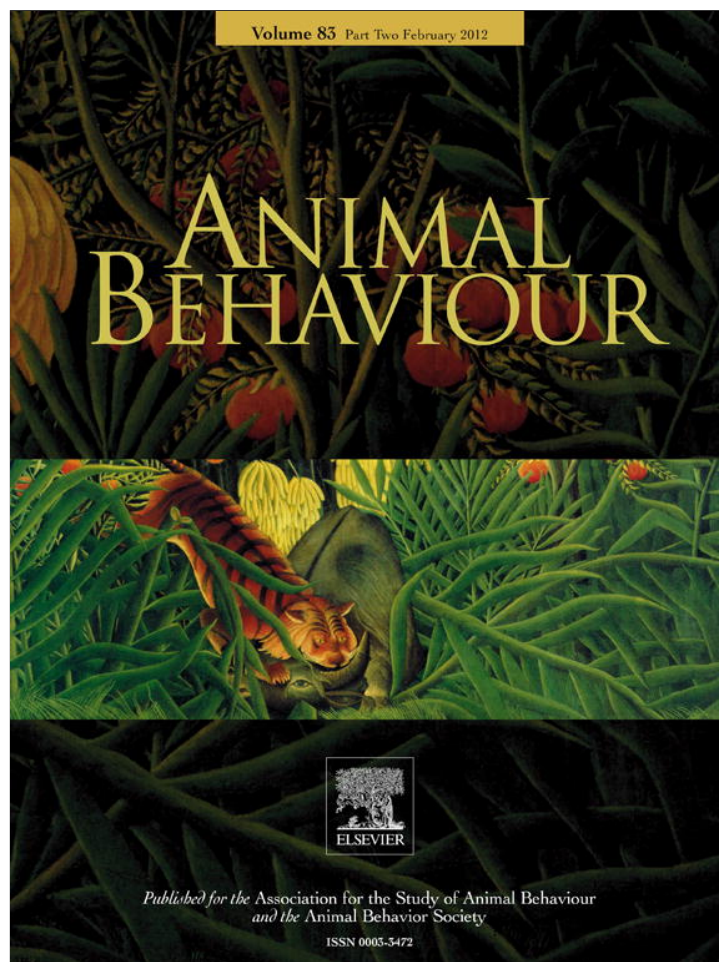


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Experimentally increased egg production constrains future reproduction of female house wrens

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Theory predicts that investment in current offspring should negatively influence a parent's ability to invest in future offspring. Despite extensive interest in documenting reproductive costs in birds, evidence for fitness-related costs of egg production to breeding females is scarce. In this study, we used an egg-removal experiment on the house wren, *Troglodytes aedon*, to test the hypothesis that producing eggs is reproductively costly. By removing eggs from the nest as they were laid, we induced females to produce more eggs than normal, although experimental and control females incubated clutches of similar size. Females producing extra eggs paid steep fitness costs for their increased effort; relative to controls, females with increased egg-laying demands were less likely to reproduce again within the same breeding season, and those that did took longer to do so, had a smaller clutch size and produced fewer fledglings from their subsequent broods. Although females in each treatment group were equally likely to return to breed the following summer, experimental females produced fewer eggs than control females in their next breeding season. This is the first demonstration that increased effort in egg production reduces the future reproductive output of passerine birds.

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Essential to our understanding of parental investment is the role played by trade-offs in life-history evolution (Roff 1992; Stearns 1992). Generally, any investment increasing the reproductive value of individual offspring necessarily reduces a parent's ability to invest in other offspring (Trivers 1972), leading to a classical trade-off between the number and quality of offspring (Smith & Fretwell 1974; Trivers 1974; Pianka 1976; Daan & Tinbergen 1997). However, individuals of iteroparous species must also balance their investment in current versus future offspring to maximize fitness (Williams 1966; Trivers 1972; Charnov & Krebs 1974), as increased investment should reduce an individual's residual reproductive value (Williams 1966; Lessells 1991).

