Differential maternal investment across European starling (*Sturnus vulgaris)* clutches

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Abstract

Most passerine species begin incubating their clutches once the penultimate egg is laid, allowing for asynchronous hatching of nestlings. This results in delayed development by 24 hours or more of the nestling hatching from the last-laid egg. The brood survival hypothesis postulates that maternal investment into this last-laid egg increases such it will be larger relative to other eggs in the clutch to compensate for delayed development initiated by asynchronous hatching. Alternatively, the brood reduction hypothesis states that maternal investment into the last-laid egg decreases such that it will be smaller relative to the rest of the clutch, allowing this smallest nestling to act as an insurance policy whose survival depends on food availability during the breeding season. Ample food will allow this nestling to survive, while food scarcity will cause it to die without risking the entire brood. European starlings (*Sturnus vulgaris*) are cavity-nesting, ground-feeding passerines who eat terrestrial invertebrates. They exhibit biparental care of asynchronously-hatched young and typically have two clutches in a season. The objective of this study was to determine whether female European Starlings laid a larger or smaller last-laid egg relative to the rest of the clutch. I predicted that females would follow a brood reduction strategy due to unpredictable food availability. I studied 189 clutches over five years and found that, as predicted, the last-laid eggs laid by females were significantly smaller than other eggs in the clutch. Late clutches were significantly smaller than early-season clutches, but last-laid eggs did not differ in mass from last-laid eggs in early clutches, nor did the mean mass of all other eggs differ. Adoption of the brood reduction strategy in European starlings is likely due to fluctuating food availability; a decrease in clutch size may reflect the typical downward trend of food abundance throughout the breeding season.

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INTRODUCTION

Passerine parental investment

The survival of young in avian species requires the investment of parental care (Burley and Johnson 2002). Passerine young are altricial and thus especially require this care – born with eyes closed and little down covering, these offspring are entirely dependent on care by their parents to grow successfully and eventually fledge (Ricklefs 1993). Due to this inevitable vulnerability of offspring, passerine (songbird) species normally exhibit biparental care, in which both the male and female parents contribute to providing offspring with the necessary elements to survive, though specific responsibilities differ between the sexes (Clutton-Brock 1991). Social monogamy, in which a long-term, but not exclusive, arrangement exists between the male and female in a pair, is thought to exist in 81% of all birds (Cockburn 2006). This evolution of biparental care affords offspring the safety of being continuously monitored by both parents (Wesolowski 1994). In some passerines, polygynous mating also occurs whereby a male pairs with multiple females resulting in enhanced opportunities for mating but decreased benefits of paternal care (Webster 1991). Differences in the degree of parental investment exhibited by either sex in either mating system arise due to trade-off that occurs between investing in reproductive effort and offspring quality, and forfeiting aspects of adult fitness as a result of this investment (such as less successive mate pairings and future breeding season survival) (Slagsvold and Lifjeld 1989).

Adult fitness plays an important role in both the timing and intensity of reproduction – or, the laying date and size of clutch produced (Verhulst and Nilsson 2008). Clutch size varies with respect to laying date, in that, as resource availability fluctuates throughout the season, the number of young that can be reared with adequate care changes (Klomp 2002). Early breeders generally have higher reproductive success than later breeders, due to the availability of resources that have not yet been diminished by breeding pairs. There is also the increased possibility of re-nesting for early breeders within that same season which allows more than one opportunity to produce surviving offspring (Verhulst and Nilsson 2008). This second attempt at nesting, known as double-brooding, occurs in species whose territory quality or abundance of food for their offspring is adequate enough to attempt reproduction a second time in the season. Female black-throated blue warblers (*Setophaga caerulescens*) are found to attempt double-brooding when food availability is higher, the density of conspecific neighbors is lower, and the mass of nestlings produced in the earlier brood was heavier (Nagy and Holmes 2005). Turner (2010) found that 62-92% of pairs of barn swallows (*Hirundo rustica*) in Europe are double-brooded. Producing multiple broods in a season increases total reproductive output, but creates a greater toll on parental investment and therefore risks offspring survival in subsequent broods (Lack 1954).

Several avian species also perform conspecific (intraspecific) brood parasitism, laying eggs in conspecific's nests in order to avoid having to invest their own parental care to the resulting offspring. Instead, they rely on the investment of the conspecific to rear their offspring (Romagnano et al. 1990). This method of forfeiting parental responsibility is found to occur in at least 234 avian species (Lyon and Eadie 2008).

It is apt then that Trivers (1972) defines parental investment as "any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring". Avian parental investment typically constitutes building a nest, laying eggs (by females), defending eggs and nestlings, and provisioning the offspring (Balshine 2012). Additionally, incubation of the eggs is an important responsibility typically conducted by the female in most species, but designated to both sexes in other species (Slagsvold and Lifjeld 1989). The timing of incubation is particularly integral in determining the onset of development for the offspring, and once begun must continue and maintain adequate temperature or else the embryo will die (White and Kinney 1974; Magrath 1990).

