EFFECTS OF FOREST FRAGMENTATION ON REPRODUCTIVE EFFORT AND PRODUCTIVITY OF INDIGO BUNTINGS (*PASSERINA CYANEA*)

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DANA L. MORRIS

Dr. John Faaborg, Dissertation Supervisor

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The undersigned, appointed by the Dean of the Graduate School,
have examined the dissertation entitled.

EFFECTS OF FOREST FRAGMENTATION ON NESTING SUCCESS, BODY CONDITION, AND REPRODUCTIVE OUTPUT OF INDIGO BUNTINGS (*PASSERINA CYANEA*)

Presented by Dana Morris

A candidate for the degree of Doctor of Philosophy

And hereby certify that in their opinion it is worthy of acceptance.

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ABSTRACT

Forest fragmentation lowers reproductive success of breeding songbirds through edge effects that attract nest predators. The increased reproductive effort required to renest after predation may have physiological consequences for females that may influence their ability to invest in high quality offspring. However, relatively little is known about the physical health or body condition of breeding female passerine birds and how this translates into current and future reproductive success. Additionally, during the breeding season, migratory passerine birds must adjust to energetic demands associated with territorial defense, reproduction, and molt. How successful they are at making these adjustments may depend on the quality of breeding sites. To determine if predation-induced renesting lowers body condition and subsequently raises stress hormones, I measured body mass, baseline corticosterone, and acute corticosterone of female Indigo Buntings (*Passerina cyanea*) breeding in a fragmented and a contiguously forested landscape in Missouri. To determine if renesting or a decline in maternal condition reduces productivity and offspring quality, I measured nesting success, clutch size, nestling quality, and brood sex ratios. I studied buntings from the pre-breeding to the post-breeding period to determine if fragmentation influences the physiological condition or behavior of males and females between the pre-breeding, breeding, and post-breeding stages.

Nest success did not differ significantly between landscapes, however, the effort to produce offspring was higher in the fragmented landscape; only 34% of females in the fragmented landscape produced broods on their first attempt but 61% produced

broods on their first attempt in the contiguous forest. Renesting females had lower body condition indices (-0.505 ± 0.07) than females that were successful on their first attempt (-0.065 ± 0.08). As maternal condition declined with nesting attempt, stress hormone levels increased, suggesting poor-conditioned females lack the energetic reserves to meet increased demands. In addition, females in poor condition produced smaller clutches and poorer-conditioned nestlings than females in good condition. Brood sex ratios were female-biased in both landscapes (proportion males in unfragmented: 0.41 ± 0.05, $n = 37$ vs. fragmented: 0.37 ± 0.07, $n = 27$) and this skew seems to be due to a large proportion of nests containing all female nestlings. In the fragmented landscape, 40% (11/27) of nests contained only female nestlings, whereas 19% (7/37) of nests were all-female in the unfragmented landscape, indicating a bias in production of the smaller, less profitable sex in the fragmented landscape. Males and females exhibited different patterns of body condition and corticosterone secretion in relation to their breeding activity. Males had lower body condition and higher stress hormone levels than females during pre-breeding, presumably due to increased energetic demands associated with territory acquisition. Males and females had similar body condition during the breeding stage and stress hormone levels declined during this period, presumably as a strategy to prevent disruption of reproductive behavior. Breeding females in the fragmented landscape were in poorer condition (body condition index: -0.53 ± 0.12) than females in the unfragmented landscape (body condition index: -0.25 ± 0.07), likely due to increased breeding effort in the fragmented landscape. However, post-breeding females in the fragmented landscape recovered body condition at a higher rate than did birds in the

unfragmented landscape, suggesting post-breeding habitat in the fragments is higher quality than post-breeding habitat in the unfragmented landscape.

This study demonstrates that maternal condition and productivity decline with increased reproductive effort and late-season broods are smaller and lower in quality. Measuring the effect of female condition on nestling condition and brood sex ratios provides new insight about the quality of offspring produced under different large-scale breeding conditions. Furthermore, fragmentation influenced breeding effort and foraging opportunities that further affected patterns in physiological responses of males and females as they transitioned from the pre-breeding to the post-breeding period. This study takes our understanding of fragmentation effects further by determining the proximate effects of fragmentation and nest predation on female condition and fitness and quantifies differences in physiological condition of birds occupying fragmented and unfragmented landscapes throughout the reproductive season.

OVERVIEW

Nest predation has been identified as an important factor limiting breeding populations of Neotropical migratory birds. Edges and openings, especially in fragmented landscapes, attract a variety of predators that eat or destroy nest contents and force birds to renest or forgo further breeding attempts within a season. Predation-induced renesting likely imposes physiological constraints associated with the predation event itself, as well as increased energetic effort required to build an alternate nest and produce a new clutch of eggs. Recent studies show that elevated egg production negatively affects maternal condition and reduces the ability to rear successful offspring. This may have important consequences to females that nest in fragmented landscapes where they experience higher rates of nest predation and are forced to renest multiple times. However, little is known about the physical health of breeding female passerine birds and how fragmentation affects individual fitness because fragmentation studies have usually measured the performance of males given that they are generally easier to observe. However, the implications of a prolonged breeding season due to nest predation may be lower maternal quality, thereby impacting the ability of females to produce high quality offspring. Consequently, the negative effects of fragmentation may have the greatest impact on females, which ultimately limit populations. I describe the costs to adults and offspring of breeding in unfavorable habitats and discuss how these costs may be mediated by the degree and context of fragmentation.

In Chapter one, I measure the effects of fragmentation on maternal quality and productivity of female Indigo Buntings (*Passerina cyanea*) breeding in a fragmented and a contiguously forested landscape in Missouri. I used body mass and corticosterone, a hormone linked to energetic condition, to assess whether predation-induced renesting reduces maternal quality by lowering body condition and raising corticosterone levels. To determine if renesting or a decline in maternal condition reduces productivity and offspring quality, I measured nesting success, clutch size, and nestling quality. My results indicate that reproductive effort was higher in the fragmented landscape and this increased effort is associated with a decline in maternal quality. Furthermore, females in poor condition produced smaller clutches and poorer-conditioned nestlings than females in good condition.

In Chapter two, I collaborated with Dr. Don Dearborn of Bucknell University and Angela Anders of Pennsylvania State University to investigate whether maternal condition or environmental conditions associated with fragmentation affect maternal investment in offspring, such that females in poor condition produce more female offspring, the smaller, cheaper sex. Although brood sex ratios were not directly related to maternal condition, broods were female-biased in both the fragmented and unfragmented landscapes. However a disproportionate number of nests in the fragmented landscape contained only female offspring. This chapter also describes differences in body condition between male and female offspring and demonstrates that evaluating brood sex ratios may further indicate relationships between habitat quality and productivity.

Chapter three focuses on the broader impact of fragmentation on the physiological condition of birds throughout the reproductive season. I measured body condition and corticosterone levels of males and females occupying fragmented and unfragmented sites from the pre-breeding period through the post-breeding period. I demonstrate that males and females exhibited different patterns of body condition and corticosterone secretion in relation to their breeding activity. Additionally, I show that fragmentation positively influenced the availability of post-breeding habitat for adults and juveniles and discuss how landscape context may compensate for prolonged breeding seasons.

My findings show that while nest predation causes decreased nesting success, nesting pairs will frequently renest after the loss of a clutch or brood, eventually resulting in the successful production of offspring. However, increased breeding effort has implications to maternal quality and productivity. These results indicate that nest predation and prolonged breeding efforts impose additional costs of fragmentation to maternal condition and offspring quality that should be considered when evaluating habitat quality.

CHAPTER 1

INFLUENCE OF FOREST FRAGMENTATION AND RENESTING ON MATERNAL CONDITION AND PRODUCTIVITY OF INDIGO BUNTINGS

Dana L. Morris

Division of Biological Sciences, 110 Tucker Hall, University of Missouri, Columbia,
MO 65211 USA.

ABSTRACT

Forest fragmentation lowers reproductive success of breeding songbirds through edge effects that attract nest predators. The increased reproductive effort required to renest after predation may have physiological consequences for adults and their offspring. To determine if predation-induced renesting is associated with a decline in condition of breeding females and reduces productivity and offspring quality, I measured maternal condition (body mass and stress hormones) and reproductive output (clutch size, nestling quality, nest success) of Indigo Buntings (*Passerina cyanea*) breeding in a fragmented and contiguously forested landscape in Missouri. Overall, nest success did not differ significantly between landscapes, however, the effort to produce fledglings was higher in the fragmented landscape because only 34% of females in fragments produced broods on their first attempt whereas 61% in the contiguous forest produced broods on their first attempt. The majority of birds in both populations were single brooded (5/192 females attempted a second brood after successfully raising a first brood). Females forced to renest had lower body condition indices (-0.505 ± 0.07) than females that were successful on their first attempt (-0.065 ± 0.08). As maternal condition declined with nesting attempt, acute corticostererone levels increased. Increased acute levels in poor-conditioned females suggests they lacked the energetic reserves to meet increased energetic demands. Additionally, females in poor condition produced nestlings in poor condition. Females that nested once produced a greater proportion of 4-egg clutches while females that were forced to renest produced 2-3 egg clutches ($\chi^2 = 29.08$, $P < 0.0001$, $df = 2$). This study demonstrates that maternal condition and productivity decline with increased reproductive effort and late-season broods are smaller and lower in quality. I describe these costs, to adults and offspring, of breeding in unfavorable habitats and discuss how costs may be mediated by the degree and context of fragmentation.

INTRODUCTION

Forest fragmentation creates "edge" or early-successional habitat that some breeding songbirds require for nesting. However, edge habitat also attracts nest predators (Dijak and Thompson 2000, Chalfoun et al. 2002) that destroy nest contents and force birds to renest. When birds are successful at raising young in fragments, it is often the result of repeatedly renesting after nest predation. Predation-induced renesting prolongs the breeding season and likely imposes higher energetic demands on adults, particularly females that incur the costs of reproduction and provide the majority of offspring care in birds (Clutton-Brock 1991, Ligon 1999). Predation-induced renesting likely increases physiological demand associated with the predation event itself, in addition to the increased energetic effort required to choose an alternate nesting site, rebuild a nest, and produce a new clutch of eggs. Thus, the increased reproductive effort associated with nesting in fragmented landscapes may pose further physiological costs to females, in addition to the costs of fragmentation that have been previously documented, such as lower nesting success (Robinson et al. 1995, Porneluzi and Faaborg 1999), higher brood parasitism by Brown-headed Cowbirds (Donovan et al. 1998), and decreased pairing success of males (Gibbs and Faaborg 1990, Bayne and Hobson 2001, Villard et al. 1993).

Hypotheses concerning renesting stress have been evaluated in the context of assessing the effect of renesting on the probability of mortality and return rates to breeding sites for short-distance migrants (Haas 1998) and a long-distance migrant (Hoover 2003). However, I am aware of no study that has simultaneously evaluated the effect of predation-induced renesting stress on maternal condition and reproductive output within a breeding season. Only recently have avian studies investigated the costs of egg production and incubation on the fitness of breeding females (Monaghan et al. 1995, Monaghan and Nager 1997). Monaghan et al. (1998) demonstrated that experimentally elevating egg production negatively affects maternal condition and reduces the ability to rear successful offspring. Experimental treatments by Visser and Lessells (2001) showed that increased egg production and incubation reduces female survival and fitness costs may be dependent on environmental conditions. This may have important consequences for females that nest in poor quality habitat where they experience higher rates of nest predation and are forced to renest multiple times. Nest predation also causes the breeding period to be prolonged and this may have implications for parental and offspring fitness due to changing food supplies (Perrins 1970, Verhulst and Tinbergen 1991, Nagy and Holmes 2005). Furthermore, late-season offspring and their parents may have little time for self-maintenance, molt, and fat deposition prior to migration (Evans-Ogden and Stutchbury 1996, Svensson and Nilsson 1997), thereby influencing their survival and recruitment into breeding populations (Verboven and Visser 1998). Consequently, the deleterious effects of fragmentation may have the greatest impact on female productivity and survival; however, these factors have rarely been the focus of fragmentation studies.

Increased rates of brood parasitism associated with forest fragmentation (Robinson et al. 1995, Donovan et al. 1997, Porneluzi and Faaborg 1999) may also increase the energetic costs to breeding females. Payne and Payne (1998) detected no long-term effects of brood parasitism on future reproductive success or survival of adult Indigo Buntings (*Passerina cyanea*). However, Dearborn et al. (1998) found evidence that brood parasitism likely increases energetic demand because adult buntings provisioned nestlings in parasitized nests at a higher rate than in nonparasitized nests. Furthermore, begging calls at parasitized nests were louder and more frequent than at unparasitized nests, perhaps leading to increased risk of predation (Dearborn 1999). Females breeding in fragmented landscapes likely experience higher energy demands than those in unfragmented landscapes because of repeated nesting attempts after nest predation and from caring for much larger Brown-headed Cowbird nestlings in addition to their own offspring.

The physiological stressors associated with both renesting and brood parasitism may elicit a stress response from the adrenocortical-hypothalamic-pituitary axis resulting in secretion of stress hormones (corticosterone) from the adrenal gland (Siegel 1980, Harvey et al. 1984, Wingfield 1994). The adrenocortical response to stress has been used to evaluate the ability of wildlife to handle adverse environmental conditions (Wingfield 1988), to measure population health (Wingfield et al. 1997), and to indicate habitat quality (Marra and Holberton 1998, Millspaugh et al. 2001). In the presence of short-term fluctuations in the environment, elevated corticosterone increases the potential for survival by redirecting behavior from nonessential activities, such as reproduction, into activities that promote survival, such as foraging (Sapolsky 1987, Wingfield 1988, Astheimer et al. 1992). A robust acute stress response may allow birds in poor physiological condition to respond to unpredictable stressors more rapidly when the effects of corticosterone are most needed (Holberton and Wingfield 2003). The adrenocortical response to stress may also serve to prepare an organism for predictable stressors (Sapolsky et al. 2000, Romero 2002). If an organism can predict exposure to stressful stimuli such as severe weather, competition for territories and mates, and nest predation, then increases in the frequency of adverse events may result in higher basal glucocorticoid concentrations. However, if the stress response is repeatedly activated over the short term, elevated basal levels of glucocorticoids may result in disruption of reproductive function (Wingfield 1988, Wingfield et al. 1998), suppression of the immune system (Harvey et al. 1984), protein loss (Holmes and Phillips 1976) and neuronal cell death (Sapolsky 1992).

To determine if predation-induced renesting influences the condition of breeding females and subsequently results in a decline in productivity and offspring quality, I measured multiple indices of maternal condition (body mass, stress hormones, and hematocrit) and reproductive output (clutch size, nestling quality, nest success) of Indigo

Buntings breeding in a fragmented and a contiguously-forested landscape and in permanent and temporary edge types in Missouri. We expected predation pressure to be higher in the fragmented landscapes and in permanent edge types, where human disturbance is likely greatest. I determine if predation-induced renesting elicits a decline in maternal condition and an increase in maternal stress hormones. I compare the condition and productivity of females breeding in fragmented and unfragmented forests and attempt to increase our understanding of the impacts of fragmentation by examining the costs of nest predation on maternal condition and annual productivity.

METHODS

Study Sites and Study Species

I studied breeding populations of Indigo Buntings in two landscapes (unfragmented and fragmented) and two "edge" types (temporary and permanent). In the unfragmented landscape in southeast Missouri, I studied Indigo Buntings during three breeding seasons (2000-2002) at three sites in the Current River Conservation Area (lat: 37.19^{o}; long: 91.00 ^{o}) within approximately 40,000 ha of contiguous oak-hickory forest in Reynolds and Shannon counties (Fig. 1). In the fragmented landscape in central Missouri, I studied buntings during one breeding season (2003) at two sites: Davisdale (1125 ha; lat: 39.00 ^{o}; long: 92.62 ^{o}) and Rudolf Bennit (1460 ha; lat.: 39.25^{o}; long: 92.45^{o}) Conservation Areas in Howard, Boone, and Randolph counties, where the landscape is characterized by 30% forest cover (Fig. 1). Each site is owned and managed by the Missouri Department of Conservation and within each landscape, sites were separated by > 5 km. Temporary edge habitat within the forest interior consisted of 4-6 year-old clearcuts (3-13 ha) and group-selection cuts (21-43 m diameter) allowed to regenerate (see Brookshire and Shifley 1997 for a more complete description of sites in the contiguous forest). Permanent edge included forest-interior roadsides (>20 m wide) and wildlife food plots (0.25-2 ha) maintained by continued disturbance such as mowing and disking. Each site contained multiple temporary and permanent openings.

Indigo Buntings are Neotropical migratory birds that nest in early successional habitat throughout the eastern U.S. from April-August and overwinter throughout Central America and northern reaches of South America (Payne 1992). Their open-cup nests are generally placed 0.5 – 2 m above the ground in small shrubs such as blackberry (*Rubus allegheniensis*) and coralberry (*Symphoricarpos orbiculatus*), and in small saplings of oak (*Quecus spp*.) and hickory (*Carya spp*.) (Payne 1992, D.M. pers. obs.).

Reproductive Measures

At each site my field assistants and I mapped territories, searched for nests, and monitored Indigo Bunting territories and nests every 1-3 days throughout the season (late April-August) to quantify clutch size, number of nesting attempts, nestling quality, nest success, and number of fledglings produced per territory. Arrival to breeding territories and nest initiation began two weeks earlier in southeast Missouri than in central Missouri. We found no evidence to indicate females delay their first nesting attempt after arrival. We attempted to follow individual nest attempts for each female, but we could not find renests for all. To increase sample size, I divided nesting attempts into two categories (first, renest) based on the average date of the first fledglings of the season and the time to renest after predation. Nests initiated after May 29 in the contiguous forest or June 9 in the fragments were considered renests. Females typically spend 2-8 days building nests, 3-4 days laying, 12 days incubating, and about 10 days feeding nestlings before fledging (Payne 1992; per. obs., D.M.).

To characterize predation pressure between landscapes, daily mortality rates for nests in each landscape were calculated using the midpoint method (Manolis et al. 2000) for nests that reached the egg-laying stage and nest success was computed as daily survival (1-daily mortality) raised to the exponent of the number of days in the nesting cycle (Mayfield 1975), which is 26 days for Indigo Buntings. We assumed constant predation pressure across the entire nest stage. Nest fate was determined by sighting banded (or unbanded) parents feeding fledglings or sighting banded fledglings near the nest on day 10-11, along with examining the condition of the nest (i.e. fecal sacs on outside rim of nest). I was interested in determining rates of nest predation; thus, nests that had unknown fates were not included in my estimates of nest success. Additionally, I quantified proportion of territories that produced offspring and the proportion of females that were successful on their first nesting attempt vs. those that were forced to renest in each landscape.

Physiological Measures

To determine if landscape, edge, or renesting affect physiological condition, I measured body mass corrected for structural size, corticosterone levels, and hematocrit of adults when their nestlings were 5-7 days old. Adults were captured at their nests using 6 m mist nets placed within 1-3 m of the nest. To avoid the effects of disturbing the female, I set the nets up the day prior to capture so the parents could acclimate to the presence of the nets. Presumably, any corticosterone secretion elicited by this disturbance would be physiologically cleared by the following day (Wingfield et al.

1992). On the morning of capture, to avoid detection by the female, I opened the mist net during her foraging trips and waited for her to enter the net while returning to the nest with food. Males were caught incidentally or with a song playback after blood samples were taken from females. I collected blood (50 µL) from the brachial vein, using a 26 g needle, into heparinized microcapillary tubes within 1-5 minutes of capture. I used this initial sample as a measure of baseline corticosterone level. Birds were held in cloth bags and a subsequent sample collected at 30 minutes post-capture provided a profile of the acute stress response to capture and handling (Wingfield et al. 1992, Wingfield 1994). Blood samples for corticosterone were kept on ice until centrifuged in a microhematocrit centrifuge (10 minutes) within 2-5 hours of collection. Plasma was drawn off using a Hamilton syringe and stored at -20 ^{0}C until assayed. Hematocrit (packed red blood cells/total volume) was measured on each baseline sample using calipers before plasma was collected.

To determine maximal corticosterone secretion, in my first season, I bled four birds at 1-5 (T1), 30 (T2), and 60 (T3) minutes post-capture. Corticosterone increased up to 30 minutes but the rate of increase was slower after 30 minutes (T1, 60.18 ng/mL ± 14.4; T2, 87.32 ± 11.3; T3, 100.89 ± 32.1). This gave me confidence that I was detecting near-maximal hormone levels after 30 minutes and I could prevent additional stress to individuals by processing and releasing them in less than one hour.

After blood samples were taken, adults and nestlings were aged, sexed, measured (unflattened wing chord; tarsus, to 0.1mm; and fat score), weighed (nearest 0.5 g), banded with colored plastic and numbered USFWS bands, and returned to the nest. All breeding females that were captured during this study had fat scores of zero (n = 177).