Although considerable effort has been devoted to documenting the costs of reproduction, the stage(s) of reproduction at which costs arise and the mechanisms generating them remain incompletely understood (Roff 1992; Stearns 1992; Williams 2005; Harshman & Zera 2007). In birds, the provisioning of nestlings and, to a lesser extent, the incubation of eggs have been traditionally viewed as more demanding on parents than egg production (Monaghan & Nager 1997). However, there is accumulating

evidence that egg production itself imposes reproductive costs (reviewed in Nager 2006). For example, Visser & Lessells (2001) showed that female great tits, *Parus major*, that received 'free eggs', and thus avoided having to produce them, had higher fitness estimates than control females producing a clutch of normal size, an effect arising through the increased return rates of 'free-egg' females in the next breeding season. Similarly, Nager et al. (2001) used an egg-removal experiment on lesser black-backed gulls, *Larus fuscus*, to induce females to lay more eggs than they normally would, and they found that, although the production of supernumerary eggs did not affect future clutch sizes, those with increased egg production in one year had lower return rates and produced smaller eggs the next breeding season. Egg-removal experiments can also provide insight into trade-offs within a reproductive attempt; Monaghan et al. (1995) and Nager et al. (2000) showed that supernumerary eggs decline in mass relative to normal, earlier-laid eggs of the clutch, and also contain disproportionately lower lipid content (Nager et al. 2000). Not surprisingly, the nestlings hatching from these eggs are less likely to survive to fledging than those from earlier-laid eggs (Monaghan et al. 1995; Nager et al. 2000), illustrating a within-brood trade-off between offspring quality and number (see also Heaney & Monaghan 1995). Similarly, Mänd et al. (2007) performed an egg-removal experiment in great tits and also found a decline in egg mass among supernumerary eggs compared with earlier-laid eggs.

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In this study, we experimentally induced female house wrens, *Troglodytes aedon*, to produce more eggs than they normally would to test the hypothesis that egg production is reproductively costly to breeding females. House wrens are 'addition indeterminate' egg layers that will lay additional eggs beyond the expected clutch size if eggs are removed from the nest during the egg-laying period (see [Methods](#)). Thus, by removing eggs from females' nests during egg laying, we caused them to produce more eggs (9–10 total eggs) than they normally would (typically 6–7 eggs). We then removed eggs from experimental nests upon clutch completion, causing females in each treatment group to incubate a similar number of eggs. We predicted that if producing supernumerary eggs imposes reproductive costs on females, those with increased egg production should face greater fitness-related consequences of that increased effort relative to controls through: (1) a reduced likelihood of producing a second brood, and reduced clutch size and fecundity of second broods later in the same breeding season and (2) reduced return rates and fecundity of females the following summer.

METHODS

Study Site and Species

We studied a population of house wrens breeding during 2010–2011 in McLean County, Illinois, U.S.A. (40°40'N, 88°53'W). This habitat is characterized by secondary deciduous forest with a dense, herbaceous understory. Nestboxes ($N = 820$) were spaced 30 m apart along north–south-oriented transects separated by 60 m (5.4 nestboxes/ha). The nestboxes were placed on 1.5 m metal poles and rested atop aluminium discs (48.3 cm diameter) to deter predators; details of nestbox construction materials and dimensions can be found in [Lambrechts et al. \(2010\)](#).

House wrens are small (10–12 g), migratory songbirds distributed widely across the midsection of North America. Males typically arrive on the study area in late April, and select and defend a nestbox in which they construct a platform of large sticks. The later-arriving females select a mate and complete the nest before laying one egg/day until their clutch of four to eight eggs is complete (first brood mean = 6.7–7.0; see Table 1 in [Dobbs et al. 2006](#)). Approximately half of the females that complete a successful breeding attempt in May attempt a second brood on the study area, with peak egg production in early May for early-season broods, and early July for late-season broods. House wrens are well suited for manipulation of egg-laying effort because females lay additional eggs if they are removed from the nest during egg laying (see [Procedures and Experimental Design](#)). Unlike many species, house wrens routinely destroy eggs and kill nestlings of conspecifics ([Belles-Isles & Picman 1986](#); E. K. Bowers, personal observation), so females are thought to have been selected for the ability to replace missing eggs if they are removed or depredated during egg production ([Kennedy & Power 1990](#)).

