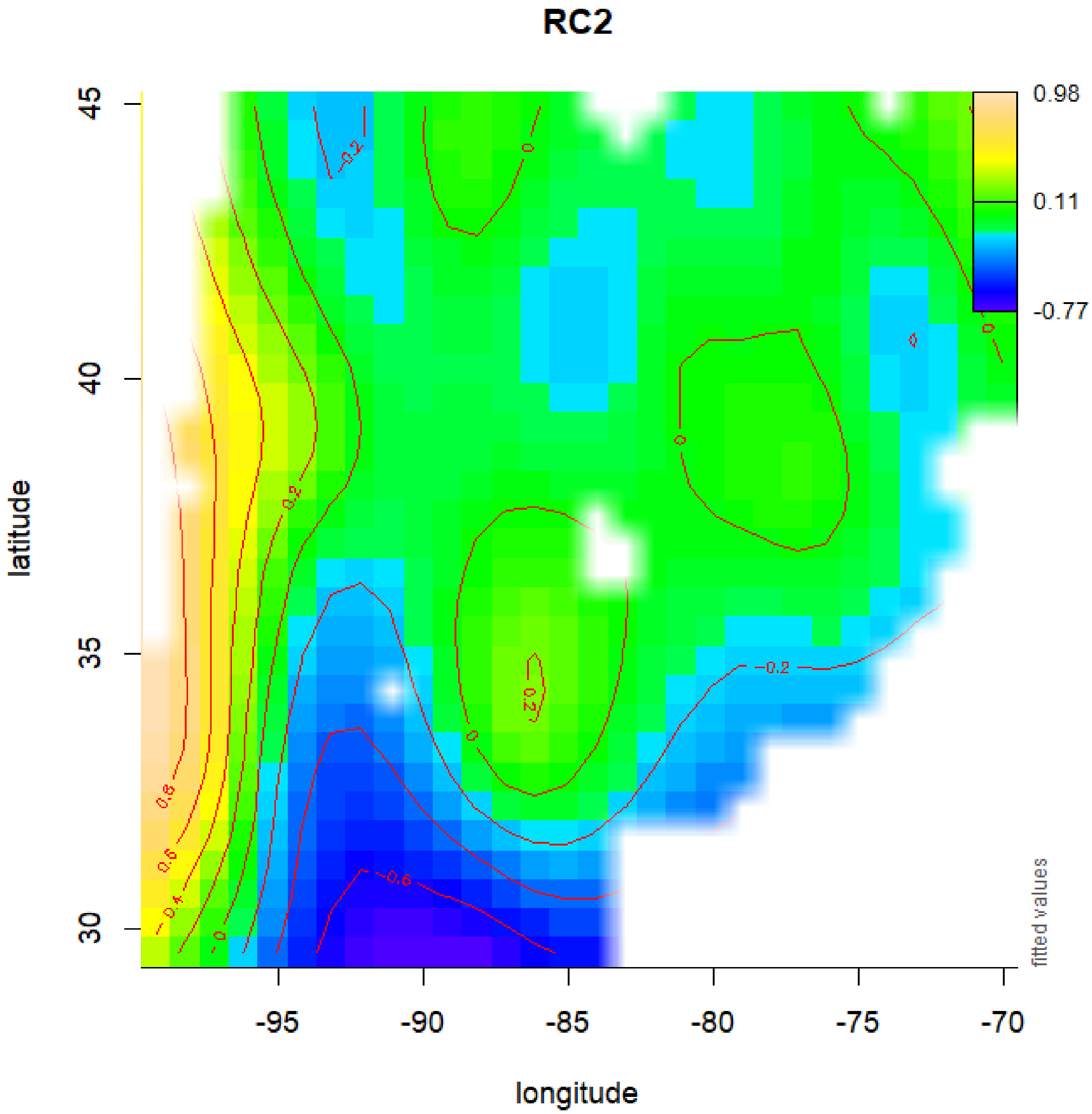


Figure 2.8 Predictions of geographic variation in speckling (PC2) from a GAMM.