To determine body mass corrected for structural size, I entered wing and tarsus length into a principal components analysis. Body weight was then regressed against PC1 and the residuals from this analysis are used as estimates of mass corrected for body size (Green 2001) and presented as an index of body condition. Individuals falling above the expected values are considered heavy, given their size, and those falling below expected values are lean, given their size. All blood samples and body measurements were taken between 0600 – 1000 hours to prevent bias in measurements due to daily fluctuations in body mass and corticosterone. Blood samples and handling were conducted under federal and state permits and were approved by the University of Missouri Animal Care and Use Committee.

Hormone Assays

I assayed blood samples for corticosterone in duplicate using a commercially available I^{125} radioimmunoassay (RIA) kit (Biomedicals, Costa Mesa, CA). I followed the manufacturer's method, except I halved the volume of all reagents and samples were diluted 1:50 with steroid dilutent prior to assay. Parallelism and recovery of exogenous corticosterone validation assays were conducted to validate the utility, accuracy, and precision of this particular RIA kit for use with a suite of passerine birds (Washburn et al. 2002). I ran separate assays for each year of the study (2000-2003) and interassay variation was 12.5%, 3.7%, 8.9%, and 15% respectively. Additionally, I ran a control from the RIA kit in each assay and had an interassay variation across all years and assays of 6.9%. Intra-assay variation calculated from 60 randomly chosen samples across all assays was 3.9%.

Data Analysis

I fitted general linear models with maternal body mass, baseline corticosterone, acute corticosterone, and hematocrit, and nestling body mass as dependent variables and used Akaike's Information Criterion for small sample sizes (AIC_c) to select optimal statistical models for inference. Models were parameterized with environmental variables including nesting attempt (first vs. renest), landscape, edge type, and brood parasitism (y/n), and maternal age (second year, after second year). In the models for maternal hormone levels, hematocrit, and nestling body mass, I included maternal mass as a covariate (Tables 1-4). Sample sizes were not large enough for all combinations of landscape and edge so I removed edge from the models for maternal hormone levels and hematocrit. Baseline and acute corticosterone levels were log-transformed to correct normality. I present parameter estimates from only the best models (or model-averaged over a confidence set of models where Akaike weights were ≥ 0.90) with minimal AIC_c scores and highest Akaike weights, thereby balancing parsimony and goodness of fit to the data (Burnham and Anderson 2002). Goodness of fit for each of the model sets was assessed with likelihood ratio goodness of fit tests comparing the difference between the -2 Log-likelihood value and the degrees of freedom of the global model (full model) and a null model containing the intercept only.

Initial analysis revealed no effect of *site* on maternal ($F_{4,176} = 1.55$, $P = 0.19$) or nestling ($F_{4,149} = 1.3$, $P = 0.27$) corrected mass so data were pooled across sites within landscape types. The effect of *year* within the contiguous landscape was not significant for maternal ($F_{2,130} = 0.12$, $P = 0.88$), acute corticosterone ($F_{2,91} = 0.80$, $P = 0.45$) or nestling ($F_{2,149} = 1.06$, $P = 0.35$) condition. Year had some effect on baseline corticosterone ($F_{2, 93} = 4.66$, $P = 0.01$), however, 95% confidence intervals overlap [2000: 3.95 ng/mL^{-1} (3.1 – 3.5); 2001: 2.9 ng/mL^{-1} (2.7 – 3.1); 2002: 3.4 ng/mL^{-1} (3.1 – 3.6)]. Thus, additionally because the fragmented sites were sampled only in 2003, I did not include *year* in the candidate models.

I used nestling condition averaged over all nestlings within a brood to account for the variance within a brood while keeping the nest or individual female as the sampling unit. Females produced nestlings that vary widely in condition such that the smallest nestling within a brood is much leaner (-0.44 ± 0.05, $n = 160$) than the largest (0.33 ± 0.05, $n = 178$). To characterize this variation further, I used a log-linear model using CATMOD in SAS (SAS Inst. 2004) to determine if lean or fat mothers produce the same proportion of lean and fat nestlings.

I observed clutch sizes of 2, 3, and 4 eggs and therefore, used separate log-linear models (2 x 3 contingency tables) to assess relationships between clutch size and landscape, nesting attempt, age, and female condition (heavy, lean). I used Bonferroni corrections for multiple tests on clutch size and rejected null hypotheses if the critical values for individual tests was $\leq \alpha/\eta$ or 0.05/4 (0.0125). I also used a log-linear model to test whether the proportion of inviable eggs differed between the fragmented and unfragmented landscape. Data are presented as adjusted means (least square) ± standard error and/or 95% confidence intervals.

RESULTS

Reproductive Effort

These populations of Indigo Buntings are largely single-brooded (2.6% or 5/192 females in the unfragmented landscape attempted >1 brood). Brood parasitism was relatively low with 11.2% (35/311) of nests parasitized in the unfragmented forest and 21.1% (22/104) in the fragmented forests. Nest success was low in both landscapes and did not vary significantly with landscape or edge type (Table 5). The proportion of successful nests was higher in the unfragmented landscape (46.8%, 175/374) compared to the fragmented landscape (31.6%, 32/101); however, the proportion of territories that eventually produced fledglings differed to a lesser degree (45.1% in unfragmented *v.* 38.8% in fragmented). Additionally, the effort to produce offspring was greater in the fragments given that only 34% (25/103) of females raised a brood early in the season, whereas 61% (217/353) of females fledged young early in the unfragmented landscape. As a result, most birds finished breeding by early July in the unfragmented landscape while the majority of females in the fragmented landscape continued attempts to produce one successful brood into August.

Maternal Condition

The best model to explain maternal body condition contained *nesting attempt* only (Table 6). Females that renested one or more times after nest predation were in poorer condition than females that nested once successfully (Fig.2A). There was little difference in body condition indices between females in the unfragmented forest (-0.05 ± 0.05) and the fragmented forest (-0.09 ± 0.07). Further *post-hoc* analysis indicates that declining maternal condition is due to renesting, rather than seasonal declines. Females caught on the early in the season while provisioning nestlings had lower body condition than females provisioning fledglings on the same day (provisioning nestlings: 0.04 ± 0.06, *n* = 3; provisioning fledglings: 0.59 ± 0.18, *n* = 3). Additionally, renesting females and post-breeding females caught during the same week in mid-August in the fragmented sites show a dramatic difference in body condition (breeding: -0.26 ± 0.14, *n* = 7; post-breeding: 1.01 ± 0.14, *n* = 33).

The best model explaining baseline corticosterone contained maternal condition and acute corticosterone with an Akaike weight of 0.97 (Table 7). Baseline corticosterone was positively related to acute levels of corticosterone (parameter estimate: 0.59 ± 0.10; 95% CI: 0.39 – 0.79). The effect of maternal condition on baseline corticosterone was small, given the parameter estimate includes zero (parameter estimate: 0.02 ± 0.06; 95% CI: -0.10 – 0.14).

Maternal condition and baseline corticosterone were the most important parameters influencing acute corticosterone levels (Table 8). Acute levels of corticosterone were positively related to baseline levels (parameter estimate: 0.33 ± 0.06; 95% CI: 0.21 – 0.45). Acute corticosterone levels were negatively related to maternal condition (parameter estimate: -0.12 ± 0.04; 95% CI: -0.20 – 0.04; Fig. 3).

The best model explaining maternal hematocrit was *landscape*, but with an Akaike weight of just 0.67, there was some degree of model selection uncertainty (Table 9). Therefore, I present model-averaged estimates for two models: *landscape* and *landscape and maternal mass*. Hematocrit was lower in the fragmented landscape (parameter estimate: 0.47 ± 0.009; 95% C.I. = 0.45-.48) than in the unfragmented landscape (parameter estimate: 0.53 ± 0.005; 95% C.I. = 0.517-.537), indicating possible nutritional deficiency in birds in the fragments. However, the effect of maternal mass on hematocrit was negligible since the parameter estimate was very low and the 95% confidence interval includes zero (parameter estimate: 0.006 ± 0.004; 95% C.I. = -0.002-0.014).

Reproductive Output

I found a greater proportion of inviable eggs in nests in the fragmented landscape (30.4%, 14/46) than in the unfragmented landscape (15.7%, 22/140; $\chi^2 = 4.47$, $P = 0.03$, $df = 1$). The proportion of nests with 2, 3, and 4 egg clutches differed between landscape ($\chi^2 = 26.45$, $P = 0.0001$, $df = 2$; Fig. 4a) and nest attempt ($\chi^2 = 29.08$, $P < 0.0001$, $df = 2$; Fig. 4b). The proportion of nests with 2, 3, and 4 egg clutches also varied with age (ASY: 7.0%, 45.2%, and 47.6% respectively; SY: 20.5%, 57.8%, 21.7%; $\chi^2 = 15.19$, $P = 0.0005$, $df = 2$). Clutch size tended to vary with maternal condition ($\chi^2 = 5.50$, $P = 0.06$, $df = 2$) with heavy females producing a larger proportion of 4-egg clutches (2-egg,12%; 3-egg, 40%; 4-egg, 48%) and lean females producing a larger proportion of 3-egg clutches (2-egg, 14.5%; 3-egg, 56.4%, 4-egg, 29.1%).

Maternal mass was the most important predictor of nestling quality (Table 10) with females in good condition producing nestlings in good condition (Fig. 5). Overall, 55% of nestlings from heavy females were heavy, given their structural size, but only 45% of nestlings from lean females were heavy ($\chi^2 = 3.99$, $P = 0.04$, $df = 1$; Fig. 6). Additionally, the model with *maternal mass*, *nesting attempt*, and *parasitism* received a small amount of support. Nestlings tended to be smaller in parasitized nests (-0.410 ± 0.22; 95% C.I. = -0.858-0.037) than in unparasitized nests (-0.056 ± 0.05; 95% C.I. = -0.050-0.162), and there was a trend for nestling mass to decline with nesting attempt (first: -0.065 ± 0.13; late: -0.289 ± 0.13).

DISCUSSION

These findings demonstrate that while nest predation causes decreased nesting success, Indigo Bunting pairs will repeatedly renest after the loss of a clutch or brood. Although nest success tended to be lower in the fragmented landscape, the percent of pairs raising young in both landscapes was similar, demonstrating the importance of renesting on season-long productivity of individuals. Renesting increases the chances of successfully producing offspring, however females must incur the costs of this additional effort. Maternal body condition was lower for females that renested one or more times after nest failure. Additionally, acute corticosterone levels were higher for females in poor condition, indicating a physiological cost to this prolonged breeding effort. Females often renested within 3-5 days after nest failure and may not have the opportunity to regain condition lost while incubating or brooding her previous clutch. Maternal condition tended to be lower in fragmented than contiguous forest, although the difference was not great. Perhaps because both landscapes had relatively high rates of nest predation, differences in maternal condition may not be distinct. Or, perhaps local nesting conditions that I did not measure, such as food availability or vegetation structure, have more influence on maternal breeding condition than large-scale effects of landscape type. However, hematocrit was lower among females in the fragmented landscape than in the unfragmented landscape, suggesting that birds in the fragments were nutritionally stressed.

Prolonged breeding effort in both landscapes was associated with a decline in reproductive output. Clutch size has been shown to decline seasonally in relation to timing of breeding (Nilsson 1994, Norris 1993, Verhulst et al. 1995), food supply (Daan et al. 1989), and maternal condition (Verhulst et al. 1995, Christians et al. 2001). Alternatively, adjustment of clutch size may be a strategic tradeoff between available resources and prospects for late-season offspring (Winkler and Allen 1996). I found that clutch size was larger in the unfragmented forest where nest success was slightly higher and females were generally more successful on their first nesting attempt. Similarly, Suarez et al. (1997) found that Indigo Buntings nesting in exterior openings (<50 m from forest edge) laid smaller clutches and experienced higher nest predation than those nesting in interior openings (>50 m from forest edge), indicating that nesting females may be unwilling to risk losing larger clutches to nest predation near edges.

Nestling quality was most strongly associated with maternal condition and lean females tend to produce a greater proportion of lean nestlings. Although the condition within broods varied widely, heavy females produce more nestlings in good condition than lean females. I expected to see a stronger effect of renesting on nestling quality. However, as females adjusted clutch size with renesting attempts, nestlings raised in smaller broods later in the season were of similar quality as those of earlier larger broods. Because females lose mass with increased nesting attempts, I expected to see an interaction between maternal mass and nesting attempt in the top models for nestling condition. However, if females adjust clutch sizes with nest attempt in order to maintain nestling quality, then smaller clutches may contain few, but high quality young. Fledging poor quality offspring late in the season has been associated with lower post-fledging survival (Hochachka 1991) and lower recruitment into breeding populations (Verboven and Visser 1998). Whether these adjustments in clutch size and investment in offspring are due to seasonal behavioral shifts, bet-hedging, or physiological constraints is unclear without further experimental evidence.

Surprisingly, I found no effect of brood parasitism on maternal condition, even though female Indigo Buntings have been shown to provision nestlings at a higher rate in parasitized nests (Dearborn et al. 1998). However, nestlings in parasitized nests were leaner than those in unparasitized nests; a finding in agreement with several studies showing decreased growth rates when host nestlings must compete with cowbird nestlings (Dearborn et al. 1998, Lichtestein and Sealy 1998, reviewed by Lorenzana and Sealy 1999).

Maternal mass was negatively related to acute corticosterone levels. As maternal mass declines with increasing nest attempts, a more robust response to capture stress may be necessary because females have lower energy reserves to compensate for the physiological disturbance of capture. Furthermore, the finding that acute corticosterone is positively related to baseline corticosterone indicates that birds with higher basal levels may lack energy reserves and thus, require higher acute effects of corticosterone during handling stress. The acute stress response has been described as an adaptive strategy to deal with energetic challenges in unpredictable environments where food shortages or inclement weather interfere with physiological maintenance or homeostasis (see Wingfield 1994). In addition, birds seem to be able to modulate the response in relation to seasonal changes in life-history strategies (Romero and Wingfield 1998, Romero 2002). For Indigo Buntings, I suggest the breeding period may be a costly life-history stage due to the unpredictable, but constant, effects of nest predation and cowbird parasitism rather than food availability or weather.

As the opportunity to breed decreases due to frequent renesting, individuals may be able to modulate the adrenocortical stress response to maximize breeding success (Wingfield et al. 1995, O'Reilly and Wingfield 2001). Baseline corticosterone varied less than expected between early-nesting or renesting females. Surprisingly, I saw no difference in baseline or acute corticosterone levels between landscapes. Perhaps because birds in both landscapes suffered high daily nest mortality rates, they are functioning in energetically demanding states but with baseline levels that maintain reproductive function over a prolonged period of time. Chronic stressors such as constant predation pressure or increased physiological activity may actually lead to reduced corticosterone levels through a negative feedback system that controls endogenous corticosterone (Siegel 1980, Harvey et al. 1984). Birds attempting to breed very late in the season in the fragmented forests showed dampened acute corticosterone levels (40.6 ± 6.0, $n = 6$) which were lower than for post-breeding females (which should show a decline in adrenocortical stress response) caught at the same time in mid-late August (57.5 ± 7.9, $n = 16$). However, these late breeders were still very lean (-0.26 ± 0.14, $n = 7$) and produced lean broods (average nestling: -0.373 ± 0.22, $n = 6$). Three of these late-breeding females were abandoned by their mates shortly after egg-laying, leaving them to complete their breeding attempts without paternal help. These late-breeders may be experiencing suppression of sensitivity of the hypothalamic-pituitary-adrenal axis due to seasonal changes in adrenal sensitivity (Romero and Wingfield 1998) or availability of corticosteroid-binding proteins (Deviche et al. 2001) or receptors (Breuner and Orchinik 2001) that bind corticosterone and regulate the availability of corticosterone to target tissues.

Changes in maternal body condition appear to be the result of prolonged breeding and not of seasonal declines since post-breeding females were captured in better condition, sometimes within days of fledging young (Morris 2005). Additionally, renesting females caught during mid-August were in poorer condition than post-breeding females caught during the same week. Furthermore, birds in the fragmented sites tend to recover body mass more quickly during the post-breeding period (Morris 2005), suggesting that food resources are plentiful late in the season, but prolonged breeding effort prevents females from maintaining body mass throughout the season. The extensive matrix of early-successional habitat, like agricultural fields and wildlife foodplots, which fragment mid-Missouri forests may actually provide better foraging opportunities for post-breeding and dispersing birds.

This study shows that while nest predation causes decreased nesting success, nesting pairs will frequently renest after the loss of a clutch or brood, eventually resulting in the successful production of offspring. Studies that compare reproductive success based on rates of nest predation without considering season-long productivity may underestimate actual productivity. However, predation-induced renesting elicits a decline in maternal condition of female Indigo Buntings and this decline is associated with lower clutch sizes and lower offspring quality. The post-breeding period may be very important for self-maintenance, fat deposition, and molt prior to migration (Evans-Ogden and Stutchbury 1996, Svensson and Nilsson 1997, Pagen et al. 2000, White et al. 2005) but the amount of time that birds have available during this period is limited by their breeding effort and surrounding landscape characteristics. Perhaps the costs of prolonged breeding may be ameliorated in sites, such as the fragmented landscape, where second-growth habitat and a surrounding matrix of agriculture provide greater post-breeding foraging opportunities and better protection from predators (Anders et al. 1998, Vega Rivera et al. 1998, Fink 2003).

Increasing fragmentation can be expected to lead to declines in populations by reducing fecundity. These results indicate that nest predation and prolonged breeding efforts impose additional costs to maternal condition and offspring quality that should be considered when evaluating habitat quality. Whether these effects have long-term consequences to survival is unknown, but a delay in migration or arrival to wintering sites in poor condition may reduce access to high quality wintering habitat (Marra and Holberton 1998) and influence timing of arrival to breeding sites the following year (Marra et al. 1998, Norris et al. 2004). However, although the wintering ecology of Indigo Buntings has not been studied in detail, wintering birds are known to show site fidelity to sites with second-growth habitat, but they are not territorial (Payne 1992). This behavior contrasts markedly from the highly territorial behavior of wintering species of warblers that have been studied to date. Such non-territorial behavior may reduce the costs of late arrival on the wintering grounds for Indigo Buntings.

This study provides a comprehensive evaluation of the interactions between habitat quality, reproductive success, and physiology that has rarely been considered in fragmentation studies. As habitat becomes increasingly fragmented and migratory birds are forced to compete for limited amounts of high quality habitat, it will be critical to understand how variations in habitat quality affect a bird’s ability to survive and raise offspring in order to manage habitat for diverse requirements and life-history stages.

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Table 1. Candidate set of models used to evaluate effects of environmental (landscape, edge, nest attempt, and brood parasitism – BHCO) and biological factors (maternal age) on maternal body condition of Indigo Buntings breeding in Missouri (2000-2003).

Model	**Model Structure**
Global	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Edge) + β_4 (BHCO) + β_5 (Age)
Environmental Main Effects	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Edge) + β_4 (BHCO)
Reduced Environmental Main Effects	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Edge)
Attempt and Landscape	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape)
Attempt and Edge	$\beta_0 + \beta_1$ (Attempt) + β_2 (Edge)
Maternal Age and Attempt	$\beta_0 + \beta_1$ (Age) + β_2 (Attempt)
Maternal Age and Parasitism	$\beta_0 + \beta_1$ (Age) + β_2 (BHCO)
Attempt	$\beta_0 + \beta_1$ (Attempt)
Landscape	$\beta_0 + \beta_1$ (Landscape)
Null	β_0

[a] Asterisk indicates two-way interaction.

Table 2. Candidate model set for environmental factors (landscape, nest attempt), and maternal factors (age, corrected mass) affecting maternal baseline corticosterone and acute corticosterone of breeding female Indigo Buntings (2000-2003) in a fragmented and unfragmented landscape in central and southeast, Missouri, respectively. Within models, sign (+/-) indicates predicted direction of effect.

Model	**Model Structure**
Global	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (BHCO)[a] + β_4 (Age) – β_5 (Mass) + β_6 (LogCort2)[b] + β_7 (Attempt*Mass)[c] + β_8 (LogCort2*Mass)
Environmental Effects	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (BHCO)
Reduced Environmental Effects	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape)
Environmental and Maternal Interactions	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Mass) + β_4 (Attempt*Mass)
Environmental Effects and Mass	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Mass)
Maternal Effects	$\beta_0 + \beta_1$ (Age) - β_2 (Mass) + β_3 (LogCort2)
Reduced Maternal Effects	$\beta_0 - \beta_1$ (Mass) + β_2 (LogCort2)
Nesting Attempt and Mass	$\beta_0 + \beta_1$ (Attempt) - β_2 (Mass)
Landscape and Mass	$\beta_0 + \beta_1$ (Landscape) - β_3 (Mass)
Age and Mass	$\beta_0 + \beta_1$ (Age) - β_2 (Mass)
Maternal Mass	$\beta_0 + \beta_1$ (Mass)
Nesting Attempt	$\beta_0 + \beta_1$ (Attempt)
Landscape	$\beta_0 + \beta_1$ (Landscape)
Null	β_0

[a] BHCO indicates brood parasitism.