Procedures and Experimental Design

Beginning in May 2010, we checked nestboxes at least twice weekly for evidence of female settlement. Once a nest was complete, we made daily visits to the nest, numbered eggs as laid using nontoxic, permanent markers, and weighed them to the nearest 0.001 g with an electronic balance (Acculab Pocket Pro 2060D). We captured females approximately halfway through incubation (mean \pm SE = 7.3 \pm 0.3 days into incubation; hatching begins after 12–13 days of incubation) and were, thus, unable to obtain data on female condition prior to implementing the egg-removal treatment. Capture was delayed until this point because females are likely to abandon the nest if captured prior to clutch

completion and the onset of incubation (C. F. Thompson, unpublished data). We visited nests daily when hatching was expected to begin to determine the date of hatching, banded nestlings 11 days after hatching, and subsequently visited nests each day to determine the date of fledging.

We established our egg-removal treatments at neighbouring sites on our study area, the East Bay and Mackinaw sites, 1400 m apart, between which individuals freely move. Females were assigned either to a control treatment ($N = 61$, Mackinaw site), in which they were allowed to complete their clutches naturally, with weighing and marking of eggs representing the only disturbance to the nest, or to an experimental treatment ($N = 31$, East Bay site), in which eggs were removed on the day they were laid. We established our treatments in this way to avoid manipulating nonexperimental females at the Mackinaw site, which were part of a longer-term, nonmanipulative study. Comparing reproductive parameters of females on the two plots during the two previous breeding seasons showed no differences for any of the breeding parameters investigated in this study, including the date of clutch initiation, clutch size, probability of being double-brooded, the interbrood interval, or the difference in clutch size between broods of double-brooded females (all $P > 0.35$); clutch initiation dates for each treatment group also did not differ in this study ($P = 0.39$). Such findings are identical to those of earlier studies comparing females at the two sites ([Drilling & Thompson 1988, 1991](#)), confirming that there are no site-specific differences in female reproductive performance within the population. For the experimental treatment, we removed a total of four eggs (numbers 2–5 in the laying sequence) from the nest on the morning each was laid, leaving one egg in the nest throughout this time to avoid female abandonment, and stored them in the laboratory at room temperature in artificial nests lined with soft polyester filling. When egg 6 was laid, we returned eggs 2–5 to the nest and continued daily visits to number and weigh subsequent eggs, which most females continued to produce for several days. It is generally thought that the tactile stimulation of eggs on the brood patch signals the female to cease egg production and commence full incubation as part of a neuroendocrine process involving maternal hormones, such as prolactin ([Mead & Morton 1985](#); [Lea & Klandorf 2002](#); [Vleck 2002](#)); this process is not instantaneous, so females produce supernumerary eggs.

When experimental females completed egg laying, we replaced eggs 5–10 (if present) with artificial eggs, so that females in each treatment would incubate a similar number of eggs (experimental: 6.0 ± 0.2 eggs, $N = 61$ nests; control: 5.6 ± 0.3 eggs, $N = 31$ nests; unequal variances $t_{39,9} = 1.05$, $P = 0.3$), although the number of eggs they incubated was less than the number they actually produced (see [Results](#)) and was not identical because many (43 of 92) females lost at least one egg from their nest (e.g. from egg destruction by conspecifics). Experimental females did not all respond the same way to the egg-removal treatment; after we returned eggs 2–5 to the nest, nine of 31 experimental females simply ceased egg production and commenced full incubation (eggs warm to the touch), while the rest continued to produce eggs (see [Results](#), [Fig. 1](#)). We retained these nine females in the experimental group for statistical analyses because we could not be certain that they did not, in fact, produce more eggs than they otherwise would. For example, the experimental females that appeared to discontinue egg production upon laying egg 6 may have otherwise produced a four- or five-egg clutch, just as did some control females ([Fig. 1](#)). In any case, removing these nine experimental females from our analyses did not influence the outcome of statistical tests (not shown).