Synchronous and asynchronous hatching

The onset of incubation is influenced by a multitude of both internal and external factors, including parental hormones and threats to both parental and offspring survival (Stoleson and Beissinger 1995). Environmental cues such as changes in the photoperiod and the onset of rainfall cause follicle-stimulating hormone to be released and begin growth stimulation of the ovarian follicles. Incubation factors such as whether both sexes incubate, intensity of predation, threats to adults and nestlings and prevalence of brood parasitism are all taken into consideration when determining the timing of incubation onset (whether incubation starts before or after laying is complete) (Stoleson and Beissinger 1995). Differences in the timing of incubation onset dictate whether synchronous or asynchronous hatching ensues. Synchronous hatching occurs when incubation begins following the laying of all eggs, and results in offspring all hatching within a few hours of each other (Clark and Wilson 1981; Rumpf 1992). Conversely, hatching asynchrony results when parents initiate incubation prior to laying the last egg, resulting in the eggs hatching over a period of one or more days or even several weeks, with last-laid eggs being the final eggs to hatch (Lack 1954; Stoleson and Beissinger 1995).

Most passerine species begin incubation after laying the penultimate (second-last laid) egg, and thus have asynchronously hatching eggs (Stenning 1996). Several hypotheses currently exist for the evolution of asynchronous hatching. Hahn (1981) asserted that hatching asynchrony reduces rivalry amongst siblings as food is less readily obtained by those offspring who hatch later, and are thus weaker due to delayed development. Hussell (1972) postulated that asynchrony allows for peak loads of food requests from offspring to be spread out over time. Clark and Wilson (1981) predicted that asynchronous hatching of eggs allows for lower overall nest predation as nestlings hatch and develop in a staggered manner before fledging (leaving the nest). Each of these hypotheses expands on Lack's hypothesis of brood reduction, which suggests that parents raise offspring at staggered developmental stages in order to adapt to the resources available for consumption (Lack 1954).

Brood reduction hypothesis versus brood survival hypothesis

An optimal brood size will allow for the adaptive consumption of resources whose availability cannot be precisely inferred but for which is instead prepared (Magrath 1990). If the clutch size is too large, it is possible that provided nutrients will be insufficient to feed all members of the brood, and parents will have to work harder to provide more resources to their offspring, lowering their own survivorship odds and future fecundity (Magrath 1990; Mock and Forbes 1994). With clutches of smaller sizes, though parental strain decreases, so too does the number of maturing offspring, thus decreasing parental reproductive output (Magrath 1990). Thus, parents will invest in a brood size that will be able to adapt to the environment in which it will be raised, under conditions that allow for optimal survival of both the offspring and the parents themselves.

Avian species have two methods with which to manipulate this condition of asynchronous hatching – through the brood survival strategy or the brood reduction strategy. The brood survival strategy constitutes increased female investment in the last-laid egg of the clutch (Hillström 1999). This approach to parental investment provides a mechanism with which to combat asynchronous hatching in years when it is likely that resource availability will be ample, as well as to favour the competitive ability of later-hatching offspring versus that of their older siblings, ensuring a better opportunity for all offspring within the brood to survive (Howe 1978; Clark and Wilson 1981). Nestlings raised from last-laid eggs with females of species adopting the brood survival strategy are found to have higher growth rates and eventual greater success when fledging than their siblings from earlier-laid (You et al. 2009).

In contrast, brood reduction strategy implies decreasing investment of parental resources into the later-laid eggs of a clutch (Magrath 1990). The adaptive brood reduction strategy invokes younger offspring hatched from later-laid eggs acting as an insurance policy based on resource availability, wherein should a lack of resources arise, weaker chicks hatching from later-laid eggs will die, allowing for resources to be allocated preferentially to their stronger siblings. However, should resources be ample, the weaker chicks will receive enough food to survive (Stoleson and Beissinger 1995). Hatching asynchrony facilitates this adaptive brood reduction in that nestlings hatching from last-laid eggs will be at least 24 hours younger than their siblings (Stoleson and Beissinger 1995). The smallest hatchlings are more likely to die in the time period between hatching and fledging as a result of factors including starvation, being trampled in the nest, and sibling competition. Their normal-sized siblings will thrive on the extra food that this resulting decrease in brood size affords them (Stenning 1996). Further, the weakest hatchlings, subjected to adaptive brood reduction, will have markedly decreased fledgling success if they do not die early on in development (Stenning 1996).

Brood reduction can arise as a result of sibling behaviour in passerine species, including competitive food begging and attack by siblings, termed “siblicide” (Mock 1985). In competitive food begging, it is found that the largest offspring will rise to top of the nest above its siblings and only retreat once satiated, resulting in their being fed more often and with greater priority than the smaller siblings. This behaviour has been observed in both the blackbird (*Turdus merula*) and the great tit (*Parus major*) (Rydén and Bengtsson 1980), among others.

Maternal investment strategies

The female parent, who constructs the eggs from which offspring will hatch, has a unique responsibility in the clutch size manipulation towards either the brood reduction or brood survival strategy. Differential allocation of maternal resources into each egg has the ability to change the competition for the environment in which offspring will be raised (Benton et al. 2005). By using the brood survival or brood reduction strategy, females modify the mass of last-laid eggs she produces so that larger, heavier eggs will produce larger nestlings (Williams 1994).