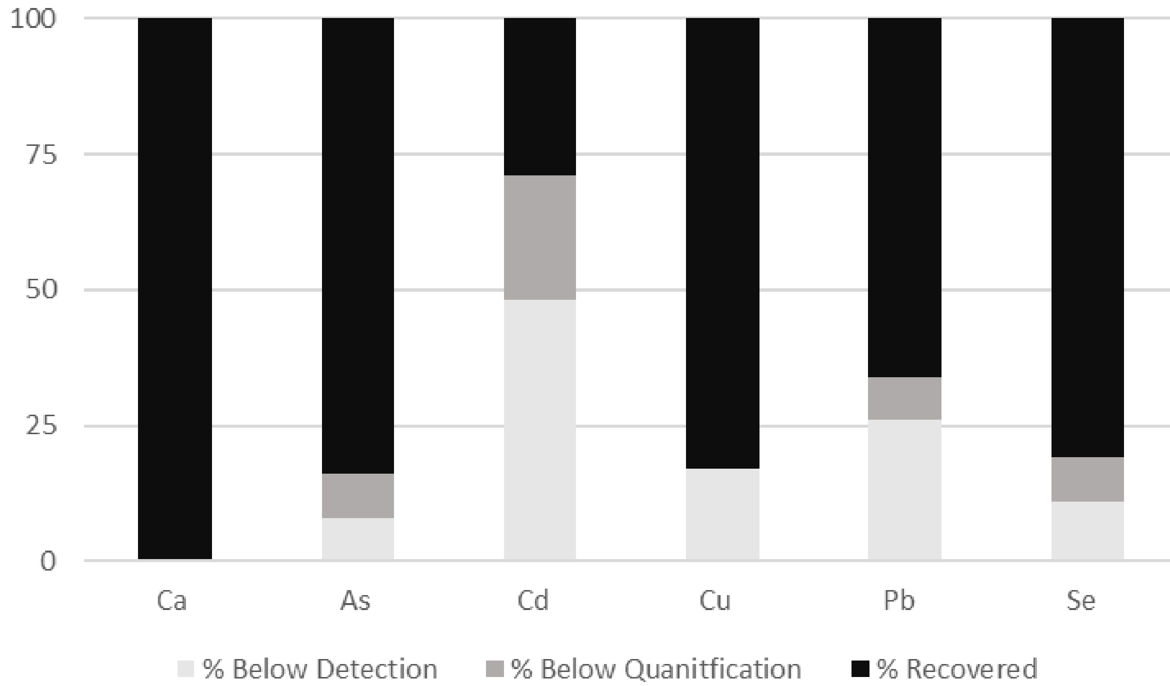


Figure 2.9 Recovery rates (%) of metals in the House Sparrow eggshells. (n = 100)