Data Analysis

All analyses were performed using SAS statistical software (SAS 9.2, SAS Institute, Cary, NC, U.S.A.), and all tests were two tailed.

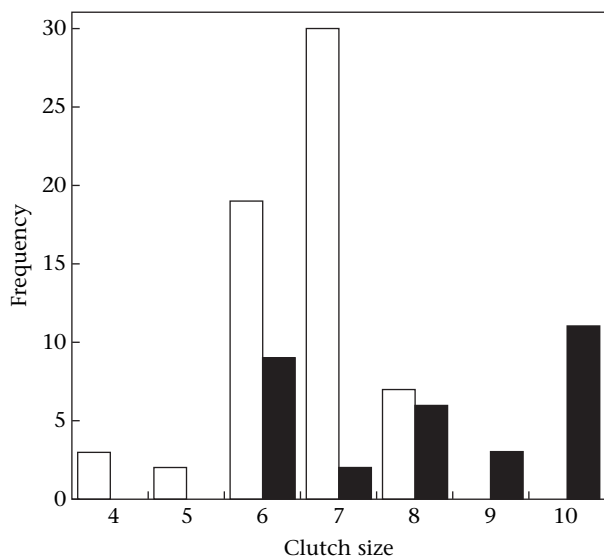


Figure 1. Frequency distribution of clutch sizes for control (□) and experimental (■) female house wrens.

We used data only for females with completed clutches (i.e. those for which the female completed egg production and the identity of the female was known), and no female had more than one nest in these analyses, except for the repeated measures analysis of clutch size, to prevent pseudoreplication. Parsimonious models were obtained by stepwise elimination of nonsignificant ($P > 0.1$) effects from full models, beginning with removal of two-way interactions. Thus, all analyses presented in the **Results** are for reduced models, with interactions reported only where significant.

We first determined how treatment affected the total number of eggs produced by control ($N = 61$) and experimental ($N = 31$) females using a two-sample t test. We then investigated how our treatment affected future breeding attempts by first determining the probability that a female would be double-brooded in 2010 (i.e. attempt a second brood after her first brood left the nest) using logistic regression with a binomial response and logit link (PROC GENMOD). Eighty-two of the original 92 females successfully fledged offspring; thus, our sample for this analysis was 82. This model initially included an index of female body condition (the residual of a $\log(\text{mass}) \times \log(\text{tarsus})$ linear regression) and the initiation date of the female's first clutch as covariates. After removing nonsignificant two-way interactions, the effect of clutch initiation date remained nonsignificant and was removed. We also used survival analysis (PROC PHREG) to analyse the effect of treatment on a female's interbrood interval (the time elapsed between the fledging of the female's first brood and the initiation of her second). This model initially included the size of a female's first brood because eggs were removed from experimental but not control nests; thus, experimental females eventually fledged fewer young (mean \pm SE = 3.1 ± 0.2 , $N = 25$) than control females (5.3 ± 0.2 , $N = 52$) from their first brood (unequal variances $t_{67.8} = 9.26$, $P < 0.001$), and the size of a female's first brood often influences the time at which she is able to initiate her second brood (Kluyver et al. 1977; Finke et al. 1987; Smith et al. 1987). We tested for this by determining whether brood size differentially influenced the time that control and experimental females took to initiate their second broods, but these effects were not significant (brood size: $P = 0.8$; treatment \times brood size: $P = 0.4$) and were removed. This model also initially included our index of female body condition and the initiation date of the female's first clutch as a covariate but, after removal of nonsignificant interaction terms, the effect of

clutch initiation date remained nonsignificant and was removed. We analysed first and second clutch sizes of double-brooded females ($N = 42$) in relation to treatment using repeated measures ANOVA (PROC MIXED), including clutch initiation date and the size of a female's first brood as a covariate, and used the SLICE option as a follow-up test to compare first and second clutch sizes of control and experimental females; this procedure teases apart two-way interactions by performing F tests for simple effects, in this case testing for differences between control and experimental clutch sizes within first and second clutches. Because of the unbalanced sample, we used the Satterthwaite degrees of freedom method, which can result in noninteger degrees of freedom. We then investigated the probability that females would return to breed in 2011 and, if so, the time until they began producing eggs in relation to treatment using logistic regression and survival analysis, respectively (as above), and we analysed the number of eggs they produced over the course of the 2011 breeding season using a two-sample t test.

