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SEASONAL TRADEOFFS IN REPRODUCTIVE INVESTMENT IN A MULTI-BROODED PASSERINE

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Abstract. Because investment in eggs is costly for birds, a female's body condition is expected to influence that investment. Moreover, resource availability can fluctuate by year, and older and younger females may acquire or allocate resources differently. Additionally, in multi-brooded species, females may make tradeoffs in investment in first and second clutches. Because environmental conditions often change through the breeding season and early maturity of second broods can increase females' fitness, patterns of females' allocation to each clutch may change with the season. Here, we investigate associations between females' quality (body condition and age) and pre-hatching investment (clutch size, average egg mass per clutch, and total clutch mass) in adults of the Eastern Bluebird (*Sialia sialis*), a multi-brooded cavity-nesting passerine. In the two years of the study, relationships differed; in 2003, females that were heavier relative to their body size produced larger eggs and invested in greater overall clutch mass, and younger females produced larger clutches. In 2004, females whose first clutches were greater in overall mass laid second clutches proportionately smaller in overall clutch mass. Together, these data suggest that overall clutch mass may represent a significant cost to female bluebirds. We also found variation in investment in eggs within the season; second clutches tended to be smaller than first clutches but tended to contain larger eggs. By increasing resources per offspring late in the breeding season, females may increase the survival or condition of late-season offspring.

Key words: *Eastern Bluebird*, *egg mass*, *egg size*, *life-history evolution*, *multi-brooded*, *seasonal investment*, Sialia sialis, *total clutch mass*, *trade-offs*.

Soluciones de Compromiso Estacionales en la Inversión Reproductiva en un Ave Paseriforme de Nidadas Múltiples

Resumen. Debido a que los huevos son una inversión costosa para las aves, se espera que la condición del cuerpo de las hembras influencie esta inversión. Más aún, la disponibilidad de recursos puede fluctuar anualmente, por lo que las hembras pueden asignar los recursos de modo diferente dependiendo de la edad de éstas. Adicionalmente, en las especies de nidadas múltiples, las hembras pueden realizar soluciones de compromiso entre las inversiones de la primera y segunda nidada. Debido a que las condiciones ambientales usualmente cambian a lo largo de la estación reproductiva, y a que la madurez temprana de las segundas nidadas puede aumentar la adecuación de las hembras, los patrones de asignación de las hembras a cada nidada pueden cambiar con la estación. Aquí investigamos las asociaciones entre la calidad de las hembras (condición corporal y edad) y la inversión efectuada antes de la eclosión (tamaño de la nidada, peso medio del huevo por nidada y peso total de la nidada) en adultos de *Sialia sialis*, un ave paseriforme de nidadas múltiples que anida en cavidades. Durante los dos años del estudio, las relaciones fueron distintas. En 2003, las hembras que fueron más pesadas en relación a sus tamaños corporales produjeron huevos más grandes e invirtieron en mayores pesos totales de la nidada, y las hembras más jóvenes produjeron nidadas más grandes. En 2004, las hembras cuyas primeras nidadas presentaron mayores pesos totales de la nidada pusieron segundas nidadas proporcionalmente menores en cuanto al peso total de la nidada. En conjunto, estos datos sugieren que el peso total de la nidada puede representar un costo significativo a las hembras de *S. sialis*. También encontramos variación en la inversión en los huevos dentro de una misma estación; las segundas nidadas tendieron a ser menores que las primeras nidadas pero tendieron a contener huevos más grandes. Mediante un incremento en los recursos por cría al final de la estación reproductiva, las hembras pueden incrementar la supervivencia o la condición de los pichones al final de la estación.

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INTRODUCTION

Within a population, the size of females' eggs and clutches varies considerably. Females alter the distribution of resources among eggs, often laying either large clutches of small eggs or small clutches of large eggs (reviewed by Christians 2002). Because individuals often maximize lifetime reproductive success by maximizing the number of surviving young per breeding attempt, females are predicted to invest resources into the greatest number of the smallest eggs capable of producing viable young (Clutton-Brock 1991). Accordingly, studies of avian reproductive investment have often focused on egg size, clutch size, and egg composition. However, females' investment before hatching can also be measured by total clutch mass, the product of both egg size and clutch size.

Investment in eggs is likely expensive; studies of passerines often demonstrate that females' body condition and age can influence egg size and clutch size (reviewed by Christians 2002). Females in better body condition likely have acquired more resources to allocate to reproduction (Smith et al. 1993). In most cases, breeding performance improves with age in the early years of life and reaches a maximum at middle age (reviewed by Sæther 1990). Life-history hypotheses that explain age-dependent fecundity include selection (inferior birds do not survive to old age), constraint (young birds are not physically or socially mature), and restraint (young birds defer maximal reproductive effort until a later age) (Martin 1995).