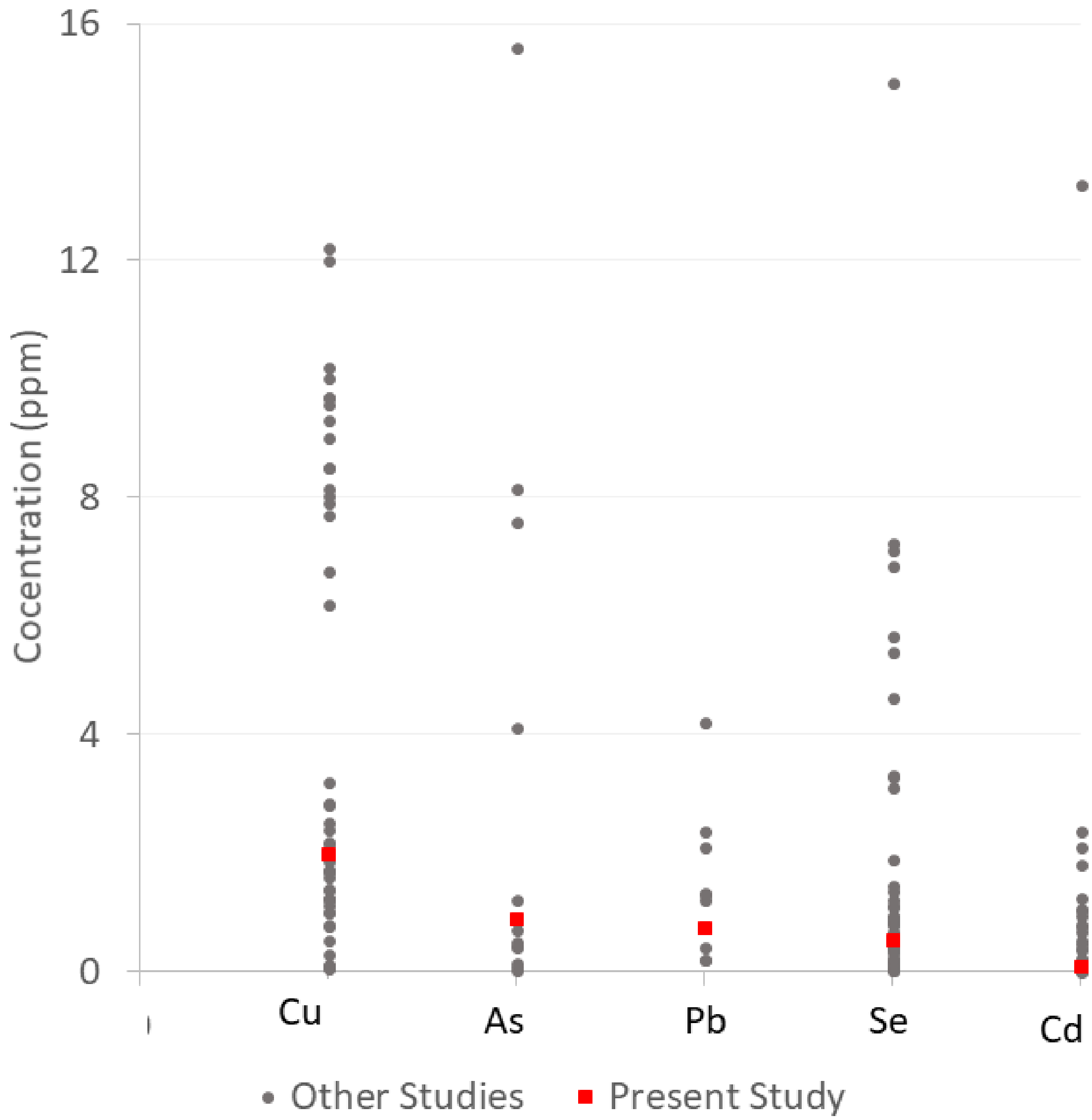


Figure 2.10 Comparison of mean concentrations of metals in House Sparrow eggshells to other studies. Concentrations of metals in House Sparrow eggshells fall within the range of concentrations found in the eggshells of various bird species.

APPENDICES

Appendix 1.1 Sparrow Swap Project Management

General Overview

Sparrow Swap is a citizen science project that engages nestbox monitors from across the United States to participate in scientific research surrounding House Sparrow management. After a pilot year (2015) with Master Naturalists in Virginia, Sparrow Swap was expanded nation-wide from 2016-2018. In February 2016, 2017, and 2018 the Sparrow Swap Team sent an informational email to bluebird societies, ornithological societies, and Audubon chapters inviting interested nestbox monitors to participate in Sparrow Swap. In order to participate, volunteers signed up for the project via scistarter.org, a website designed to aggregate and run citizen science projects. Once joining and agreeing to the informed consent (Appendix 1.2), volunteers were able to download field protocols and datasheets for both a Swap (Appendix 1.3) and Removal (Appendix 1.4). We, the Sparrow Swap team then mailed hand-painted wooden House Sparrow replicas volunteers interested in swapping eggs (Figure 1.1). Over the course of 3 years (2016-2018) approximately 200 participants joined the project Sparrow Swap, with 84 individuals sending in datasheets on the outcomes of management.

