Anthropogenic materials and feathers incorporated in European starling (*Sturnus vulgaris*) nests and their effects on reproductive success

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A Thesis Submitted to Saint Mary's University, Halifax, Nova Scotia in Partial Fulfillment of the

Requirements for the Degree of Master of Science in Applied Science

September 2024, Halifax, Nova Scotia, Canada

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ABSTRACT

There are numerous hypotheses that may explain why certain materials are used in nest building. I tested the availability and age hypotheses on an eastern Canadian population of European starlings (*Sturnus vulgaris*). The percentage of nests containing anthropogenic materials was positively associated with number of garbage items within the surrounding area. No relationship was detected between anthropogenic materials within nests and age of either parent. I found no effect of the amount of anthropogenic materials on nestling brood condition; however, a negative relationship between fledging success and cigarette butts within nests existed. When determining if feather colour effected hatching success, I found a positive relationship with pigmented feathers. Previous research showed species have a preference for white coloured nesting materials. When offered different coloured nesting materials, starlings frequently choose white and silver. These findings increase our understanding of how nest materials and their colours affect avian reproduction.

Date: September 3rd, 2024

ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge and give thanks to my wonderful supervisor Dr. Colleen Barber who has dedicated so much of her time helping me navigate my Master of Science degree. I would not have been able to achieve this degree without her kindness, support, and patience throughout this journey. She has helped me find my deep passion for birds and showed me what I achieve to be someday. I will carry the plentiful knowledge she has taught me for the rest of my life.

I would like to give thanks to my committee members Dr. Laura Weir, Dr. Erin Cameron and Dr. Matthew Reudink for all their support and helping me navigate the slippery slopes of figuring out how to do my stats, plus giving me great suggestions for my research.

I would like to thank my lab mate Alyssa as she has been working by my side throughout all of the crazy field days while gathering data in all various weather conditions and helping me achieve in getting all of the data I would need for this thesis. I would like to thank my previous lab mate Seth for all the support he has given me as we worked together during our Honours and for coming to visit me even after he graduated while I spent the long hours in the lab dissecting nests.

I would also like to give thanks to my mother and brother for all their support and cheering me on throughout this journey. Along with my friends Lexy, Sarah, Morgan and Clara for all their support and listening to me talk endlessly about my research and birds.

Lastly, I would like to give thanks to my partner Jordan who has been tremendously supportive of me and has given me lots of encouragement throughout this process.

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CHAPTER 1: General Introduction

Anthropogenic materials

Use of anthropogenic materials such as plastic increased after World War II when technology advanced to produce them more easily than metal, glass and ceramics, and at a much lower cost (Kedzierski et al. 2020). The increase in the world's population since the 1950s when plastic was first made readily available helped to accelerate their use (Kedzierski et al. 2020). Historically, 50% of plastic produced was for single use, while only 20-25% was produced for long-term use (Kedzierski et al. 2020). A global analysis completed by Geyer at al. (2017) estimated that 6300 Megatons (Mt) of plastic waste was produced between 1950 and 2015. Of that 6300 Mt, an estimated 4600 Mt (60%) has been discarded in landfills or the environment, with another 800 Mt (12%) incinerated and 600 Mt (9%) recycled. Geyer et al. (2017) estimate that by 2050, the amount of plastic in landfills and the environment will increase to 12 000 Mt. As anthropogenic materials remain in the environment, they accumulate in places ranging from oceans, farmlands, forests (Kedzierski et al. 2020), and even our own blood in the form of nanoplastics (Lamoree 2022). These materials are often picked up by wildlife, including birds as they forage and them sometimes incorporate them into their nests (Feare 1984; Heldbjerg et al. 2017).

Materials in avian nests

Nests are critical for most avian species to successfully reproduce as they provide shelter for the eggs and offspring (Hansel 2000). Avian nests are typically constructed of both natural and anthropogenic materials that are collected from the surrounding environment (Clark and Mason 1985; Feare 1984). Natural materials include feathers, twigs or dried plants such as leaves and grasses and flowers (Feare 1984; Ruiz-Castellano et al. 2018), while anthropogenic materials include fabrics, plastics, fishing supplies and strings/ropes. (Feare 1984; Garcia-Cegarra et al. 2013; Townsend and Barker 2014). Cigarette butts are also incorporated into some bird nests, and studies suggest that this is a learned parental behaviour that can result in benefits to offspring (Suárez-Rodríguez et al. 2013; Suárez-Rodríguez & Garcia 2014). For example, Suárez-Rodríguez & Garcia (2014) demonstrated that cigarette butts decreased the abundance of nest ectoparasites, resulting in a greater weight gain among House finches (*Carpodacus mexicanus*). However, they also found a positive relationship between cigarette butts in a nest and the number of nuclear abnormalities in the red blood cells as well as a greater degree of genotoxicity damage (Suárez-Rodríguez & Garcia 2014).

Several studies have investigated the incorporation of anthropogenic materials in the nests of avian species from both marine (Brentano et al. 2020; Grant et al. 2018; Lee et al. 2015; Garcia-Cegarra et al. 2020; Montevecchi 1991), and terrestrial environments (Antczak et al. 2010; Briggs et al. 2023; Francila et al. 2023; Hammer et al. 2017; Harvey et al. 2020; Hudecki et al. 2020; Jagiello et al. 2018; Jagiello et al. 2019; Jagiello et al. 2022; León-E et al. 2024; Sergio et al. 2011; Suárez-Rodriguez et al. 2013; Tariq et al. 2024; Townsend and Barker 2014; Vasquez et al. 2022; Wang et al. 2009). Hypotheses for why birds use anthropogenic materials are numerous. The availability hypothesis suggests that species choose materials because it is what is available to them in their surrounding environment (Wang et al. 2009). The amount of anthropogenic materials within a nest has been positively correlated with their abundance in the surrounding environment (Antczak et al. 2010; Harvey et al. 2021; Jagiello et al. 2018; Jagiello et al. 2020; Jagiello et al. 2022; Tariq et al. 2024; Vasquez et al. 2022; Wang et al. 2009). The signaling hypothesis proposes that anthropogenic nesting materials could serve as an ornament or decoration (Sergio et al. 2011) and is selected to either attract a partner (Borgia 1985; Ruiz-

Castellano et al. 2018) or repel intruders (Sergio et al. 2011). The adaptive hypothesis (Clayton and Wolf 1993) suggests that anthropogenic materials are incorporated into nests to decrease the abundance of parasites or other arthropods (Hammer et al. 2017; Suárez-Rodríguez et al. 2013). Finally, the age hypothesis states that older parents incorporate more anthropogenic materials into the nest as was found in female White storks (*Ciconia ciconia*; Jagiello et al. 2018). The incorporation of anthropogenic materials in nests has been suggested to be due to courtship signaling as seen among Black kite (*Milvus migrans*) nests, where the amount of debris increased with parental age up to 12 years (Sergio et al. 2011).

The occurrence of anthropogenic materials within avian nests is concerning due to the possibility of entanglement or ingestion. Townsend and Barker (2014) found that 5.6% (11/195) of American crow (*Corvus brachyrhynchos*) nestlings died due to entanglement from anthropogenic materials within the nest. Other studies (Antczak et al. 2010; Hudecki et al. 2020; Jagiello et al. 2018; Montevecchi 1991; Ryan 2018) have reported similar findings highlighting the potential serious consequences of incorporating certain types of anthropogenic materials to their nests. Another concern is the threat of ingesting these materials which could lead to physiological changes (Cunha et al. 2022; Fry et al. 1987) and/or suffocation (Jagiello et al. 2018). Jagiello et al. (2019) revealed that among 25 published studies on anthropogenic materials incorporated into nests, 20% had reports of ingestion, 36% of nests had reports of entanglement and 12% reported both occurring.

The role of feathers within nests and whether feather colour has an impact on reproduction

Feathers occur naturally in the environment and are often incorporated into nests (Hansel 2000). Feathers may be beneficial to nestling development within the nest. Lombardo et al.

(1995) and Stephenson et al. (2009) found that Tree swallow (*Tachycineta bicolor*) nestling development, measured as mass on day 12 of the nestling period, was positively associated with the number of feathers in a nest. The increase in body mass could be due to the thermoregulation benefits that feathers provide before the nestlings have developed their own feathers. Furthermore, Barn swallows (*Hirundo rustica*; Peralta-Sanchez et al. 2011) and Spotless starlings (*Sturnus unicolor*; Ruiz-Castellano et al. 2018) chose white over pigmented feathers for their nests. This preference may be due to possible anti-microbial properties found in white feathers that decrease the harmful bacteria load on eggshells (Peralta-Sanchez et al. 2010; Peralta-Sanchez et al. 2011; Peralta-Sanchez et al. 2014), resulting in intrinsic benefits such as increased hatching success (Peralta-Sanchez et al. 2011).

White nesting materials appear to be the most preferred by adults of several species (Brentano et al. 2020; Briggs et al. 2023; Cunha et al. 2022; Garcia-Cegarra et al. 2020; Sergio et al. 2011; Tavares et al. 2019). This colour preference has been demonstrated in both anthropogenic materials (Brentano et al. 2020; Briggs et al. 2023; Cunha et al. 2022; Garcia-Cegarra et al. 2020; Sergio et al. 2011; Tavares et al. 2019) and feathers (Peralta-Sanchez et al. 2011; Ruiz-Castellano et al. 2018) incorporated into nests. This choice may be based on the availability of materials in the surrounding environment (e.g. Garcia-Cegarra et al. 2020).

Study Species

European starlings are an urban-dwelling, non-native species found living in urban and rural environments (Barton et al. 2020). They are a double-brooded passerine (Dunnett 1955; Feare 1984; Kessel 1957) who lay an average of five eggs per clutch which are incubated for 12 days (Feare 1984; Kessel 1957). Nestlings remain in the nest for 20-24 days, receiving care from both parents (Dunnett 1955; Feare 1984; Kessel 1957). Parents forage for food and nest materials up to 500 m from their nests (Feare 1984; Heldbjerg et al. 2017). Starlings are an ideal species for this study as they incorporate both natural and anthropogenic materials within their cavity nests (Clark and Mason 1985).

Objectives

My study aims to determine possible reasons why European starlings incorporate anthropogenic materials and feathers from other species into their nests, and the consequences of incorporating these items. I also examined if there is a colour preference for nesting material (feathers, ribbons) and if feather abundance (as measured by mass) and colour affect brood success.

My second chapter focuses on determining why starlings incorporate anthropogenic materials in their nests by exploring the availability and the age hypotheses. This chapter adds to the growing research on whether nesting material selection in avian species is based on what is available in their surrounding environment (Antczak et al. 2010; Harvey et al. 2021; Jagiello et al. 2018; Jagiello et al. 2020; Jagiello et al. 2022; Tariq et al. 2024; Vasquez et al. 2022; Wang et al. 2009), or is a learned behaviour that increases with age (Jagiello et al. 2018; Sergio et al. 2011).

My third chapter concentrates on the potential effects that anthropogenic materials within the nests have on brood success. I explore whether there is an impact on hatching success, brood condition and fledging success.

My fourth chapter determines whether the abundance of feathers within nests has a positive effect on nestling growth (Lombardo et al. 1995; Stephenson et al. 2009). I will also determine if feather colour has an impact on hatching success (Peralta-Sanchez et al. 2011) and if there is a colour preference in nesting materials as has been demonstrated in other studies (Brentano et al. 2020; Briggs et al. 2023; Cunha et al. 2022; Garcia-Cegarra et al. 2020; Sergio et al. 2011; Tavares et al. 2019) by performing an experimental study on whether starlings have a colour preference for ribbons when offered simultaneously to them.