[b] LogCort2 indicates the log of acute corticosterone used in models predicting baseline corticosterone; LogCort1 was used as a parameter in the models for acute corticosterone.

[c] Asterisk indicates two-way interaction.

Table 3. Candidate model set for environmental factors (landscape, edge, nest attempt, brood parasitism), and maternal factors (age, corrected mass) affecting hematocrit of breeding female Indigo Buntings (2000-2003) in a fragmented and unfragmented landscape in central and southeast, Missouri, respectively. Within models, sign (+/-) indicates predicted direction of effect.

Model	**Model Structure**
Global	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Edge) + β_4 (BHCO) + β_5 (Age) + β_6 (Mass) + β_7 (Attempt*Mass)[a] + β_8 (Landscape*Mass) + β_9 (Mass*Age)
Environmental Effects	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Edge) + β_4 (BHCO)
Reduced Environmental Effects	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Edge)
Environmental and Maternal Interactions	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Mass) + β_4 (Attempt*Mass)
Environmental Effects and Mass	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Mass)
Nesting Attempt and Landscape	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape)
Nesting Attempt and Edge	$\beta_0 + \beta_1$ (Attempt) + β_2 (Edge)
Nesting Attempt and Mass	$\beta_0 + \beta_1$ (Attempt) + β_2 (Mass)
Landscape and Mass	$\beta_0 + \beta_1$ (Landscape) + β_3 (Mass)
Maternal Effects	$\beta_0 + \beta_1$ (Mass) + β_2 (Age)
Maternal Mass	$\beta_0 + \beta_1$ (Mass)
Nesting Attempt	$\beta_0 + \beta_1$ (Attempt)
Landscape	$\beta_0 + \beta_1$ (Landscape)
Null	β_0

[a] Asterisk indicates two-way interaction

Table 4. Candidate model set for factors (landscape, edge, nest attempt, parasitism – BHCO, maternal age, maternal mass) affecting body condition of Indigo Bunting nestlings in a fragmented and unfragmented landscape in central and southeast, Missouri, respectively from 2000-2003. Within models, sign (+/-) indicates predicted direction of effect.

Model	**Model Structure**
Global	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Edge) + β_4 (BHCO) + β_5 (Age) + β_6 (Mass)
Environmental Effects w/ Interactions	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Edge) + β_4 (BHCO)
Environmental Effects w/o Interactions	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Edge) + β_4 (BHCO)
Reduced Environmental and Maternal Effects	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Mass) + β_4 (Age)
Reduced Environmental and Mass	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Mass)
Attempt and Landscape	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape)
Nesting Attempt, Parasitism, and Mass	$\beta_0 + \beta_1$ (Attempt) + β_2 (BHCO) + β_3 (Mass)
Mass and Landscape	$\beta_0 + \beta_1$ (Mass) + β_2 (Landscape)
Mass and Nesting Attempt	$\beta_0 + \beta_1$ (Mass) + β_2 (Attempt)
Mass and Parasitism	$\beta_0 + \beta_1$ (Mass) + β_2 (BHCO)
Maternal Effects	$\beta_0 + \beta_1$ (Mass) + β_2 (Age)
Maternal Mass	$\beta_0 + \beta_1$ (Mass)
Attempt	$\beta_0 + \beta_1$ (Attempt)
Null	β_0

[a] Asterisk indicates two-way interaction.

Table 5. Nesting success and productivity (± 95% CI) of Indigo Buntings in a fragmented landscape (2003) and unfragmented landscape (2000-2002) in central and southeast Missouri, respectively. Nest success was computed as daily survival (1-daily mortality) raised to the exponent of the number of days in the nesting cycle (26). Territory success was calculated as the percent of territories fledging at least one host offspring. Productivity was computed as the mean number of fledglings per territory.

Environmental Variable	**Observation Days**	**No. Nests Success/Total**	**Nest Success**	**Territory Success**	**Productivity**
Landscape					
Fragmented	1195	32/101 (.316)	0.17 (0.007-0.32)	38.8	0.98 (0.55-1.41)
Contiguous	3703	175/374 (.468)	0.26 (0.19-0.32)	45.1	1.27 (0.97-1.56)
Edge Type					
Permanent	2834	106/270 (.392)	0.19 (0.12-0.25)	41.6	1.29 (0.90-1.68)
Temporary	2064	101/205 (.493)	0.31 (0.21-0.41)	47.5	1.15 (0.77-1.53)

Table 6. Model selection results for maternal body mass. Models are ranked in ascending order by Akaike's Information Criterion adjusted for small samples (AIC$_c$). The maximum likelihood value (multiplied by -2), the number of parameters (k), AIC$_c$ value, difference between the best model and subsequent models (ΔAIC$_c$), and Akaike weights (w_i) are given for each model. The global model fits the data ($\chi^2 = 20.3$, $df = 10$, $P < 0.05$, $n = 174$).

Model	**(-2Loglike)**	***k***	**AIC$_c$**	**ΔAIC$_c$**	**w_i**
Attempt	388.0	3	400.1	0.0	0.77
Attempt, Edge	383.6	5	404.0	3.8	0.12
Null	401.6	1	405.6	5.5	0.05
Attempt, Landscape	386.5	5	406.9	6.7	0.03
Attempt, Age	387.8	5	408.2	8.0	0.01
Landscape	396.3	3	408.4	8.3	0.01
Attempt, Landscape, Edge	381.5	7	410.2	10.0	0.01
Age	400.9	3	413.0	12.9	0.00
BHCO	401.6	3	413.7	13.6	0.00
Age, BHCO	381.5	9	418.6	18.4	0.00
Landscape, Attempt, Edge, BHCO,	400.9	5	421.3	21.1	0.00
Global	381.3	11	426.9	26.8	0.00

Table 7. Model selection results for maternal baseline corticosterone. Models are ranked in ascending order by Akaike's Information Criterion adjusted for small samples (AIC_c). The maximum likelihood value (multiplied by -2), the number of parameters (k), AIC_c value, difference between the best model and subsequent models (ΔAIC_c), and Akaike weights (w_i) are given for each model. The global model fits the data ($\chi^2 = 41.2$, $df = 13$, $P < 0.001$, $n = 124$).

Model	**(-2Loglike)**	***k***	**AIC_c**	**ΔAIC_c**	**w_i**
Mass, LogCort2	196.0	3	208.2	0.0	0.97
Mass, LogCort2, Age	194.5	5	215.0	6.8	0.03
Null	224.5	1	228.5	20.3	0.00
Nest Attempt	219.8	3	232.0	23.8	0.00
Mass	223.9	2	232.0	23.8	0.00
Landscape	223.2	3	235.4	27.2	0.00
Nest Attempt, Mass	219.8	4	236.1	27.9	0.00
Landscape, Mass	222.9	4	239.2	31.0	0.00
Age, Mass	223.5	4	239.8	31.6	0.00
Nest Attempt, Landscape	219.7	5	240.2	32.0	0.00
Global	183.3	14	243.1	34.9	0.00
Nest Attempt, Landscape, Mass	219.7	6	244.4	36.2	0.00
Nest Attempt, Landscape, BHCO	219.7	7	248.6	40.5	0.00
Nest Attempt, Landscape, Mass, Attempt*Mass Interaction	218.1	8	251.3	43.1	0.00

Table 8. Model selection results for maternal acute corticosterone. Models are ranked in ascending order by Akaike's Information Criterion adjusted for small samples (AIC_c). The maximum likelihood value (multiplied by -2), the number of parameters (*k*), AIC_c value, difference between the best model and subsequent models (ΔAIC_c), and Akaike weights (w_i) are given for each model. The global model fits the data ($\chi^2 = 46.2$, $df = 13$, $P < 0.001$, $n = 126$).

Model	**(-2Loglike)**	***k***	**AIC_c**	**ΔAIC_c**	**w_i**
Mass, LogCort1	127.3	3	139.5	0.0	0.97
Mass, LogCort1, Age	125.9	5	146.4	6.9	0.03
Mass	153.9	2	162.0	22.5	0.00
Null	161.9	1	165.9	26.4	0.00
Landscape, Mass	152.7	4	169.0	29.5	0.00
Age, Mass	153.3	4	169.6	30.1	0.00
Nest Attempt, Mass	153.4	4	170.7	31.2	0.00
Nest Attempt	159.4	3	171.6	32.1	0.00
Landscape	161.7	3	173.9	34.4	0.00
Global	115.7	14	175.3	35.8	0.00
Nest Attempt, Landscape, Mass	151.2	6	175.9	36.4	0.00
Nest Attempt, Landscape	158.0	5	178.5	39.0	0.00
Nest Attempt, Landscape, Mass, Attempt*Mass Interaction	145.8	8	179.0	39.5	0.00
Nest Attempt, Landscape, BHCO	157.8	7	186.7	47.2	0.00

Table 9. Model selection results for maternal hematocrit. Models are ranked in ascending order by Akaike's Information Criterion adjusted for small samples (AIC$_c$). The maximum likelihood value (multiplied by -2), the number of parameters (*k*), AIC$_c$ value, difference between the best model and subsequent models (ΔAIC$_c$), and Akaike weights (w_i) are given for each model. The global model fit the data ($\chi^2 = 33.9$, $df = 18$, $P < 0.025$).

Model	**(-2Loglike)**	***k***	**AIC$_c$**	**ΔAIC$_c$**	**w_i**
Landscape	-402.7	3	-390.5	0.0	0.67
Landscape, Mass	-405.3	4	-389.0	1.5	0.31
Attempt, Landscape, Mass	-403.4	5	-382.9	7.6	0.02
Attempt, Landscape	-405.5	6	-380.9	9.7	0.01
Attempt, Landscape, Edge	-404.8	7	-375.9	14.6	0.00
Mass	-382.1	2	-374.0	16.5	0.00
Null	-377.5	1	-373.5	17.1	0.00
Attempt, Landscape, Mass, Attempt*Mass	-406.5	8	-373.4	17.1	0.00
Attempt	-382.6	3	-370.4	20.1	0.00
Attempt, Mass	-385	4	-368.7	21.8	0.00
Age, Mass	-383.1	4	-366.8	23.7	0.00
Attempt, Edge	-385.5	5	-365.0	25.5	0.00
Attempt, Landscape, Edge, BHCO	-405.6	10	-363.9	26.6	0.00

[a] Asterisk indicates interaction between variables.

Table 10. Model selection results for nestling mass. Models are ranked in ascending order by Akaike's Information Criterion adjusted for small samples (AIC_c). The maximum likelihood value (multiplied by -2), the number of parameters (k), AIC_c value, difference between the best model and subsequent models (ΔAIC_c), and Akaike weights (w_i) are given for each model. The global model fit the data ($\chi^2 = 21.6$, $df = 11$, $P < 0.05$).

Model	**(-2Loglike)**	***k***	**AIC_c**	**ΔAIC_c**	**w_i**
Mass	277.2	2	285.3	0.0	0.84
Attempt, Mass, BHCO	273.4	4	289.7	4.4	0.09
Attempt	280.2	3	292.4	7.1	0.02
Null	289.0	1	293.0	7.7	0.02
Mass, BHCO	268.9	6	293.5	8.2	0.01
Attempt, Landscape, Mass	272.6	6	297.2	11.9	0.00
Attempt, Mass, Interaction	272.7	6	297.3	12.0	0.00
BHCO	285.2	3	297.4	12.1	0.00
Landscape	286.2	3	298.4	13.1	0.00
Age, Mass, Interaction	275.2	6	299.8	14.5	0.00
Landscape, Mass, Interaction	279.5	5	299.9	14.6	0.00
Age	276.0	6	300.6	15.3	0.00
Attempt, Mass, BHCO, Attempt*Mass	288.6	3	300.8	15.5	0.00
Attempt, Landscape, Mass, Age	268.6	8	301.6	16.3	0.00
Landscape, Attempt, Interaction	271.4	8	304.4	19.1	0.00
Attempt, Landscape, Edge, BHCO, Attempt*Landscape[a]	275.1	9	312.4	27.1	0.00
Global	267.4	12	317.7	32.4	0.00

[a] Asterisk indicates interaction between variables.

Figure. 1. Location of sites where Indigo Buntings were studied in central Missouri (2003) and in southeast Missouri (2000-2003). Shaded areas represent forest cover. Map adapted from Porneluzi and Faaborg (1999).

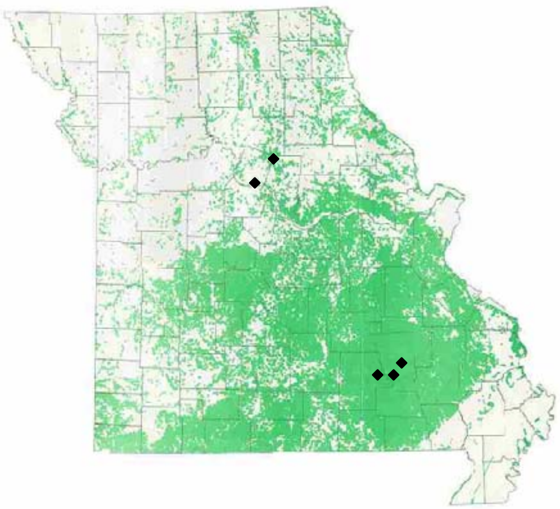


Figure 2. Breeding condition of female Indigo Buntings breeding successfully on their first attempt versus females renesting after nest predation (n=177; parameter estimates (dot), SE (box), 95% CI (line).

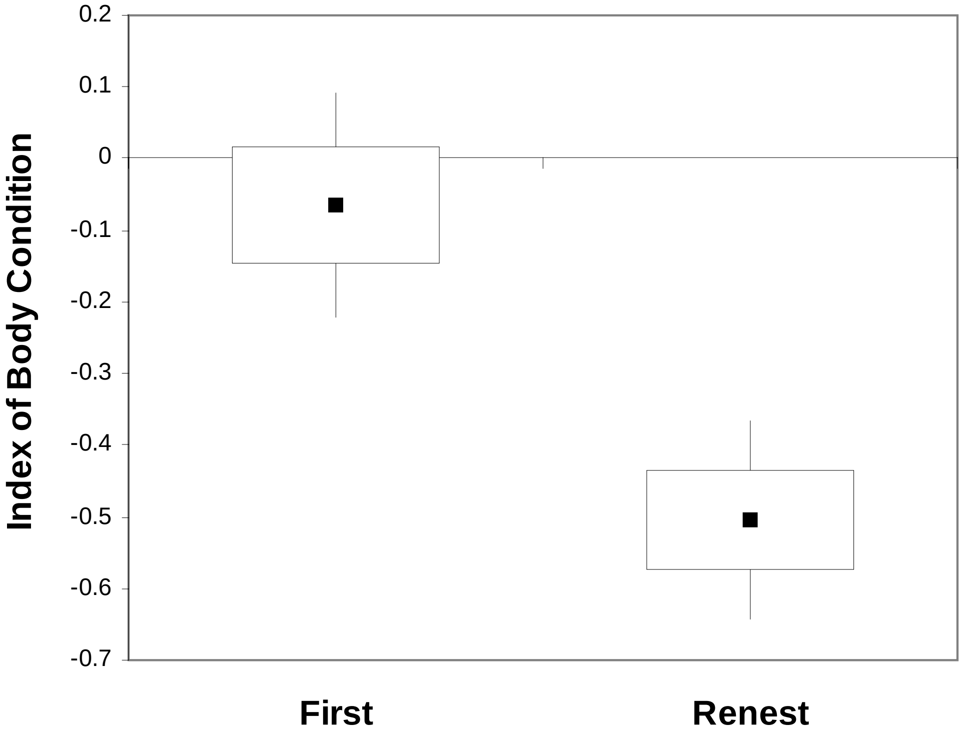

0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
Index of Body Condition
First
Renest

Figure 3. Acute corticosterone is negatively related to maternal body condition [Log(Cort2) = 3.99 – 0.12 (maternal body condition)] of female Indigo Buntings breeding in a fragmented and unfragmented landscape in Missouri (2000-2003).

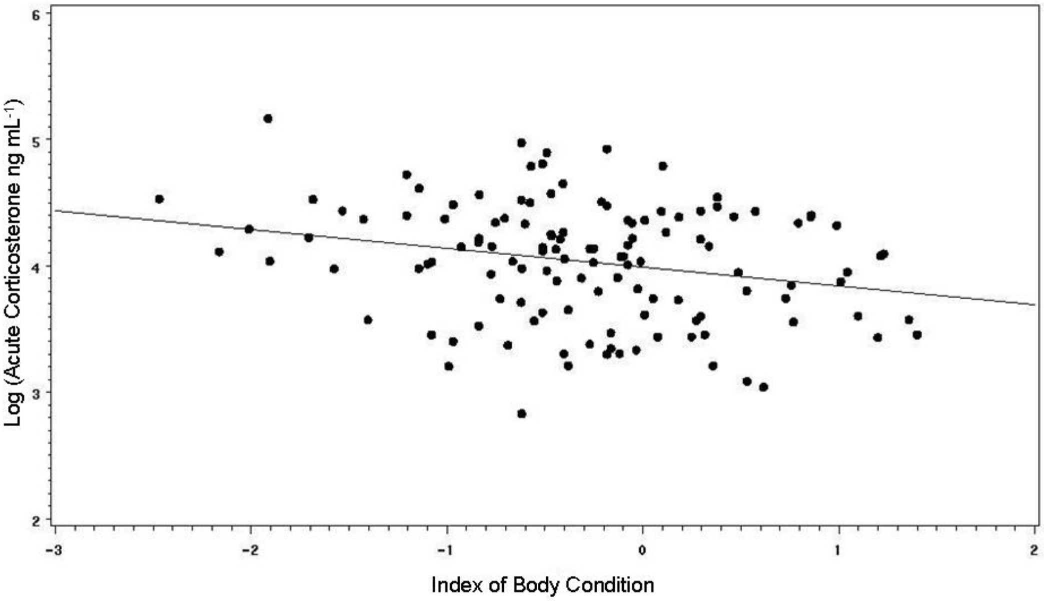

Log (Acute Corticosterone ng mL⁻¹)
6
5
4
3
2
-3
-2
-1
0
1
2
Index of Body Condition

Figure 4. Relationship between landscape and nesting attempt on reproductive output of Indigo Buntings. (**A**) The proportion of 3-egg clutches is higher in the fragmented landscape ($\chi^2 = 26.45$, $P < 0.001$, $df = 2$, $n = 168$) and (**B**) in renests ($\chi^2 = 29.08$, $P < 0.001$, $df = 2$, $n = 168$).