Life-history theory proposes that the effort an organism expends on current reproduction will reduce energy available for future reproduction (Williams 1966, Levins 1968), entailing a trade-off in resource allocation (Roff 1992, Stearns 1992). Many species of birds rear more than one brood of young per season and thus are faced with the challenge of allocating resources between first and second broods in a manner that maximizes reproductive success (Tinbergen 1987, Verhulst et al. 1995). Indeed, second broods may be particularly costly to females, as maintaining or increasing clutch size in later broods of the season has been found to reduce adults' fitness (Boyce and Perrins 1987).

Resources incorporated into individual eggs provide the only resources available for development before hatching. Thus, nestlings hatching from large eggs are typically heavier at hatching and possess greater energetic reserves than those hatching from small eggs (reviewed by Williams 1994). Moreover, large eggs can provide young with a survival advantage during early development after hatching (Magrath 1992, Styrsky et al. 1999).

Later in the season, both environmental conditions and parental energetic reserves often deteriorate (Styrsky et al. 1999). Late-season offspring may face increased risk of predation, load of parasites, and pressures of fall molt, so the value of later clutches may be lower (Smith et al. 1989). To increase the likelihood that late-season nestlings survive, females may need to allocate more resources to individual offspring by increasing egg size and reducing clutch size. For example, in response to decreased prey available to second broods, House Wrens (*Troglodytes aedon*) invest in fewer, larger eggs (Styrsky et al. 1999). In both the House Wren and in the population of the Eastern Bluebirds (*Sialia sialis*) we studied, cross fostering of clutches of large and small eggs demonstrates that egg size has a greater influence on the fitness of offspring in the late season (Styrsky et al. 1999; Robinson et al., unpubl. data). These results indicate that the influence of egg size on nestling development can vary with environmental conditions through the season.

This study explores relationships between egg investment and breeding season (spring–summer) in the Eastern Bluebird. This species is a good subject for a study of strategies of reproductive investment because it uses nest boxes readily, breeds repeatedly in the same location, and tolerates considerable disturbance at the nest. It is a socially monogamous passerine and typically raises two broods in a breeding season (Gowaty and Plissner 1998). In Arkansas, the breeding season occurs between April and early August. Late-season offspring face increased risks of predation and loads of parasites (Robinson et al., unpubl. data) and likely experience increased pressures of fall molt, which begins in September (Gowaty and Plissner 1998).

In an Alabama population of the Eastern Bluebird, both body condition and age influence reproductive investment. Females that are heavier for their body size commence egg laying earlier in the season, provision offspring more often, and produce larger fledglings (Siefferman and Hill 2005). Older females lay eggs earlier in the season and fledge more young, but younger females feed offspring more often (Siefferman and Hill 2005). Variation in eggs within a clutch and between first and second clutches has been described in a South Carolina population. Egg mass tends to increase with the order in which eggs are laid. Eggs of second clutches tend to average larger than those of first clutches, and clutch size tends to decrease in second clutches. Hatching is synchronous with most nestlings hatching within 1 day; however, hatching asynchrony is greater in second broods when incubation is shortened (Gowaty and Plissner 1998).

To investigate how females invest in clutches, we measured clutch size, average egg mass within a clutch, and total egg mass of the clutch. First, we predicted that a female's body condition should influence her investment in a clutch, such that females heavier for their body size should lay larger eggs, larger clutches, or heavier clutches. Second, we predicted that if investment in first broods reflects a cost of reproduction, females that laid heavier first clutches should lay relatively lighter second clutches. We also predicted that overall clutch mass should decrease through the breeding season. Last, we expected females to invest in first and second clutches differently. Specifically, we predicted that, in second clutches, females should lay larger eggs in smaller clutches to maximize resources available to individual young and thus offsetting late-season environmental stress.

METHODS

We monitored 200 boxes in which Eastern Bluebirds nested in 2003 and 2004 in Craighead County, Arkansas (35° 54′ N, 90° 40′ W). Boxes were mounted on 1.5-m steel poles and separated by at least 100 m. We monitored boxes weekly for nest building, and when complete nests were found, we monitored them daily for the laying of the first egg. Eastern Bluebirds usually lay one egg daily between 07:00 and 10:00 (Meek and Robertson 1995), so we labeled freshly laid eggs after 10:00 to determine the sequence of laying. After the clutch was complete, we measured the length and breadth of each egg with dial calipers (±0.01 mm). We measured nine eggs twice to assess the repeatability of measurements (Lessells and Boag 1987); both measurements were significantly repeatable (length: $r = 0.9999$, $P < 0.001$; breadth: $r = 0.9994$, $P < 0.001$).