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CHAPTER 2: Mechanisms driving the use of anthropogenic materials in European starling (*Sturnus vulgaris*) nests

ABSTRACT

Avian nests often contain both natural materials and anthropogenic materials. Anthropogenic materials can include plastics, paper, string/twine, fishing supplies and cigarettes. Several hypotheses including the availability and age hypotheses exist on why avian species incorporate anthropogenic materials within their nests. The availability hypothesis suggests that species choose materials based on what is available in their surrounding environment, while the age hypothesis proposes a positive relationship between the quantity of anthropogenic materials in a nest and the age of either one or both parents. My first objective was to determine if areas within Nova Scotia, Canada having higher amounts of garbage pollution also had a greater frequency of European starling (*Sturnus vulgaris*) nests with anthropogenic materials. The frequency of nests containing anthropogenic materials was greater at sites containing higher amounts of garbage pollution. Likewise, nests with a decreased probability of having anthropogenic materials were located at sites with lower amounts of garbage pollution present. Secondly, I examined whether the amount of anthropogenic materials (measured by mass) within nests increased with age of either the male or female parent but found no such relationship. It remains unknown why starlings choose anthropogenic materials for their nests, but our results suggest the incorporation may be related to what is available in the surrounding area.

INTRODUCTION

Avian nests are built from natural materials such as parts of plants and feathers but also sometimes contain anthropogenic materials such as plastics, fabrics, twine/string, paper, fishing supplies and cigarette butts. There are several hypotheses as to why birds incorporate anthropogenic materials into their nests. The availability hypothesis suggests that species choose materials based on availability in their surrounding environment. Several studies support this hypothesis because of finding a positive correlation between the amount of anthropogenic materials within avian nests and the amount of anthropogenic materials available in the surrounding environment (Antczak et al. 2010; Harvey et al. 2021; Jagiello et al. 2018; Jagiello et al. 2020; Jagiello et al. 2022; Tariq et al. 2024; Vasquez et al. 2022; Wang et al. 2009). For example, Lee et al. (2015) studied the conservation of Black-faced spoonbills (*Platalea minor*) off the west coast of South Korea by determining how the proportion of nests containing plastic changed after providing natural materials (tree branches and rice straws). Prior to natural material being provided, 71% (20/28) of nests contained plastic. Two years after providing natural materials this proportion decreased to 33% (14/43), along with the number of nests increasing from 28 to 43 (Lee et al. 2015).

The age hypothesis suggests a relationship between the quantity of anthropogenic materials in a nest and the age of either one (Jagiello et al. 2018), or both parents (Sergio et al. 2011). Sergio et al. (2011) suggested the level of "decorations" (anthropogenic materials) in Black kite (*Milvus migrans*) nests increases with time, hitting a maximum at 10 -12 years of age after which it decreased (Sergio et al. 2011). Similarly, the probability of finding anthropogenic materials in White stork (*Ciconia ciconia*) nests was positively correlated with the age of the female parent, but not with that of the male parent (Jagiello et al. 2018). The above two studies suggest that incorporating anthropogenic materials within nests might be a learned behaviour.

Although not examined in this study, anthropogenic materials could potentially be incorporated into avian nests as a display to either attract a partner (Borgia 1985; Ruiz-Castellano et al. 2018) or repel intruders (Sergio et al. 2011) as suggested in the signaling hypothesis. For example, the male Satin bowerbird (*Ptilonorhynchus violaceus*) displays materials within his bower to attract a partner (Borgia 1985). This material usually consists of feathers or blue objects such as plastic bottle tops (Wojcieszek et al. 2006). Another example is demonstrated among Black kites as they use anthropogenic materials to display a threat against intruders (Sergio et al. 2011). Of all Black kite nests, 77% (n = 127) contained anthropogenic materials which appeared within the 20 days before egg laying (Sergio et al. 2011). Increased decoration levels in the nest correlated with the parents increased attack rate and success on fending off any intruders that came close to the nest territory. Sergio et al. (2011) suggested that these nest decorations signal territory and individual quality, along with the level of dominance seen in social interactions due to the cost of decorations in the nest being higher for lower-quality individuals.

The adaptive hypothesis suggests that anthropogenic materials are incorporated into nests based on intrinsic benefits such as decreasing the abundance of parasites or other arthropods. Hammer et al. (2017) examined the mass of anthropogenic materials incorporated into Great Tit (*Parus major*) and Blue Tit (*Cyanistes caeruleus*) nests and their effect on arthropod diversity within the nest. They reported that 84% (77/94) of Great and Blue Tit nests contained anthropogenic materials and found a reduction in the overall diversity of arthropods within nests (Hammer et al. 2017). Suárez-Rodríguez et al. (2013) performed an experimental study determining if cigarette butts found incorporated into House sparrow (*Passer domesticus*) and House finch (*Carpodacus mexicanus*) nests reduced their ectoparasite load. They found that smoked cigarettes resulted in significantly fewer ectoparasites within these nests (Suárez-Rodríguez et al. 2013). However, physiological changes associated with cigarette butts can occur. For example, the presence of cigarette butts was associated with nuclear abnormalities in the red blood cells of House finch nestlings (Suárez–Rodríguez and Garcia 2014).

In my study I tested the availability and age hypotheses on European starlings (*Sturnus vulgaris*). Starlings are cavity nesters (Kessel 1957) typically having two clutches between April and July (Dunnett 1955; Feare 1984; Kessel 1957). Females produce an average clutch size of five eggs that are incubated by both parents for approximately 12 days after the last egg is laid (Feare 1984; Kessel 1957). After hatching, nestlings remain in the nest receiving care from both parents until fledging occurs around days 20 – 24 of the nestling period (Dunnet 1955; Feare 1984; Kessel 1957). Males build the nest structure out of dried vegetation to attract a female for mating (Feare 1984). The cup lining of the nest is composed of softer materials including feathers and anthropogenic materials such as cloth, string, paper, and wool. By late March or April, more nest materials are incorporated either by the male or female. Marples (1936) found that males decorated the nest, even during the incubation period. However, Feare (1984) found that once pair formation occurred, the female completed nest construction and then solicited copulations from that male.

The first objective of this study was to determine the percentage of nests containing anthropogenic materials at four different locations of varying garbage pollution in Nova Scotia, Canada. I then tested whether there was a positive relationship between the percentage of nests containing anthropogenic materials and the level of garbage pollution in the surrounding area, predicting there would be a positive relationship. Secondly, I tested whether there was a difference in the mass of anthropogenic materials in nests between the younger verses older breeding adults (male or female parent), predicting a significant difference with older parents having higher masses of anthropogenic materials.

METHODS

Study sites

Four different sites were used for this study. My first study site was located at Saint Mary's University in Halifax, Nova Scotia, Canada (44.6313° N, -63.5815° W). The campus of Saint Mary's University is located in the downtown area of Halifax over a large city block with many old trees and green spaces. This site is home to a population of starlings breeding in approximately 40 nestboxes installed 2-3 m off the ground on mature trees. This breeding population has been monitored annually since 2007.

The other three sites were located in different areas around Nova Scotia with each site containing 16 nest boxes. Nova Scotia Hospital is located in Dartmouth, Nova Scotia (44.6511° N, -63.5491° W) and is located on the hospital grounds, surrounded by trees, green spaces and a community vegetable garden. Otter Lake is located at the city landfill, Nova Scotia (44.6171° N, -63.7342° W) and contains grassy fields surrounded by forests and garbage disposal areas. The third site is located at Graves Island Provincial Park in Chester, Nova Scotia (44.5611° N, -64.2083° W) which is a small camping island with a road connecting it to the mainland. The area has many trees, and green spaces. All data were collected from 2021-2023, spanning April until July of each year.

Data collection and analysis – level of garbage pollution

I determined the level of garbage pollution at the four sites by counting anthropogenic materials along four different 50 m transects at each site during the year of 2023. Starting from the center of the nest box colony, the 50 m transects were laid out in four directions (north, east, south, west). I counted garbage items that occurred within 1m of either side of the transect line. Garbage items were only counted if they were deemed a reasonable size for starlings to use as nesting materials. I did a chi-square test to determine if the total number of garbage items counted from each site was statically different than expected (based on all sites being the same). Statistical analysis was done using Quick Cals (GraphPad) and results were considered significant when $P < 0.05$.

Data collection – nests

Nests were monitored throughout the egg laying, incubation and nestling periods. I closely monitored the nests from days 20-24 of the nestling period to determine fledging at Saint Mary's University in 2021-2023. I collected the nests from the other three sites at two separate periods during the breeding season (late April and mid-July). After fledging was complete or the nest had been abandoned, I collected the nest in a large plastic bag and stored it in a -20 C freezer. Before dissection of the anthropogenic materials began, I removed the nest from the freezer and allowed it to thaw and dry out. I then moved the dry nest to a 2-layer sieve for dissection, running warm water over it. The openings of the top layer of the sieve were 1.27 cm while those of the bottom layer were 4 mm to allow better separation of the nest materials. I then placed the nest materials on a tray to dry for approximately 2-3 days at room temperature. The anthropogenic materials were weighed on a Scout Pro SP202 scale to the nearest 0.01 gram and stored in plastic bags for future use.

Data collection and analysis –anthropogenic materials vs age

Adult starlings were caught at the Saint Mary's University site from 2021-2023 while their offspring were in the nest boxes, using one of two different methods. A Swiffer mop was used when the nest box had many trees or bushes in the nearby vicinity. This method involved hiding near the nestbox and waiting for the adult to visit with food for their offspring. Once the parent went inside the box, I would run to the nestbox and quietly cover the hole of the nest box with the Swiffer mop. Another person would use a ladder to carefully remove the adult from the nest box. If the nest was in an area without any nearby trees or parked cars, I used a mo-trap (Stutchbury and Robertson 1986), which consists of a trapdoor (a square piece of wood on a metal hinge) placed inside the nest box. The trapdoor was triggered to close by a propped-up twig that would move when the adult flew into the nestbox.

After catching the adult, she/he was banded with a Canadian Wildlife Service band on the right leg along with either a yellow (male) or a pink (female) coloured plastic band to indicate the sex. The left leg was banded with two coloured plastic bands whose colour combination was unique to that individual for identification. Seven hackle (throat) feathers were removed and placed in a labeled plastic bag for age group categorization (either SY, second year or ASY, after second year adults; Kessel 1951; Barber and Wright 2017). ASY females and males have longer iridescence lengths on their hackles than SY adults. A female was considered SY if the iridescence length was < 6.5 mm and ASY if the iridescence length was > 6.5 mm. A male was considered SY if his iridescence length was < 11.0 mm and ASY if the iridescence length was >

11.0 mm (Kessel 1951; Barber and Wright 2017). There were six SY females and two SY males, along with 96 ASY females and 68 ASY males.

Due to data not being normally distributed I completed a Mann-Whitney test comparing the mass of anthropogenic materials within nests for SY vs ASY females, and again for SY vs ASY males. Statistical analyses were completed using R (version 2024.04.2+764, R Core Team). Mean $\pm$ SE are presented. Results were considered significant when $P < 0.05$.

RESULTS

Level of garbage pollution

I found a difference in the number of anthropogenic materials counted to quantify garbage pollution at each of the four sites ($X^2 = 162.004$, df = 3, P = 0.0001; Table 1.1). Graves Island and Saint Mary's University had significantly less anthropogenic materials than expected. Nova Scotia Hospital and Otter lake had significantly more anthropogenic materials than expected.

Table 1.1 Summary of the level of garbage pollution at each of the four Nova Scotia study sites in 2023.