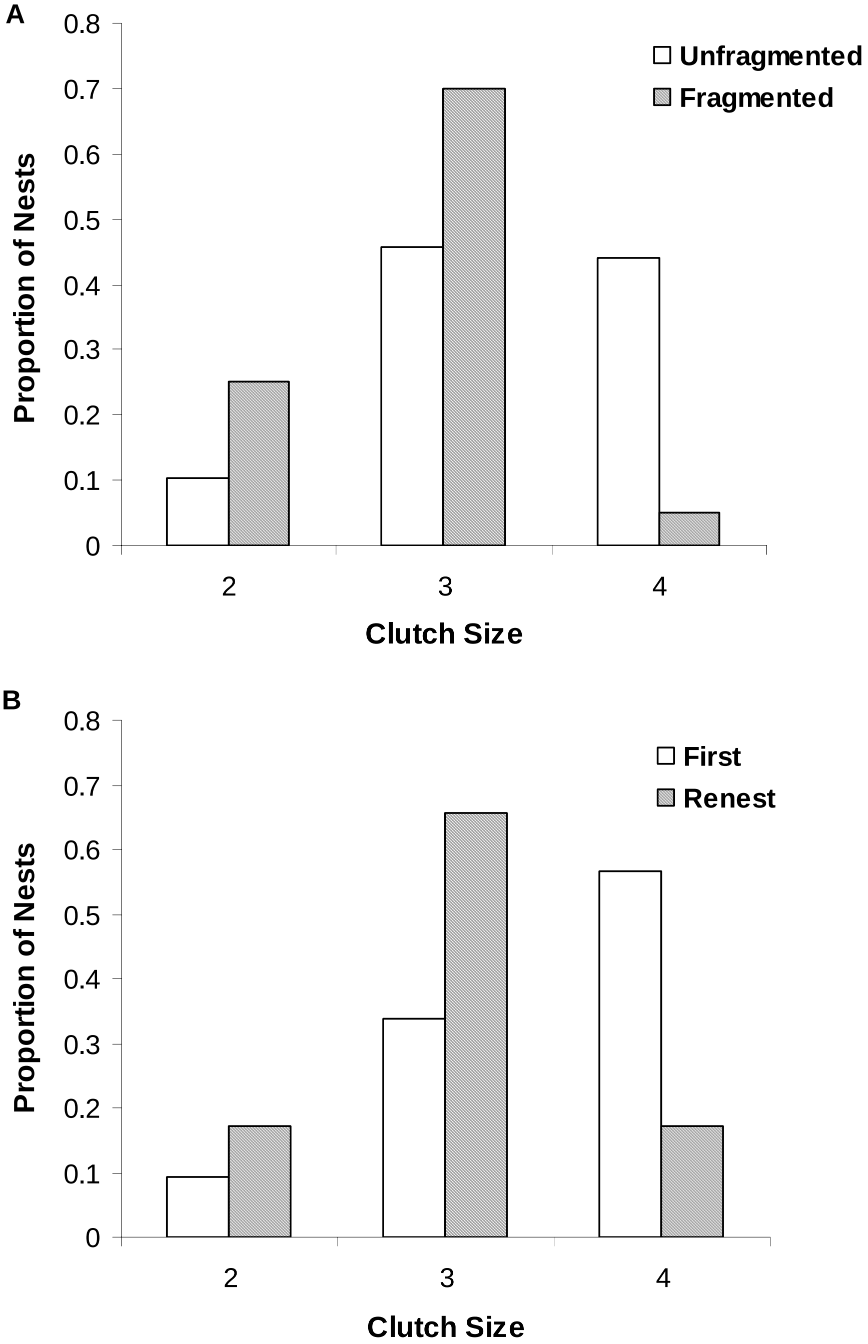

A
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion of Nests
2
3
4
Clutch Size
☐ Unfragmented
▨ Fragmented
B
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion of Nests
2
3
4
Clutch Size
☐ First
▨ Renest

Figure 5. Nestling condition is positively related to maternal condition of Indigo Buntings (*n* = 149; parameter estimate: 0.18 ± 0.06; 95% CI: 0.06 – 0.30).

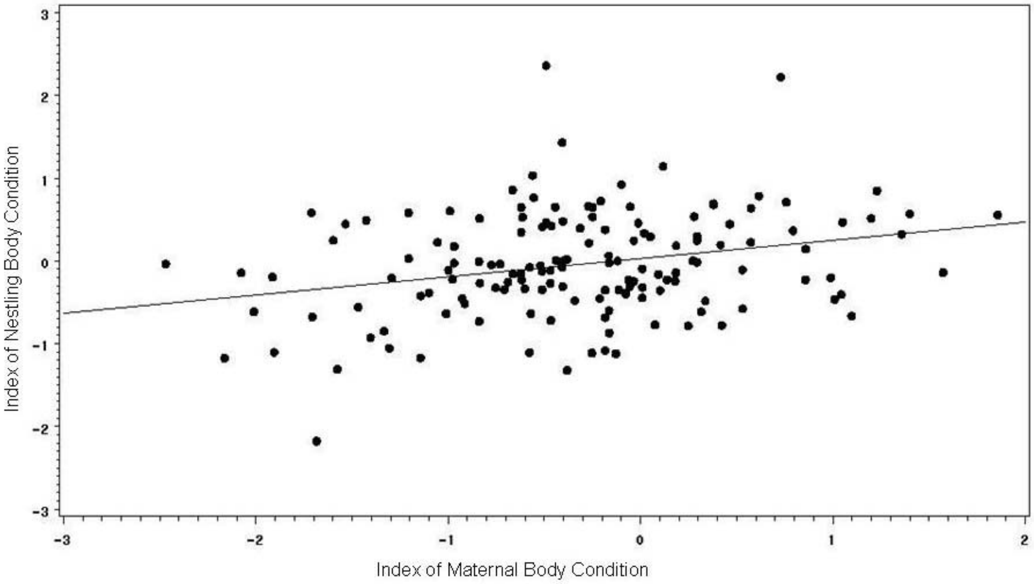
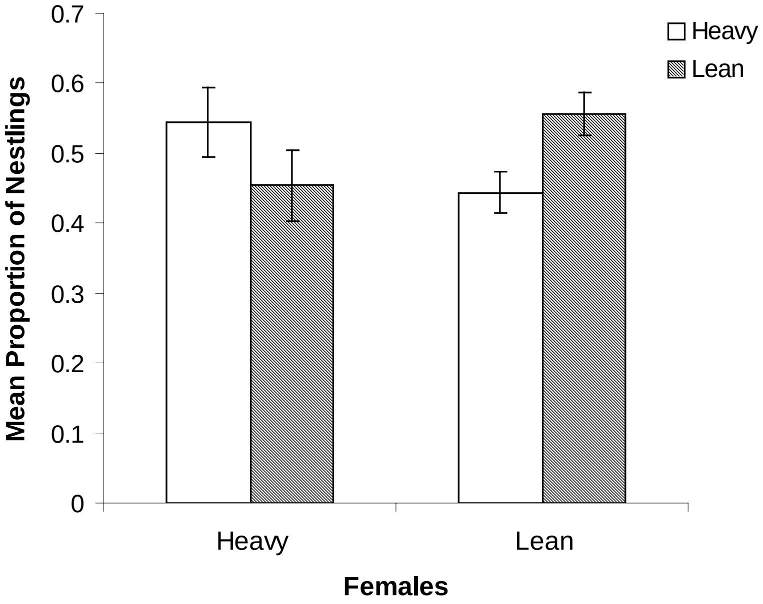

3
2
1
0
-1
-2
-3
Index of Nestling Body Condition
-3
-2
-1
0
1
2
Index of Maternal Body Condition

Figure 6. Lean female Indigo Buntings tend to produce a greater proportion of lean offspring than heavy females ($\chi^2 = 4.0$, $P = 0.04$, $df = 1$).


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mean Proportion of Nestlings
☐ Heavy
▨ Lean
Heavy
Lean
Females

CHAPTER 2

BROOD SEX RATIOS IN RELATION TO FOREST FRAGMENTATION, NEST PREDATION, AND MATERNAL CONDITION OF INDIGO BUNTINGS

Dana L. Morris

Division of Biological Sciences, 110 Tucker Hall, University of Missouri, Columbia,
MO 65211 USA.

ABSTRACT

Forest fragmentation creates edge habitat that attracts nest predators that lower reproductive success and force breeding birds to renest. The increased energetic effort required for renesting imposes additional constraints on females and likely influences their ability to invest in high quality offspring. I investigated whether forest fragmentation, renesting, and maternal effort interact to influence offspring sex ratios in Indigo Buntings (*Passerina cyanea*) breeding in a fragmented and unfragmented landscape. Brood sex ratios were female-biased in both landscapes (proportion males in unfragmented: 0.41 ± 0.05, *n* = 37 nests vs. fragmented: 0.37 ± 0.07, *n* = 27 nests) and this skew seems to be due to a large proportion of nests containing all female nestlings. In the fragmented landscape, 40% (11/27) of nests contained only females, whereas in the unfragmented landscape, 19% (7/37) of nests were all-female. However, the generalized linear models failed to detect important effects of environmental factors, renesting, or maternal condition on the proportion of males in broods. Although adult

male buntings are structurally larger than females, I found that this difference is not as pronounced in 6-day old nestlings, as male nestlings were only slightly heavier and tended to have longer wings than females. Male nestlings tended to be in better condition than female nestlings, although maternal condition did not seem to affect the condition of sons or daughters differently. However, the condition of male nestlings tended to decline with renesting attempts, suggesting that high quality males become more difficult for adult females to produce or maintain later in the breeding season. Additionally, with declines in clutch size later in the season, brood sex ratios tended to become more female-biased. These results suggest that the increased reproductive effort associated with renesting in fragmented landscapes may pose costs, in addition to lower nesting success, that result in skewed primary sex ratios.

INTRODUCTION

Forest fragmentation may reduce habitat quality for breeding songbirds through effects of decreased food availability (Burke and Nol 1998, Zanette et al. 2000), altered microclimate (Chen et al. 1993), and increased nest predation and interspecific brood parasitism (Robinson et al. 1995, Donovan et al. 1998, Porneluzi and Faaborg 1999). Additionally, forest fragmentation has been associated with physiological stress in an old-growth forest passerine, with nestling condition negatively related to forest patch size (Suorsa et al. 2003a). When birds are successful at raising young in fragments, it is often the result of repeatedly renesting after nest predation. Predation-induced renesting prolongs the breeding season and likely imposes higher energetic demands on adults, particularly females, which provide the majority of offspring care (Clutton-Brock 1991, Ligon 1999). Breeding females that are forced to renest respond to this environmental stressor by losing body condition, secreting higher levels of stress hormones, reducing clutch sizes, and producing lower quality offspring (Morris 2005).

Under poor environmental conditions, parental ability to invest in offspring may be limited and a potential consequence of renesting and declining maternal condition may be facultative adjustment of offspring sex ratios. Recent studies have shown that the costs of egg production and incubation affect female condition and her ability to rear successful offspring (Monaghan et al. 1995, Monaghan et al. 1998). Additionally, experimental increases in egg production reduce female survival, and the fitness costs to breeding females and their offspring may be dependent on local environmental conditions (Visser and Lessells 2001). This may have important consequences to females that nest in poor quality habitat where they experience higher rates of nest predation and are forced to renest multiple times. However, we know little about how large-scale environmental characteristics and breeding habitat quality potentially influence maternal condition and offspring sex allocation. In one of the few studies demonstrating an association between breeding habitat quality and sex ratios, Suorsa et al. (2003b) found that primary sex ratios of the Eurasian treecreeper (*Certhia familiaris*) were constrained by the amount of forest edge habitat, with female-biased broods associated with highly fragmented stands of pine forests.

Optimal sex allocation theory predicts that parents should adjust the sex ratio of offspring based on their own condition and the reproductive potential of their offspring in order to maximize parental and offspring fitness (Trivers and Willard 1976; Charnov 1982). Under favorable environmental conditions, healthy females may produce more of the profitable sex, especially if that sex is more costly to produce or experiences differential mortality (Fisher 1930). Additionally, Trivers and Willard (1976) proposed that although males are generally larger and may be costlier to produce, they have higher reproductive potential. Therefore, females in good condition should adjust the sex ratio of their offspring toward the sex with higher reproductive return, relative to females in poor condition.

With the development of molecular sexing techniques, a recent surge of studies has shown support for adaptive sex allocation in relation to maternal condition (Nager et al. 1999, Nager et al. 2000) and reproductive effort (Kalmbach et al. 2001). Sex ratio adjustments have been found under a variety of ecological conditions where females in better condition produce more of the larger or costly sex (Velando 2002) particularly when environmental conditions are good (Komdeur et al. 1997; Komdeur et al. 2002); when early breeders are in better condition than late-breeders (Velando et al. 2002; Olsen and Cockburn 1991); and when environmental conditions deteriorate, females overproduce the smaller or cheaper sex (Kalmbach et al. 2001).

Despite numerous studies that demonstrate control of sex determination in birds, the precise mechanisms behind this control are unknown (but see Pike and Petrie 2003 and Komdeur et al. 2002). Environmental factors that have been suggested to influence primary sex ratios (prey abundance, Appleby et al. 1997; rainfall, Burley et al. 1989; habitat quality Komdeur et al. 1997; diet, Bradbury and Blakey 1998; breeding attempt, Velando et al. 2002; and resource availability, Kalmbach et al 2001) likely act by influencing the condition of breeding females. Parental condition has been implicated in influencing sex ratios and the condition of young from pre-ovulatory processes through the period of brood rearing (see review by Pike and Petrie 2003). Changes in maternal condition may alter maternal endogenous hormone levels that may potentially influence reproductive physiology associated with follicular growth (Sturkie 2000), yolk production (Williams 1999), segregation of sex chromosomes at meiosis (Dijkstra et al. 1990, Petrie et al. 2001), sex-selective resorption of the ova (Emlen 1997), and sex-specific allocation of resources to embryos (Krackow 1995, Petrie et al. 2001). Sex ratio bias may also occur as a result of sex-specific mortality that may be due to poor-conditioned females allocating inappropriate resources to the brood (Velando 2002, Nager et al. 2000) or a difference in competitive ability between male and female nestlings (Oddie 2000, Lessells et al. 1996). Furthermore, the condition of laying females has been shown to affect offspring growth through the influence of stress hormones (corticosterone) (Hayward and Wingfield 2004) and androgens (testosterone) transferred to the yolk during egg production (Eising et al. 2001, Verboven et al. 2003).

I identified the extent to which environmental quality and maternal effort interact to influence productivity and offspring sex ratios of Indigo Buntings (*Passerina cyanea*). Indigo Buntings are of particular interest for studying sex ratios because they are frequently polygynous (Westneat 1988; Carey and Nolan 1975, 1979), with male offspring presumably of higher reproductive potential, thereby allowing for investigation of whether high quality females adjust sex ratios to the more profitable sex. I determined if Indigo Buntings adjust sex ratios toward females as a reproductive strategy in response to unfavorable breeding conditions due to fragmentation or increased nesting attempts. In these study populations, adult males are 4% and 6% larger in wing and mass, respectively, than females (Morris, unpubl. data). I determined if male nestlings are larger and, therefore, more costly to produce than females; if nestling body condition varies with gender; and if maternal condition has a greater effect on the condition of sons than daughters.

METHODS

Study Sites

I studied Indigo Buntings during three breeding seasons (2001-2003) in three sites in an unfragmented forest within approximately 40,000 ha of contiguous oak-hickory forest in southeast Missouri (lat: 37.19°; long: 91.00 °) and two sites (1125 ha, 1460 ha) in a fragmented landscape in central Missouri (lat: 39.00 °, long: 92.62 °; lat.: 39.25°; long: 92.45°, respectively). Each site is owned and managed by the Missouri Department of Conservation and separated by > 5 km. In these sites, Indigo Buntings nest in early successional habitat created by forestry practices, agriculture, mowing, and natural disturbances such as wind storms.

Physiological Measures

Female Indigo Buntings were captured upon arrival to breeding sites (late April-early May) or at their nests using song playbacks and mist nets or by intensive mist-netting in the selected study sites. I quantified female quality by measuring body mass corrected for size, baseline corticosterone, and acute corticosterone. My field assistants and I measured birds and collected blood samples from adults and nestlings when nestlings were 5-7 days old. Birds respond to stressful environmental stimuli by secreting corticosterone, the major glucocorticoid in birds (Harvey et al. 1984), which increases the potential for short-term survival by redirecting behavior from nonessential activities such as reproduction, into activities that promote survival, like foraging (Wingfield 1988, Astheimer et al. 1992). The adrenocortical response to stress has been used to evaluate the ability of wildlife to handle adverse environmental conditions (Wingfield et al. 1994, Holberton and Able 2000), to measure an organism's physical condition, and to indicate habitat quality (Marra and Holberton 1998, Millspaugh et al. 2001). Blood (50 µL) was collected from the brachial vein into heparinized microcapillary tubes within 1-5 minutes of capture. This sample was used as baseline corticosterone level and a subsequent sample collected at 30 minutes after capture provides a profile of the acute stress response (Wingfield et al. 1992, Wingfield 1994). Baseline levels of corticosterone (measured before circulating hormone levels rise due to capture stress) indicate the physical history of an individual bird. Low baseline levels paired with large body mass may indicate a healthy individual that has energy reserves in the form of fat (Harvey et al. 1984). High baseline levels paired with small body mass indicate an individual has chronically elevated corticosterone levels and is likely catabolizing fat or protein reserves for energy (Harvey et al. 1984, Marra and Holberton 1998). A robust acute response to stress may indicate an individual's poor physiological condition that requires the physiological boost from the effects of corticosterone in response to stressors (Holberton and Wingfield 2003).

An extra 50 µL of blood was taken from adults and nestlings and stored in Longmire's lysis buffer and later analyzed to determine brood sex ratios. Birds were aged, sexed, measured (unflattened wing chord; tarsus, to 0.1mm; fat score), weighed (to 0.5 g), and banded with colored plastic and numbered USFWS bands. All blood samples and body measurements were taken between 0600 – 1000 hours to prevent bias in measurements due to daily fluctuations in body mass and corticosterone. To determine body mass corrected for structural size, wing and tarsus length were entered into a principal components analysis. Body weight was then regressed against PC1 and the residuals from this analysis are used as estimates of mass corrected for body size (Green 2001) and presented as an index of body condition.

Hormone Assays

Blood samples for corticosterone were kept on ice until centrifuged at 10,000 rpm for 10 minutes. Plasma was drawn off and stored at -20 ^{0}C until assayed using commercially available I^{125} radioimmunoassay (RIA) kits (ICN Biomedicals, Costa Mesa, CA). I followed the manufacturer's method, except I halved the volume of all reagents and samples were diluted 1:50 with steroid dilutent prior to assay. Parallelism and recovery of exogenous corticosterone validation assays were conducted to validate the utility, accuracy, and precision of this particular RIA kit for use with a suite of species of passerine birds (Washburn et al. 2002). Assays were run separately for each year of the study (2000-2003) and interassay variation was 12.5%, 3.7%, 8.9%, and 15% respectively. Additionally, a control from the RIA kit was run in each assay in all years and had an interassay variation of 6.9%. Intra-assay variation calculated from 60 randomly chosen samples across all assays was 3.9%.

Identification of Nestling Sex

I extracted DNA from nestling blood samples using either standard phenol-chloroform methods (Sambrook and Russell 2001) or an alcohol-based DNA precipitation technique (L. Gibbs, unpubl. protocol). In the latter method, I digested the samples with proteinase K, then precipitated and discarded cell protein waste using ammonium acetate. I used isopropanol to precipitate DNA from the remaining solution and then I washed the DNA in ethanol and air dried it before re-suspending it in either 1X TE or 10 mM Tris. In 3 cases, I sampled tissue from nestlings that had died of natural causes. Tissue samples were frozen at –80°C, ground in lysis buffer with a sterile mortar and pestle, and then subjected to phenol-chloroform DNA extraction.

I determined the sex of all nestlings using P2/P8 primers from Griffiths et al. (1998), amplifying a region of the CHD-1 gene on the avian sex chromosomes. A total volume of 20µL was used for the PCR reaction, with the following conditions: 1X Promega Mg-free buffer, 1.75 mM $MgCL_2$, 200µM of each dNTP, 1.0µM of each primer, 0.5 U of *Taq* polymerase, and 100-300 ng of genomic DNA. PCR amplifications were performed under the following thermal-cycling conditions: an initial denaturing step at 95°C for 2 min. followed by 34 cycles of 95°C for 30s, 48°C for 1 min., and 72°C for 2 min., with a final cycle of 72°C for 5 min. After isolation and amplification, the PCR products were separated for 3 h at 7 V/cm on 3% high-resolution agarose gels and visualized with ethidium bromide staining. I used DNA from eight known-sex adult Indigo Buntings (4 male, 4 female) as positive controls, and each gel included a negative control to detect potential contamination by foreign DNA. There was none.

Data Analysis

To determine if the overall proportion of male offspring or proportion of males in broods differs from parity, I used two-sided binomial tests (FREQ procedure in SAS). To examine the effect of maternal condition and environmental effects on brood sex ratios I used Akaike's Information Criterion (AIC) to select the most parsimonious model for statistical inference. I fitted sets of generalized linear models with binomial errors and logit-link functions, with number of males as the response variable and brood size as the binomial denominator. Therefore individual nests were the units of measure. Models were parameterized with variables including nesting attempt, landscape, brood parasitism, maternal corrected mass, baseline corticosterone, and acute corticosterone levels. Nesting attempt was defined as first (nests initiated before 29 May in the contiguous forest or 9 June in the fragmented landscape due to differences in arrival dates) or renest (initiated after these dates) based on the average date of the first fledglings of the season in each landscape and the time to renest after predation. Baseline and acute corticosterone levels were log-transformed to establish normality. Because sample size was small (n = 55 broods), I was not able to use a global model that included all possible relevant interaction terms (Table 1). The models were fit to the data using the GENMOD procedure (SAS Inst. 2004). Because residual variation of the fitted logistic regression model often deviates from the binomial assumption of the model (Krackow and Tkadlec 2001, Wilson and Hardy 2002), as was the case with these data, I estimated the dispersion parameter (deviance of global model/degrees of freedom of global model) and used a quasi-likelihood method for model selection (Burnham and Anderson 2002). This estimate of overdispersion from the global model was incorporated into the calculation of QAIC_c values (adjusted for small samples) for each model. Models with low QAIC_c values and high Akaike weights are considered to have the most support.