We calculated each egg's mass by the following formula developed by Hoyt (1979), where mass (g) = $K \times L \times B^2$ and L = length (mm), B = breadth (mm), and $K = 5.41 \times 10^{-4}$. We determined the species-specific constant K by using a portable electronic scale (Acculab PP2060D) to weigh a subset of 31 eggs to the nearest 0.001 g within 2 hr of being laid. These eggs were distributed throughout the sequence of laying, and only one egg was weighed per clutch. Volume calculated from linear measurements was an accurate predictor of actual egg mass ($R^2 = 0.96$, $F_{1,30} = 822.41$, $P < 0.001$). We measured length and breadth of each egg and used the mass predicted from these measurements as our index of egg size. Hereafter, we use egg mass when referring to our calculated index.

We monitored the first and second clutches of every pair and calculated the average mass of eggs in each nest. Total clutch mass was calculated as the combined mass of all eggs in the nest. To reduce the possibility of nest abandonment, we used a nest-box trap to attempt to capture all females during late incubation or early chick rearing (day 14–31 after the laying of the first egg in their first nest) (Robinson et al. 2004). All females were given a unique combination of color bands to allow for easy identification of individuals in the field. On the basis of the shape of the 10th primary, we estimated the age of all newly banded females as either second year (having undergone only one post-nestling molt) or after second year (Pitts 1985). We measured body mass (±0.25 g) and tarsus length (±0.01 mm). Larger females were heavier ($R^2 = 0.09$, $F_{1,63} = 6.05$, $P = 0.02$). The residuals of a regression of mass on tarsus length are a commonly used index of body condition (Jakob et al. 1996). However, we also detected a negative relationship between body condition and stage of breeding; females captured later in incubation or chick rearing were heavier for the body size ($R^2 = 0.36$, $F_{1,61} = 33.03$, $P < 0.0001$). Therefore, we standardized our measure of body condition by using the residuals of a regression of day of capture on body condition (Whittingham and Dunn 2000). We consider our proxy of condition to be a measure of the amount of resources available for egg laying at the beginning of the reproductive season.

STATISTICAL METHODS

We used SAS (version 9.1) to analyze data, and all statistical tests were two tailed. We used Shapiro–Wilk tests to assess the normality of variables, and all data conformed to normal distributions. For nests in which we measured the mass of all eggs in the clutch, we performed repeated-measures ANOVA with post hoc tests of least significant differences to analyze how egg mass within a clutch changed with the order in which it was laid. We captured 30 females in 2003 and 33 in 2004; 8 were captured in both years. To analyze the effects of clutch size on average egg mass, we devised a mixed-effect model with random effects, specifying clutch order (first versus second) and year as fixed factors, clutch size as the covariate, and the female's identity as the random factor. To analyze the effects of the female's body condition, age, and clutch order on average egg mass, clutch size, and total egg mass, we devised three mixed-effect models with random effects. All models a priori included the clutch order, year, and age as fixed factors, the female's body condition as a covariate, and the female's identity as a random factor. To simplify models, we used a stepwise backward procedure and first tested for interactions between clutch order and year. In all models, we found significant interactions between clutch order and year on clutch size ($F_{1,62} = 8.78$, $P = 0.004$), average egg mass ($F_{1,62} = 3.35$, $P = 0.01$), and total clutch mass ($F_{1,62} = 9.02$, $P = 0.004$), suggesting that females responded to clutch order differently in 2003 and 2004. Because models had significant interaction terms, we analyzed each year separately. Next, to further investigate the influence of seasonality on individual females' average investment in an egg, we used paired *t*-tests to compare clutches in the first and second nests. To investigate the similarity of first and second clutches, we used paired correlations. For these paired analyses, we analyzed each year separately.

As total clutch mass is a good proxy for pre-incubation investment, we used this measure to investigate tradeoffs that individual females may make through the breeding season. To investigate whether the total mass of first clutches influenced investment in the mass of second clutches, we used a regression of total mass of the first clutch on the difference in total mass between first and second clutches (second clutch minus first).