Great tits (*Parus major*) are known to exhibit a brood survival strategy, wherein the last-laid egg is larger and permits nestlings hatched from these eggs to have growth and survival rates similar to those siblings who had hatched earlier as a result of asynchronous hatching (You et al. 2009). Hillström (1999) found that European pied flycatchers (*Ficedula hypoleuca*) also adopt a brood survival strategy in which egg size increases with laying order, to ensure increased survival in the later-laid eggs of a clutch. In contrast, female European swifts (*Apus apus*) have a last-laid egg that weighs significantly less than the rest (O'Connor 1979). This is believed to be due to an increase in adverse weather patterns that result in a diminished food supply of insects (O'Connor 1979).

Avian egg composition

Eggs consist of lipids, water, minerals, antibodies, and proteins, all of which are vital for the developing embryo (Pilz et al. 2003). Lipids are the dominant form of mobilizable energy for embryos, and high lipid levels within the yolk are found to be associated with an accelerated growth rate of body mass in hatchlings (Ardia 2005). Water resources are implicated in the humidity of incubation and facilitate some transport of oxygen and carbon dioxide through the egg. Low humidity in the egg is associated with desiccation of the embryo, and high humidity is associated with prevention of development, suggesting that water resources within the egg and pore formation in the egg shell are both extremely important (Rahn 1981).

Minerals are integral to producing the egg shell and act as the primary source of calcium for the developing embryo (Terepka 1963). Antibody IgY, derived from the mother, provides the embryo with its initial form of humoural immunity and increases the embryo's developmental combat against the onset of disease (Morales et al. 2006).

Eggshell proteins also play an important role in immune defense, responsible for antimicrobial activity during embryonic development (Mine et al. 2003).

Yolk androgens are implicated in hatchling success. They are naturally produced androgenic steroidal hormones that, depending on their concentration, result in a wide range of phenotypic variation affecting body mass, fetal growth rate and fetal behavior and physiology (Pilz et al. 2003; Love et al. 2008). Female birds can manipulate the allocation of these resources when making their eggs *in utero*. For example, house sparrow (*Passer domesticus*) eggs had higher yolk androgen levels when the number of occupied neighbouring nest sites increased, while tree swallows (*Tachycineta bicolor*) produced eggs with higher yolk androgen levels as reflected by higher levels of maternal aggression in response to nest intrusions (Whittingham and Schwabl 2002; Mazuc et al. 2003). Female European starlings (*Sturnus vulgaris*) have increased androgen levels during periods of high breeding activity, which usually occur in the pre-laying period and will thus result in increased levels of androgens in the yolks of eggs being produced at this time (Eising et al. 2008).

Egg size is largely implicated in offspring success as a component of maternal effort (Braasch and Becker 2019). Larger eggs are composed of greater amounts of these resources, including larger yolks, and as a result have increased yolk androgens which provide an additional advantage to hatchling success (Smith et al. 1995; Pilz et al. 2003). It is an important predictor of hatchling mass with larger eggs producing hatchlings that are physically larger, of better condition and therefore having increased survival (Smith et al. 1995; Potti 1999; Braasch and Becker 2019).

***Sturnus vulgaris* as a model system**

The European starling is a member of the family *Sturnidae*, known scientifically as *Sturnus vulgaris*. This species generally has two broods: the early brood occurring from late April to early June, and the late brood, which occurs from late May/early June to late July. European starlings are cavity nesters; nest building and sexual activity occur in the early morning, as do copulations, which occur close to the nest (Feare 1984). Laying of eggs also occurs in the morning, with one egg per day deposited in the nest at some time before 1000h.

European starling eggs are ovoid with slight variations in shape; some are rounder or more pointed at the tip than others. Some eggs are spotted as the result of parasitic behavior by a hematophagous ectoparasite (fly) *Carnus hemapterus*, which feeds on the blood of parental brood patches, and as a result is are squashed against the eggs during incubation, creating spotting patterns on the egg shells (López-Rull et al. 2007). Eggs laid in a single clutch are very similar in colour and shape if laid by the same female (Feare 1984).

Clutch size ranges from 4 to 6 eggs, with 7 eggs being found in rare cases (Feare 1984). Clutch sizes are generally larger in the early-season nests than in the later-season nests (Kessel 1957). Adults initiate incubation after the penultimate egg is laid, resulting in asynchronous hatching between the last-laid egg and the earlier-laid eggs. Both parents incubate the eggs and provision the offspring (Pinxten et al. 1994). Nestlings fledge from the nest at approximately 21 days of age (Kessel 1957).

European starlings are a species subject to intraspecific brood parasitism. A study conducted by Smith (1993) on intraspecific brood parasitism found that it occurs in 31.7% of starling nests.

Objectives and predictions

The purpose of this study is to determine whether differential maternal investment exists within European starling clutches by examining egg size as assessed through egg mass. Passerines are known to exhibit different brood adaptation strategies in response to hatching asynchrony and resource availability, exhibiting differing levels of maternal investment in last-laid eggs as a result. With the adaptive brood survival, females invest preferentially in the last-laid egg such that it is larger, thereby compensating for hatching asynchrony and providing better competitive odds for the hatchling against its older siblings (You et al. 2009). Alternatively, with the adaptive brood reduction strategy, resources are withheld from the last-laid egg thereby making it smaller, which facilitates brood reduction should resource availability become poor over the nestling period (Stoleson and Beissinger 1995).