Site	Total count of garbage	Expected (%)	Observed (% of total)
Graves	4	25%	1.48%
SMU	33	25%	12.27%
NSH	94	25%	34.94%
Otter Lake	138	25%	51.3%

I found a difference in the number of nests containing anthropogenic materials at each of the four sites ($X^2 = 25.65$, df = 3, $P < 0.0001$; Table 1.2). Graves Island, Nova Scotia Hospital, and Otter Lake had less nests than what was expected with Graves Island having the least amount. While Saint Mary's University had double the number of nests containing anthropogenic materials than what was expected.

Table 1.2. Summary of the number of nests containing anthropogenic materials at each of the four Nova Scotia study sites in 2023.

Site	Percent of nests with garbage	Expected (%)	Observed (% of total)
Graves	46.2%	25%	8.82%
SMU	95%	25%	50.00%
NSH	92.3%	25%	17.65%
Otter Lake	94.1%	25%	23.53%

Anthropogenic materials were present in 6/13 nests at Graves Island, 34/36 nests at Saint Mary's University, 12/13 nests at Nova Scotia Hospital and 16/17 nests at Otter Lake, (Table 1.2, Fig. 1.1)

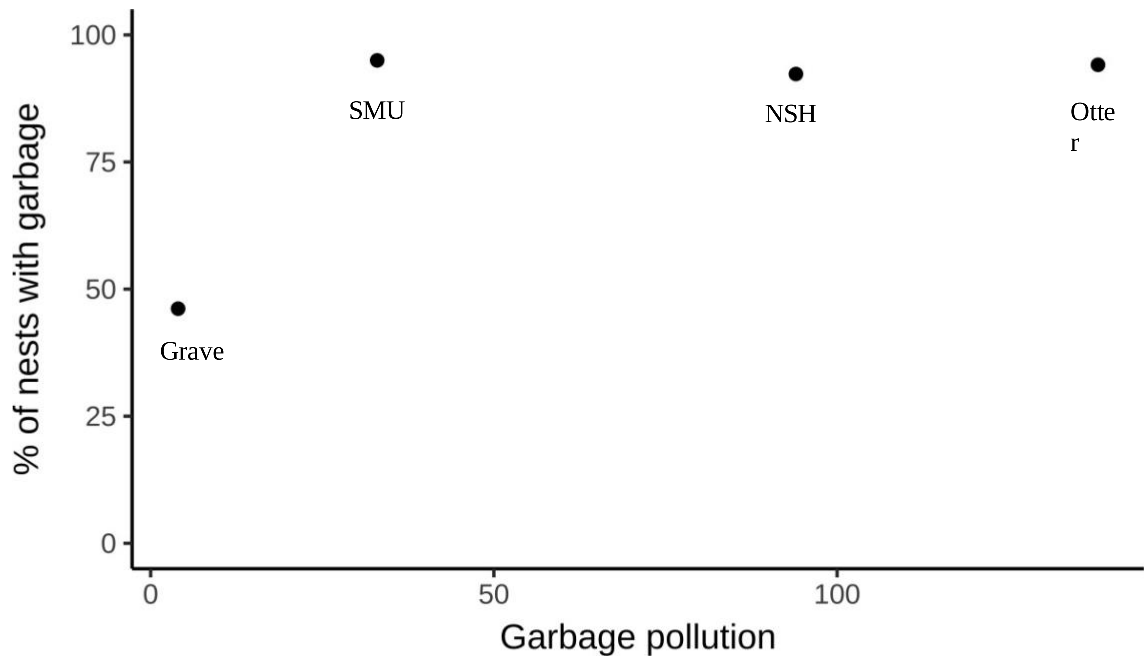

Figure 1.1 A scatterplot showing the percent of nests with anthropogenic materials versus the level of garbage pollution (number of garbage items on transects) at the four Nova Scotia sites (Graves Island, Otter Lake, Nova Scotia Hospital (NSH) and Saint Mary's University (SMU) respectively).

Anthropogenic materials vs age

I found no significant difference in the mass of anthropogenic materials within nests between ASY females (1.34 grams $\pm$ 0.15) and SY females (1.14 grams $\pm$ 0.44; Mann U = 96, n_1 = 96, n_2 = 6, P = 0.92; Fig 1.2) or between ASY males (1.28 grams $\pm$ 0.16) and SY males (0.30 grams $\pm$ 0.025; Mann U = 29, n_1 = 68, n_2 = 2, P = 0.17; Fig 1.3).

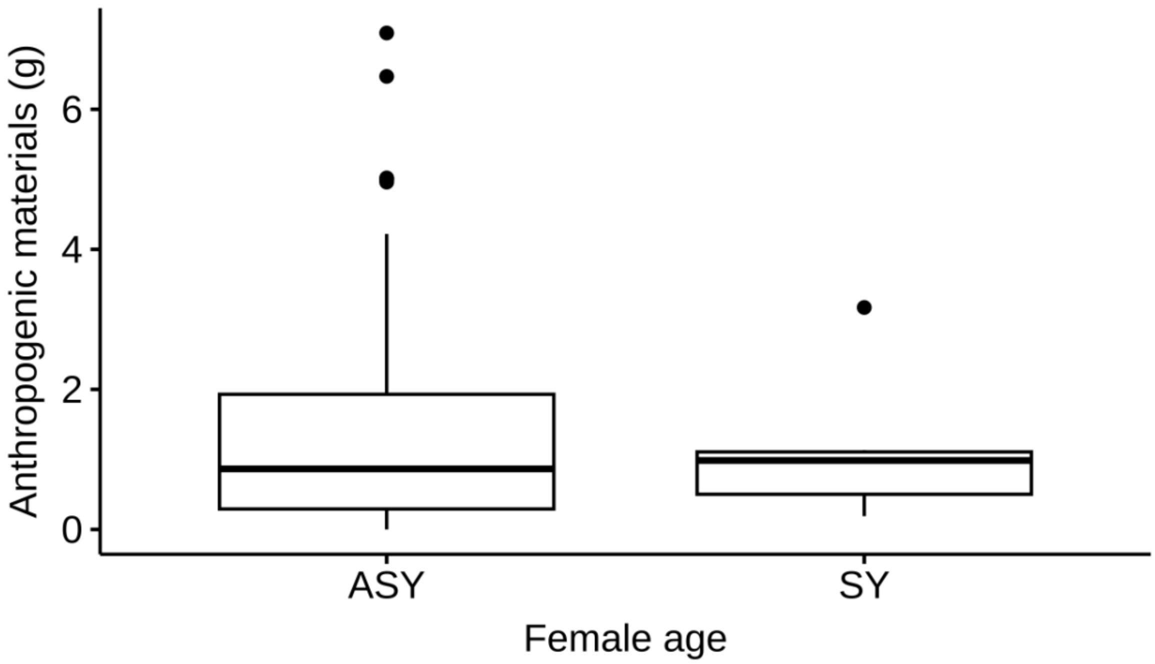

Figure 1.2 A boxplot showing the mass of anthropogenic materials (g) in nests of both ASY (n = 96) and SY (n = 6) females at Saint Mary’s University from 2021-2023.

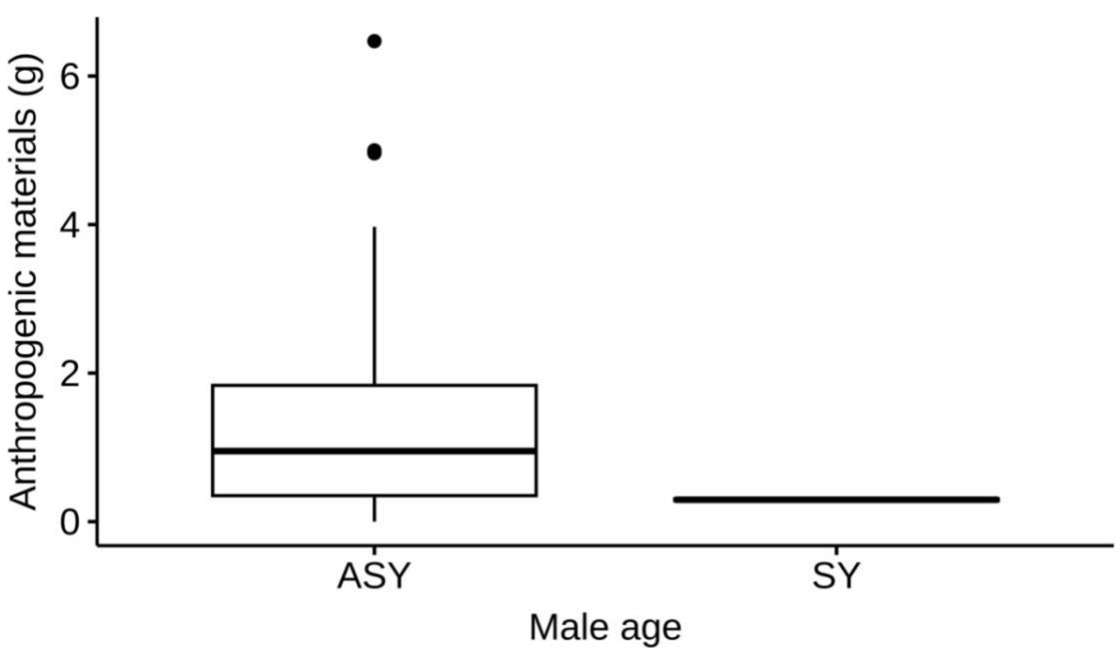

Figure 1.3 A boxplot showing the mass of anthropogenic materials (g) in nests of both ASY (n = 68) and SY (n = 2) males at Saint Mary’s University from 2021-2023.

DISCUSSION

All four Nova Scotia sites had nests containing anthropogenic material in 2023. Graves Island had the lowest percentage of nests containing anthropogenic materials while Saint Mary's University had the highest. I predicted that areas containing higher amounts of garbage pollution would result in an increased percentage of nests having anthropogenic materials incorporated into them. In support of my prediction, Graves Island had the least number of nests containing anthropogenic materials as well as the fewest garbage items detected at the study site. Although the level of garbage pollution varied among the other three sites (Saint Mary's University, Nova Scotia Hospital and Otter Lake), these sites generally had much higher levels than did Graves Island. These three sites also had a high percentage of nests containing anthropogenic materials ranging from 92.31% -95%. These findings support the availability hypotheses and are similar to results in other studies (e.g. Antczak et al. 2010; Harvey et al. 2021; Jagiello et al. 2018; Jagiello et al. 2020; Jagiello et al. 2022; Lee et al. (2015); Tariq et al. 2024; Vasquez et al. 2022; Wang et al. 2009) in that areas with increased levels of garbage pollution have a higher probability of nests containing garbage. Similar to our study, Harvey et al. (2021) compared the nests of urban Darwin finches (*Geospiza fuliginosa*) to those in nonurban environments and found that every nest in the urban environment contained anthropogenic materials while the nonurban nests had none.

Contrary to my prediction, the mass of anthropogenic materials within nests did not differ between SY and ASY females or males. Jagiello et al. (2018) found a positive relationship between the probability of debris in a nest and the age of female White Storks, while Sergio et al. (2011) found that the level of decorations (anthropogenic materials) in nests increased up to ages 10-12 years in Black kites. My findings could be due to the small sample size of SY adults breeding at Saint Mary's University; only six SY females and two SY males had nests over the three years examined, whereas I had nests from 96 ASY females and 68 ASY males. The positive relationship that Jagiello et al. (2018) found was not detected in male storks, likely due to their small sample (20 males compared to 33 females). Another possible reason for the lack of a relationship with adult age in my population could be due to anthropogenic materials being plentiful at the Saint Mary's University site. My result showed that 95% of Saint Mary's University nests contained anthropogenic materials incorporated into them suggesting that it is common for this population of starlings to use these as nesting materials. Perhaps if I examined age at another site containing a lower percentage of nests containing anthropogenic materials, I would find a relationship between adult age and anthropogenic materials within the nests. Lastly, perhaps my lack of finding a relationship could be related to the limitation of determining age in my study as an adult could only be classified as SY or ASY. ASY is a brood determination of age as the adult could range anywhere from two to at least eight years of age (Barber and Wright 2017).