RESULTS

During both years of our study, Eastern Bluebirds' clutches ranged from 3 to 6 eggs (mean ± SD = 4.71 ± 0.04, $n = 237$). An egg's mass varied significantly with the order in which it was laid ($F_{5,722} = 25.15$, $P < 0.001$), and eggs within a clutch were similar in size ($F_{198,722} = 20.10$, $P < 0.001$). Egg mass tended to increase with order of laying. On average, the first egg was lighter than all other eggs in the clutch (all $P \leq 0.001$). Eggs 2 and 3 were lighter than eggs 4 and 6 (all $P < 0.05$) but not lighter than egg 5 (all $P < 0.33$). Moreover, egg 2 was not lighter than egg 3 ($P = 0.46$) and egg 4 was not lighter than eggs 5 or 6 (all $P < 0.33$).

TABLE 1. Effects of clutch order (first versus second), age, and female's body condition on clutch size, average egg size, and total clutch mass of Eastern Bluebird eggs in 2003 and 2004.

Trait	Year	Factor	Estimates	SE	df	*F*	*P*
Clutch size	2003	Clutch order[a]	0.32	0.10	27	9.63	0.004
		Age[b]	0.27	0.14	27	4.13	0.05
		Body condition	0.03	0.05	27	0.34	0.56
Clutch size	2004	Clutch order[a]	0.74	0.10	30	51.53	<0.0001
		Age[b]	−0.13	0.20	30	0.44	0.51
		Body condition	−0.03	0.08	30	0.18	0.68
Average egg mass (g)	2003	Clutch order[a]	−0.12	0.02	27	63.55	<0.0001
		Age[b]	0.04	0.08	27	0.24	0.63
		Body condition	0.09	0.03	27	11.27	0.002
Average egg mass (g)	2004	Clutch order[a]	−0.03	0.03	30	1.03	0.32
		Age[b]	0.02	0.07	30	0.09	0.77
		Body condition	0.04	0.03	30	2.30	0.14
Total clutch mass (g)	2003	Clutch order[a]	0.56	0.37	27	2.37	0.14
		Age[b]	0.99	0.52	27	3.66	0.07
		Body condition	0.54	0.18	27	9.35	0.005
Total clutch mass (g)	2004	Clutch order[a]	2.08	0.35	30	36.05	<0.0001
		Age[b]	−0.30	0.68	30	0.19	0.66
		Body condition	0.12	0.27	30	0.19	0.67

[a] Estimates are relative to second clutches.
[b] Estimates are relative to after-second-year birds.

Although there was a significant interaction between clutch order and year ($F_{1,67} = 4.07$, $P = 0.05$), in neither year did we detect a significant relationship between egg mass and clutch size (2003: $F_{1,28} = 2.20$, $P = 0.15$; 2004: $F_{1,31} = 0.00$, $P = 0.98$), indicating that, within a clutch, a female does not trade off between egg mass and clutch size.

EFFECTS OF CLUTCH ORDER, FEMALE'S AGE, AND FEMALE'S BODY CONDITION

We found no significant relationship between females' body condition and clutch size in either year (Table 1, Fig. 1a), but in 2003 second-year females laid larger clutches than did older females (Table 1). In both years, we found that first clutches were larger than second clutches, but the effect was stronger in 2004 than in 2003 (Tables 1 and 2, Fig. 2a). Next, we used a paired analysis of females that laid both early and late in the season to determine whether an individual female's clutch size decreased. We found that first clutches were larger, but the trend was stronger in 2004 than in 2003 (2003: paired $t_{30} = 3.07$, $P < 0.005$; 2004 paired $t_{33} = 7.28$, $P < 0.0001$). The size of a female's first clutch and that of her second clutch, however, were significantly correlated (paired-samples correlations: 2003: $r_{30} = 0.38$, $P = 0.04$; 2004: $r_{30} = 0.53$, $P = 0.002$).

A female's age did not predict average egg mass (Table 1). Females that were heavier for their body size (better body condition) laid significantly heavier eggs in 2003 but not in 2004 (Table 1, Fig. 1b). Eggs in second clutches averaged heavier than those in first clutches in 2003 but not in 2004 (Tables 1 and 2, Fig. 2b). Next, we used a paired analysis of females that laid two clutches in a year to determine whether egg mass increased later in the breeding season. In 2003, eggs in a female's second clutch were heavier than those in her first clutch (paired $t_{30} = 7.65$, $P < 0.0001$), but in 2004 the difference was not significant (paired $t_{33} = 1.31$, $P = 0.20$). The average mass of an egg in a female's first clutch and that in her second clutch, however, were significantly correlated (paired-samples correlations: 2003: $r_{30} = 0.93$, $P < 0.0001$; 2004: $r_{33} = 0.70$, $P < 0.0001$).