Previous literature has suggested that the brood reduction hypothesis is the most appropriate explanation for hatching asynchrony (Stouffer and Power 1991). Brood reduction creates a mechanism by which parents can maximize their nestling's quality such that last-laid nestlings are less likely than their older siblings to survive when food supplies are low (Clark and Wilson 1981; Stouffer and Power 1991). To adopt the brood reduction hypothesis, females would change their investment in the rates of yolk, albumen and shell produced such that decreased rates would result in smaller egg size (Williams 1994). It is assumed that the last-hatched egg is laid last, as has been indicated in previous studies (Zach 1982; Smith 1988). In years of poor food supply, brood reduction through hatching asynchrony would decrease the brood size to comply with food abundance, inducing the smaller last-laid nestling's death to allow greater food availability to its older siblings (Amundsen and Slagsvold 1996). In seasons with plentiful food resources, the success of the entire brood would be unaffected by hatching pattern and brood reduction (Amundsen and Slagsvold 1996). Egg mass has a significant effect on hatchling success (Smith et al. 1995), and previous studies indicate that larger egg mass is significantly correlated with larger morphological characteristics of hatchlings (Rofstad and Sandvik 1987).

As European starlings have decreasing clutch sizes over the season (Christians et al. 2001), and the abundance of food is unpredictable, I predict that the last-laid egg will be smaller (weigh less) than the eggs produced before it, due to differential maternal investment within European starling clutches in order to facilitate this adaptive brood reduction.

METHODS

Study species

The European starling is a songbird species and a member of the family Sturnidae. This species originates from Europe as well as parts of Russia, and was originally introduced to North America in Central Park, New York City in 1800 (Linz et al. 2007). It is estimated that anywhere between 5 and 50 million European starlings are currently present in Canada (Government of Canada 2014). However, their populations are in steep decline in their native range and are also rapidly declining in North America (Rosenberg et al. 2019). Adult European starlings are small-to-medium sized passerines, varying in length from 20 to 23 cm, in weight from 60 to 96 g and in wingspan from 30 to 40 cm (Feare 1984; Cabe 1993).

Study site

This study was conducted on the campus of Saint Mary's University (44°37'54. 07" N, 63° 34' 47.09" W) in Halifax, Nova Scotia, Canada, bfrom late April through July over all study years (Table 1). Data were accumulated from the 2013, 2014, 2015, 2016 and 2019 field seasons. Sample sizes for each field season are shown in Table 1.

Table 1. Number of early and late clutches over the years of study.

Field Season	Early Clutches	Late Clutches
2013	25	19
2014	23	Not assessed
2015	21	14
2016	25	19
2019	20	23

Females laid their eggs in the 45 wooden nest boxes attached two to three meters off the ground to trees distributed around campus (Figure 1). This campus population has been studied since 2007. The campus has many trees and shrubs surrounded by patches of grass, parking lots and buildings.