In conclusion, a higher percentage of nests containing anthropogenic materials were located in areas having a relatively higher level of garbage pollution among this eastern Canadian population of European starlings. Contrary to what has been reported in other species, no difference existed between the mass of anthropogenic materials incorporated in the nest and the age of either the male or female parent. Future research is needed to determine if this finding is due to the small sample size of SY adults or some other factor.

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CHAPTER 3: How anthropogenic materials affect European starling (*Sturnus vulgaris*) reproductive success

ABSTRACT

Avian nests are made up of both natural materials consisting of greenery and feathers as well as sometimes with anthropogenic materials which include plastic, paper, string/twine, fabric, fishing supplies and cigarette butts. Anthropogenic materials within avian nests can result in serious detrimental effects such as nestling entanglement or accidental ingestion of materials. My first objective was to determine if the abundance (as measured by mass) of anthropogenic materials within a nest has an impact on brood condition and fledging success of European starling (*Sturnus vulgaris*) nestlings in eastern Canada. Second, I examined whether brood condition, hatching success and fledging success were impacted by the abundance of cigarette butts found within nests. I found there to be no relationship existing between brood condition or fledging success and the abundance of anthropogenic materials present in nests. Similarly, no relationship existed between brood condition or hatching success and the abundance of cigarette butts. However, I found a negative relationship detected between fledging success and cigarette butt abundance, indicating potential adverse effects of this type of anthropogenic material.

INTRODUCTION

Nests are a vital part for nestling development among avian species. They provide shelter from potential predators and weather events (Hansel 2000) while also offering insulation during development (Lombardo et al 1995). Nests typically consist of natural materials that include twigs, leaves, flowers, feathers (Hansel 2000) and often also include anthropogenic materials such as plastic, fabric, paper, twine/string (Antczak et al. 2010; Briggs et al. 2023; Hudecki et al. 2020; Jagiello et al. 2018; Lee et al. 2015; Montevecchi 1991; Tariq et al. 2024) and cigarette butts (Pérez-Beauchamp et al. 2024; Suarez-Rodriguez et al. 2013; Suárez-Rodríguez and Garcia 2014; Suárez–Rodríguez and Garcia 2017). Although the use of anthropogenic materials has been widely recorded in the nests of several avian species worldwide (Antczak et al. 2010; Brentano et al. 2020; Briggs et al. 2023; Francila et al. 2023; Garcia-Cegarra et al. 2020; Girão et al. 2024; Hammer et al. 2017; Harvey et al. 2021; Hudecki et al. 2020; Jagiello et al. 2018; Jagiello et al. 2022; Lee et al. 2015; Montevecchi 1991; Ryan 2018; Tariq et al. 2024; Townsend and Barker 2014; Vasquez et al. 2022; Wang et al. 2009), it is concerning due to the potential problems of entanglement and ingestion by nestlings. Jagiello et al. (2019) examined 25 published journal articles on the effects of anthropogenic materials, and found that 36% of studies reported entanglement, 20% reported ingestion and 12% reported both entanglement and ingestion occurring among 24 avian species.

Entanglement by anthropogenic materials residing in the nest can cause serious harm to both the nestlings and the adults, sometimes resulting in death. Montevecchi (1991) reported that 97% (722/741) of Northern gannet (*Sula bassana*) nests contained plastic. He witnessed 15 nestlings and three adults becoming entangled in the plastic and managed to free all except one adult (Montevecchi 1991). Townsend and Barker (2014) reported entanglement of 11 American crow (*Corvus brachyrhynchos*) nestlings that later died in urban and agricultural landscapes. They found anthropogenic materials in 85.2% (46/54) of the nests. Synthetic rope, twine and string were the most abundant materials, followed by plastic strips, tape and wire. The possibility of entanglement increased with the total length of material found in the nests, with odds increasing 7.55 times per meter of materials (Townsend and Barker 2014). A global review paper by Ryan (2018) focused on bird entanglement from both published and unpublished papers and reported that a total of 265 species of birds from 53 different families were entangled by anthropogenic materials. Fishing line and netting were the most common materials causing entanglement of 83% of the reported species. Due to the presence of fishing supplies, entanglement rates are higher for avian species residing in marine environments (147/265) compared to those in freshwater/coastal regions (69/265) and terrestrial environments (49/265) (Ryan 2018). Many published papers (e.g. Antczak et al. 2010; Harvey et al. 2020; Hudecki et al. 2020; Jagiello et al. 2018; Montevecchi 1991; Ryan 2018; Townsend and Barker 2014) report deaths occurring due to entanglement, with rates as high as 18% among Darwin finch (*Geospiza fuliginosa*) nestlings (Harvey et al. 2021).

Ingestion of anthropogenic materials within nests can lead to internal problems including biochemical changes (Cunha et al. 2022), mechanical damage through perforation and impaction within the digestive system (Fry et al. 1987), and death due to suffocation (Jagiello et al. 2018). Certain bird species including Yellow-legged and Lesser black-backed gulls (*Larus michahellis* and *Larus fuscus*; Lopes et al. 2021, 2022), Black vultures (*Coragyps atratus*; Cunha et al., 2022), and Albatrosses (*Phoebastria immutabilis* and *Phoebastria nigripes*; Gray et al. 2012) had plastic debris either within regurgitated food pellets that were collected from breeding sites or found inside their digestive systems. When focused on ingestion Albatrosses are known for consuming anthropogenic materials (Fry et al. 1987). Gray et al. (2012) found that 63.8% (30/47) of Laysan albatross (*Phoebastria immutabilis*) and Black-footed albatross (*Phoebastria nigripes*) recovered as by-catch in the North Pacific Ocean contained plastic within their digestive system. Not surprisingly, nestlings with more ingested plastic were in poorer condition (Fry et al. 1987). Similarly, Cunha et al. (2022) found that 43.95% of the 51 dissected adult Black vultures from landfill sites in Brazil had plastic within their stomach. Of that plastic, 89.9% was greater than 5 mm in diameter and 56.2% had a diameter between 10-30 mm. Cunha et al. (2022) discovered that plastic bags and food packaging are the most common anthropogenic materials ingested by birds and other animals feeding at landfill sites. Vultures that consumed plastic suffered biochemical changes suggesting the plastic led to the induction of oxidative stress. These biochemical changes may have affect their muscles, leading to locomotor issues, along with fitness, reproduction and behaviour problems (Cunha et al. 2022).

Cigarette butts are commonly incorporated into avian nests of several species including those of Darwin finches (*Geospiza fuliginosa*, *Geospiza fortis*, and *Geospiza scandens*) (Pérez-Beauchamp et al. 2024), House sparrows (*Passer domesticus*) and House finches (*Haemorhous mexicanus*) (Suárez-Rodríguez at al. 2013). Suárez-Rodríguez at al. (2013) used a thermal trap containing either smoked cellulose from smoked cigarettes or cellulose from non-smoked, intact cigarettes and found that parasite abundance was negatively associated with the cellulose from the smoked cigarettes. This result suggested that the incorporation of smoked cigarette butts within a nest was beneficial for reducing the number of ectoparasites in the nest. Suárez–Rodríguez and Garcia (2014) found that nestlings were heavier in nests with more cellulose from smoked cigarettes, likely due to the reduced ectoparasite load. Their study also revealed that the amount of cellulose from smoked cigarettes found in nests was positively associated with hatching and fledging success (Suárez–Rodríguez and Garcia 2014). Greater fledging success was likely due to their increased weight, whereas the reasoning for the increased hatching success was unknown but could be related to a decreased chance of parental abandonment to the nest during incubation due to the decreased ectoparasite loads. However, negative physiological changes associated with cigarette butts can occur. For example, the presence of cigarette butts was associated with nuclear abnormalities in the red blood cells of House finch nestlings (Suárez–Rodríguez and Garcia 2014). So, although cellulose from smoked cigarettes within nests resulted in fewer ectoparasites, it also resulted in greater genotoxic damage to the nestlings.

In my study I examined the effects of anthropogenic materials on European starling (*Sturnus vulgaris*) nestlings. Starlings are an urban-dwelling species (Barton et al. 2020) that incorporates both natural (Clark and Mason 1985) and anthropogenic materials into their cavity nests. They are double brooded with females laying an average of five eggs per clutch (Dunnett 1955; Feare 1984; Kessel 1957). The eggs are incubated by both parents for 12 days before hatching (Feare 1984; Kessel 1957), with nestlings remain in the nest for 20-24 days, receiving care from both parents before fledging (Dunnett 1955; Feare 1984; Kessel 1957).

The first goal of this study was to determine whether a relationship existed between brood condition and fledging success vs the mass of anthropogenic materials within nests. I predicted that brood condition and fledging success would each be negatively correlated with the mass of anthropogenic materials. My second objective was to determine if a relationship existed between brood condition, hatching success and fledging success vs the mass of cigarette butts from smoked cigarettes, predicting all three would be positively correlated with cigarette butt mass.

METHODS

Study site

I conducted this study at Saint Mary's University in Halifax Nova Scotia, Canada (44.6313° N, 63.5815° W) from April-July 2021-2023. The campus is located in the downtown area of Halifax and consists of many green spaces including trees, bushes and greenery. About 40 nest boxes containing a population of breeding starlings are located across the Saint Mary's University campus attached to trees 2-3 m from the ground. The nest boxes have been monitored each year since 2007.

Data Collection – Field

I monitored the nests daily when egg laying began. I numbered eggs in each nest box on the day they were laid and recorded the number of eggs hatched. I then measured nestlings on days 5 and 11 of the nestling period, with day 0 representing the day of first hatch. I weighed the nestlings with a Pesola spring scale to the nearest 0.5g and measured the right tarsus length using Fowler Sylvac digital calipers to the nearest 0.1mm. Each nestling in a brood received a different coloured band on their right tarsus on day 5 of the nestling period to distinguish siblings from each other. I replaced this band with a Canadian Wildlife Service Band on their right tarsus on day 11. I monitored the nest boxes up until all nestlings had fledged the nest (~ days 20-24) to determine fledging success at each nest.

Data Collection – Lab

I collected the nests from nest boxes following fledging or after abandonment/failed fledging due to the eggs not hatching or nestlings dying prematurely. Following fledging a total of 104 nests were collected, while 11 nests were collected before fledging due to nestling death and 17 nests were collected before hatching due to nest abandonment.

I stored each nest in a large plastic bag in a -20 C freezer. Before nest dissections began, I thawed the nests and dried them on baking trays in a 4 C fridge. I then placed each nest in a two-layer sieve with the openings of the top sieve being 1.27 cm and the bottom layer being 4 mm to separate the nest materials for easier dissection, running warm water over the nests. I sorted the nest material on trays and allowed it to dry for 2-3 days at room temperature. The anthropogenic materials including cigarette butts were weighed on a Scout Pro SP202 scale to the nearest 0.01 gram after drying before storing them in plastic bags at room temperature. Cigarette butts were also weighed separately in the same manner.

Hatching/fledging success and brood condition

Hatching and fledging success data was only collected in 2022 and 2023. I determined hatching success by dividing the number of hatched nestlings by the number of eggs laid. I determined fledging success by dividing the number of nestlings that fledged by the number of nestlings that originally hatched. I determined brood condition over all three years by doing a linear regression of mass against tarsus length of nestlings on day 11 of the nestling period (Whittingham and Dunn 2000). I averaged the residuals for each nest and used them as an indication of condition. Negative values indicated poor brood condition whereas positive values indicated good brood condition.