To determine if body size (tarsus, wing) or weight varies between male and female nestlings I used 1-way mixed models with nestling sex as the predictor variable and brood identity as the random effect. To determine if body condition varies between male and female nestlings or if maternal condition has a different effect on the condition of sons and daughters, I used a general linear mixed model with corrected mass of nestlings as the dependent variable and brood identity as the random effect. Fixed factors included nestling sex, nesting attempt, and maternal corrected mass plus all two-way interactions with nestling sex. All general linear models were weighted by brood size. Data are presented as least square means ± standard error (SE).

RESULTS

I determined the sex of 249 nestlings from 93 broods, but I present data on brood sex ratios from only 64 complete broods that did not contain inviable eggs or lose eggs or nestlings to nest predation before the brood was sampled. I found no evidence that the partial loss of nestlings was due to starvation and recent video studies of nest predation show that predators frequently take one or more nestlings without causing complete failure (Stake and Cimprich 2003). Overall, 80 out of 192 (41.6%) nestlings were male, and this varies significantly from a binomial distribution ($Z = 2.31$, $P = 0.01$). The mean proportion of males in complete broods was 0.40 ± 0.04, which varies significantly from 0.5 (2-tailed binomial test: $Z = -3.37$, $P < 0.0004$, $n = 64$; Fig. 1).

Even though these sex ratios differed from binomial, the overall fit of the global model showed no severe lack of fit or overdispersion (heterogeneity factor = 1.8 for the global model). A likelihood ratio goodness of fit test comparing the residual deviance and degrees of freedom between the global model and a null model shows that the global model fit the data ($\chi^2 = 19.32$, $df = 10$, $P < 0.05$). The best model to explain variation in brood sex ratios was the null model (Table 2). However, models within 1-2 QAIC_c units of the best model are considered to have some support. The model containing the log of baseline corticosterone had some support with an Akaike weight of 0.25. The direction of the effect is in the direction predicted, such that lower baseline levels of corticosterone are associated with higher proportion of males in broods, however, the 95% confidence intervals of the parameter estimate include zero (-0.03 ± 0.28; 95% CI: -0.57 – 0.52) indicating no evidence for a statistical effect. Additionally, the model containing maternal condition has some support with an Akaike weight of 0.18. Maternal condition is negatively associated with the proportion of males in broods, however the 95% confidence intervals about the parameter estimate include zero (parameter estimate: -0.15 ± 0.20; 95% CI: -0.54 – 0.24).

Brood sex ratios (proportion male) tended to be female-biased to the same extent in both landscapes with 0.41 ± 0.05 (n = 37) in the unfragmented and 0.37 ± 0.07 (n = 27) in the fragmented landscape. However, a log-linear test provides evidence that sex ratio and landscape are not independent ($G = 12.6$, $P = 0.02$, $df = 5$). In the fragmented landscape, 40.7% (11/27) of all nests in the sample contained all female offspring, compared to 18.9% (7/37) in the unfragmented landscape (Fig. 2a). The loglinear model is non-significant for nesting attempt and sex ratio ($G = 7.9$, $P = 0.16$, $df = 5$). However, 30% of all renests contained females only (Fig. 2b). A *post hoc* analysis of mean proportion of males in broods indicated that the proportion of males tended to be higher in larger clutches (Fig. 3), which were common among high quality females and early nesting attempts (Morris 2005).

Male nestlings weighed only slightly more (9.12 ± 0.12 g) than female nestlings (8.9 ± 0.10 g), although this difference is not significant ($F_{1,133} = 1.21$, $P = 0.27$). There was no difference in tarsus length ($F_{1,133} = 0.03$, $P = 0.86$; Fig. 4) between female or male nestlings, although wing length tended to be longer in males than females ($F_{1,133} = 2.6$, $P = 0.10$; Fig. 4). The body condition index of nestlings did not differ with nestling sex ($F_{1,180} = 0.48$, $P = 0.48$) or nest attempt ($F_{1,180} = 0.66$, $P = 0.41$), although it appears that female nestlings tend to gain condition between first attempts and renests but males lose condition between nesting attempts (attempt*nestling sex interaction: $F_{1,180} = 2.91$, $P = 0.08$; Fig 5). Maternal condition had a positive affect on nestling condition ($F_{1,180} = 6.98$, $P = 0.009$), but there is no strong evidence that maternal condition has a differential effect on male than female offspring (maternal condition*nestling sex interaction: $F_{1,180} = 0.86$, $P = 0.35$). I used the ESTIMATE statement in GENMOD to generate predicted values of condition of male and female nestlings under different levels of maternal condition. Male nestlings tend to be in better condition at each level of maternal condition than female nestlings, although the variance of these estimates is high (Fig. 6).

DISCUSSION

Nesting success was low in both landscapes during the course of this study (unfragmented: 0.26 ± 0.03, $n = 374$ vs. fragmented: 0.17 ± 0.16, $n = 101$; Morris 2005, Chp. 1). However, reproductive effort was considerably higher in the fragments, given that only 34% (25/103) of all females raised a brood early in the season, whereas 61% (217/353) of females fledged young early in the unfragmented landscape (Morris 2005). As a result, most birds finished breeding by early July in the unfragmented landscape while the majority of females in the fragmented landscape continued attempts to produce one successful brood into August. Furthermore, the fact that 40% of all nests in the fragmented landscape contained all-female nestlings suggests that fragmentation impacted reproduction.

In both landscapes, brood sex ratios were skewed toward females. However, the candidate set of models failed to show strong relationships between sex ratios and maternal condition, baseline corticosterone, acute corticosterone, landscape type, or nesting attempt. However, given the small sample of complete broods ($n = 55$), the large number of parameters in my models, and the degree of overdispersion, it is likely that the models lacked power to detect these patterns.

In Indigo Buntings, early-breeding females are in better condition than those that are forced to renest after nest predation (Morris 2005), and therefore should be able to invest more in males, as would be expected in a polygynous system where males are assumed to be the more profitable sex. I found no important differences in sex ratios between first attempts and renests; however, most of the data points for first attempts were from the unfragmented forest. Of the 27 broods in the fragments that were used in the analysis of sex ratios, only 3 were sampled from first attempts due to high predation rates early in the season (Morris, unpubl. data). Therefore, it is likely that the high frequency of all-female broods in the fragmented landscape reflects a trend for female-biased broods with renesting attempts. Very few studies demonstrate brood sex ratios as biased as these; however, Velando et al. (2002) found that early-breeding European Shags (*Phalacrocorax aristotelis*) were in better condition and produced more male-biased broods (0.63), but late broods were heavily female biased (0.36).

I found that male nestlings were only slightly larger than females at six days post-hatching and maternal condition did not significantly affect the condition of sons and daughters differently, as I would have predicted if sons were more costly to produce. However, male nestlings tended to be leaner than female nestlings late in the season, thereby suggesting that males become more costly to produce, especially for renesting females that are in poorer condition. Perhaps the condition of male nestlings is more dependent on maternal condition, and as maternal condition declines with renesting attempts, high quality male nestlings become more difficult to maintain (Anderson et al. 1993). When resources or maternal condition declines, parents should produce fewer of the sex that is more dependent on the resources of the parents (Trivers and Willard 1973). Additionally, under adverse environmental conditions females may forego the cost of producing high quality males, especially later in the season, if they lack the resources necessary to rear males or the probability of survival of male offspring decreases (Hochachka and Smith 1991, Verboven and Visser 1998). Furthermore, adjusting the sex ratios toward females as clutch size declines with renesting attempts is beneficial to females in this study because it allows them to maximize their own fitness and the fitness of their offspring as reproductive effort increases and their chances for renesting decrease in the current breeding season (Myers 1978).

In general, the condition of male nestlings tended to be higher than the condition of female nestlings. In 65.2% (30/46) of all nests with mixed-gender broods, the leanest nestling in the brood was female, suggesting that female nestlings are at a competitive disadvantage. It is likely that male nestlings compete better at begging than females (Magrath 1990) and may be provisioned at higher rates or adults are preferentially provisioning male nestlings (Oddie 2000, Smiseth et al. 2003). Zanette et al. (2005) showed that in Song Sparrows (*Melospiza melodia*), female nestlings are poorer competitors compared to their male siblings and to Brown-headed Cowbird nest mates. Consequently, sex-specific mortality of female Song Sparrows in parasitized nests led to male-biased broods. I had very few parasitized nests in my samples and there was no evidence that broods were male-biased in parasitized nests. However, I found a trend for female nestlings to be heavier later in the season, suggesting that as fewer males were produced, competition with male siblings decreased and allowed female nestlings greater access to food resources.

The surprising trend for maternal condition to increase with increasing proportion of males in broods may further suggest that male nestlings are more costly to produce or that females trade-off their own condition in order to raise males. Or perhaps, because I measured adult females after the clutch was laid, I detected that males are more costly to raise, rather than produce. As indicated above, males may beg for food more intensely than female nestlings, thus requiring greater provisioning effort by adult females. Hence, because I measured adults during nestling provisioning instead of during egg formation, I have little evidence for a causal relationship between maternal condition and sex allocation.

An alternative hypothesis to explain why I did not detect females positively adjusting brood sex ratios in relation to their own condition could involve a behavioral strategy in relation to environmental conditions. In many species of passerines, females tend to be the more dispersing sex (Greenwood and Harvey 1982, Payne and Payne 1993). If breeding females can gauge resources and have control of offspring sex determination, it may be adaptive to produce more females when local environmental conditions or food resources are unfavorable. Breeding populations of migratory birds often have male-biased sex ratios (Aldrich 1973), suggesting females have low survival (Nolan 1978, Payne 1989). Although the direct cause of this discrepancy in survival is not known, it has been related to costs of reproduction (Sherry and Holmes 1992, Greenwood et al. 1995, Visser and Lessells 2001) and limitations of winter habitat in passerines (Robbins et al. 1989, Rappole et al. 1992, Marra and Holmes 2001). Biased production of offspring may alter demography of breeding populations, depending on life history traits, and seems particularly notable for species where first-year birds return to natal sites to breed. Consequently, breeding females may be able to compensate for increased mortality of females by skewing offspring sex ratios, which may be especially important under adverse breeding conditions, such as in the fragmented landscape studied here.

I saw no direct evidence supporting the prediction that maternal condition or stress hormones influence brood sex ratios, despite the bias in sex ratios. Whether this skew in brood sex ratios is due to physiological constraints or seasonal behavioral shifts or strategies is unclear. However, higher reproductive effort for females breeding in the fragmented landscape is associated with a decline in maternal condition and reproductive output. The patterns shown here are indicative that breeding females adjust the sex ratio of their broods in response to environmental conditions and certainly warrant further experimental investigation.

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Table 1. Candidate set of models used to evaluate effects of environmental (landscape, nest attempt, and brood parasitism – BHCO) and maternal factors (mass, log of baseline corticosterone, log of acute corticosterone) on brood sex ratios of Indigo Buntings breeding in Missouri (2000-2003).

Model	**Model Structure**
Global	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (BHCO) + β_4 (Mass) - β_5 (LogBaseline) - β_6 (LogAcute) + β_7 (Attempt*Mass[a]) + β_8 (Mass*LogAcute)
Main Effects	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (BHCO) + β_4 (Mass) - β_5 (LogBaseline) - β_6 (LogAcute)
Maternal Effects	$\beta_0 + \beta_1$ (Mass) - β_2 (LogBaseline) - β_3 (LogAcute)
Environmental Effects	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (BHCO)
Environmental and Maternal	$\beta_0 + \beta_1$ (Attempt) + β_2 (Landscape) + β_3 (Mass)
Maternal Mass	$\beta_0 + \beta_1$ (Mass)
Maternal Corticosterone	$\beta_0 - \beta_1$ (LogBaseline) - β_2 (LogAcute)
Maternal Baseline Corticosterone	$\beta_0 - \beta_1$ (LogBaseline)
Maternal Acute Corticosterone	$\beta_0 - \beta_1$ (LogAcute)
Maternal Mass and Acute Corticosterone	$\beta_0 + \beta_1$ (Mass) - β_2 (LogAcute)
Null	β_0

[a] Asterisk indicates two-way interaction.

Table 2. Model selection results for evaluating effects of environmental variables and maternal effects on the proportion of males in broods. Models are ranked in ascending order by Akaike's Information Criterion adjusted for overdispersion and small samples ($QAIC_c$). The maximum likelihood value (multiplied by -2), the number of parameters (k), $QAIC_c$ value, difference between the best model and subsequent models (ΔAIC_c), and Akaike weights (w_i) are given for each model.

Model	**(-2Loglike)**	k	$QAIC_c$	$\Delta QAIC_c$	w_i
Null	88.67	1	52.34	0.00	0.35
Maternal Baseline Corticosterone	86.76	2	53.43	1.09	0.20
Maternal Mass	87.90	2	54.06	1.73	0.15
Maternal Acute Corticosterone	88.60	2	54.45	2.12	0.12
Maternal Corticosterone	86.04	3	55.27	2.93	0.08
Maternal Mass & Acute Cort.	86.50	3	55.53	3.19	0.07
Maternal Effects	85.87	4	57.51	5.17	0.03
Environmental & Maternal	86.99	6	63.08	10.74	0.00
Environmental Effects	88.50	7	66.55	14.21	0.00
Main Effects	85.13	10	73.29	20.96	0.00
Global	84.93	13	83.06	30.72	0.00

Figure 1. Proportion of males in all complete Indigo Bunting broods ($n = 64$) sampled from a fragmented and unfragmented landscape in Missouri (2001-2003).

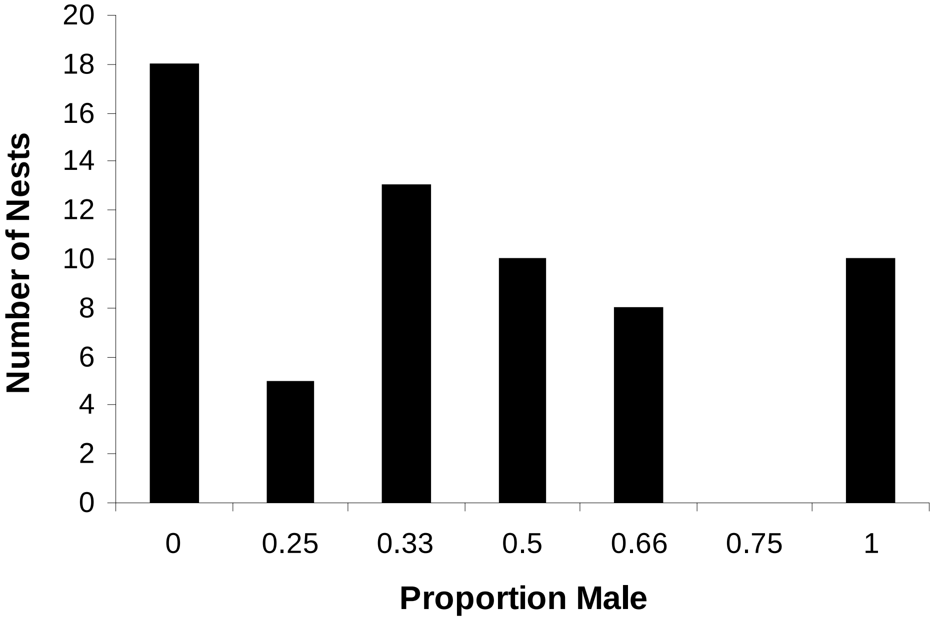
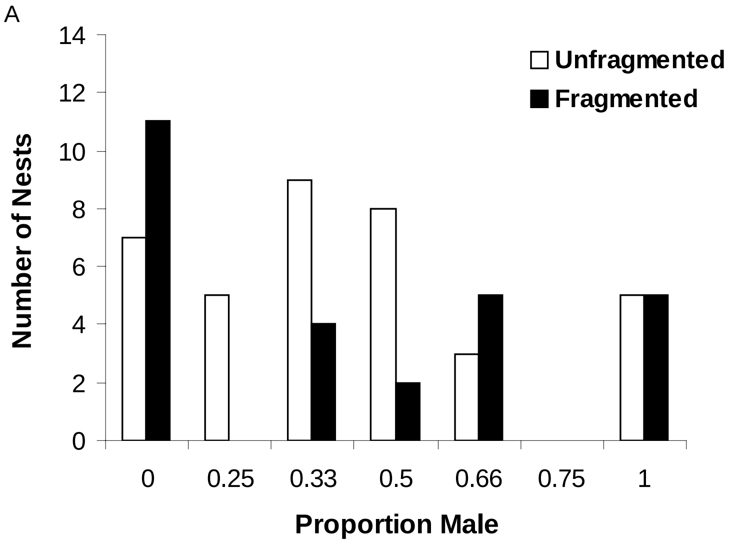

Number of Nests
20
18
16
14
12
10
8
6
4
2
0
0
0.25
0.33
0.5
0.66
0.75
1
Proportion Male

Figure 2. (**A**) Frequency distributions of brood sex ratios differed between landscapes (*G* = 12.6, *P* = 0.04, *df* = 5). In the fragmented landscape, 40.7% (11/27) of nests contained only female offspring, 40.7% (11/27) were mixed females and males, and 18.6% (5/27) were all male compared to 18.9% (7/37), 65.5% (25/37), and 13.5% (5/37) respectively, in the unfragmented landscape. (**B**) Frequency distributions of brood sex ratios were not significantly different between early nests and renests (*G* = 7.92, *P* = 0.21, *df* = 5).


A
14
12
10
8
6
4
2
0
Number of Nests
☐ Unfragmented
■ Fragmented
0
0.25
0.33
0.5
0.66
0.75
1
Proportion Male

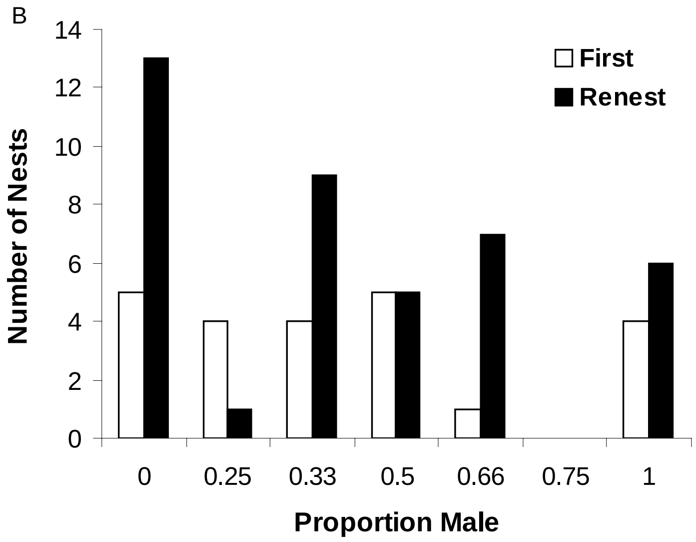

B
14
12
10
8
6
4
2
0
Number of Nests
☐ First
■ Renest
0
0.25
0.33
0.5
0.66
0.75
1
Proportion Male

Figure 3. Brood sex ratios in relation to clutch size (two, *n* = 13; three, *n* = 35; four, *n* = 15) of Indigo Buntings with mean (dot) ± SE (box) and 95% confidence intervals (line).