We analysed within-clutch variation in egg mass for control and experimental clutches ($N = 657$ eggs from 92 females) using mixed-model ANOVA (PROC MIXED), with egg number (i.e. position in the laying sequence) and treatment as fixed effects and individual female as a random effect. We used the SLICE option as a follow-up to compare the mass of eggs in similar laying positions within control and experimental clutches.

Ethical Note

All research activities, including banding of birds, were performed in accordance with Illinois State University Institutional Animal Care and Use Committee (Protocol No. 05-2010), United States Fish and Wildlife Service (USF&WS) banding permit 09211 and USF&WS collecting permit MB692148-0.

RESULTS

Clutch Size and Reproductive Costs

The clutch sizes of experimental females were significantly larger than those of control females (means \pm SE: experimental = 8.2 ± 0.3 , $N = 31$; control = 6.6 ± 0.1 , $N = 61$; two-sample unequal variances t test: $t_{39.4} = 4.86$, $P < 0.001$; Fig. 1). Experimental females were subsequently less likely to attempt a second brood (logistic regression: parameter estimate \pm SE = 1.7 ± 0.7 , Wald $\chi^2_1 = 6.39$, $N = 82$, $P = 0.012$; Fig. 2a), and those experimental females that attempted a second brood took longer than control females to do so following the fledging of their first brood (survival analysis: parameter estimate = 1.3 ± 0.5 , Wald $\chi^2_1 = 6.50$, $N = 79$, $P = 0.011$; Fig. 2b). Females with higher condition indices were more likely to be double-brooded than those with lower condition indices (logistic regression: parameter estimate = 38.1 ± 15.1 , Wald $\chi^2_1 = 6.37$, $N = 82$, $P = 0.012$) and also took less time to initiate their second brood than those in poorer condition (survival analysis: parameter estimate = 17.3 ± 8.2 , Wald $\chi^2_1 = 4.45$, $N = 79$, $P = 0.035$); there was also a slight, but nonsignificant, negative correlation between female condition and the initiation date of the female's first clutch (Pearson correlation: $r_{56} = -0.23$, $P = 0.07$).

Our repeated measures analysis of double-brooded control ($N = 34$) and experimental ($N = 8$) females revealed a decline in clutch size from the first to the second brood, and this decline was greater for experimental females than for control females (repeated measures ANOVA: treatment \times brood number interaction: $F_{1,35.2} = 29.21$, $P < 0.001$; Fig. 3; clutch initiation date: parameter estimate = -0.02 ± 0.007 , $F_{1,26.6} = 7.55$, $P = 0.011$; brood size: parameter estimate = 0.47 ± 0.06 , $F_{1,34.7} = 59.6$, $P < 0.001$). Follow-