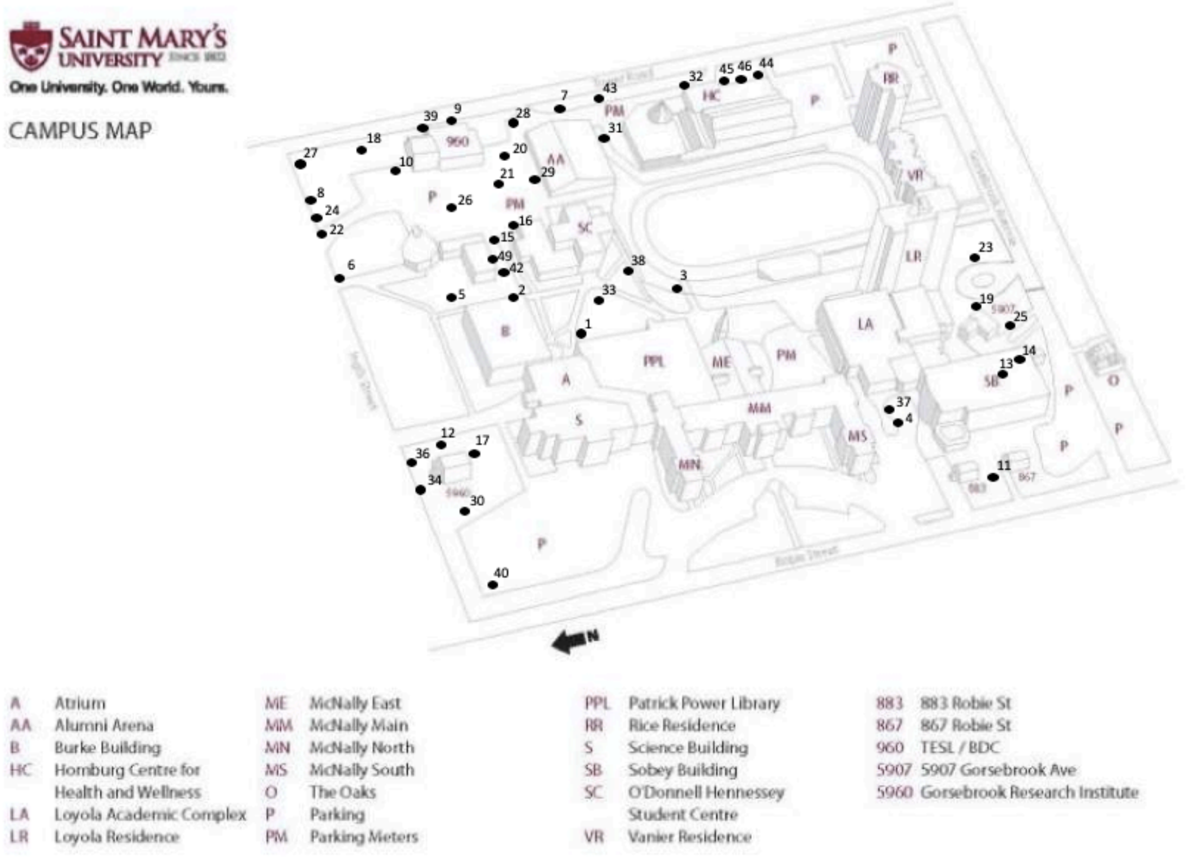

Figure 1. Map delineating the locations of 45 nest boxes at Saint Mary's University campus, Halifax, Nova Scotia, Canada.

Data Collection

Nests were checked daily for the presence of eggs after 1030 h. European starlings are considered to be late layers compared to other passerines, laying one egg a day between 0800 and 1000 h (Kessel 1957). From the nest box (Figure 2), I removed each egg on the day it was laid, numbered it according to its laying order on the rounded end with a waterproof sharpie and weighed it with an American Weigh digital pocket scale (AMW-

250) to the nearest 0.1 gram. I then placed it back in the nest. In two of the years (2015 and 2019), nest-checks started later in April for logistical reasons, and so often the earlier-laid eggs in a clutch were not weighed on the same day as they were laid but were still weighed within a maximum of three to four days of laying. It should be noted that due to this delayed measurement of some of the eggs, mass data collected may not have precisely reflected the initial weight of the egg, as water loss occurs during the first 2-3 weeks of incubation (Boerjan 2010). However, because delayed measurement only occurred for a small number of eggs and was still collected within the first 4 days of laying, this initial egg weight loss should be negligible. Occasionally, two eggs appeared on the same day which indicated intraspecific brood parasitism, and I excluded those clutches from data analysis. I considered laying to be done when a minimum of 24 hours had elapsed since the last egg was laid with no new eggs appearing.

Figure 2. European starling nest box containing a clutch of 5 eggs, with one egg just beginning to hatch.

Statistical analysis

Normality of all data was tested using the D'Agostino Pearson Omnibus normality test with GraphPad Prism (v.6.0 software). A one-way ANOVA test was used to assess if the mass of last-laid eggs as well as the mean mass of other eggs within a clutch varied significantly among years, and to determine that these data could be pooled over all five years (see Results). Next, I tested to determine if I could pool early- and late-season clutches by conducting a Mann-Whitney test to analyze the mass of the last-laid eggs between early and late clutches, and an unpaired *t*-test to analyze the mean mass of other eggs in the clutch between early and late clutches. No significant differences were found (see Results) so last-laid eggs were combined over both early-season and late-season clutches as were the mean masses of other eggs. Finally, I used a Wilcoxon matched-pairs signed rank test to determine if the mass of last-laid eggs differed significantly from the mean mass of all other eggs within a clutch. All tests were two-tailed, and results were considered to be significant when $p \leq 0.05$.

RESULTS

Data was found to be normally distributed across years of collection. The one-way ANOVA found no significant differences in mean egg mass by year ($F_{4, 184} = 0.541$, $p = 0.71$; Figure 3), permitting pooling of egg mass over all five years.

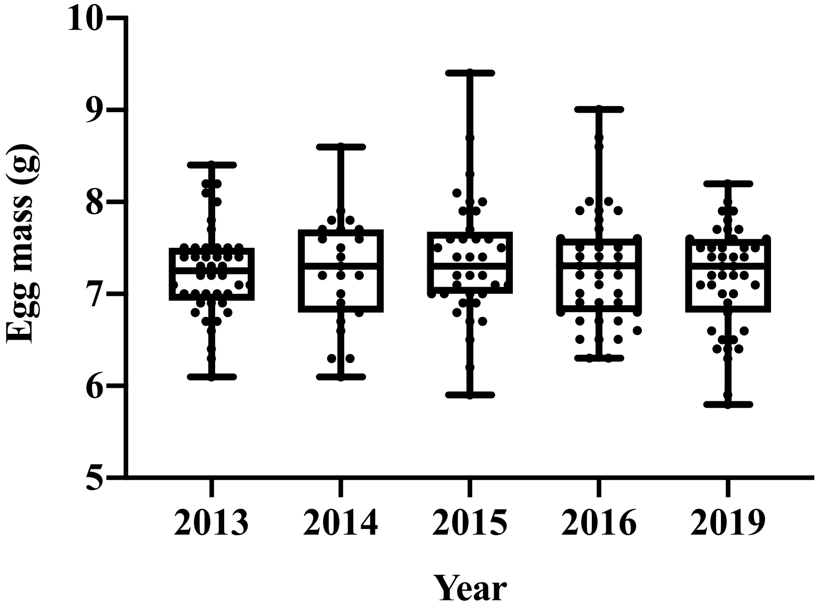

Figure 3. Mass of European starling eggs grouped by year of collection. A one-way ANOVA showed that mean egg masses did not differ significantly between years. Black bars represent the median and black dots are individual data points of egg mass in each year.