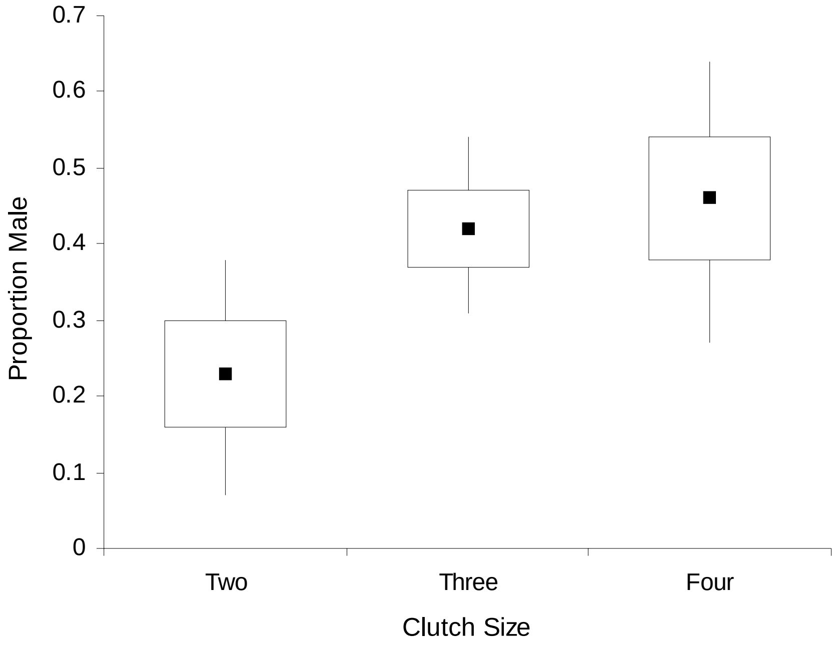
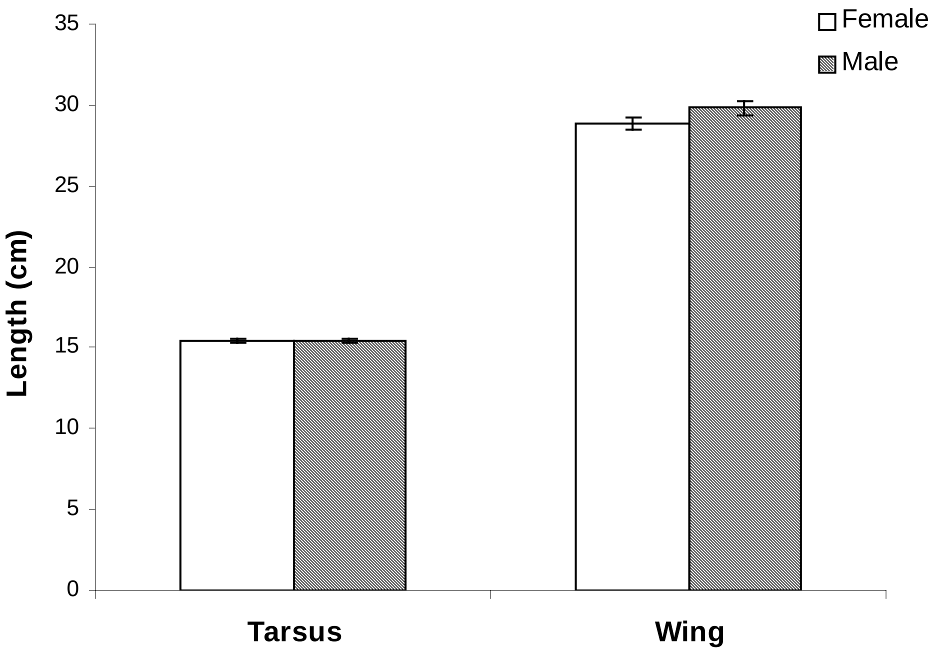
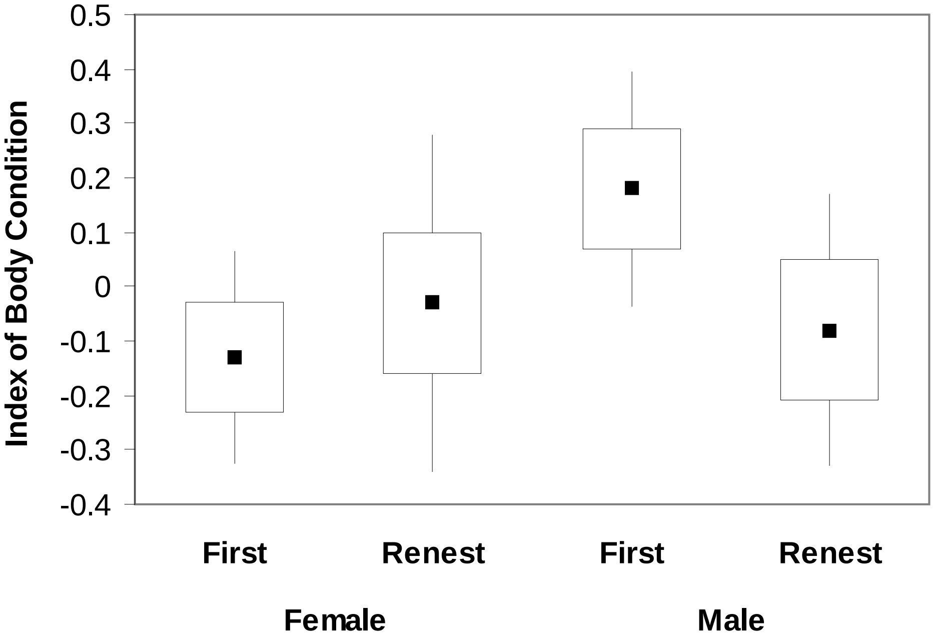

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion Male
Two
Three
Four
Clutch Size

Figure 4. Differences in body size between female (*n* = 81) and male (*n* = 55) nestlings at 6-days post-hatching. Standard error bars are shown with each bar.



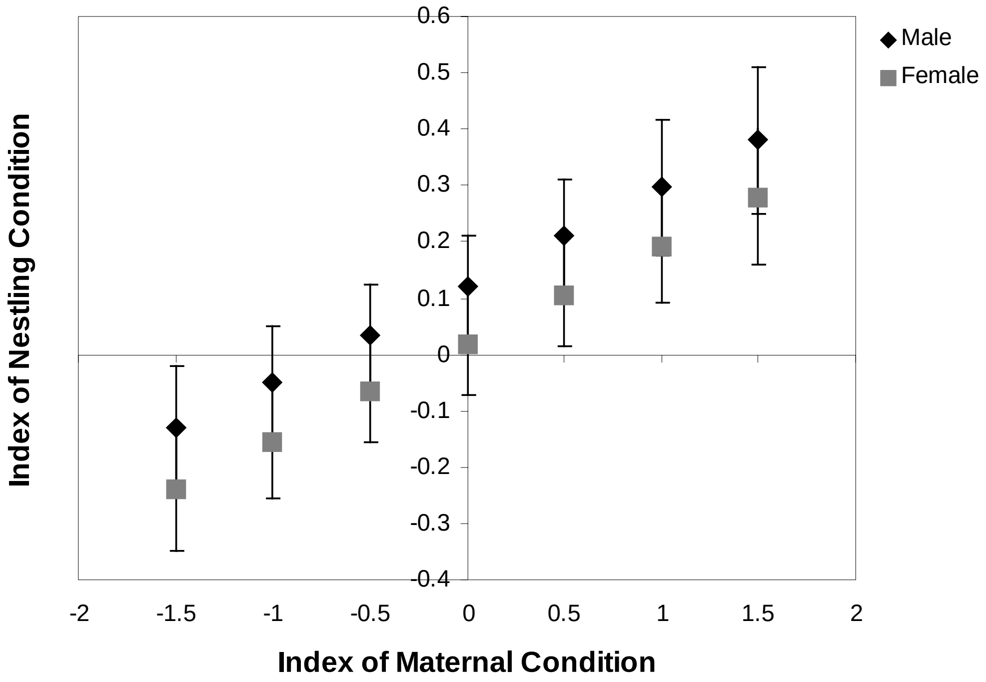
35
30
25
20
15
10
5
0
Length (cm)
Tarsus
Wing
☐ Female
▨ Male

Figure 5. Differences in body condition of male and female nestlings with renesting attempts. Mean (dot) ± SE (box) and 95% confidence intervals (line).



Index of Body Condition
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
First
Renest
First
Renest
Female
Male

Figure 6. Predicted values of male and female nestling condition under different levels of maternal body condition. Mean (dot) ± SE (line).



Index of Nestling Condition
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
Index of Maternal Condition
◆ Male
■ Female

CHAPTER 3

EFFECTS OF GENDER, BREEDING STAGE, AND FOREST FRAGMENTATION ON BODY CONDITION AND ADRENOCORTICAL RESPONSE OF INDIGO BUNTINGS (*PASSERINA CYANEA*)

Dana L. Morris

Division of Biological Sciences, 110 Tucker Hall, University of Missouri, Columbia,
MO 65211 USA.

ABSTRACT

During the breeding season, migratory passerine birds must adjust to energetic demands associated with territorial defense and reproduction. How successful they are at making these adjustments may depend on environmental conditions and the quality of breeding sites. I studied Indigo Buntings (*Passerina cyanea*) from pre-breeding to the post-breeding period to determine if males and females show different patterns in body condition and stress hormone levels between the pre-breeding, breeding, and post-breeding stages and if these patterns vary with the degree of forest fragmentation on the breeding grounds. During the pre-breeding stage, males had lower body condition and higher baseline and acute corticosterone levels than females. In addition, pre-breeding males had higher levels of baseline and acute corticosterone in the fragmented landscape than the unfragmented landscape, possibly resulting from higher densities of conspecifics. Breeding males had higher levels of acute corticosterone than breeding females. Breeding females in the fragmented landscape were in poorer condition (body condition index: -0.53 ± 0.12) than females in the unfragmented landscape (body condition index: -0.25 ± 0.07), likely due to high rates of nest predation and increased breeding effort. Among post-breeding birds, there was no difference in baseline or acute corticosterone levels between genders or landscapes. Post-breeding females in the fragmented landscape recovered body condition at a higher rate than did birds in the unfragmented landscape. I suggest that in Indigo Buntings, males and females exhibit different patterns of corticosterone secretion in relation to their breeding activity. Furthermore, fragmentation influenced breeding effort and foraging opportunities that further affected patterns in physiological responses.

INTRODUCTION

Migratory birds face many challenges associated with behavioral and physiological shifts in relation to life-history stage. Within the reproductive season, males and females may face different constraints associated with arrival, offspring care, post-breeding maintenance, and molt. For example, the pre-breeding stage may be more costly to males than females because of their increased activity associated with territorial defense. Males are typically the territorial sex and often precede non-territorial birds or females on arrival to breeding grounds (Francis and Cook 1986, 1990, Stewart et al. 2002). Upon arrival, territorial males must establish and defend territories and attract mates. These behaviors are often accompanied by elevated energy expenditure and testosterone levels (Wingfield 1985, Ketterson et al. 1991, Silverin 1993, Ketterson and Nolan 1999) that allow them to sustain high levels of aggression necessary for territory maintenance. However, elevated testosterone is also associated with a decrease in self maintenance (Ketterson et al. 1992) and increase in sensitivity to stressors (Schoech et al. 1999). Females often arrive after males have established territories and do not generally participate in expensive pre-breeding behaviors (Carey 1982, Francis and Cook 1986). The breeding stage is likely more costly for females since they produce the clutch and provide the majority of offspring care (Clutton-Brock 1991; Ligon 1999). Increased egg production is correlated with a decline in maternal condition (Monaghan et al. 1998, Visser and Lessells 2001) and may limit maternal survival (Visser and Lessells 2001). Less is known about the physiological demands of the post-breeding stage. The post-breeding stage may be especially important for fat deposition and recovery of body mass prior to molt and migration (Evans-Ogden and Stutchbury 1996, Svensson and Nilsson 1997). Emerging evidence shows that some adults and juveniles make short-distance dispersals from their breeding territories to habitat with dense vegetation where foraging opportunity is high (Anders et al. 1998, Vega Rivera et al. 1998, White 2005). However, prolonged breeding seasons and constraints on foraging sites may limit opportunities for post-breeding birds to prepare for migration (Fink 2003, Norris et al. 2004).

Glucocorticoid secretion, which is activated by the adrenocortical-hypothalamic-pituitary axis under stress, is one mechanism by which metabolism and behavior are regulated. In birds, secretion of the stress hormone, corticosterone, from the adrenal gland (Siegel 1980, Harvey et al. 1984, Wingfield 1994), alters behavior and metabolism thereby increasing the likelihood of immediate survival when birds are faced with energetically demanding conditions such as severe storms (Wingfield et al. 1983), predation (Scheuerlein et al. 2001), and periods of food limitation (Astheimer et al.

1992). Recent studies show that as energetic demands change with stages of the life cycle, some birds are able to seasonally modulate the stress response. Thus, organisms may prevent the deleterious effects of chronically elevated glucorticoid secretion such as suppression of reproductive and immune function (Munck et al. 1984, Silverin 1986, Wingfield 1988), protein loss (Holmes and Phillips 1976, Gray et al. 1990), and neuron death (Sapolsky 1992), depending on environmental and seasonal constraints.

The majority of studies that have documented seasonal modulation of the stress response in birds have investigated sub-arctic, arctic, or desert-breeding species (Wingfield et al. 1992, Romero and Wingfield 1998, Romero et al. 1998a, Romero 2003). Few studies have investigated seasonal patterns of corticosterone secretion in temperate-breeding passerines, presumably because temperate latitudes exhibit more favorable environmental conditions than sub-arctic or arctic latitudes. For instance, breeding birds in temperate latitudes may be less likely to experience short breeding seasons or severe storms that threaten food availability and immediate survival than birds at higher latitudes. However, temperate breeders may experience environmental quality that fluctuates seasonally and varies regionally due to the effects of forest fragmentation and habitat alteration.

Evidence from across the mid-western and eastern U.S. shows that forest fragmentation reduces habitat quality for breeding songbirds through effects of decreased food availability (Burke and Nol 1998, Zanette et al. 2000), altered microclimate (Chen et al. 1993), and increased nest predation and interspecific brood parasitism (Robinson et al. 1995, Donovan et al. 1995, Donovan et al. 1998, Porneluzi and Faaborg 1999). Additionally, forest fragmentation has been associated with physiological stress in breeding females (Morris 2005) and nestlings (Suorsa et al. 2003). Forest fragmentation leads to higher nest predation rates for many species of breeding songbirds by increasing the amount of edge habitat for nest predators (Chalfoun et al. 2002; Dijak and Thompson 2000). Nest predation decreases reproductive success and forces individuals to renest multiple times, thereby prolonging the breeding season and imposing higher energetic demands on breeding birds. Birds that experience prolonged breeding seasons may face trade-offs concerning reproductive success and molt and migration (Sanz 1999, Hemborg et al. 2001, Norris et al. 2004), incur lower fitness of adults and nestlings (Martin 1995, Svensson and Nilsson 1997), and recruit fewer juveniles into breeding populations (Verboven and Visser 1998, Visser and Verboven 1999). Therefore, the potential to modulate sensitivity to environmental stressors seems important for temperate breeding populations of songbirds where environmental conditions and habitat quality may be particularly unpredictable both spatially and temporally.

Based on different behavioral strategies used by females and males during the breeding season, I expected females and males to exhibit different physiological profiles of body condition and stress hormones. I investigated differences in body condition and stress hormone levels of Indigo Buntings (*Passerina cyanea*) between the pre-breeding, breeding, and post-breeding stages and determine if patterns vary with gender. Females provide the majority of offspring care, typically spending 2-8 days building nests, 12 days incubating, and ~ 10 days feeding nestlings before fledging (Payne 1992; per. obs., D.M.). Males rarely feed nestlings (5%-10% of visits, Payne 1992, D.M. per. obs.) but they often share or take over the care of fledglings (Payne 1992, D.M. per. obs.). I also determined if forest fragmentation affects these patterns by studying populations breeding in a large contiguous forest and in a fragmented landscape. Previous work in these two populations shows that nest success is low in both landscapes [unfragmented: 0.26 (95% CI: 0.19-0.32); fragmented: 0.17 (95% CI: 0.007-0.32)]. However, repeated renesting after nest predation forces 66% of females in the fragmented landscape to continue renesting through July and August, whereas, only 39% of females in the unfragmented landscape must continue attempts to produce one successful brood through mid-July (Morris 2005). Therefore, reproductive effort is greater in the fragmented landscape where I expected to see lower body condition and higher corticosterone levels of breeding females and males. I predict that because prolonged breeding limits the amount of time birds have for foraging, fat deposition, and pre-basic molt, post-breeding birds in the fragmented landscape would be in poorer condition and show higher adrenocortical responses to stress than birds in the unfragmented forest.

METHODS

Study Site

I studied Indigo Buntings in a contiguously-forested landscape in southeast Missouri and in forest fragments in central Missouri. In the unfragmented landscape in southeast Missouri, I studied Indigo Buntings during three breeding seasons (2000-2002) in three sites in the Current River Conservation Area (lat: 37.19^{o}; long: 91.00^{o}) within approximately 40,000 ha of contiguous oak-hickory forest. In the fragmented landscape in central Missouri, I studied buntings during one breeding season (2003) in two sites; Davisdale (1125 ha; lat: 39.00^{o}; long: 92.62^{o}) and Rudolf Bennit (1460 ha; lat.: 39.25; long: 92.45^{o}) Conservation Areas where the landscape is characterized by 30% forest cover. Each of the five sites is owned and managed by the Missouri Department of Conservation and within each landscape, sites were separated by > 5 km. In these sites, Indigo Buntings nest in early successional habitat created by forestry practices, agriculture, mowing, and natural disturbances such as wind storms.

Pre-breeding females and males were captured with mist-nets upon their arrival to breeding grounds in late-April or early May, before nest initiation. Arrival to breeding territories and nest initiation began two weeks earlier in southeast Missouri than in central Missouri. Most of these birds were captured in wildlife foodplots or grassy areas where they congregated to feed in the morning hours. As part of a broader study on the effect of nest predation on condition of breeding females, I used mist-nets to capture breeding adults at their nests when nestlings were six-days old. I captured post breeding birds that were beginning to show signs of molt and/or recession of brood patches and cloacal protuberances, while they flocked to forage on seeds in wildlife food plots during late July (2002 & 2003) in the contiguous forest and during the latter half of August (2003) in the fragmented sites. Very few individuals were repeatedly sampled during each stage, therefore, birds in each stage represent independent samples.

Physiological Measures

To determine if landscape, breeding stage, or gender affect physiological condition, I measured body mass (corrected for structural size), corticosterone levels, and hematocrit of adults. I used 26 g needles and heparinized microcapillary tubes to collect 50 µL of blood from the brachial vein within 1-5 minutes of capture. This sample was used as a baseline level of corticosterone (time-0) and indicates hormone levels prior to capture disturbance. Birds were held in cloth bags, and a subsequent sample collected at

30 minutes post-capture (time-30) provided a profile of the acute stress response to capture and handling (Wingfield et al. 1992, Wingfield 1994). Blood samples for corticosterone were kept on ice until centrifuged in a microhematocrit centrifuge (10 minutes) within 2-5 hours of collection. Plasma was drawn off using a Hamilton syringe and stored at -20 ^{0}C until assayed. Hematocrit (packed red blood cells/total volume) was measured on each baseline sample using calipers before plasma was collected.

After blood samples were taken, adults were aged, sexed, measured (unflattened wing chord; tarsus, to 0.1mm; and fat score), weighed (nearest 0.5 g), and banded with colored plastic and numbered aluminum USFWS bands. To determine body mass corrected for structural size, wing and tarsus length were entered into a principal components analysis. Body weight was then regressed against PC1 and the residuals from this analysis were used as estimates of mass corrected for body size (Green 2001) and presented as an index of body condition. Individuals falling above the expected values are considered heavy, given their size, and those falling below expected values are lean, given their size. In these study populations, adult males are 4% and 6% larger in wing and mass, respectively, than females (Morris, unpubl. data); therefore, I ran separate regressions for males, females, and juveniles. All blood samples and body measurements were taken between 0600 – 1000 hours to prevent bias in measurements due to daily fluctuations in body mass and corticosterone. Blood samples and handling were conducted under federal and state permits and were approved by the University of Missouri Animal Care and Use Committee.

Hormone Assays

Blood samples for corticosterone were assayed in duplicate using a commercially available I^{125} radioimmunoassay (RIA) kit (Biomedicals, Costa Mesa, CA). I followed the manufacturer's method, except I halved the volume of all reagents and samples were diluted 1:50 with steroid dilutent prior to assay. Parallelism and recovery of exogenous corticosterone validation assays were conducted to validate the utility, accuracy, and precision of this particular RIA kit for use with a suite of passerine birds (Washburn et al. 2002). Assays were run separately for each year of the study (2000-2003) and interassay variation was 12.5%, 3.7%, 8.9%, and 15%, respectively. Additionally, a control from the RIA kit was run in each assay and had an interassay variation across all years and assays of 6.9%. Intra-assay variation calculated from 60 randomly chosen samples across all assays was 3.9%.

Data Analysis

To examine patterns in corticosterone secretion within each gender, I ran separate 2-factor repeated measures analysis of variance tests with corticosterone levels at time-0 and time-30 as the repeated measure (time) and landscape and breeding stage as main effects. Besides examining mean baseline and acute hormone levels, the repeated factor indicates whether patterns in corticosterone secretion differ with landscape or breeding stage. Baseline and acute corticosterone levels were log-transformed to correct non-normality. I tested for differences in mean baseline and acute corticosterone levels with gender and landscape within each breeding stage using a 2-factor ANOVA. Within the post-breeding stage, a repeated measures ANOVA was used to determine if patterns of corticosterone secretion vary for males or females undergoing molt versus those that were not molting.

Body condition was examined separately for males and females because the indices used to derive their condition were based on separate regression lines. Within each sex, I tested for effects of landscape and breeding stage on body condition. To determine if baseline or acute corticosterone levels are dependent on body condition, I ran separate linear regressions for each gender within each breeding stage where baseline or acute corticosterone levels were response variables and body condition was the predictor variable. Differences in hematocrit with landscape, gender, or breeding stage were determined using a 3-way ANOVA. I also tested for a landscape effect on body condition of juvenile birds caught during the post-breeding stage using ANOVA. All tests were performed in SAS (SAS Inst. 2004). P-values less than 0.05 indicate statistical significance. Unless otherwise noted, data are presented as adjusted (least square) means ± standard error (SE).