Data analysis

Data were tested for normality using a Shapiro-Wilk normality test. I compared brood condition for early (May) and late (June) nests using a two-tailed t-test or a Mann-Whitney test to determine if a significant difference existed between the two for potential data pooling. I also compared brood condition over each of the three breeding seasons using a one-way ANOVA test to determine if a significant difference existed among years.

I compared the mass of anthropogenic materials over each of the three breeding seasons using a Kruskal-Wallis test to determine if a significant difference existed among years. I used Spearman rank correlation tests comparing brood condition to the mass of anthropogenic materials and cigarette butts for the three breeding seasons. I also used Spearman rank correlation tests to compare fledging success to the mass of anthropogenic material and cigarette butts. Similarly, I used Spearman rank correlation tests to compare hatching success to the mass of smoked cigarette butts. All statistical analyses and graphs were completed using R (version 2024.04.2+764, R Core Team). Results were considered significant when $P < 0.05$.

RESULTS

Brood condition

No significant difference was detected in brood condition between early (Mean $\pm$ SE) (0.75 $\pm$1.18) and late (-1.82 $\pm$ 0.83) season nests of 2021 ($t = 1.83$, $df = 36$, $P = 0.074$). Similarly, no significant difference was found in brood condition between early (-0.059 $\pm$ 0.35) and late (0.64 $\pm$ 1.08) season nests of 2022 ($U = 212$, $n_1 = 27$, $n_2 = 17$, $P = 0.61$), or between early (-0.0036 $\pm$1.23) and late (0.28 $\pm$ 2.46) season nests of 2023 ($t = -0.11$, $df = 21$, $P = 0.91$). I therefore pooled early and late broods for each year. When comparing brood condition among each of the three years, I detected no significant differences (F = 0.75, df = 2, 102, P = 0.39; Fig. 2.1), and so pooled the three years as well.

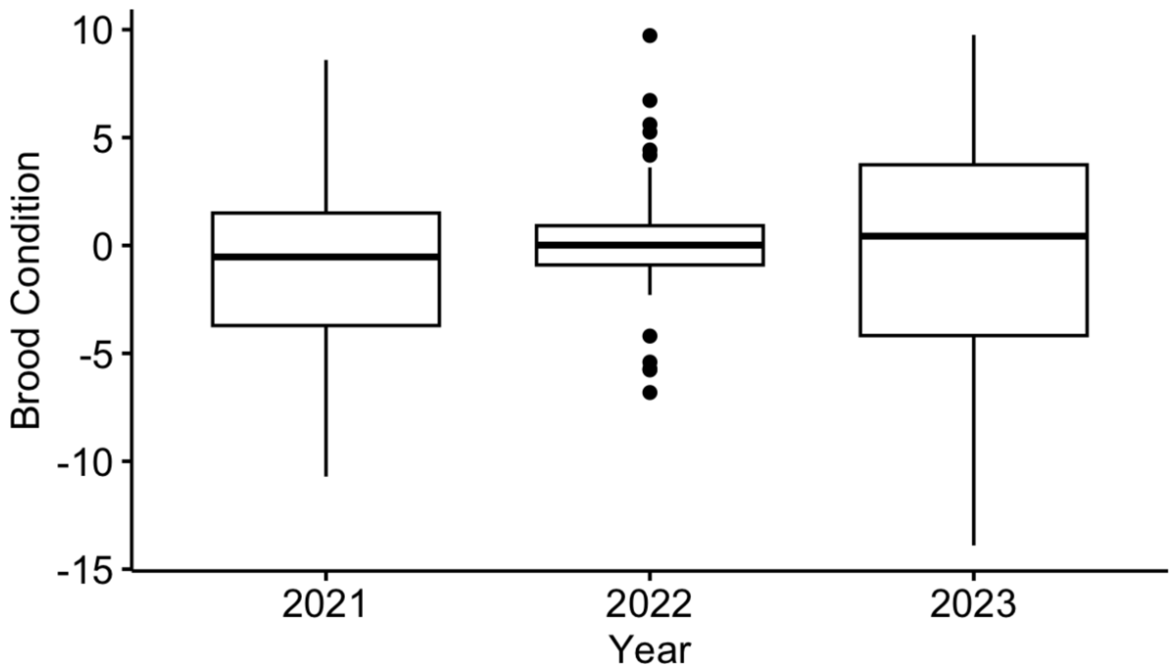

Figure 2.1 A boxplot showing the average brood condition of nestlings located in nest boxes at Saint Mary’s University, Halifax for 2021 (n = 38), 2022 (n = 44) and 2023 (n = 23).

Anthropogenic materials

A total of 96.99% (129/133) of European starling nests contained anthropogenic materials at Saint Mary’s University between 2021-2023. Nests in each of the three years contained similar amounts of anthropogenic materials (H = 89.02, df = 2, P = 0.42; Fig. 2.2).

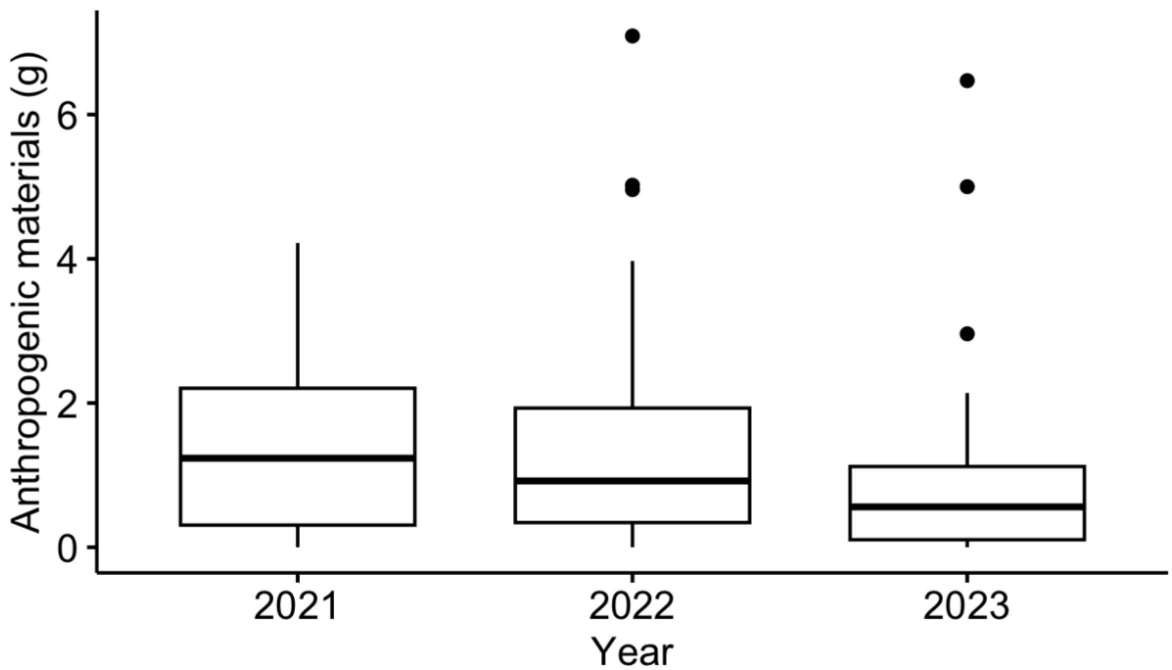

Figure 2.2 A boxplot showing the average mass of anthropogenic materials located in nests for 2021 (n = 38), 2022 (n = 44) and 2023 (n = 23).

Brood condition and fledging success vs anthropogenic material

There was no significant relationship when comparing average brood condition with the abundance of anthropogenic materials within nests (r_s = -0.096, n = 105, P = 0.33).

Fledging success did not differ significantly between 2022 (0.65 $\pm$ 0.05) and 2023 (0.57 $\pm$ 0.075) (Mann U = 1129.5, n_1 = 59, n_2 = 35, P = 0.43) allowing the data to be pooled. Fledging success was not correlated with the mass of anthropogenic materials in a nest (r_s = 0.055, n = 94, P = 0.60).

Brood condition, hatching & fledging success vs cigarette butts

There was no significant difference when comparing the mass of cigarette butts among the three years ($H = 12.23$, $df = 2$, $P = 0.51$). Brood condition was not correlated with mass of cigarette butts in a nest ($r_s = -0.13$, $n = 105$, $P = 0.19$).

Hatching success did not differ significantly between 2022 (0.74 ± 0.048) and 2023 (0.62 ± 0.069) (Mann $U = 1225$, $n_1 = 59$, $n_2 = 35$, $P = 0.11$). There was no significant relationship when comparing hatching success and the mass of cigarette butts within nests ($r_s = -0.013$, $n = 94$, $P = 0.90$). Finally, fledging success was significantly negatively correlated with the mass of cigarette butts ($r_s = -0.20$, $n = 94$, $P = 0.049$; Fig. 2.3).

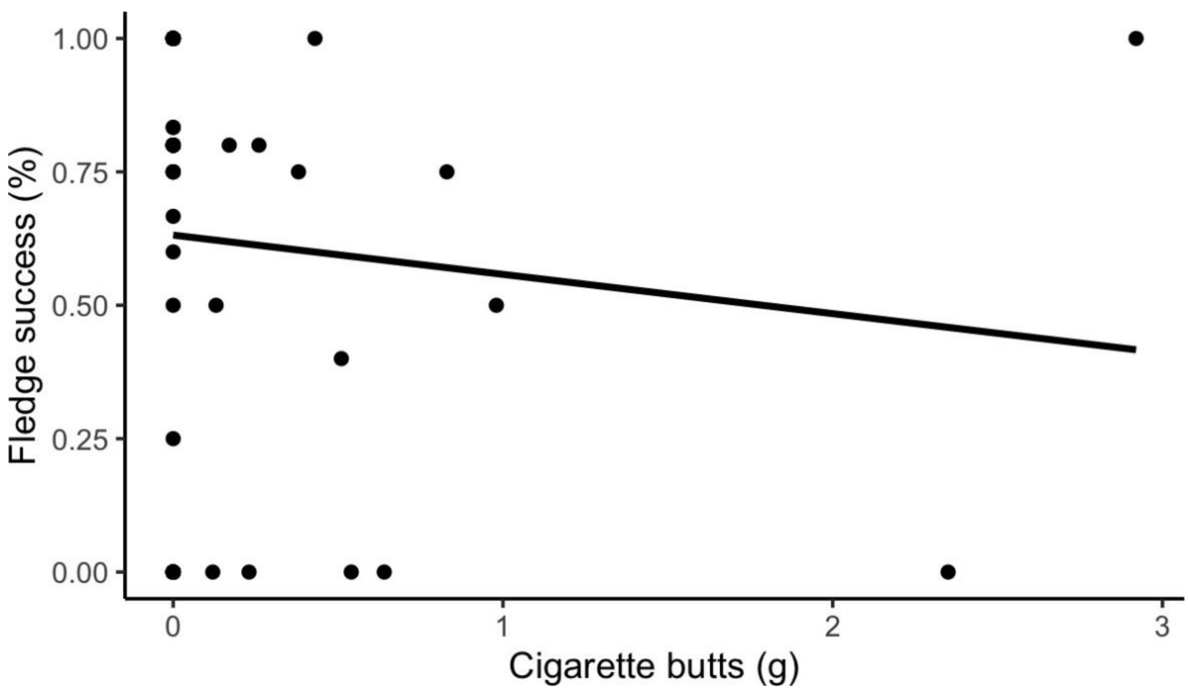

Figure 2.3 A scatterplot with a line of best fit showing fledging success vs the mass of cigarette butts (g) in a nest ($n = 94$ nests).