Recruitment

Each spring in February and March interest letters were sent to leaders of bluebird groups and other bird focused organizations across the country requesting that they share information on Sparrow Swap to their members. We specifically targeted bird focused organizations, and specifically organizations that were focused on bluebirds or maintained trails of nestboxes. The intent of targeting these organizations was to better capture an audience of potential citizen scientist who already have the skill and knowledge to correctly identify and manage House Sparrow nests.

Joining the Project

Participants joined the project using SciStarter.org, an online citizen science platform that aggregates citizen science projects. Sparrow Swap also used SciStarter's tools to manage sign-ups and messaging to participants through SciStarter. In order to participate, the citizen scientists were asked to agree to an informed consent in compliances with Institutional Review Board requirements. After joining the project, participants could download datasheets and contact the Sparrow Swap Team to request wooden house sparrow eggs be mailed to them. When requesting eggs, we asked participant to estimate the number of House Sparrow nests they intended to swap.

Communication

When datasheets and eggs were received in the mail, participants were notified that the eggs had been received along with any other relevant information. If the data sheet was incomplete or inconsistent, the Sparrow Swap Team would try to clarify any information with the participant. In addition to individual communications, regular mass updates about the project were sent on approximately a three-week basis throughout the breeding season to inform participants of the status of the project. Messages includes important information about participating in the project, updates on scientific research goals, new stories and other relevant media relating to any aspect of the project. In addition, relevant information provided in the messages were also posted to a blog on sparrowswap.org to allow anyone, including those who did not sign up for the project to learn more about the project. These regular updates provided opportunities for participants to ask questions and interact with the Sparrow Swap Team. In the summer of 2018, a question and answer webinar was also conducted to provide an opportunity to engage participants in the project. Yearly updates (2017-2018) were sent to participants in the form of report backs which remained available in the Downloads section of the Sparrow Swap website (www.sparrrowswap.org)

*Volunteer Advisory Boards***.**

In 2018, we created a Sparrow Swap Advisory Board consisting of six volunteers who were chosen to represent the diversity of types of participants in Sparrow Swap. The goal of the advisory board was to get feedback on report backs, changes in datasheets, and general feedback to better understand how to improve both communication and the research goals of Sparrow Swap.

Appendix 1.2 Informed consent for Sparrow Swap Participants

The purpose of Sparrow Swap is to (1) build the egg collection at the North Carolina Museum of Natural Science to support scientific studies of eggshell characteristics and any environmental contaminants the eggs may contain, and (2) determine the most effective ways for volunteer bluebird monitors of minimizing damage by House Sparrows.

By enrolling in Sparrow Swap, you will be collecting, chilling, packaging, and shipping House Sparrow eggs according to specified protocols, while providing information on the location of where House Sparrow eggs originated. We keep all information about you, including precise information on nestbox locations, confidential and individual identities anonymous in all reports and publications. Project data are stored on a secure server in a password-protected database, accessible only to the researchers. Your identity will not be directly associated with any contaminants possibly found in eggs.

There is no compensation for participating; the benefit of participating is a better understanding of how the eggs of House Sparrows in your nest boxes compare to eggs elsewhere, and how House Sparrows in your next nestbox respond to egg removals compared to House Sparrows in the nest boxes of other participants.

Please remember that wild bird eggs, like any raw animal materials such as raw meats from the grocery store, may contain pathogens. Store House Sparrow eggs in their own container in the refrigerator and always wash your hands after handling eggs.

If you have questions about the research, please contact caren.cooper@naturalsciences.org. If you feel you rights as a citizen scientist in Sparrow Swap have been violated during the course of this project, you may contact Deb Paxton, Regulatory Compliance Administrator, Box 7514, NCSU Campus (919/515-4514).