RESULTS

Females

I sampled 19 females during the pre-breeding period, 186 breeding females, and 61 post-breeding females in both landscapes combined. Corticosterone profiles of females differed significantly with breeding stage (main effect of stage: $F_{2,161} = 3.86$, $P = 0.02$). Baseline and acute corticosterone levels were highest in the pre-breeding stage (3.80 ± 0.30 ng mL^{-1}; 4.43 ± 0.26 ng mL^{-1}, respectively), declined during the breeding stage (3.24 ± 0.05 ng mL^{-1}; 4.03 ± 0.04 ng mL^{-1}), and declined again during the non-breeding stage (3.11 ± 0.15 ng mL^{-1}; 3.68 ± 0.13 ng mL^{-1}). There was no effect of landscape or the interaction between landscape and stage on corticosterone profiles (Table 1). Plasma levels of corticosterone increased in response to capture and handling (effect of time: $F_{1,161} = 31.57$, $P < 0.0001$). There was no difference in the pattern of corticosterone secretion with landscape or breeding stage (Table 1). However, there was a significant interaction of the within-comparison factors (time*landscape*stage: $F_{2,161} = 3.80$, $P = 0.02$). During pre-breeding, the baseline corticosterone levels were slightly more elevated in females in the fragmented landscape (3.91 ± 0.20 ng mL^{-1}) than females in the unfragmented landscape (3.69 ± 0.58 ng mL^{-1}). However, the acute corticosterone response to capture stress was somewhat more elevated in females in the unfragmented landscape (4.51 ± 0.50 ng mL^{-1}) than in the fragmented landscape (4.35 ± 0.17 ng mL^{-1}; Fig. 1). Among breeding females, baseline and acute corticosterone levels were very similar between landscapes, but again, the pattern of corticosterone secretion was slightly higher for females in the unfragmented landscape (Fig. 1). During the post-breeding stage, the acute response to capture stress was lower for females in the unfragmented landscape (3.38 ± 0.22 ng mL^{-1}) than in the fragmented landscape (3.98 ± 0.13 ng mL^{-1}).

Males

Overall I sampled 69 pre-breeding males, 125 breeding, and 150 post-breeding males. Corticosterone profiles of males differed significantly with breeding stage ($F_{2,135} = 33.9$, $P = 0.0001$). Pre-breeding males had higher baseline and acute corticosterone levels than breeding or post-breeding males (Fig. 2). There was no effect of landscape on baseline corticosterone (unfragmented: 3.25 ± 0.09 ng mL^{-1}; fragmented: 3.45 ± 0.08 ng mL^{-1}). However, the interaction between breeding stage and landscape was significant

($F_{2,135}$ = 9.62, P = 0.0001). Baseline corticosterone levels during pre-breeding were higher in the fragmented landscape than in the unfragmented landscape (Fig. 2) otherwise; corticosterone levels were similar between landscapes in each stage (Fig. 2). Plasma levels of corticosterone increased in response to capture and handling (effect of time: $F_{1,135}$ = 234.3, $P < 0.0001$). There was no difference in the pattern of corticosterone secretion with landscape or breeding stage (Table 1; Fig. 2).

Pre-breeding Stage

Among pre-breeding birds, males had significantly higher levels of baseline corticosterone than females (3.93 ± 0.07 ng/mL^{-1} vs. 3.53 ± 0.12 ng/mL^{-1}; Table 2, Fig. 3a) and higher acute corticosterone than females (4.76 ± 0.07 ng/mL^{-1} vs. 3.88 ± 0.18 ng/mL^{-1}; Table 2, Fig. 3a). Pre-breeding birds in the fragments had higher baseline levels than pre-breeding individuals in the unfragmented sites (4.16 ± 0.09 ng/mL^{-1} vs. 3.30 ± 0.11 ng/mL^{-1}) and higher acute corticosterone levels (4.69 ± 0.10 ng/mL^{-1} vs. 3.95 ± 0.17 ng/mL^{-1}; Table 2).

Breeding Stage

Breeding birds did not show an effect of landscape ($F_{1,252}$ = 0.16, P = 0.69) or gender ($F_{1,252}$ = 0.18, P = 0.67) on baseline corticosterone (Table 3). However, males had significantly higher acute corticosterone levels than females (4.29 ± 0.05 ng/mL^{-1} vs. 4.03 + 0.04 ng/mL^{-1}; Fig. 3b).

Post-breeding Stage

Among post-breeding birds, neither baseline corticosterone nor acute corticosterone varied with landscape ($F_{1,44}$ = 0.85, P = 0.36; $F_{1,39}$ = 0.84, P = 0.36, respectively) or gender ($F_{1,44}$ = 1.53, P = 0.22, $F_{1,39}$ = 1.38, P = 0.24, respectively; Fig.

3c). However, for acute corticosterone, there was a significant interaction between landscape and gender (Table 4) with females in the fragmented sites secreting higher levels (3.98 ± 0.16 ng/mL^{-1}) than females in the unfragmented sites (3.38 ± 0.26 ng/mL^{-1}), although males in the fragmented sites tended to secrete lower levels (3.81 ± 0.15 ng/mL^{-1}) than males in the unfragmented sites (4.04 ± 0.21 ng/mL^{-1}; Fig. 3c).

Among post-breeding females that had started their pre-basic molt, baseline corticosterone levels were similar for females that had not begun to molt (3.05 ± 0.20 ng/mL^{-}1 vs. 3.09 ± 0.16 ng/mL^{-}1) but acute levels were lower for females that had begun to molt (molt: 3.38 ± 0.24 ng/mL^{-1}; no molt: 4.10 ± 0.19 ng/mL^{-1}; $F_{1,16} = 5.37$, $P = 0.03$). Males, on the other hand, did not show a difference in baseline or acute corticosterone with the onset of molt (time*molt: $F_{1,19} = 0.88$, $P = 0.36$).

Body Condition

Female body condition varied in response to breeding stage ($F_{2,265} = 43.9$, $P <$ 0.0001) with females in good condition during prebreeding, declining during breeding, and rebounding during the post-breeding stage (Fig. 4a). Additionally, body condition differed within breeding stages between landscapes (breeding stage*landscape: $F_{1,265} =$ 4.89, $P = 0.008$). In the fragmented sites, breeding females were in lower body condition (body condition index: -0.53 ± 0.12) than in the unfragmented sites (body condition index: -0.25 ± 0.07); however post-breeding females in the fragments were in better body condition (body condition index: 1.01 ± 0.14) than post-breeding females in the unfragmented sites (body condition index: 0.48 ± 0.15; Fig. 4a). Body condition of males tended to slowly increase from the pre-breeding to the post-breeding stage (main effect of breeding stage, $F_{2,243} = 5.03$, $P = 0.007$; Fig. 4b); however, there was no difference in body condition between landscapes ($F_{1,243} = 0.58$, $P = 0.45$).

Fat scores of males and females coincide with changes in body condition throughout the season. In females, 9.1% (2/23) of pre-breeding females, 0.57% (1/173) of breeding females, and 9.4% (5/53) of post-breeding females had fat scores above 0.5. In males, the pattern generally followed their increase in body condition as the season progressed (pre-breeding:11.1% (8/72); breeding: 13.3% (16/120); post-breeding: 8.5% (10/118). Juvenile birds caught in both landscapes during the post-breeding stage did not vary significantly in body condition ($F_{1,115} = 0.30$, $P = 0.58$).

Among females, baseline corticosterone was not related to body condition, regardless of breeding stage (pre-breeding: $r = 0.008$, $df = 12$, $P = 0.76$; breeding: $r = 0.006$, $df = 153$, $P = 0.31$; post-breeding: $r = 0.02$, $df = 19$, $P = 0.58$). Acute corticosterone was negatively related to body condition of breeding females ($r = 0.05$, $df = 145$, $P = 0.008$, parameter estimate: -0.13 ± 0.04), but not pre- or post-breeding females ($r = 0.36$, $df = 9$, $P = 0.06$; $r = 0.01$, $df = 17$, $P = 0.67$, respectively).

In males, baseline corticosterone was negatively related to body condition during the post-breeding stage ($r = 0.15$, $df = 23$, $P = 0.05$, parameter estimate, -0.44 ± 0.21) but not during the pre-breeding ($r = 0.01$, $df = 33$, $P = 0.53$) or breeding stages ($r = 0.01$, $df = 92$, $P = 0.39$). Acute corticosterone was not related to male body condition regardless of breeding stage (pre-breeding: $r = 0.01$, $df = 38$, $P = 0.52$; breeding: $r = 0.001$, $df = 92$, $P = 0.77$; post-breeding: $r = 0.02$, $df = 21$, $P = 0.49$).

Hematocrit

Hematocrit was higher in birds in the unfragmented landscape (0.511 ± .01) than in the fragmented landscape (0.469 ± 0.01; main effect of landscape: $F_{1,344} = 20.81$, $P < 0.0001$) and decreased as the breeding season progressed (breeding stage: $F_{2,344} = 6.49$, $P = 0.002$). However, hematocrit in the fragmented landscape tended to increase as the breeding season progressed, while hematocrit in the unfragmented landscape decreased from pre-breeding to post-breeding (landscape*stage interaction: $F_{2,344} = 9.06$, $P = 0.0001$; Fig. 5). There was no effect of gender on hematocrit ($F_{1,344} = 0.79$, $P = 0.37$).

DISCUSSION

Males and females had different patterns in body condition and sensitivity to stressors with changes in breeding status and parental activity and these patterns appear to be affected, to some degree, by environmental constraints. In general, female body condition was high pre-breeding, declined during breeding, and rebounded after breeding. Males, on the other hand, showed poor body condition during pre-breeding and tended to recover body condition as the season progressed. Baseline and acute corticosterone levels of females and males were highest during pre-breeding and declined steadily with each successive stage. Similarly, Pereyra and Wingfield (2003) found that corticosterone levels declined from pre-nesting to initiation of clutches for female and male Dusky Flycatchers (*Empidonax oberholseri*), perhaps in order to prevent conflict between high corticosterone and reproduction.

Although not well understood, seasonal regulation of corticosterone secretion may occur in response to changes in body condition (Wingfield et al. 1994), pituitary sensitivity to adrenocorticotropin hormone (ACTH) released from the hypothalamus

(Romero et al. 1998c, Romero and Wingfield 1998), adrenal sensitivity to ACTH (Romero and Wingfield 1998, Romero et al. 1998b, Romero et al. 1998c), and changes in sensitivity or availability of corticosterone receptors (Breuner and Orchinik 2001) and binding proteins (Deviche et al. 2001, Romero et al. 1998b). Modulation of the stress response may protect organisms from harmful effects of elevated corticosterone, thereby facilitating physiological or behavioral responses during different life-history stages (see review by Romero 2002) or under different ecological conditions (Wingfield et al. 1995). For example, birds that experience short breeding seasons at high latitudes exhibit reduced adrenocortical responses to stress than birds at lower latitudes (Wingfield et al. 1994, Wingfield et al. 1995, Wilson and Holberton 2004), possibly as a way to balance the conflict between responsiveness to stress and reproductive success. Results for Indigo Buntings indicate the acute adrenocortical response to capture stress varies with stage of breeding in relation to changes in the degree of parental activity and energy expenditure.

Within the pre-breeding stage, males had lower body condition and higher baseline and acute corticosterone levels than females. Lower body condition and increased sensitivity to stress of pre-breeding males suggests they were energetically stressed due to their high level of activity associated with territory establishment and defense. Testosterone levels of territorial males have been shown to be highest during territory establishment and decline once nesting has begun (Silverin 1993). On the other hand, females had just arrived to the breeding sites and were choosing mates and territories but had not begun to lay clutches, and thus, were not likely to be expending as much energy in breeding activities yet.

Although there were no differences in body condition or corticosterone secretion by pre-breeding females between landscapes, pre-breeding males had higher levels of baseline and acute corticosterone in the fragmented landscape than the unfragmented landscape. Silverin (1998) found higher corticosterone and testosterone levels in male Pied Flycatchers (*Ficedula hypolueuca*) breeding in higher population densities, due to increased rates of territorial intrusion and persistent territorial maintenance. A *post-hoc* analysis of distance to nearest neighbor (measured during the breeding stage) reveals that Indigo Bunting territories were closer together in the fragmented landscape (72.7 ± 14.28 m, 95% C.I.: 44.4 – 101.1 m; $F_{1,101} = 20.4$, $P < 0.0001$) than in the unfragmented landscape (149.1 ± 9.0, 95% C.I.: 131.2 – 166.9 m). Therefore, with higher breeding densities, competition for territories was likely more intense during the prebreeding stage in the fragmented landscape and males suffered costs from this increased energetic effort.

Among breeding birds, males had higher levels of acute corticosterone than females. However, body condition of actively breeding females was lower than males during this stage, suggesting the effort associated with breeding is higher for females. Although females in poor condition may benefit from a more robust response to environmental stressors during this period of high energy expenditure, they show a lower acute response to capture stress than males. This pattern coincides with other studies that have shown that the gender with the greatest parental effort or population breeding under more severe environmental constraints tends to show a reduced response to acute stressors during breeding (Wingfield et al. 1995, Silverin and Wingfield 1998, O’Reilly and Wingfield 2001, Holberton and Wingfield 2003, Wilson and Holberton 2004). These studies suggest that lower sensitivity to stressors prevents elevated corticosterone levels from disrupting reproductive function (Greenberg and Wingfield 1987, Wingfield 1988). Nevertheless, breeding female Indigo Buntings exhibit a negative relationship between body condition and the acute stress response. This suggests that poor-conditioned females, despite their reproductive status, are dependent on the adrenocortical response during capture stress because they lack the energy reserves necessary to “ride out” the disturbance (Wingfield et al. 1994).

Breeding effort for females was higher in the fragmented landscape due to repeated renesting after nest predation (see Morris 2005, Chp. 1). Lower body condition indices of females breeding in the fragmented landscape reflect this effort, even though corticosterone levels do not differ in females breeding in either landscape. When faced with more severe constraints on nesting, females in the fragmented landscape may lose body condition while modulating corticosterone levels in order to cope with a sustained breeding effort while retaining reproductive function (Greenberg and Wingfield 1987, Wingfield 1988).

Among post-breeding birds, there was no difference in baseline or acute corticosterone levels between males or females. However, during this stage, females that had begun to molt showed a dampened stress response, although males did not exhibit this reduced response, even though a similar proportion of males (8.5%) and females (9.4%) had begun to molt. However, the small sample size of males that were sampled for corticosterone during molt (n = 4) likely prevented detection of a difference. Molt is considered to be energetically costly (Murphy and King 1992) and several studies have shown that sensitivity of the adrenocortical response is lower during molt (Astheimer et al. 1994, Romero et al. 1998a, Romero et al. 1998b). A decrease in the adrenocortical response to capture stress in birds during molt may prevent protein mobilization and other costly physiological responses in addition to the constraints of molt (Astheimer et al. 1995, Romero et al. 1998b). Interestingly, male Indigo Buntings mated to late-breeding females in the fragmented sites often abandoned their territories, presumably in favor of starting molt and pre-migratory fattening (D. M., pers. obs.). Prolonged breeding seasons have been shown to limit the time available for pre-migratory molt and lead to combining molt and migration in American Redstarts (*Setophaga ruticilla*), leading to production of lower quality feathers (Norris et al. 2004). This trade-off between breeding and pre-basic molt may be more important in populations that experience prolonged breeding periods and constraints on the timing of molt (Sanz 1999), such as the bunting population in the fragmented landscape.

Overall, post-breeding birds showed no difference in corticosterone secretion with landscape, although males and females tended to show opposite trends by landscape. Females in the fragmented landscape secreted higher levels of corticosterone in response to capture stress than females in the unfragmented landscape. However, males in the fragments secreted lower levels of acute corticosterone than males in the unfragmented sites. Despite the prolonged breeding period, post-breeding females and males in the fragmented landscape recovered body condition at a higher rate than did birds in the unfragmented landscape. The fact that females in the fragmented landscape were in better physical condition, yet had higher adrenocortical responses to capture stress is perplexing. In the fragmented landscape, wildlife food plots were in closer proximity to breeding territories than in the contiguous forest. In these sites, I may have captured some females feeding in these plots while they were still caring for young, thereby reflecting higher body condition, but not the seasonal downward shift in corticosterone.

Hematocrit levels of males and females combined were lower in the fragmented landscape during pre-breeding and breeding. Low hematocrit levels during reproduction may indicate nutritional deficiencies (Jones 1983, Polo et al. 1992). During post-breeding, hematocrit levels showed a marked decline in birds in the unfragmented landscape. A decline in hematocrit has been observed in ducks (Driver 1981) and passerines undergoing post-nuptial molt (Chilgren and DeGraw 1977, DeGraw and Kern 1985), presumably because blood volume increases during molt but the total number of circulating erythrocytes does not (Chilgren and DeGraw 1977). Therefore, the decline in hematocrit among post-breeding birds in the unfragmented landscape could be due to a larger proportion of birds undergoing molt, rather than to nutritional stress.

Recent studies suggest songbirds require diverse habitats during each stage of reproduction to meet their resource needs. Besides nest-site requirements, species may also require or benefit from particular habitat adjacent to breeding territories that provides food and cover for newly-fledged or dispersing offspring (Anders et al. 1998, White et al. 2005), post-breeding adults (Pagen et al. 2000), and molting birds (Vega Rivera et al. 1998a). The extensive matrix of early-successional habitat, like agricultural fields and wildlife food plots, which fragment central Missouri forests, may actually provide better foraging opportunities for post-breeding and dispersing birds. Survival of juvenile Wood Thrush (*Hylochichla mustelina*) has been shown to be higher in fragmented forests than contiguous forests (Fink 2003). Additionally, post-breeding families of Wood Thrush and Swainson’s Thrush move into early successional or scrub habitat with greater shrub cover for predator avoidance and better foraging opportunities (Anders et al. 1997, Vega Rivera et al. 1998b, White et al. 2005). Similarly, forests with some mix of agricultural edge have been shown to be important stopover habitats for fall migrants (Rodewald and Brittingham 2004). Moreover, I found no difference in body condition of juvenile Indigo Buntings caught during the post-breeding stage in either landscape despite the fact that most juveniles caught in the fragmented sites likely fledged several weeks later than juveniles in the unfragmented sites. Therefore, proximity or abundance of habitat beneficial for post-breeding activities may compensate for the costs of increased nest predation and prolonged breeding efforts in fragmented landscapes.

These results indicate that environmental factors such as breeding densities, predation pressure, and availability of open grassy areas and agricultural habitat contribute to variation in energetic condition of breeding Indigo Buntings. Seasonal modulation of corticosterone secretion in these populations seems to be related to energetic costs incurred differently by males and females during different stages of the breeding season. Although environmental conditions in the fragmented landscape seem less favorable due to the increased breeding effort by females, post-breeding foraging opportunities allow birds to quickly recover body condition and perhaps compensate for a prolonged breeding season. Further study is needed on the differences in reproductive effort between males and females under different environmental conditions to provide greater insight into the effect of environmental constraints on physiological responses and reproductive success.

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Table 1. Results of repeated measures ANOVA testing for between-subject factors (landscape: fragmented, unfragmented; breeding stage: pre-breeding, breeding, post-breeding) and within-subject factors (time: repeated measure of baseline and acute corticosterone) and interactions for female and male Indigo Buntings in Missouri (2000-2003).

Source	*df*	Mean Square	*F-Statistic*	*Prob > F*
Females				
Between				
Landscape	1	0.12	0.30	0.58
Breeding Stage	2	1.61	3.86	0.02
Landscape*Breeding Stage	2	0.12	0.29	0.74
Error	161	0.42		
Within				
Time	1	5.49	31.57	< 0.0001
Time*Landscape	2	0.01	0.09	0.76
Time*Breeding Stage	2	0.15	0.86	0.42
Time*Landscape*Stage	2	0.66	3.80	0.02
Error	161	0.17		
Males				
Between				
Landscape	1	0.71	1.98	0.16
Breeding Stage	2	12.09	33.90	0.001
Landscape*Breeding Stage	2	3.43	9.62	0.001
Error	135	0.35		
Within				
Time	1	44.1	234.3	< 0.0001
Time*Landscape	2	0.23	1.23	0.27
Time*Breeding Stage	2	0.12	0.62	0.53
Time*Landscape*Stage	2	0.32	1.69	0.18
Error	135	0.18		

Table 2. ANOVA results of baseline and acute corticosterone of Indigo Buntings during the pre-breeding stage. Landscape = fragmented or unfragmented. Gender = male or female.

Source	*df*	Mean Square	*F-Statistic*	*Prob > F*
Baseline Corticosterone				
Landscape	1	6.99	32.49	< 0.0001
Gender	1	1.51	7.01	0.01
Landscape*Gender	1	0.03	0.13	0.72
Error	47	0.21		
Acute Corticosterone				
Landscape	1	2.93	13.15	0.0007
Gender	1	4.29	19.26	< 0.0001
Landscape*Gender	1	0.24	1.09	0.30
Error	45	0.22		

Table 3. ANOVA results of baseline and acute corticosterone of Indigo Buntings during the breeding stage. Landscape = fragmented or unfragmented. Gender = male or female.