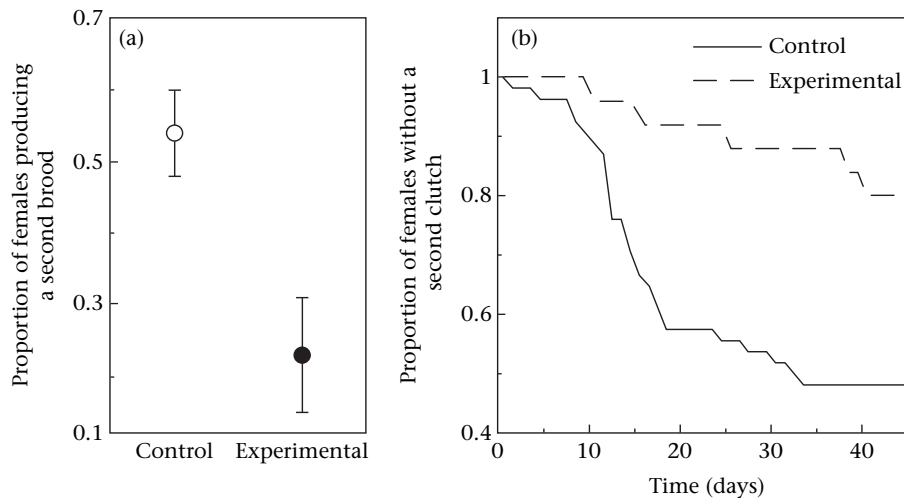


Figure 2. (a) Mean \pm SE proportions of control and experimental female house wrens that initiated a second brood after fledging of their first brood. (b) Mean \pm SE proportions of control and experimental females without an active nest (i.e. a clutch of eggs) in relation to the number of days since fledging of their first brood.

up tests showed that, although experimental females had larger first clutches than control females ($F_{1,35.7} = 22.19, P < 0.001$), their second clutches were smaller than those of control females ($F_{1,26.4} = 9.56, P = 0.005$; Fig. 3). Of these 42 females, 29 successfully fledged offspring from their second broods, with experimental females fledging fewer offspring than control females (two-sample t test: $t_{27} = 2.19, P = 0.037$).

Although experimental females tended to be less likely to return to breed in 2011 (2 of 31 experimental females versus 11 of 61 control females), the difference was not significant (logistic regression: parameter estimate = 1.09 ± 0.81 , Wald $\chi^2_1 = 1.83, P = 0.176$), and there was no association between a female's treatment in 2010 and when she began producing eggs in 2011 (survival analysis: parameter estimate = 1.04 ± 0.77 , Wald $\chi^2_1 = 1.83, P = 0.176$). However, among those females that returned, experimental females produced significantly fewer eggs than control females over the course of the breeding season (means \pm SE: control: $9.4 \pm 0.9, N = 11$; experimental: $5.0 \pm 1.0, N = 2$; two-sample t test (log-transformed): $t_{11} = 2.31, P = 0.041$).

Egg Mass Variation within Control and Experimental Nests

Egg mass increased with laying order, but after experimental females laid egg 6 and had eggs 2–5 returned to the nest, the mass of subsequent eggs declined (treatment \times egg number: $F_{7,549} = 3.95, P < 0.001$; Fig. 4). Mass of eggs 1–6 did not differ between control and experimental clutches, but eggs 7 and 8 of experimental females were lighter than those of control females (egg 7 difference: $F_{1,182} = 5.55, P = 0.020$; egg 8 difference: $F_{1,385} = 12.41, P < 0.001$; Fig. 4).

DISCUSSION

Our egg-removal treatment successfully increased the number of eggs that experimental females produced and, compared with controls, reduced the likelihood that experimental females would attempt a second brood on our study area. Experimental females that did attempt a second brood took longer to do so and produced

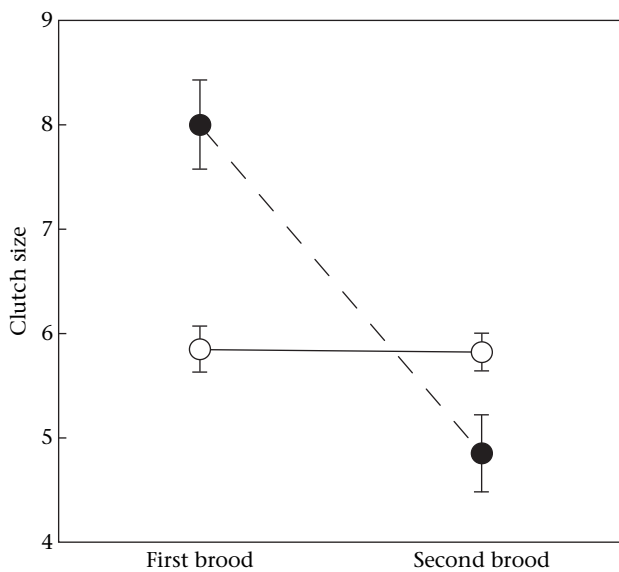


Figure 3. First and second clutch sizes (LS means \pm SE) of double-brooded control (○) and experimental (●) female house wrens.