Appendix 1.3 Swapper Instructions and Datasheet

Sparrow Swap Project, North Carolina Museum of Natural Science
11 West Jones St. Raleigh, NC 27601

SPARROW SWAP

Swapper Instructions

Questions? contact us at sparrowswap@ncsu.edu

Why be a Swapper? As a Swapper, the information provided in the follow-up visits will help determine effective management strategies for minimizing damage done by house sparrows.

Step 1: PREPARE CLUTCH CARRIER- Fill container of choice (Tupperware container will do) with rice or birdseed as cushioning to bring with you into the field for egg collection.

Step 2: REMOVE & SWAP FAKE EGGS- To increase adoption of replica eggs, remove and swap eggs AFTER the house sparrow (HOSP) has finished completely laying her clutch (typically around 4-6 eggs). During this step, try to be undetected by the female, but record on the datasheet under comments whether you see a sparrow and whether the female flushes from nest during your visit. Warm egg replicas in your hand or pocket, remove and place real eggs into carrier and swap the same number of warmed replicas into the nest. To ensure that, on subsequent nest visits, you are able to discern whether the nest or eggs have been attended, either photograph the replica clutch in the nest and/or place a small object (a piece of grass or yarn) over the clutch. **If you are uncertain whether this nest belongs to a HOSP do not remove the eggs.**

THIS IS VISIT #1 – FILL IN TOP SECTION OF SWAP DATASHEET

Step 3: LABEL & CHILL CLUTCH- Be sure to keep each clutch separate by only placing one clutch per container and labeling each container with the nestbox name and collection date. Place the whole container into a fridge while you complete the follow-up observations.

Step 4: FOLLOW-UP OBSERVATIONS- Please observe the nestbox for 3 visits (21 days) unless any bird lays an egg or something removes all of the replicas from the nest. Record the a) species using the nestbox, b) nestbox contents, c) whether you see the parents, and d) whether the nest is attended or abandoned during a follow-up visit about 7 days after the initial swap **(Visit #2)**. Visit again about 14 days **(Visit #3)** and about 21 days **(Visit #4)** after the initial swap. Count the replicas at each visit to make sure no replicas are missing. If another HOSP egg(s) is laid after the initial swap, please remove the replicas and end that datasheet. On the same visit choose if you want to be a Collector, Remover, or Swapper for the new clutch and start a new datasheet accordingly. If no new eggs have been laid by Visit #4, remove the replicas and the nest from the nestbox.

Step 5: PACKAGE & SHIP HOSP EGGS, REPLICAS, AND DATASHEET(S)- Before shipping let eggs sit at room temperature for about an hour. Wipe off any condensation that might have formed on the shell. Download the packaging protocol PDF from our SciStarter page or follow the instructions shown in the video at http://bit.do/sparrowswapvideos

You'll need the following materials to package your eggs:

- Plastic Easter eggs
- Paper towels
- Cushioning material (ex. newspaper)
- Press'n Seal plastic wrap
- Egg cartons
- Box to ship to the Museum

Ship box to the museum at:

Caren Cooper
ATTN: Sparrow Swap
North Carolina Museum of Natural Science
11 West Jones St.
Raleigh, NC 27601-1029

Appendix 1.3 Swapper Instructions and Datasheet (continued)

[ID:]	**SWAPPER DATASHEET**	[Lab Use Only: NCSM]

Data to be collected on Visit #1				
	Contact Information	Full Name:	SciStarter Username:	
		Email Address:		
		Home Address:		
	Nest Box Information	Nest Box Name:		**If you cannot access lat./long. information, please note here the closest address to where the box is located.** ____________ ____________ ____________
		HOSP Deterrents: Halo__ Spooker__ None__ Other__________		
		*Box Latitude: __________ *Box Longitude: __________ *Can be found via handheld GPS device or free Google Maps smartphone app OR list the closest address in the box to the right		
	Collection Information (Visit #1)	Swap Date:	Clutch Size:	1st Egg Date:
		Comments:	Did you see a HOSP during the visit? **Y / N** If yes, did a HOSP flush from the nestbox? **Y / N**	

Data On Nestbox Activity After Swap (Visits #2, #3, #4)