DISCUSSION

Most of the nests (96.99%) in my focal European starling population contained anthropogenic materials over the three-year period examined. Contrary to my prediction, brood condition was not related to the abundance of anthropogenic materials in a nest. This finding is also different from the negative relationship I had previously found when examining only one year (2021) (Armstrong 2022). In a study on albatross nestlings, Fry et al. (1987) found that those who ingested higher amounts of plastic were in poorer condition compared to nestlings with less plastic in their system (Fry et al. 1987). Our studies differ, however, in that Fry et al (1987) examined ingested anthropogenic materials whereas I only examined what was present in the nest. In 2022, I documented one case of nestling entanglement and another case of a nestling ingesting a long black plastic string. I intervened in both cases to assist the nestlings which likely had a positive effect on their condition.

Contrary to my prediction, fledging success also did not show any significant relationship with the mass of anthropogenic materials within nests. Townsend and Barker (2014) observed 11 crow nestlings failing to fledge due to entanglement within the nest, but the frequency of entanglement in my European starling population was extremely low.

Brood condition was not associated with cigarette butt mass within nests. Suárez-Rodríguez and Garcia (2014) found that House finch nestlings were heavier when in nests with higher amounts of cellulose from smoked cigarettes likely due to a decrease in ectoparasite loads as has been found in other studies (e.g. Suárez-Rodríguez et al. 2013; Suárez-Rodríguez and Garcia 2014). However, in contrast to these other studies, Pérez-Beauchamp et al. (2024) did not find a relationship between the mass of cigarette butts and ectoparasite abundance within several species of Darwin finch nests. However, instead they found that cigarette butts decreased pupation success among the avian vampire fly (*Philornis downsi*) by producing a larger pupal volume and a higher rate of deformities among the pupation stage of the flies. Although not examined here, future research should determine whether cigarette butts had an effect on ectoparasite abundance residing in starling nests. Similarly, contrary to my prediction I did not find a positive relationship between hatching success and cigarette butt mass within nests. Suárez-Rodríguez and Garcia (2014) did find this positive relationship in House finches and suggested it was possibly related to a decreased chance of nest abandonment among parents due to the lower ectoparasite load they experienced within nests.

Finally, I did not find a positive relationship between fledging success and the mass of cigarette butts, but instead detected a negative relationship. Suárez-Rodríguez and Garcia (2014) discovered a positive relationship between the two and attributed it to the increased mass of the nestlings. I found no relationship between brood condition and mass of cigarette butts, which could account for why I found no positive relationship between fledging success and cigarette butt abundance. Suárez-Rodríguez and Garcia (2017) suggested that House finches add cigarette butts to their nests in response to an increased ectoparasite load in the nest. Perhaps the negative relationship that I found between fledging success and cigarette abundance in nests was due to an increased abundance of ectoparasites that was not deterred by the cigarette butts.

In conclusion, the abundance of anthropogenic materials did not adversely affect brood condition or fledging success among this eastern Canadian population of European starlings. Similarity, hatching success and brood condition were not adversely affected by the abundance of cigarette butts in a nest. Interestingly, fledging success was negatively impacted by the abundance of cigarette butts within nests. Future research is needed to determine the causal mechanisms of this finding.

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CHAPTER 4: How does the colour of nesting materials affect European starling (*Sturnus vulgaris*) eggs and nestlings?

ABSTRACT

Feathers are a common component in the nests of many avian species. They provide insulation for the developing nestlings and a soft surface amidst the dry grasses. Several avian species prefer white feathers over pigmented ones and white feathers have been shown to increase hatching success, suggesting an intrinsic benefit. White feathers have antimicrobial benefits that prevent the accumulation of certain bacteria loads on the eggshell that cause harm to the developing embryo. The prevalence for white coloured nesting materials has been demonstrated among several avian species. The presence for white anthropogenic materials within nests is suggested to be based on what is available in the surrounding area. This selection of white anthropogenic materials highlights the availability hypothesis which states that species choose materials based on what is available to them in their surroundings. My first objective was to examine whether brood condition was impacted by feather mass and found no relationship existed between the two. My second objective was to determine if feather colour and feather mass within a nest have an impact on hatching success in European starling (*Sturnus vulgaris*) nests in eastern Canada. The mass of white feathers incorporated into nests had no significant effect on hatching success, however, a strong positive relationship existed between the mass of pigmented feathers and hatching success over the two years examined. Finally, I determine if a colour preference based on availability for ribbons existed among adults by providing them with a choice of blue, green, red, silver and white ribbons. Starlings preferred the white and silver ribbons. It is unknown why pigmented feather abundance would augment hatching success, but it warrants future research to determine if starlings prefer pigmented over white feathers and what mechanism accounts for the positive effect pigmented feathers have on hatching success.

INTRODUCTION

Avian nests commonly consist of various anthropogenic and natural materials that include plant materials and feathers. These feathers are gathered by the parents as nest lining (Hansel 2000) and are thought to provide insulation and comfort for the developing altricial nestlings (Lombardo et al. 1995). Feathers may also serve to hide eggs in a nest from other laying females, thereby preventing brood parasitism (Heinrich 2015) or function in mate attraction (Sanz and García-Navas 2011).

The presence and abundance of feathers in the nest have several functions. Feathers may enhance the thermoregulatory abilities of nestlings (Lombardo et al. 1995; Peralta-Sanchez et al. 2011; Stephenson et al. 2009) such that parents do not need to expend as much energy keeping the eggs and nestlings warm. For example, Tree swallow (*Tachycineta bicolor*) nests that contained more feathers produced larger nestlings on day 12 of development, likely due to the extra warmth they provided to nestlings before the offspring could self-regulate their body temperature (Lombardo et al. 1995). Stephenson et al. (2009) also found that Tree swallow nestling growth was positively associated with the number of feathers in their nest. Feathers may also reduce the number of ectoparasites in a nest by acting as a barrier. Winkler (1993) removed feathers from Tree swallow nests every day, creating featherless nests, and found these nests had significantly higher numbers of mites and lice than the control nests. However, Stephenson et al. (2009) observed the number of feathers in a nest had no effect on ectoparasite levels. Removal of feathers had no effect on nestling survival, but doing so significantly impacted nestling growth rates, resulting in lighter nestlings with shorter wings and tarsi (Winkler 1993).

Colour preference of feathers within nests has been examined in Barn swallows (*Hirundo rustica*; Peralta-Sanchez et al. 2011) and Spotless starlings (*Sturnus unicolor*; Ruiz-Castellano et al. 2018) showing a preference for white feathers over pigmented. Peralta-Sanchez et al. (2011) conducted an experiment in which they added feathers to Barn swallow nests such that they contained only white or pigmented feathers and found higher hatching success in nests containing only the white feathers (Peralta-Sanchez et al. 2011). White feathers appear to possess anti-microbial properties that decrease the harmful bacteria load on eggshells (Peralta-Sanchez et al. 2010; Peralta-Sanchez et al. 2014), resulting in greater hatching success. Feathers have many different species of bacteria and other microorganisms living on their surface, including various species of *Enterococcus* spp., and *Staphylococcus* spp. (Peralta-Sanchez et al. 2010) as well as *Bacillus* spp. (Burtt and Ichida 1999). Both *Enterococcus* spp. and *Staphylococcus* spp. are pathogenic and can cross the eggshell, harming the developing embryo (Bruce and Drysdale 1994).

Peralta-Sanchez et al. (2010) examined the abundance of black feathers within nests and found that towards the end of incubation, eggshells had higher bacterial densities in nests containing more black feathers. The total number of feathers within a nest also had a negative relationship with the bacteria load of *Enterococcus* and *Staphylococcus* on eggshells during incubation (Peralta-Sanchez et al. 2010).

Numerous studies (Brentano et al. 2020; Briggs et al. 2023; Cunha et al. 2022; Garcia-Cegarra et al. 2020; Sergio et al. 2011; Tavares et al. 2019) have observed that some species incorporate white coloured anthropogenic materials in their nests, suggesting there is a colour preference for nesting materials. Garcia-Cegarra et al. (2020) found that 100% of 18 Red-legged cormorant (*Phalacrocorax gaimardi*) nests contained anthropogenic materials. The most common colour was white, followed by black and green. They suggested the prevalence of the white plastic was due to the high presence of white plastic bags in the surrounding area (Garcia-Cegarra et al. 2020). Briggs et al. (2023) conducted an experimental study on Pied flycatchers (*Ficedula hypoleuca*) by offering four different colours of strings (white, yellow, orange and blue) on the first day of nest building. They found a significant number of white, yellow and orange strings were taken and incorporated into the inner layers of their nests suggesting a preference for these colours based on the availably of what colours were provided (Briggs et al. 2023).

European starlings (*Sturnus vulgaris*) are a double-brooded passerine species that typically incorporate feathers into the cup lining of their nests (Feare 1984; Kessel 1957). They are cavity nesters (Kessel 1957) and lay one egg a day in the morning (Feare 1984; Kessel 1957) until an average of five eggs are produced per clutch (Dunnett 1955; Feare 1984; Kessel 1957). Eggs are incubated by both parents for approximately 12 days before the eggs hatch (Feare 1984; Kessel 1957). The nestlings then remain in the nest receiving care from both parents until fledging occurs at around days 20-24 of the nestling period (Dunnett 1955; Feare 1984; Kessel 1957). Starlings will remove old nest material from the cavity following fledging of the first brood (Mazgajski et al. 2004). Doing so is thought to reduce the number of mites and other ectoparasites (Pacejka et al. 1996) along with various types of bacteria (Singleton and Harper 1998). Fairn et al. (2014) documented nests from this population of starlings in Halifax, Nova Scotia having many types of ectoparasites including various species of lice, mites, fleas (*Ceratophyllus gallinae*) and *Carnus hemapterus.*

My first objective was to determine if there was a relationship between brood condition and total feather abundance (measured by feather mass), within starling nests over three breeding seasons. Winkler (1993) found that featherless Tree swallow nests had significantly poorer nestling growth rates, so I predicted there would be a positive relationship between brood condition and the total mass of feathers within European starling nests. Secondly, I asked if a positive relationship exists between hatching success and the mass of white feathers among starling nests over two breeding seasons, as was found by Peralta-Sanchez et al. (2011). My third objective was to determine whether a negative relationship exists between hatching success and pigmented feather mass, as found by Peralta-Sanchez et al. (2010). My last objective was to determine if there was a colour preference among starlings when they searched for nesting materials; I predicted they would prefer white ribbons over blue, green, red and silver.

METHODS

Study site

This study was conducted at Saint Mary's University in Halifax Nova Scotia, Canada (44.6313° N, 63.5815° W) from 2021-2023. The campus is located in the downtown area of Halifax over one large city block, and consists of many green spaces with bushes, trees and grass. About 40 nest boxes across campus are attached to trees 2-3 m from the ground and are used by breeding European starlings every year.

Data Collection – Field

I visited nests daily in the morning and recorded both the number of eggs laid and the number of nestlings hatched. Nestlings within a brood were banded with a unique coloured plastic band on their right tarsus until a Canadian Wildlife Service band replaced it on day 11 of the nestling period (day 0 is day of first hatch in each nest). Doing so allowed individual identification of each nestling within the brood. I measured nestlings on days 5 and 11. These measurements consisted of weighing nestlings using a Pesola spring scale to the nearest 0.5 g, and right tarsus length measurements using a Fowler Sylvac digital caliper to the nearest 0.01 mm. The nestlings were monitored until fledging occurred (~ day 20) or until the nest was abandoned or depredated. Over three years, I collected a total of 104 of the nests after fledging, while another 11 nests were collected before fledging due to nestling death and 17 nests were collected before hatching due to abandonment or predation.