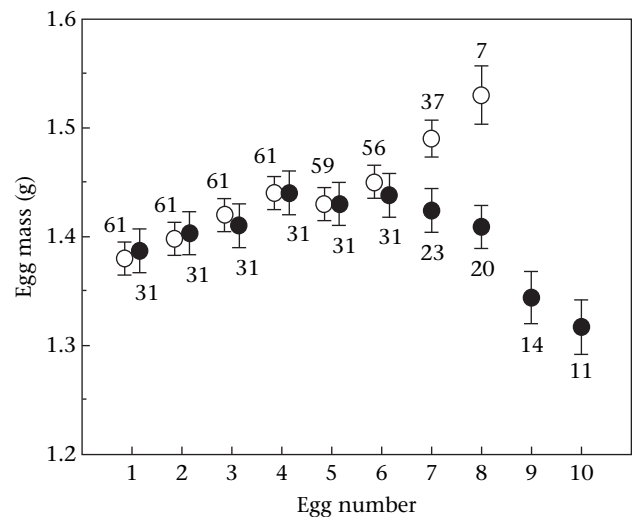


Figure 4. Mass (LS means \pm SE) of eggs in relation to their laying sequence in control (○) and experimental (●) house wren nests. Numbers above and below each mean represent sample sizes for control and experimental nests, respectively. In experimental nests, eggs 2–5 were removed and returned upon laying of egg 6, which led most females to continue egg production for several days.

smaller clutch sizes than control females. In north-central Illinois forests, abundance of arthropod prey declines over the course of the breeding season (Kendeigh 1979), contributing to the production of smaller clutches in late-season broods (Finke et al. 1987; Styrsky et al. 1999). A reduction in clutch size of second broods also occurred in this study, but to a greater extent for females with experimentally increased egg production during their first brood. This was the case after controlling for laying date, indicating that the reduced clutch size was not caused by females' delayed breeding, but instead by increased egg production in the females' first broods. The delay in starting a second brood and reduced clutch size within and between breeding seasons by experimental females is of particular importance because increased egg production per se is often assumed to represent increased parental investment; however, measures of parental effort are often assumed to represent measures of investment, but what appears to be an increased, costly investment can only be confirmed if it comes at a cost to future reproductive output (Kvarnemo 2010). Although increased effort in other reproductive stages (e.g. incubation) are known to impose fitness-related consequences (Visser & Lessells 2001; Hanssen et al. 2005; Dobbs et al. 2006), the reproductive costs documented here can only be attributed to increased egg production during the first brood because control and experimental females incubated a similar number of eggs, and experimental females actually brooded and provisioned fewer, not more, nestlings than control females.

Body condition (i.e. residual body mass) influenced whether females attempted a second brood, with those in better condition being more likely to be double-brooded than those in poorer condition (see Whittingham et al. 2002 for a similar result). Furthermore, among double-brooded females, those in good body condition took less time to produce their second clutch than those in poorer condition. This suggests that females require time to recover between breeding attempts, and increased egg production lengthens this interval. Because the number of broods that a female produces is considered the most important determinant of individual reproductive success (Poirier et al. 2004; Whittingham & Dunn 2005), body condition appears to be important in determining female fitness (see also Houston et al. 1983). For example, early reproduction provides young with a postfledging competitive advantage over later-fledging young (Nilsson 1989, 1990). It is, therefore, not surprising that recruitment rates of offspring produced earlier in the season are higher than those produced later in the season (reviewed in Verhulst & Nilsson 2008). The pattern also holds for second broods of double-brooded species, where offspring from second broods are more likely to be recruited to future breeding populations if they fledge earlier than others (Kluyver et al. 1977; E. K. Bowers, unpublished data). Hence, aside from reduced clutch size, the delayed second clutch of experimental relative to control females probably imposed further reproductive costs in the form of reduced recruitment rates of the offspring they produced.