	Visit #2	**Visit #3**	**Visit #4** (remove replicas)
Date:	__________	__________	__________
Species:	__________	__________	__________
Number of real eggs:	__________*	__________*	__________*
Number of replicas:	__________	__________	__________
What is in the nestbox? (circle)	Replicas rotated Replicas not rotated Object moved No object movement Replicas warm Replicas not warm Other________	Replicas rotated Replicas not rotated Object moved No object movement Replicas warm Replicas not warm Other________	Replicas rotated Replicas not rotated Object moved No object movement Replicas warm Replicas not warm Other________
Did you see the parents? (circle)	Flushed parents Parents nearby No parents seen Other________	Flushed parents Parents nearby No parents seen Other________	Flushed parents Parents nearby No parents seen Other________
Do you think the nestbox is (circle)...	Abandoned or Attended	Abandoned or Attended	Abandoned or Attended

Comments: (feel free to write on back)

* Stop datasheet. If new eggs are HOSP, start a new datasheet as a Remover, Swapper, or Collector.

Appendix 1.4 Remover Instructions and Datasheet

Sparrow Swap Project, North Carolina Museum of Natural Science
11 West Jones St. Raleigh, NC 27601

SPARROW SWAP

Remover Instructions

Questions? contact us at sparrowswap@ncsu.edu

Why be a Remover? As a Remover, the information provided in the follow-up visits will help determine effective management strategies for minimizing damage done by house sparrows. The house sparrow eggs you provided will be photographed and analyzed to understand variation in color and speckling of house sparrow eggs across the United States.

Step 1: PREPARE CLUTCH CARRIER - Fill container of choice (Tupperware container will do) with rice or birdseed as cushioning to bring with you into the field for egg collection.

Step 2: REMOVE CLUTCH - Remove house sparrow (HOSP) eggs AFTER the HOSP parent has finished completely laying her clutch (typically around 4-6 eggs). Collect HOSP eggs from nestbox by GENTLY rolling (not pinching) eggs into palm and placing eggs into pre-made clutch carrier. **You can choose to remove or leave the nest, but please mark on the datasheet which option you choose**. If you remove the nest, dispose of the nest far away from the nestbox to avoid attracting predators. **If you are unsure whether this is a house sparrow nest, do not remove the eggs.**

THIS IS VISIT #1 – FILL IN TOP SECTION OF REMOVER DATASHEET

Step 3: LABEL & CHILL CLUTCH- Be sure to keep each clutch separate by only placing one clutch per container and labeling each container with the nestbox name and collection date. Place the whole container into a fridge while you complete the follow-up observations.

Step 4: FOLLOW-UP OBSERVATIONS - Please observe the nestbox until another egg (HOSP or native bird) has been laid or 3 visits (21 days) after the removal, whichever comes first. Record a) the species using the nestbox, b) nestbox contents, c) whether you see the parents, and d) whether the nest is attended or abandoned during a follow-up visit about 7 days after the initial removal **(Visit #2)**. Visit again about 14 days **(Visit #3)** and about 21 days **(Visit #4)** after the initial removal. If/when a new HOSP clutch has been laid, decide whether you want to be a Collector, Remover, or Swapper for that clutch and start a new datasheet for the clutch accordingly.

Step 5: PACKAGE & SHIP HOSP EGGS AND DATASHEET(S)- Before shipping let eggs sit at room temperature for about an hour. Wipe off any condensation that might have formed on the shell. Download the packaging protocol PDF from our SciStarter page or follow the instructions shown in the video at http://bit.do/sparrowswapvideos

You'll need the following materials to package your eggs:

- Plastic Easter eggs
- Paper towels
- Cushioning material (ex. newspaper)
- Press'n Seal plastic wrap
- Egg cartons
- Box to ship to the Museum

Ship box to the museum at:

Caren Cooper
ATTN: Sparrow Swap
North Carolina Museum of Natural Science
11 West Jones St.
Raleigh, NC 27601-1029

Appendix 1.4 Remover Instructions and Datasheet (Continued)