In 2003, females that were heavier for their body size laid significantly heavier clutches (Table 1, Fig. 1c); moreover,

TABLE 2. Summary statistics (mean ± SD) of egg measurements from individual Eastern Bluebirds that laid first and second clutches in 2003 and 2004.

Clutch	Year	*n*	Clutch size	Average egg mass (g)	Total clutch mass (g)
First	2003	30	4.93 ± 0.37	2.89 ± 0.24	14.27 ± 1.58
Second	2003	30	4.63 ± 0.56	3.01 ± 0.25	13.78 ± 2.17
First	2004	33	5.15 ± 0.57	2.97 ± 0.20	15.31 ± 1.98
Second	2004	33	4.42 ± 0.61	3.01 ± 0.23	13.32 ± 2.08

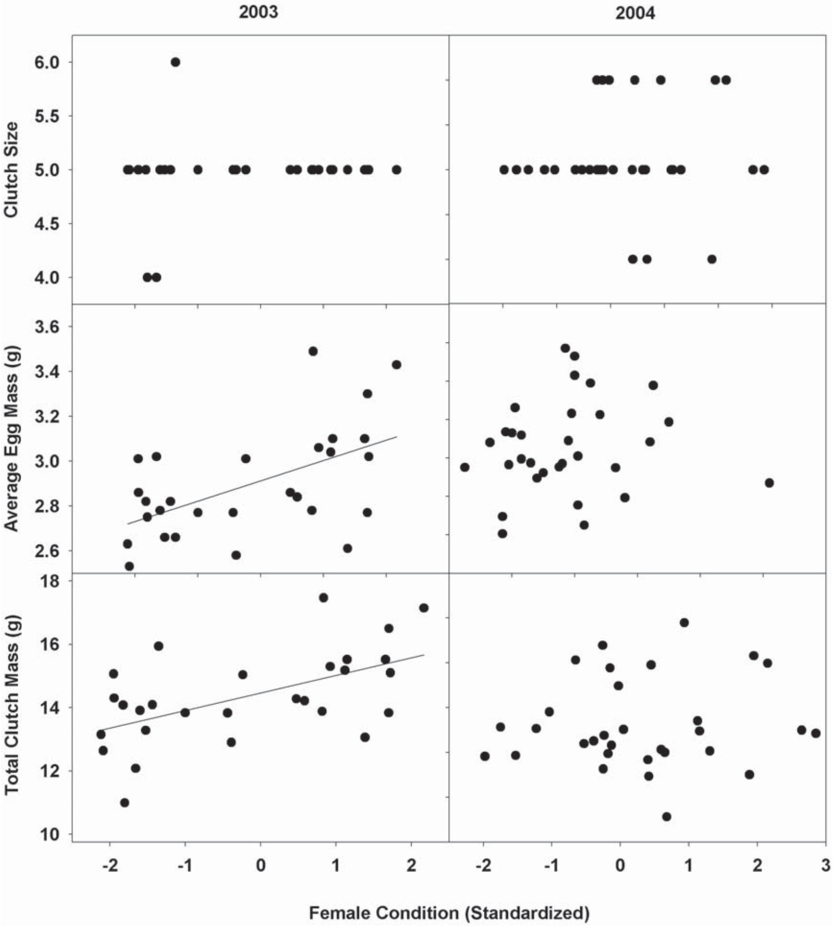

FIGURE 1. Relationship between females' body condition and (a) clutch size, (b) average egg mass, and (c) total mass of eggs in first clutches laid by Eastern Bluebirds in 2003 and 2004.

there was a trend for young females to lay heavier clutches than older females (Table 1). In 2004, we did not detect any influence of body condition or age on total clutch mass (Table 1, Fig. 1c). In 2003, we found no significant difference between first and second clutches in total clutch mass; however, in 2004, first clutches were heavier than second clutches (Tables 1 and 2, Fig. 2c). The paired analyses revealed that individual females laid first clutches significantly heavier than second clutches only in 2004 (2003: paired $t_{30} = 1.45$, $P = 0.15$; 2004: paired $t_{33} = 3.07$, $P < 0.005$). The total mass of a female's first clutch and that of her second clutch, however, were significantly correlated (paired samples correlations: 2003: $r_{30} = 0.53$, $P = 0.002$; 2004: $r_{33} = 0.56$, $P = 0.0008$).