Analysis of egg mass between early and late clutches according to egg sequence

Data was analyzed to compare egg masses in early-season clutches versus late-season clutches of all years compiled. No significant difference was found between the mass of the last-laid egg in early-season versus late-season clutches (Mann-Whitney test U = 3919, $n_1 = 114$, $n_2 = 75$, $p = 0.33$; Figure 4). Further, no significance difference was found between the average mass of other eggs in early-season versus late-season clutches (Unpaired *t*-test $t = 0.3046$, df = 187, $p = 0.76$; Figure 5). It was therefore determined that the data from early-season and late-season clutches could be pooled, as no significant difference in egg mass was found between early-season and late-season clutches with respect to last-laid eggs and the mean mass of other eggs across all years.

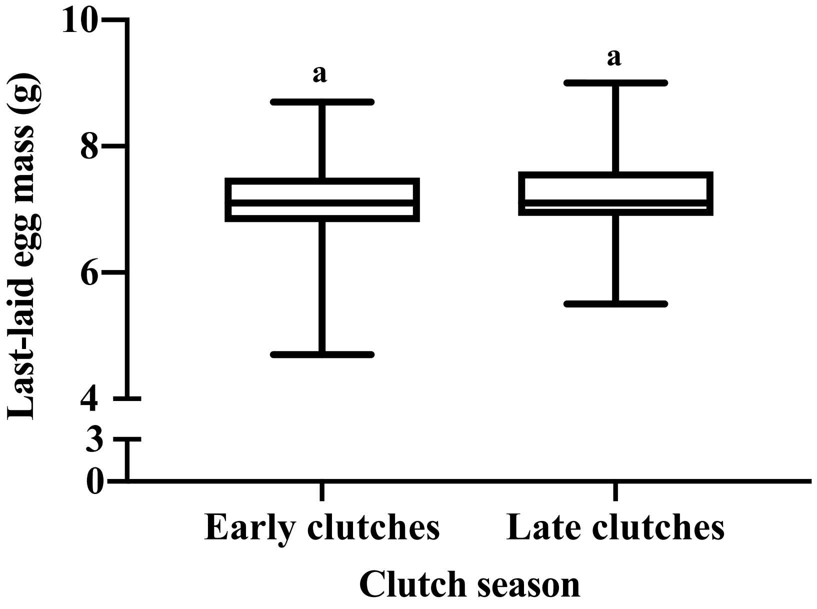

Figure 4. European starling egg masses (median, quartiles and range) of last-laid eggs of early-season clutches (n = 114) versus the last-laid eggs of late-season clutches (n = 75) over five years. Boxes with the same letters above them delineate masses not significantly different from each other.

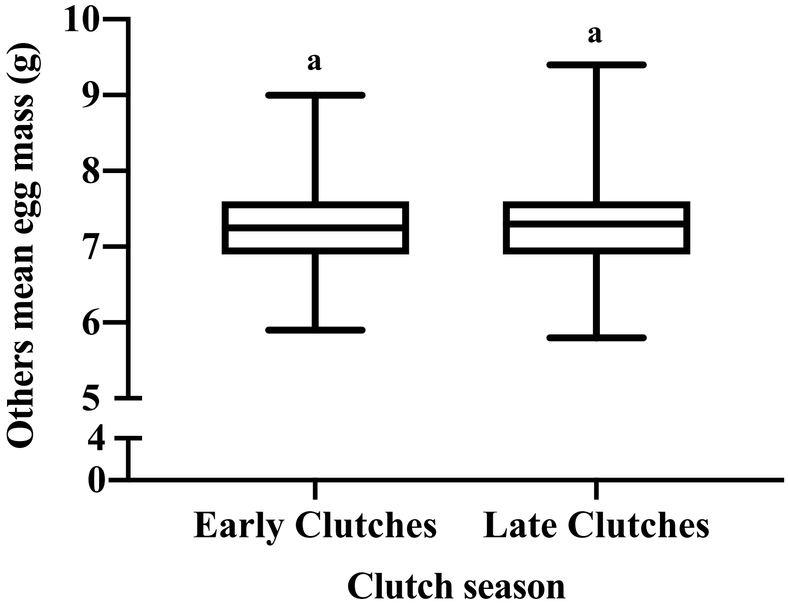

Figure 5. Mean (median, quartiles and range) European starling egg masses of other eggs in early-season clutches (n = 114) versus the mean egg masses of other eggs in late-season clutches (n = 75) over five years. Boxes with the same letters above them delineate masses not significantly different from each other.

Analysis of egg mass for all clutches

Data was analyzed to compare mass of the laid-laid egg of both early-season and late-season clutches across all years against average mass of other eggs in the clutch of early-season and late-season clutches across all years. The mass of the last-laid egg was significantly less than the average mass of other eggs in the clutch over all clutches (Wilcoxon matched-pairs signed rank test W = -4669, n = 189, p = 0.0003; Figure 6).