Experimental females were less likely than control females to attempt second broods on the study area. We do not know the precise fate of females forgoing a second brood. They may have remained on the study area and produced a second brood using a natural nest cavity, moved off the study area and attempted a second brood elsewhere, ceased breeding altogether, or died. We cannot fully distinguish among these possibilities, but we do know that more than 95% of the nests on the study area are built in nest-boxes (Drilling & Thompson 1988), making it unlikely that more than one or two females with second broods went undetected. Another possibility is that experimental females were more likely to emigrate after their first brood than were control females. However, extensive dispersal of breeders within a breeding season is rare among passerines (Greenwood & Harvey 1982). Furthermore, experimental

and control females were equally likely to fledge their first brood, and females on the study area tend not to move far after producing a successful brood (Drilling & Thompson 1988). Although reduced survival has been shown to act as a reproductive cost (Graves 1991; Cichoń et al. 1998; Visser & Lessells 2001), control and experimental females were equally likely to return to breed the following summer, and 31% (4 of 13) of the females that returned to breed in 2011 only produced one clutch in 2010, demonstrating that some single-brooded females did not necessarily fail to produce a second clutch because of increased mortality. Thus, mortality alone cannot explain the disappearance of a large number of females. Regardless of the fate of females that did not produce a second brood, neither increased emigration nor mortality of experimental females can explain the females' delay in starting a second brood or the smaller clutch sizes of their second broods in 2010, or the reduced egg production of experimental females the following breeding season.

Aside from the trade-off in fecundity among breeding attempts, we also saw a trade-off in female capacity to allocate resources to offspring within clutches, as egg mass declined sharply among supernumerary eggs (Fig. 4). Egg mass is a commonly accepted measure of parental effort in birds because it is positively correlated with nutrient content (Nager et al. 2000) and also with offspring size and growth rates, with nestlings from larger eggs typically benefiting to a greater extent than those from smaller eggs (Styrsky et al. 1999, 2000; reviewed in Krist 2011). Although the consequences of egg size variation for offspring survival are most obvious early in life (Williams 1994; Styrsky et al. 1999; Christians 2002; Krist et al. 2004), poor neonatal nutrition can result in a reduced capacity to resist oxidative damage (Alonso-Alvarez et al. 2007), delayed reproduction (Blount et al. 2006) and reduced survival later in life (Birkhead et al. 1999; reviewed in Metcalfe & Monaghan 2001). Hence, intraclutch variation in egg mass should still affect individual fitness through its influence on health in early development. That maternal effort in egg formation limits not only females' future reproduction, but also their investment among individual offspring, further suggests that trade-offs both within and among reproductive attempts interact to determine the number of offspring that females should produce at any time.

Previous egg-removal experiments on this study population induced females to produce nearly double the expected clutch size (Johnson 1998), with a similar decline in mass among supernumerary eggs (C. F. Thompson, unpublished data), and Mänd et al. (2007) found a similar pattern in great tits. The decline in mass of supernumerary eggs suggests that both energetic and nutrient limitations play a role in reducing female investment in supernumerary eggs in their first clutch and the eggs in their second clutch. House wrens are 'income breeders' (Drent & Daan 1980), needing to obtain sufficient resources for egg development from their daily diet (Winkler & Allen 1996; Meijer & Drent 1999). Given the extent to which nutrient limitation, particularly calcium, constrains egg production in small, insectivorous passerines (Graveland et al. 1994; Johnson & Barclay 1996; Tilgar et al. 2002; Mänd & Tilgar 2003) and the extent to which food availability influences the interval between first and second broods (Kluyver et al. 1977), nutrient limitation may explain the reduced mass of supernumerary eggs within first experimental nests, and the immediate fecundity costs to these females. Resource-related constraints, however, are unlikely to account for the reduced fecundity of experimental females in their next breeding season. A more likely explanation may involve pleiotropic effects of elevated reproductive hormones that experimental females experienced during their extended egg production (see Williams 2005). Thus, resource limitation is a likely factor contributing to short-term reproductive costs, whereas negative effects of elevated reproductive hormones may mediate long-term fitness costs of increased reproductive effort.

Acknowledgments

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