TRADEOFFS BETWEEN CLUTCHES

In 2003, investment in the first clutch did not influence investment in the second clutch ($R^2 = 0.05$, $F_{1,28} = 1.45$, $P = 0.24$; Fig. 3); in 2004, however, we found a significant negative relationship ($R^2 = 0.19$, $F_{1,31} = 7.13$, $P = 0.01$; Fig. 3). Females that invested more resources in their first clutch (total clutch mass) invested proportionately less in their second clutch, suggesting that investment in first clutches negatively influences the amount of resources available for late-season egg production.

DISCUSSION

We found that, in the Eastern Bluebird, reproductive investment before hatching varied with a female's body condition, age, and date of laying. However, these relationships differed in the two years of the study, suggesting that the cost of reproduction is influenced by interactions between an individual's quality and environmental conditions. Our data provide evidence that heavier and younger females invest more in clutches, that clutch-investment strategies change from first to second clutches, and that the relative change in investment from first to second clutches is influenced by investment in the first clutch. In 2003, females that were heavier for their body size laid heavier eggs and invested more in total clutch mass, consistent with the hypothesis that females that have acquired more resources can allocate more resources to reproduction. We also found evidence that investment in clutches is costly to

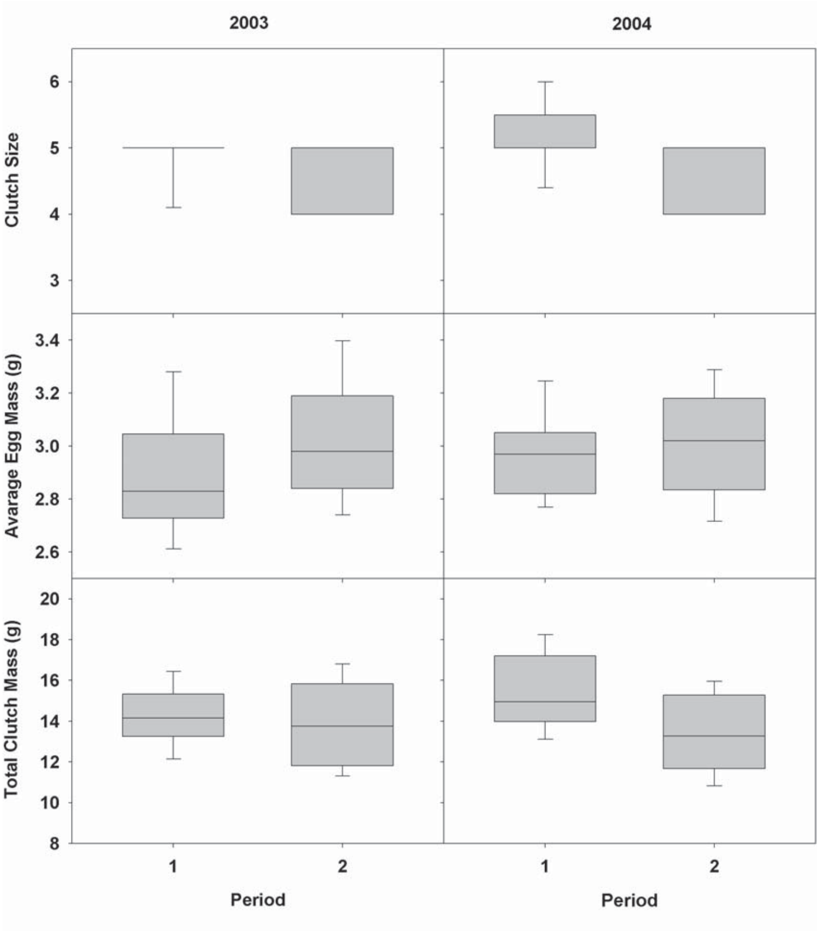

FIGURE 2. Box plots of characteristics of first and second clutches laid by female Eastern Bluebirds in 2003 and 2004. (a) Clutch size; (b) average egg mass; (c) total clutch mass. The line within each box represents the median value, the upper and lower borders of each box are the 25th and 75th percentiles, and the lower and upper bars are the 10th and 90th percentiles.