[ID:]	REMOVER DATASHEET	[Lab Use Only: NCSM]	
Data to be collected on Visit #1			
Contact Information	Full Name:	SciStarter Username:	
	Email Address:		
	Home Address:		
Nest Box Information	Nest Box Name:	**If you cannot access lat./long. information, please note here the closest address to where the box is located.** ________ ________ ________	
	HOSP Deterrents: Halo__ Spooker__ None__ Other________		
	*Box Latitude: ________ *Box Longitude: ________ *Can be found via handheld GPS device or free Google Maps smartphone app OR list the closest address in the box to the right		
Collection Information (VISIT #1)	Collection Date:	Clutch Size:	1st Egg Date:
	Did you remove the nest from the nestbox? **Y / N** Did you see a HOSP during the visit? **Y / N** If Yes, did a HOSP flush from the nestbox? **Y / N**	Comments:	

Data On Nestbox Activity After Removal (Visits #2, #3, #4)

	Visit #2	**Visit #3**	**Visit #4**
Date:	________	________	________
Species:	________	________	________
What is in the nestbox? (circle)	Empty box Nest material Eggs* Chicks* Other________	Empty box Nest material Eggs* Chicks* Other________	Empty box Nest material Eggs* Chicks* Other________
Did you see the parents? (circle)	Flushed parents Parents nearby No parents seen Other________	Flushed parents Parents nearby No parents seen Other________	Flushed parents Parents nearby No parents seen Other________
Do you think the nestbox is (circle)...	Abandoned or Attended	Abandoned or Attended	Abandoned or Attended

Comments: (Feel free to write on back)

* Stop datasheet. If the new eggs are HOSP, start a new datasheet as a Remover, Swapper, or Collector.

Appendix 2.1 Egg Packaging Protocol

Sparrow Swap Project, North Carolina Museum of Natural Science
11 West Jones St. Raleigh, NC 2760

Egg Packaging Protocol

Goal: Suspend real HOSP eggs in plastic Easter eggs using layers of Press'n Seal.

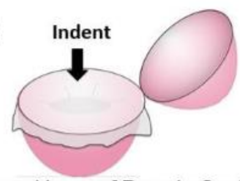
Materials Needed:

- Plastic Easter eggs
- Egg carton
- Paper towel
- Press'n Seal
- Scissors
- Masking tape
- Pen or marker
- Box
- Cushioning materials

1

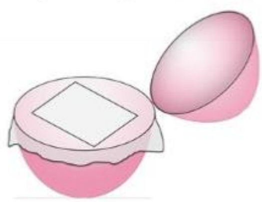
Let eggs sit a room temp. for an hour. Wipe off any condensation

2



Spread layer of Press'n Seal. Create an indent to hold the egg.

3

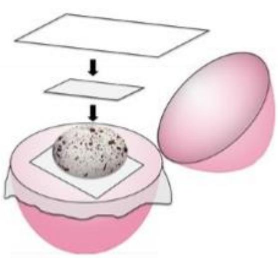


Place a square of paper towel

4

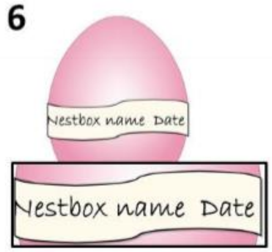
Gently place HOSP egg on top
Tip: Use egg carton to steady egg.