Source	*df*	Mean Square	*F-Statistic*	*Prob > F*
Baseline Corticosterone				
Landscape	1	0.06	0.16	0.69
Gender	1	0.07	0.18	0.67
Landscape*Gender	1	0.36	0.93	0.33
Error	249	0.38		
Acute Corticosterone				
Landscape	1	0.04	0.20	0.65
Gender	1	3.23	14.94	0.0001
Landscape*Gender	1	0.00	0.01	0.94
Error	240	0.22		

Table 4. ANOVA results of baseline and acute corticosterone of Indigo Buntings during the post-breeding stage. Landscape = fragmented or unfragmented. Gender = male or female.

Source	*df*	Mean Square	*F-Statistic*	*Prob > F*
Baseline Corticosterone				
Landscape	1	0.47	0.85	0.36
Gender	1	0.84	1.53	0.22
Landscape*Gender	1	0.12	0.22	0.64
Error	41	0.55		
Acute Corticosterone				
Landscape	1	0.28	0.84	0.36
Gender	1	0.47	1.38	0.24
Landscape*Gender	1	1.43	4.25	0.04
Error	36	0.34		

Figure 1. Corticosterone profiles of female Indigo Buntings during pre-breeding, breeding, and post-breeding stages in the fragmented and unfragmented landscape. Values represent mean baseline (Time-0) and acute (Time-30) corticosterone levels ± SE. Corticosterone levels were similar during prebreeding, however, there was a significant interaction between baseline and acute levels between landscapes. Breeding-stage corticosterone levels were similar between landscapes. Acute corticosterone was significantly lower in post-breeding females in the unfragmented than the fragmented landscape.

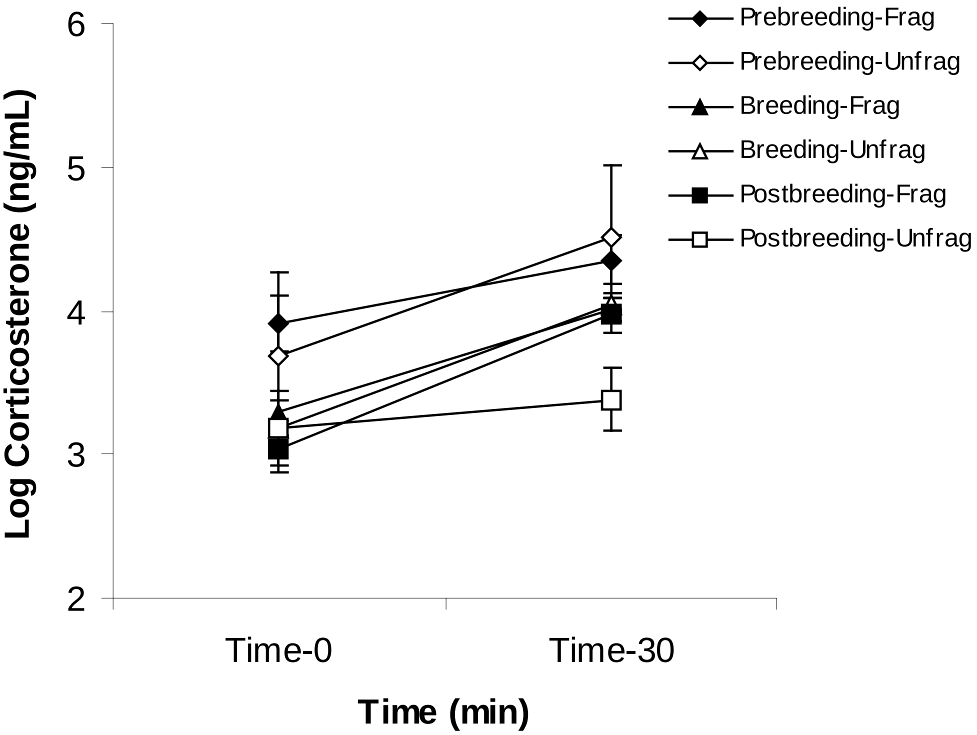

Log Corticosterone (ng/mL)
6
5
4
3
2
Time-0
Time-30
Time (min)
Prebreeding-Frag
Prebreeding-Unfrag
Breeding-Frag
Breeding-Unfrag
Postbreeding-Frag
Postbreeding-Unfrag

Figure 2. Corticosterone profiles of male Indigo Buntings decline from pre-breeding, breeding, to post-breeding stages in the fragmented and unfragmented landscape. Values represent mean baseline (Time-0) and acute (Time-30) corticosterone levels ± SE. Baseline and acute corticosterone levels are significantly higher for pre-breeding males in the fragmented landscape. There were no differences in corticosterone secretion between landscapes during breeding or post-breeding.

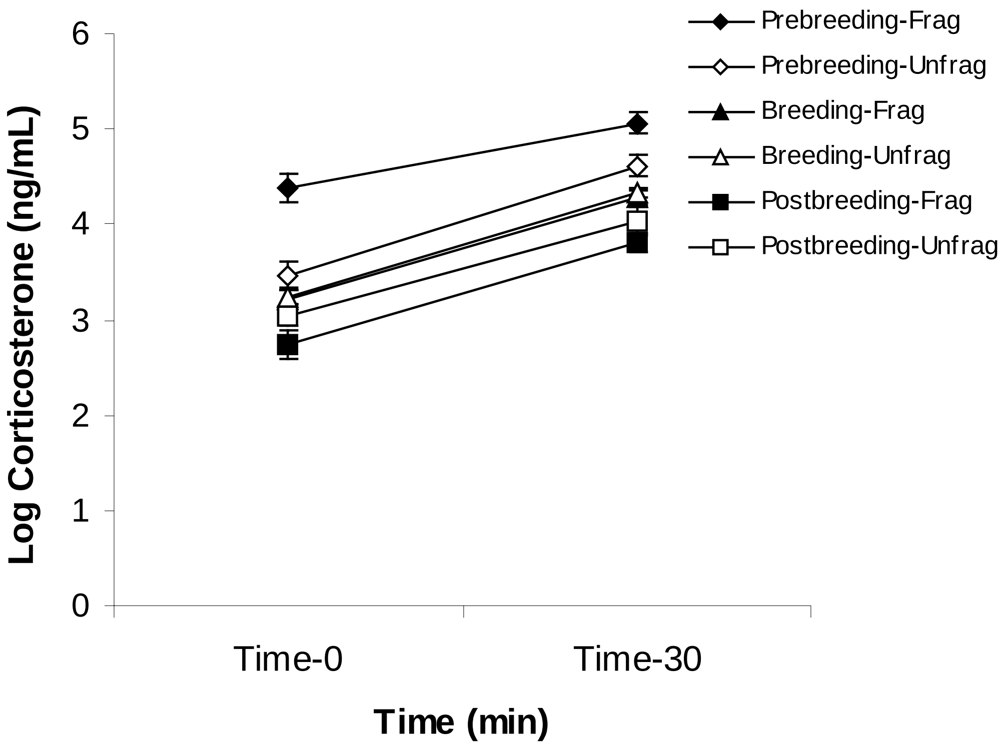
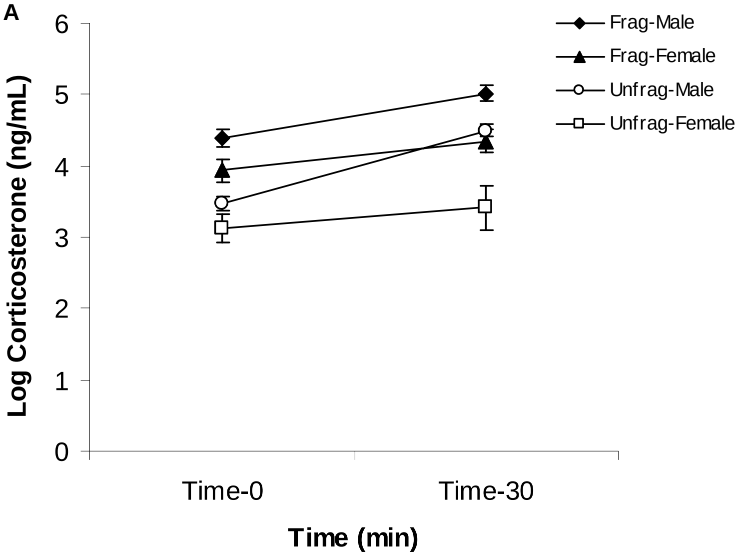
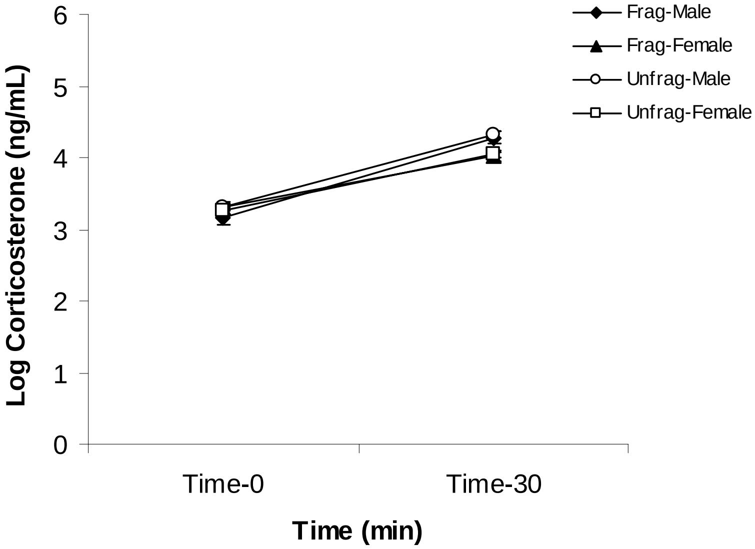

Prebreeding-Frag
Prebreeding-Unfrag
Breeding-Frag
Breeding-Unfrag
Postbreeding-Frag
Postbreeding-Unfrag
Log Corticosterone (ng/mL)
6
5
4
3
2
1
0
Time-0
Time-30
Time (min)

Figure 3. A) Mean baseline (Time-0) and acute corticosterone (Time-30) levels of prebreeding birds are higher in the fragmented landscape. B) Among breeding birds, there was no effect of landscape or gender on mean baseline corticosterone levels, although males had higher acute levels. C) Mean baseline and acute corticosterone did not vary with landscape or gender for postbreeding birds, however, females in the fragmented landscape had higher acute levels than females in the unfragmented landscape.

A


6
5
4
3
2
1
0
Log Corticosterone (ng/mL)
Time-0
Time-30
Time (min)
Frag-Male
Frag-Female
Unfrag-Male
Unfrag-Female

B


6
5
4
3
2
1
0
Log Corticosterone (ng/mL)
Time-0
Time-30
Time (min)
Frag-Male
Frag-Female
Unfrag-Male
Unfrag-Female

C

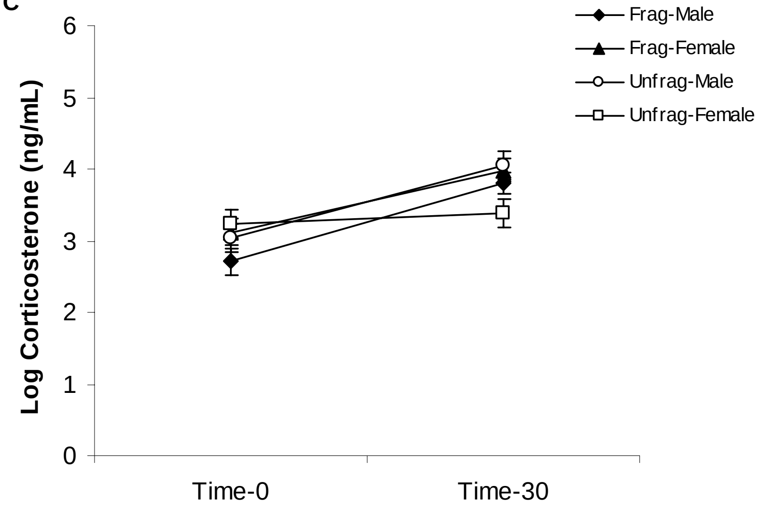

6
5
4
3
2
1
0
Log Corticosterone (ng/mL)
Time-0
Time-30
Frag-Male
Frag-Female
Unfrag-Male
Unfrag-Female

Figure 4. A) Body condition of female Indigo Buntings varies with breeding stage and post-breeding females in the fragmented landscape are significantly heavier than post-breeding females in the unfragmented landscape. B) Body condition of male Indigo Buntings varies with breeding stage but there is no effect of landscape. Values shown are mean indices of body condition ± SE.

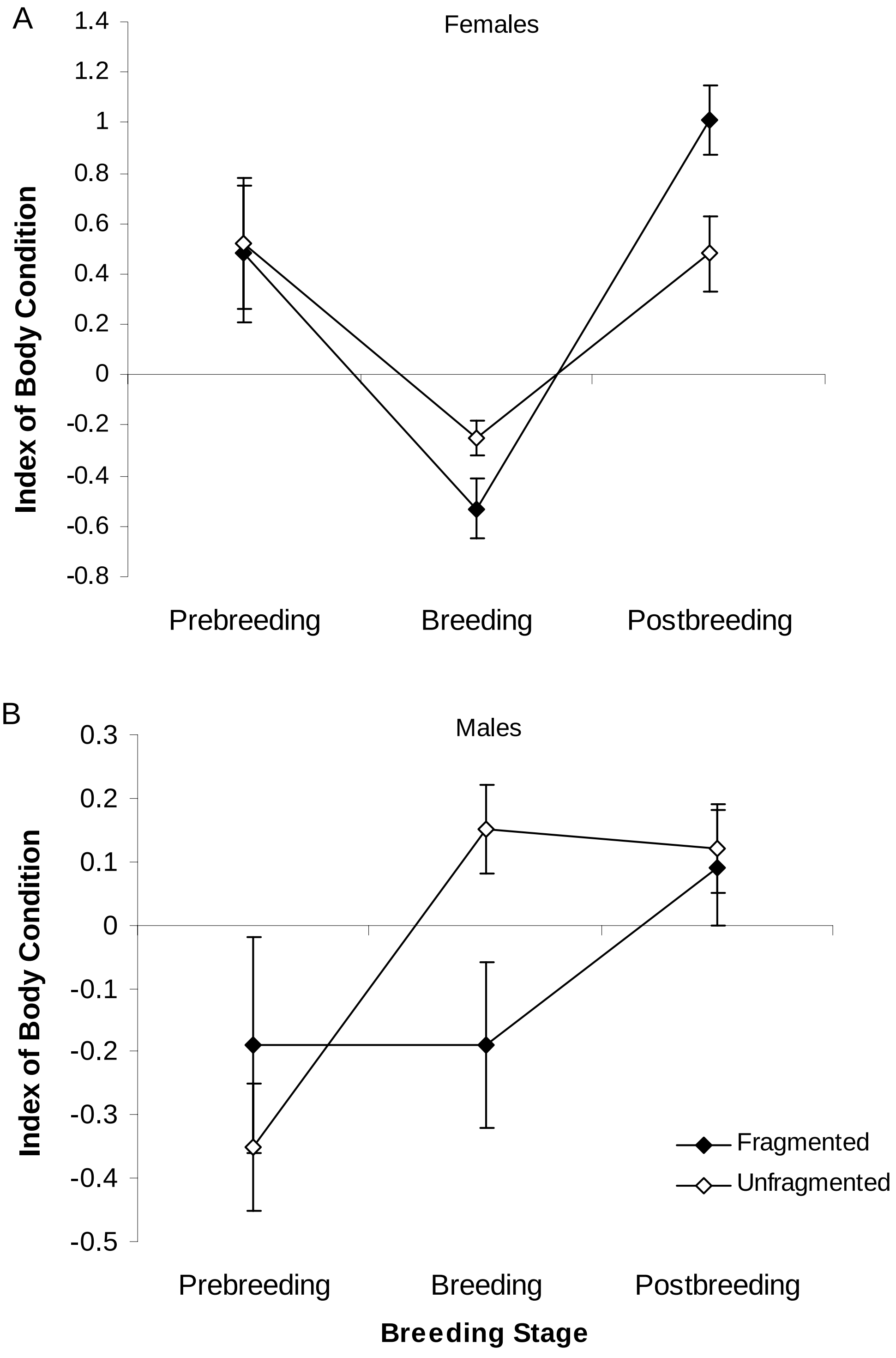
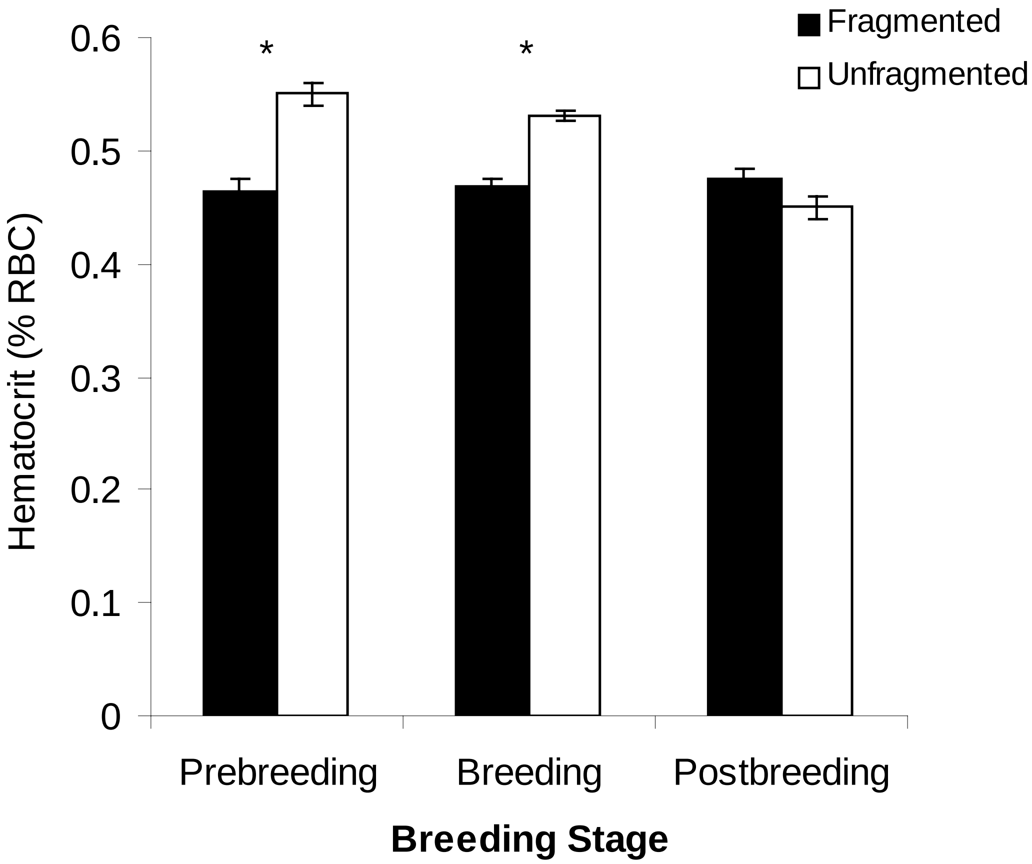

A
Females
Index of Body Condition
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
Prebreeding
Breeding
Postbreeding
B
Males
Index of Body Condition
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
Fragmented
Unfragmented
Prebreeding
Breeding
Postbreeding
Breeding Stage

Figure 5. Hematocrit of male and female Indigo Buntings varies with stage of the breeding season and landscape. Significant pairwise-comparisons are indicated with an asterisk.


■ Fragmented
□ Unfragmented
0.6
0.5
0.4
0.3
0.2
0.1
0
Hematocrit (% RBC)
*
*
Prebreeding
Breeding
Postbreeding
Breeding Stage

VITA

Dana Morris was born April 8, 1971, in Independence, Missouri. After attending Blue Springs High School, she attended Northwest Missouri State University (1989-1992) and transferred to the University of Missouri-Columbia where she received her B.S. in Fisheries and Wildlife Sciences (1993) and her M.S. in Fisheries and Wildlife Sciences (1996). She has worked for various natural resources agencies in Missouri; worked on research projects in Missouri, Virginia, and Puerto Rico; taught in public schools and private colleges; and was recently a research associate with the African Conservation Centre in Nairobi, Kenya. She is married to Paul Porneluzi (Ph.D. Biology ‘96) of Butler, Pennsylvania. She is presently on staff as an instructor in the department of Fisheries and Wildlife Sciences and is coordinator of the Mizzou Tigers for Tigers mascot conservation program at the University of Missouri-Columbia.