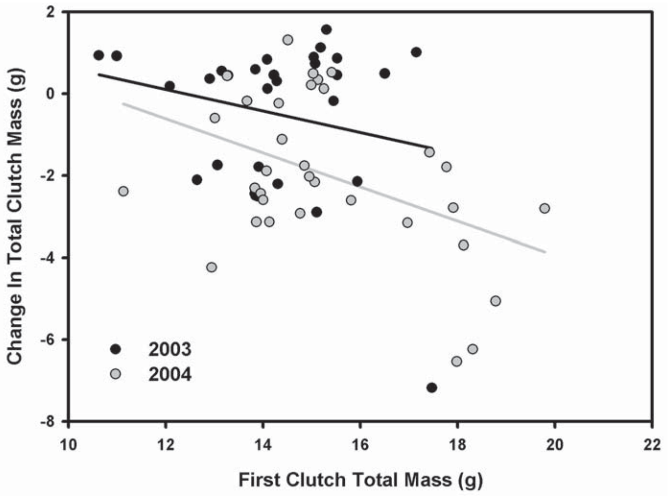

FIGURE 3. Relationship between total egg mass of first clutches and the difference in total clutch mass between second and first clutches (second minus first) of female Eastern Bluebirds in 2003 and 2004.

female Eastern Bluebirds. In 2004, females that invested relatively more in first clutches (total clutch mass) reduced their investment in second clutches more. This evidence of a cost of reproduction, however, must be interpreted with caution, as these data are correlative and not experimental. We also found that females invested differentially in eggs as the season progressed. In both years, females laid second clutches smaller than first clutches, and in 2003, females laid larger eggs in their second clutches. Finally, within a clutch we found that the size of eggs increased with the order in which they were laid, as in other species of passerines (e.g., Murphy 1994, Cichon 1997, Ardia et al. 2006, but see Slagsvold et al. 1984).

Because the associations between females' characteristics and clutch investment and seasonal changes in clutch investment in the two years changed, the relationships between environment and egg investment are clearly not simple. Unfortunately, because we did not measure habitat quality, we can only speculate about what caused these differences. Temperature and precipitation in the two years were similar (National

Climate Data Center, www.ncdc.noaa.gov). In 2003, there was little variation in the size of first clutches, so relationships between females' condition and average egg size may have been easier to detect. In 2004, the seasonal decrease in total clutch investment was driven largely by a decrease in clutch size, suggesting greater variation in habitat quality between the times of laying of first and second clutches. Moreover, a decrease in habitat quality through the breeding season of 2004 would explain the tradeoff in total clutch investment that we found within that year. Regardless of the cause, these differences by year suggest that resources available for egg production and optimal strategies of egg investment (clutch versus egg mass) likely change with environmental conditions. Perhaps differences between 2003 and 2004 should not be surprising. Annual variation in the strength and shape of age-dependent traits, for example, has been found in almost every species in which traits depend significantly on age (Martin 1995). Life-history tradeoffs depend on the interaction of selective pressure over evolutionary time and on the precise way in which physiological processes operate in each environment. Thus, in differing environments, organisms likely face and solve problems differently.

Our finding in 2003 that heavier females lay larger eggs is consistent with other studies of passerines (Styrsky et al. 2002, reviewed by Christians 2002) and with the predictions of Smith et al. (1993). Variation in levels of reproductive expenditure may result from variation in inherent individual fitness or in resource availability. Yet without an experimental manipulation of reproductive investment, it is difficult to determine whether organisms expending the most on reproduction are the individuals that can most afford to do so either because they are inherently "fitter" or because they have access to abundant resources (van Noordwijk and de Jong 1986).

In passerines, egg size is often linked to food availability (reviewed by Martin 1987). Food-supplementation experiments provide additional evidence that increased food availability during egg laying can increase egg size (Wiebe and Bortolotti 1995, Ramsay and Houston 1997). Most passerines are "income breeders," using nutrients assimilated through the day for egg production (Perrins 1996). Thus, females in better condition may forage more efficiently and invest more resources in egg production (Reynolds et al. 2003, Ardia et al. 2006). Females in better body condition may invest relatively fewer resources in body maintenance and have greater resources available for investment in clutches than females in poorer condition. Our measure of females' body condition was taken during incubation of first clutches or rearing of first broods. As we found that adults' body condition declined over the season, we used the residuals from the regression of body condition on the capture date (measured as the number of days after the initiation of egg laying) to correct for seasonal changes. Thus, body condition is a proxy for a female's condition early in the season, but we caution that investment in first clutches may have affected this estimate of body condition.