5



Gently repeat with another layer of paper towel & Press'n Seal

6



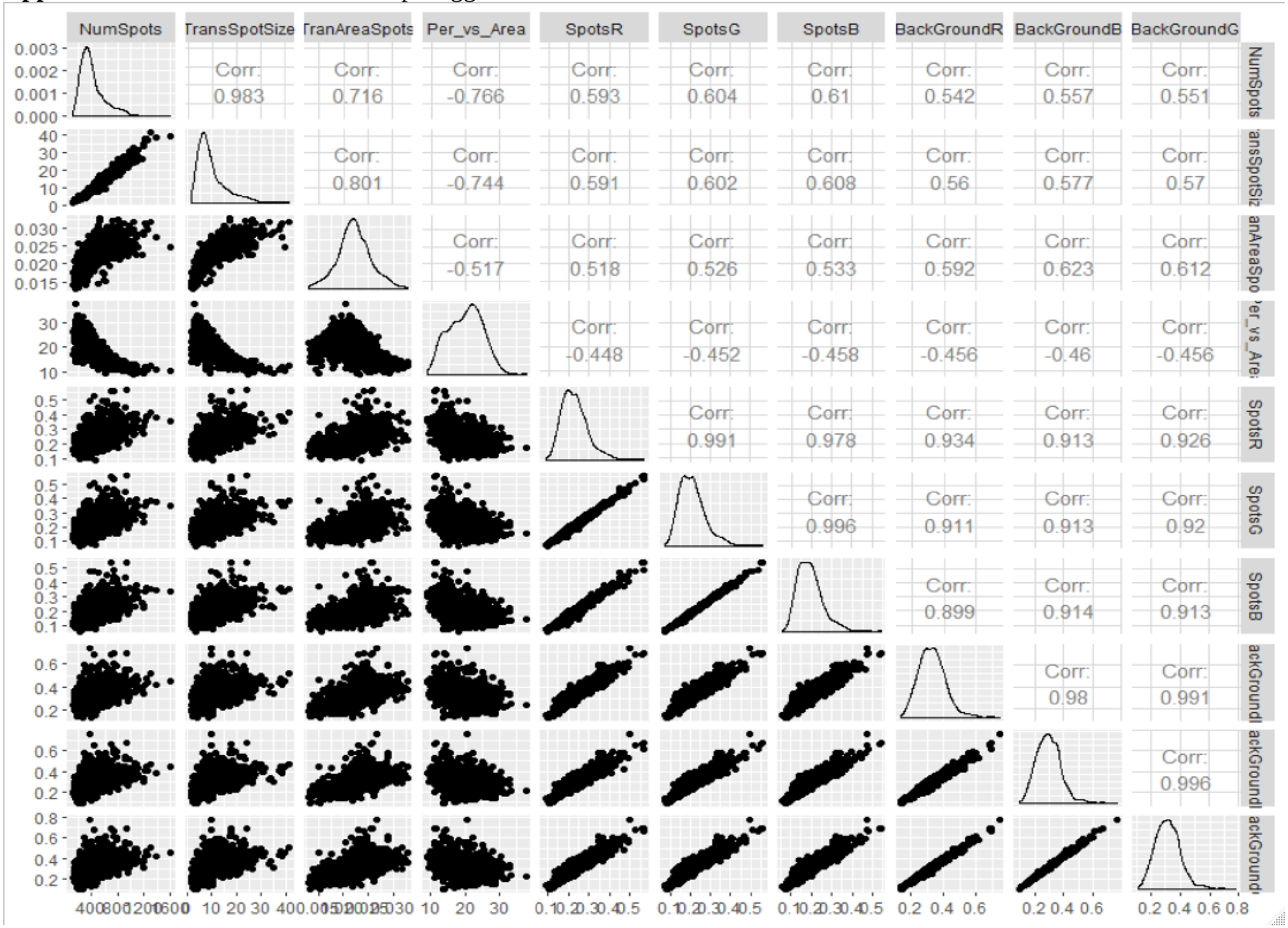
Close & tape egg shut. Label nestbox name and collection date.

7 Place labeled eggs into egg cartons. Place filled cartons, data sheets, and replicas (if applicable) into a box with enough room to add cushioning material. Fill all empty space with cushioning material such as newspaper and/or bubble wrap to secure the cartons.

Ship box to the museum at:

Caren Cooper
ATTN: Sparrow Swap
North Carolina Museum of Natural Sciences
11 West Jones St.
Raleigh, NC 27601-1029

Appendix 2.2 Correlation Matrix of SpotEgg Variables



Appendix 2.3 Correlation Matrix of Eggshell Characteristics