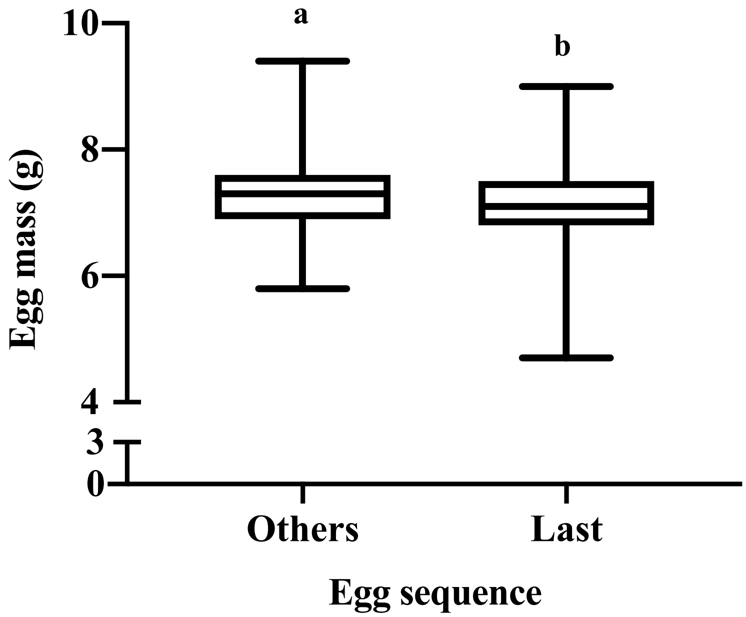

Figure 6. European starling egg mass (median, quartiles and range) of last-laid eggs versus the average mass of other eggs in the clutches for all clutches (n = 189). Boxes with different letters above them delineate masses significantly different from each other.

Analysis of clutch sizes between early and late clutches

Data was analyzed to compare clutch sizes between early-season and late-season clutches across all years. A significant difference was found between clutch sizes for early-season and late-season clutches. Late-season clutches had significantly fewer eggs than did early-season clutches (Mann-Whitney test U = 2307, $n_1 = 114$, $n_2 = 75$, $p < 0.0001$; Figure 7).

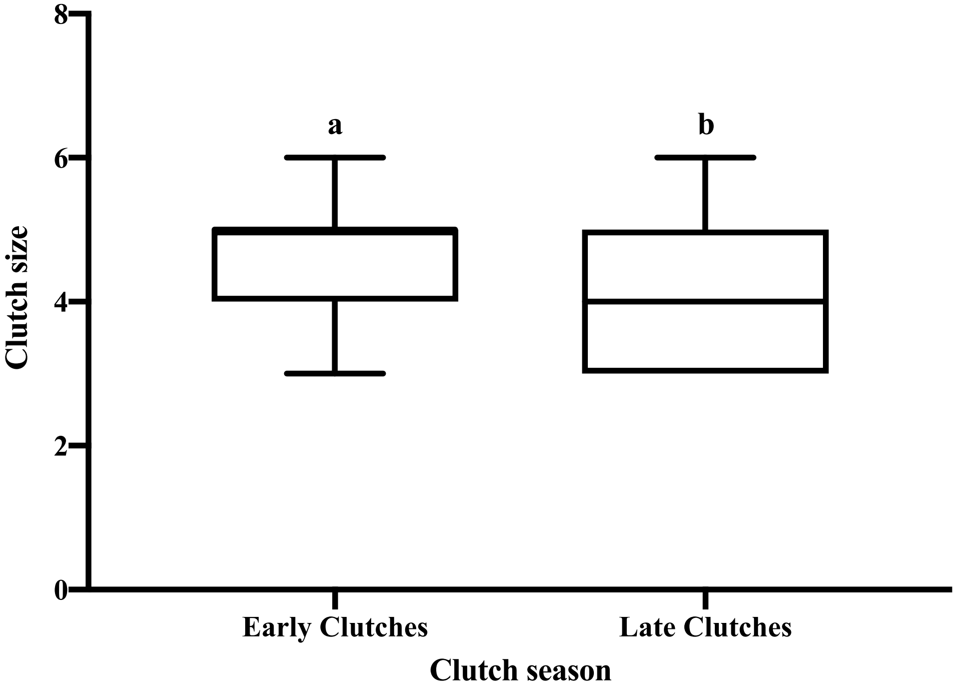

Figure 7. European starling clutch sizes (median, quartiles and range) of early-season clutches (n = 114) versus late-season clutches (n = 75). Boxes with different letters above them delineate clutch sizes significantly different from each other.

DISCUSSION

Brood adaptation in early and late clutches

The aim of this study was to determine if differential maternal investment existed within European starling clutches by examining egg size as assessed through egg mass. The major finding of the study concludes that female European starlings implement significant differential maternal investment during the laying of clutches (Figure 6), such that they invest significantly less in the last-laid egg leading to a smaller mass than the average mass of others in the clutch. Therefore, it can be stated that European starlings appear to adopt a brood reduction strategy.