Data Collection – Lab

Nests were collected on the day of fledging or early the next day. Nests were also collected for instances where all nestlings died prematurely or when the eggs did not hatch. Nests were placed in a large plastic freezer bag and stored at -20 C until they could be dissected. Before dissection, I thawed each nest and dried them on baking trays at room temperature. I then placed the nest material in a 2-layer sieve with the top layer of the sieve openings being 1.27 cm and the bottom layer being 4mm and ran warm water through them. The two layers helped separate the materials for sorting. I then dissected the nest materials and allowed them to dry for 2-3 days at room temperature. Once dry, the white and pigmented feathers were weighed on a Scout Pro SP202 scale to the nearest 0.01 gram and stored in plastic bags for future use.

Hatching success

Hatching success was determined by dividing the number of nestlings who hatched by the number of eggs that were laid for each nest.

Data Analysis

Data were tested for normality using a Shapiro-Wilk normality test. Hatching success for early (May) and late (June) nests were compared using a two-tailed Wilcoxon test to determine if a significant difference existed. Spearman correlation tests were used to compare hatching success with each of the mass of white and pigmented feathers for two of the breeding seasons (2022 - 2023; hatching success was not monitored thoroughly in 2021).

Brood condition was determined by regressing nestling mass against tarsus length on day 11 of the nestling period. These residuals were then averaged for each nest and used as an index of brood condition. Positive residuals indicated that the brood was in good condition, while negative residuals indicated poor condition. Brood condition for early and late nests were compared using a two-tailed t-test to determine if a significant difference existed between the two. Brood condition over each of the three breeding seasons was compared using a Kruskal-Wallis test to determine if a significant different existed among years. Spearman correlation tests were conducted comparing brood condition to mass of total feathers for the three breeding seasons. All data analysis and graphs were done using R (version 2024.04.2+764, R Core Team). Mean $\pm$ SE are presented. Results were considered significant when $P < 0.05$.

Data collection and analysis – experimental study

My experimental study took place at Saint Mary's University in Halifax, Nova Scotia beginning in mid-April and ending in late July of 2023. I assembled 17 stations made of wooden stakes (2-inch wide x 2-inch thick x 42-inch long) that I placed around the campus near nest boxes. I provided five different colours (blue, red, green, silver and white) of 10cm long ribbons that I attached to each side of the wooden stakes using a metal screw eye such that 10 ribbons were on each stake (five on each side), fig 3.1. I checked each stake daily, and colours were rotated down a placement on the stake each time a ribbon was missing. Rotation of the colours ensured that the ribbons were chosen based on the colour and not the position on the stake. I wrote a number on each ribbon to ensure that the ribbon was part of this study if found at a later date. I kept daily records of each missing ribbon from each of the 17 stakes for later analysis. I conducted a Chi square test to determine if there was a significant difference between the number of ribbons that were observed taken compared to the number of ribbons that were expected to be taken if all were removed at equal frequency. Statistical analysis was done using Quick Cals (GraphPad) and results were considered significant when $P < 0.05$.

Figure 3.1. A picture showing an example of how the ribbons were offered to the European starlings (Sturnus vulgaris) attached to 17 wooden stakes at Saint Mary’s University in 2023.

RESULTS

Early vs Late Nests – Brood condition

I found no significant difference in brood condition between the early (-0.059 $\pm$ 0.35) and late (0.64 $\pm$ 1.08) broods of 2022 ($W = 212$, $n_1 = 26$, $n_2 = 18$, $P = 0.61$), or between early (-0.0036 $\pm$ 1.23) and late (0.28 $\pm$ 2.46) broods of 2023 ($t = -0.1112$, $df = 21$, $P = 0.91$). However, in 2021, early broods were in significantly better condition (1.14 $\pm$ 1.17) than late (-2.27 $\pm$ 0.74) broods ($t = 2.539$, $df = 36$, $P = 0.016$). Brood condition did not differ significantly among the three years ($H = 104$, $df = 2$, $P = 0.48$); the three years were pooled together.

Brood Condition vs Total Feather Mass

When all three years were pooled together there was no relationship found between brood condition and the total feather mass within nests ($r_s = -0.04241$, $n = 105$, $P = 0.67$).

Early vs Late Clutches – Hatching Success

In both 2022 and 2023, no significant differences existed in hatching success between early and late clutches (mean $\pm$ SE) (Early 2022: 0.79 $\pm$ 0.069; Late 2022: 0.70 $\pm$ 0.067; $W = 524.5$, $n_1 = 29$, $n_2 = 30$, $P = 0.14$), (Early 2023: 0.73 $\pm$ 0.080; Late 2023: 0.47 $\pm$ 0.11; $W = 208.5$, $n_1 = 19$, $n_2 = 16$, $P = 0.13$). I therefore pooled early and late clutches for each year. Although hatching success did not differ significantly among these two years (2022: 0.74 $\pm$ 0.048; 2023: 0.62 $\pm$ 0.069; $W = 1279$, $n_1 = 59$, $n_2 = 35$, $P = 0.08$), 2022 tended to have a higher hatching success than 2023. I pooled both years together.

Hatching Success vs Feathers

Hatching success was not significantly correlated with the mass of white feathers (r_s = -0.06240, n = 95, P = 0.55), but was positively correlated with the mass of pigmented feathers (r_s = 0.2186, n = 95, P = 0.035; Fig 3.1). There was a significantly lower mass of white feathers (0.26 grams $\pm$ 0.021) than pigmented feathers (2.16 grams + 0.16) within nests (W = 8696, n_1 = 96, n_2 = 96, P $< 2.2 \times 10^{-16}$; Fig 3.2).

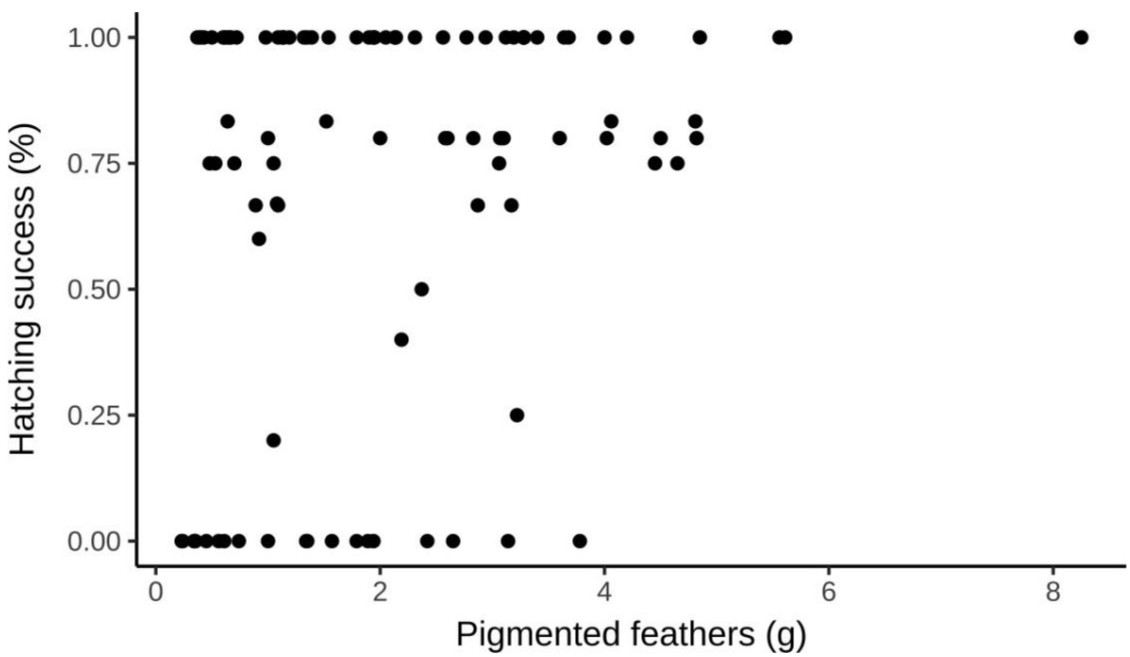

Figure 3.2 A scatterplot showing hatching success vs mass of pigmented feathers (g) in 95 European starling nests at Saint Mary's University.

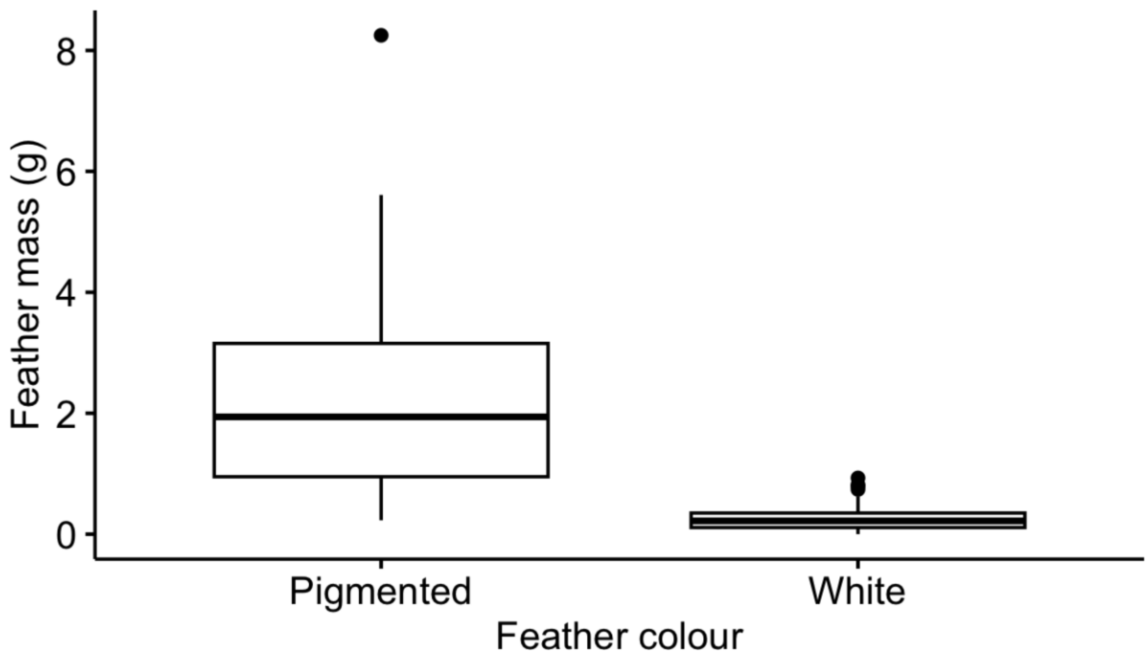

Figure 3.3. A boxplot showing the mass of pigmented feathers (g) and the mass of white feathers in 95 European starling nests at Saint Mary's University.

Ribbon experiment

I found a significant difference in the number of ribbons that were taken compared to the amount that was expected to be taken ($X^2 = 19.68$, df = 4, P = 0.0006). White and silver ribbons were selected more often than expected, whereas the blue, green and red ribbons were selected less often than expected by chance (Table 3.1). When dissecting the nests, nine white, eight silver, four blue, two red and one green ribbon were found among the nests.

Table 3.1 Summary of the ribbon experiment at Saint Mary's University in 2023.

Ribbon color	Number taken	Expected	Observed (% of total)
Blue	18	20%	16.82%
Green	9	20%	8.41%
Red	16	20%	14.95%
Silver	33	20%	30.84%
White	31	20%	28.97%

DISCUSSION

Contrary to my prediction, brood condition of European starling nestlings was not enhanced by the mass of feathers present in nests over the three years of this study. However, both Winkler (1993) and Stephenson et al. (2009) found that Tree swallow nestlings had increased growth rates in nests with more feathers. Lombardo et al. (1995) attributed this relationship to the thermoregulation benefits that feathers provide. A possible reason that I did not discover the same results as both Winkler (1993) and Stephenson et al. (2009) could be due to them using nestling growth rates while I used residuals of a linear regression of mass against tarsus length to assess brood condition.