Within a clutch, we found no evidence of a tradeoff between clutch size and egg size, consistent with the majority of studies of birds (reviewed in Christians 2002). This lack of a negative relationship between egg size and clutch size means that it is intuitive to estimate females' investment in a clutch from total clutch mass. Surprisingly, researchers sometimes overlook this measure of total clutch investment and instead focus on the tradeoff between egg number and size (Martin et al. 2006). We found that individual females laid heavier first clutches than second clutches in 2004. This late-season reduction in reproductive investment may occur because females have fewer resources available or because late-season young are less likely to survive to reproduce (Boyce and Perrins 1987, Styrsky et al. 1999). Also in 2004, we found evidence consistent with a cost of reproduction; females that laid heavier first clutches laid proportionally lighter second clutches. These data suggest that investment in egg production early in the season directly influences energy available for laying second clutches. For example, captive female Zebra Finches (*Taeniopygia guttata*) that lay larger eggs in first clutches lay proportionately smaller eggs in replacement clutches (Williams 1996). It is also possible, however, that females that lay heavier early clutches also invest relatively greater resources incubation or in parental care. Indeed, in many species, including the bluebirds we studied, parental investment before hatching is positively correlated with that after hatching; examples are penguins (Reid and Boersma 1990) and gulls (Risch and Rohwer 2000; Robinson et al., unpubl. data). Thus, to test the cost of egg laying properly, researchers must use experiments to disentangle the costs of investment before and after hatching (Visser and Lessells 2001). It may be that the female bluebirds that invested relatively more in first clutches experienced greater reductions in body condition. An optimal study design would have included capturing the females repeatedly during the breeding season. Unfortunately, because we measured females' body mass only during incubation of first clutches, we cannot distinguigh between these explanations. In the Mountain Bluebird (*Sialia currucoides*; the sister species of the Eastern Bluebird; Klicka et al. 2005), however, females' body mass declines through the breeding season (Merkle and Barclay 1996), suggesting that second clutches may be more costly than first clutches.

As have studies of other multi-brooded passerines (Tinbergen 1987, Smith et al. 1989) and a previous study of the Eastern Bluebird (Pinkowski 1977), we found that the size of second clutches declined. The reduction in clutch size could be explained by tradeoffs in resource allocation. In 2003, concurrent with this reduction in clutch size, average egg mass increased from early- to late-season clutches, also consistent with a previous study of the Eastern Bluebird (Gowaty and Plissner 1998). The increase in egg size also indicates that females change egg-investment strategies over the breeding season; the evolutionary explanation may be that females invest more resources into each young to offset late-season selection pressures. The pressures of parasites and predation are higher in the later season (authors, pers. obs.) and may have selected for earlier fledging from late-season broods. In accordance with selection for earlier fledging, the incubation of late-season clutches length is shortened, and nestlings hatch more asynchronously (Gowaty and Plissner 1998). As the season progresses, females invest more in each offspring by laying fewer, heavier eggs, possibly to increase the likelihood of the offsprings' survival. In both the House Wren and Eastern Bluebird, late-season offspring benefit more from larger eggs than do their early-season siblings (Styrsky et al. 1999; Robinson et al., unpubl. data), suggesting that this strategy of clutch investment is adaptive. Thus, by increasing the mass of eggs laid later in the breeding season, bluebirds likely increase fitness of later-hatched young. The concurrent reduction of clutch size in second clutches could be a consequence of late-season selection for larger eggs and a reduction of resources available to females for egg production.

The associations we found between total egg investment and both females' body condition and past expenditure in clutches suggests that environmental variation has differing influences on clutch size and egg mass. The fact that clutch size did not appear to be correlated with variation in the female's condition is not surprising, as past experimental enlargements of broods (increasing reproductive effort) early in the season did not influence the size of the Eastern Bluebird's late-season clutches (Siefferman and Hill 2008).

In 2003, younger females laid more eggs but clutch size was not related to female condition. These results are opposite of our prediction that after-second-year females should allocate more resources to clutches than should young females. In 2003, however, egg size was associated with the female's condition but not with her age. In another population of the Eastern Bluebird, Siefferman and Hill (2005) found that older females achieved greater annual reproductive success but did not measure egg size, and this trend appeared to be driven by older females initiating breeding earlier in the season. Perhaps by laying larger clutches, younger females are able to compensate for later nest initiation and poorer parenting ability.

Overall, investment in eggs appears to be influenced by the interaction of environmental conditions and an individual's quality. Our correlative results suggest both strategy and constraint influencing reproductive investment. The sizes of second clutches decreased, and, in one year of the study, females laid larger eggs in second clutches. Later in the season, birds may be unable to allocate energy toward overall clutch production and experience selection to maximize investment in individual late-season offspring. In one year of the study, females in better body condition laid larger eggs and heavier clutches. Moreover, in another year, females that invested relatively more resources early in the season laid clutches of disproportionately reduced mass later in the season. Further experimental work is needed to examine both the proximate and ultimate determinants of how individuals allocate resources into egg production and parental care to maximize reproductive investment.

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