Several previous studies (Ricklefs 1965; O'Connor 1979; Clark and Wilson 1981; Husby 1986; Leblanc 1987; Hillström 1999; Maddox and Weatherhead 2008; You et al. 2009; Braasch and Becker 2019) have examined the significance of brood adaptation strategies in other passerine species. The brood reduction strategy in which maternal investment, and therefore mass, *decreases* in the last-laid egg, has been found in the European swift (*Apus apus*) (O'Connor 1979) as well as in the curve-billed thrasher (*Toxostoma curvirostre*) (Ricklefs 1965). The contrasting brood survival strategy in which maternal investment in the last-laid egg *increases* relative to other eggs and results in an increase in mass of the last-laid egg has been found to exist in pied flycatchers (*Ficedula hypoleuca*) (Hillström 1999), as well as great tits (*Parus major*) (You et al. 2009).

The decrease in weight of the last-laid egg in European starling clutches is likely due to females preparing to adapt to fluctuating and therefore unpredictable resources throughout the breeding season. In years of sparse food supply, asynchronous hatching would allow for adoption of the brood reduction strategy, such that older siblings in the clutch would have a competitive advantage over the sibling hatching from the last-laid egg. This last-hatched nestling would die in poor food times and its food resources would be allocated amongst the more developed siblings (Amundsen and Slagsvold 1996). In years of increased resource availability, all offspring within the clutch would be more likely to have access to food, and therefore all would be more likely to survive (Ricklefs 1965).

Clutch size throughout the breeding season

Results also showed that clutch size decreased significantly in late-season clutches compared with early-season clutches (Figure 7). This decrease could be explained by the decline of ground invertebrate abundance throughout the breeding season (Staley et al. 2007). Abundance of ground invertebrates declines with decreasing moisture of soil, and as a result European starling feeding resources generally decline throughout the breeding season as the summer progresses (Staley et al. 2007). A decrease in the number of eggs laid in later clutches implies that fewer offspring will need access to this declining food supply, therefore justifying the production of smaller clutch sizes later in the breeding season.

Ricklefs (1965) also stated that brood reduction may be a significant factor in determining the clutch size of avian species. Forbes (1990) concluded that an insurance offspring (in this case, last-laid offspring) proves beneficial when the clutch size is larger and when the cost of offspring formation is low, both of which are more likely to occur when ample food resources exist earlier in the breeding season. Although increasing clutch size increases the reproductive value of the clutch as more offspring will be available to eventually reproduce, it is costly to the reproductive value of the parents who must now care for this increased number of offspring (Sockman et al. 2007). Therefore, mothers must make the difficult decision whether to increase clutch size; perhaps this decision is made easier when laying early clutches because food resources are more readily available early in the breeding season and parental exhaustion imposed by raising a prior clutch is not a factor. Though females will face a trade-off in decreasing their own reproductive value while investing in more offspring overall, early-season clutches are likely larger because mothers are able to produce these clutches with high resource availability while maintaining the insurance policy of a smaller, last-laid egg whose reproduction cost is lower. This way, mothers can offset the cost of their own reproductive value imposed by investing in more offspring overall by ensuring survival of the more developed and more competitive older offspring who are most likely to be reproductively successful in the future.

Research implications

A recent study conducted by Rosenburg et al. (2019) found that avian species in North America have declined by over 3 billion individuals in the past 48 years. Perhaps surprisingly, introduced and abundant urban-thriving passerines such as house sparrows (*Passer domesticus*) and European starlings have populations that are in steep decline not only in their native range, but in North America as well (Rosenberg et al. 2019). Understanding the reproductive behaviours of all passerine species is critical for ongoing conservation efforts of our rapidly declining North American avifauna. More specifically, the findings of the present study could be applied not only to conservation efforts of European starlings, but to other passerine species as well, such that understanding the conditions under which mothers choose to adopt brood adaptation strategies will allow conservation efforts to be tailored to these conditions.

Further, the health of our environment and integrity of our ecosystems can be indicated based on the success of avian species (Rosenberg et al. 2019). European starlings are an excellent indicator species due to their adaptability to urban environments (Sontag and Louette 2007), and the findings of this study contribute to the understanding of their reproductive strategies as an aspect of their success in these environments. A rapid population expansion throughout North America resulted from the initial release of 80 birds into Central Park within New York City and permanent species establishment is estimated to have taken 10 years (Linz et al. 2007). This finding highlights the outstanding ability that European starlings have to maintain their populations within heavily human-influenced habitats as urban exploiters (Sontag and Louette 2007). However, even the adaptable starlings have declining numbers (Rosenberg et al. 2019), illuminating the adversity that other native, more vulnerable species face living in habitats manipulated by humans (Sontag and Louette 2007).

Directions of future research

The conclusions of this study highlight the importance of the relationship between differential maternal investment and fluctuating resource availability throughout the years of this study. Originally proposed by Lack (1954), the adjustment of offspring production to facilitate adaptations to resource supply could be applied to future research looking specifically at the quality and quantity of resources available and examining this food supply in relation to the size of eggs produced by mothers.

Conclusion

In conclusion, maternal investment is, as predicted, differential across European starling clutches. European starlings appear to exhibit a brood reduction strategy, such that the last-laid egg is significantly lighter than the average mass of other eggs in the clutch**.** There is also a significant difference in the size of European starling clutches as the breeding season progresses; the size of late-season clutches is significantly smaller than early-season clutches. Adoption of the brood reduction strategy allows European starlings to prepare for fluctuating food availability, while a decrease in clutch size reflects a further adaptation to the typically declining trend of food abundance throughout the breeding season.

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