Hatching success was not positively correlated with white feather mass over the two years of my study. Peralta-Sanchez et al. (2011), however, found that not only did Barn swallows prefer white over pigmented feathers for lining their nest, but experimental nests with only white feathers had higher hatching success than did experimental nests with only pigmented feathers. Similarly, Ruiz-Castellano et al. (2018) found that Spotless starlings preferred white feathers when given the option of choosing between white and pigmented feathers. One possible explanation for not detecting this relationship in my study population of European starlings is that there was a significantly higher mass of pigmented feathers found in starling nests compared to white feathers. This result could potentially be due to the lack of white-feathered birds in the city of Halifax other than various seagulls (*Larus* spp.) (Armstrong 2022). Instead, there are many avian species with pigmented feathers such as Pigeons (*Columba livia*), Blue jays (*Cyanocitta cristata*), and Crows (*Corvus brachyrhynchos*). It could also be that white feathers are abundant but are not the preferred choice of European starlings. Additionally, European starlings generally have a high hatching success of 80-90% among their populations (Feare 1984) which could also explain the lack of a relationship found in this study between hatching success and the mass of white feathers.

Contrary to my prediction, I found a positive relationship between hatching success and pigmented feather mass over the two years. This positive relationship was surprising due to previous research showing that Barn swallow nests containing pigmented feathers have higher bacterial densities than nests with white feathers (Peralta -Sanchez et al. 2010), which resulted in an increased frequency of hatching failures (Peralta-Sanchez et al. 2018). Similar results were previously found by Hansen et al. (2015) when examining Greater white-fronted geese (*Anser albifrons*) embryo mortality, along with Soler et al. (2012) when examining 22 different avian species in Europe. Perhaps in our population of starlings, the pigmented feathers rather than the white feathers contained antimicrobial properties that enhanced hatching success. This would also explain why they were more abundant within nests. Another possible reason for the unexpected positive relationship found here between hatching success and pigmented feather mass could be due to factors that were not examined during this study. For example, Heinrich

(2015) reported that Tree swallows use feathers to help hide the nest contents, potentially reducing the incidence of intraspecific brood parasitism from conspecifics whereby a female will lay her egg in the host nest and remove a host egg. European starlings do engage in intraspecific brood parasitism (Feare 1984; Kessel 1957) and so could potentially be using any feathers they can find to hide their eggs. Another possible reason could be that starlings use feathers within the nests as a mating signal among partners. Sanz and García-Navas (2011) found that male Blue Tits (*Cyanistes caeruleus*) carry feathers to the nests to induce an increased clutch size from their mate and that males who participated in this feather carrying behaviour fed their offspring at higher rates than males that did not. More feathers of any colour lining the nest cup would also be beneficial in keeping eggs warm during incubation under low ambient temperatures. Capilla-Lasheras et al. (2021) conducted an observational study examining weather conditions and abundance of feathers lining Eurasian tree sparrow (*Passer montanus*) nests and found that the tree sparrows were incorporating more feathers into their nests earlier in the season when temperatures were lower.

As I predicted, European starlings preferred white coloured nesting materials (ribbons) when given the chance to choose among five colours. However, they also preferred the silver colour. Although the stage of the nest (e.g., egg laying, incubation, etc) when the ribbons were incorporated was not determined, finding the ribbons in the nests means that the ribbons were being selected from the stations as nest material by the starlings. Although some of the ribbons were found within dissected nests it does not rule out the possibility that other species could have potentially taken some of the ribbons from the stations. The preference for the colour white was found by others for various avian species (e.g. Brentano et al. 2020; Briggs et al. 2023; Cunha et al. 2022; Garcia-Cegarra et al. 2020; Sergio et al. 2011; Tavares et al. 2019). Garcia-Cegarra et al. (2020) attributed the use for white nesting materials by Red-legged cormorants to the high prevalence of white coloured plastic and polypropylene bags found near their colonies. Garcia-Cegarra et al. (2020) also found cormorants preferred green coloured nesting materials likely due to the high amount of green fishing supplies in the area. Therefore, the availability hypothesis appears to be supported by their study. A future avenue of research in my focal population would be to determine if white coloured materials are more abundant at the Saint Mary's University breeding site. Colour preference of nesting materials has also been suggested to result from what an individual bird experiences in their early life while being reared in a nest. Sargent (1965) found that Zebra finches (*Taeniopygia guttata*) raised in either brown, green or red coloured nests preferred that specific colour of material when building their own nests.

European starlings also preferred the silver ribbons which could be due to both white and silver being close together on the visible colour spectrum. Muth et al. (2013) found that Zebra finches had a blue colour preference for nesting material but also preferred green in an earlier study (Muth and Healy 2011). Muth et al. (2013) speculated that the change in colour preference was due to both blue and green being close in proximity on the visible spectrum.

In conclusion, there was no effect of the total mass of feathers on brood condition. Secondly, the mass of pigmented feathers in this eastern Canadian population of European starling nests increased hatching success. White feathers were not as abundant in nests as were pigmented feathers, nor did the mass of white feathers have an effect on hatching success. Starlings prefer white coloured nesting materials when offered the choice. Future research is needed to investigate whether European starlings therefore actually do prefer pigmented feathers over white feathers and what mechanism accounts for the positive effect pigmented feathers have on hatching success. Additionally, further examination into colour preferences by European starlings will help to determine why they appear to choose white and silver over other colours.

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CHAPTER 5: General Discussion

Anthropogenic materials are being improperly discarded in habitats all over the world (Kedzierski et al. 2020), resulting in avian species picking them up as they forage for nesting materials (Antczak et al. 2010; Brentano et al. 2020; Briggs et al. 2023; Francila et al. 2023; Garcia-Cegarra et al. 2020; Grant et al. 2018; Hammer et al. 2017; Harvey et al. 2020; Hudecki et al. 2020; Jagiello et al. 2018; Jagiello et al. 2019; Jagiello et al. 2022; Lee et al. 2015; León-E et al. 2024; Montevecchi 1991; Sergio et al. 2011; Suárez- Rodriguez et al. 2013; Tariq et al. 2024; Townsend and Barker 2014; Vasquez et al. 2022; Wang et al. 2009). These anthropogenic materials in avian nests range from plastics, paper, twine/string, fishing supplies and cigarette butts from smoked cigarettes (Feare 1984; Garcia-Cegarra et al. 2013; Pérez-Beauchamp et al. 2024; Suarez-Rodriguez et al. 2013; Suárez-Rodríguez and Garcia 2014; Suárez–Rodríguez and Garcia 2017; Townsend and Barker 2014). They cause damage to avian species through entanglement (Antczak et al. 2010; Hudecki et al. 2020; Jagiello et al. 2018; Montevecchi 1991; Ryan 2018; Townsend and Barker 2014) and ingestion (Cunha et al. 2022; Fry et al. 1987; Jagiello et al. 2018), which can both result in death.

In Chapter 2, I explored why European starlings (*Sturnus vulgaris*) incorporate anthropogenic materials in their nests. I found similarities to other studies; the percentage of nests containing anthropogenic materials in an area is comparable to the number of garbage items in the surrounding area of where the nests are located. I did not find that the mass of anthropogenic materials in the nests was related to the age of either parent. However, Jagiello et al. (2018) and Sergio et al. (2011) found a positive association between the amount of anthropogenic materials in the nest and age. This question warrants further research as my sample sizes for SY parents was small.

In Chapter 3, I determined if the mass of anthropogenic materials within starling nests had an impact on nestling brood condition and fledging success. Townsend and Barker (2014) found that 11/195 American crow (*Corvus brachyrhynchos*) nestlings became entangled in anthropogenic materials within the nest and had failed to fledge. Although, I found that 97% (129/133) of nests at my Saint Mary's University study site contained anthropogenic materials, I did not find any relationship between either nestling brood condition or fledging success compared with the mass of anthropogenic materials within nests. I speculated that this lack of a relationship might be related to only one recorded instance of entanglement and ingestion occurring in my study. I also examined whether the abundance of cigarette butts from smoked cigarettes within nests (as determined by mass) had an impact on hatching success, nestling brood condition and fledging success. Previous research (Suárez-Rodríguez et al. 2013; Suárez-Rodríguez & Garcia 2014) suggests that cigarette butts reduce the abundance of ectoparasites in the nest. Unlike the positive relationships that were found by Suárez–Rodríguez and Garcia (2014), I did not find that cigarette butts affected hatching success or brood condition

Interestingly, I did find a significant negative relationship between fledging success and the mass of cigarette butts.

Feathers are a vital component of nests in many species as they can provide nestlings with thermoregulation benefits (Lombardo et al. 1995; Peralta-Sanchez et al. 2011; Stephenson et al. 2009), can help hide eggs from brood parasites (Heinrich 2015) or can be used in mate attraction (Sanz and García-Navas 2011). Previous studies have suggested a preference for white coloured feathers among avian species (Peralta-Sanchez et al. 2011; Ruiz-Castellano et al. 2018), possibly related to increased hatching success provided from their antimicrobial effects (Peralta-Sanchez et al. 2010; Peralta-Sanchez et al. 2014). In Chapter 4, I examined whether the total mass of feathers within starling nests had an impact on nestling brood condition. Although both Winkler (1993) and Stephenson et al. (2009) found that Tree swallow (*Tachycineta bicolor*) nestlings had increased growth rates in nests with more feathers, I did not find a relationship when comparing total feathers to brood condition. I also examined whether the colour and mass of feathers within starling nests had an impact on hatching success. I found no relationship between hatching success and the mass of white feathers, but I found a positive relationship between hatching success and the mass of pigmented feathers. I suggested that the positive relationship between hatching success and the mass of pigmented feathers could be explained by an unexamined factor. I did find a preference for white and silver nesting materials (ribbons) when conducting an experimental study providing the starlings a choice of five different colours of ribbons (blue, green, red, white, silver). The preference for the white nesting materials was predicted based on other studies (e.g. Brentano et al. 2020; Briggs et al. 2023; Cunha et al. 2022; Garcia-Cegarra et al. 2020; Sergio et al. 2011; Tavares et al. 2019). Unexpectedly, however, silver nesting materials were also preferred, which, to my knowledge, has not been found in any other studies. I speculated that the preference for silver nesting materials was related to white and silver being close to each other on the visible spectrum. Muth et al. (2013) found that Zebra finches (*Taeniopygia guttata*) had a preference for blue nesting materials in one study (Muth et al. 2013) and for green in an earlier study and thought it was due to their proximity on the visible colour spectrum.

In conclusion, this thesis highlights various hypotheses explaining how anthropogenic end up in avian nests and the potential consequences of their incorporation. Although not found among this Eastern Canadian population of starlings, anthropogenic materials can result in nestling entanglement and ingestion by nestlings, leading to death. Various hypotheses exist on why avian species incorporate anthropogenic materials into their nest but there is no one answer as to why this behavior occurs. The percentage of nests in an area containing anthropogenic materials appears to be related to the number of garbage items in the surrounding area. There was also a preference for white and silver nesting materials among this starling population. There was a significant higher mass of pigmented feathers found in nests compared to white coloured feathers leading to a positive relationship with hatching success.

These findings help gain a deeper understanding on how different nesting materials affect avian reproduction. Future research is needed to explore other hypotheses for why anthropogenic materials are incorporated into nests along with gaining insight on how pigmented feathers increase hatching success.

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