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Clutch size and the costs of incubation in the house wren

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Trade-offs in the allocation of finite resources among different stages of a breeding attempt as well as between different reproductive events should shape the evolution of life-history traits. To investigate the effects of incubation effort on within-brood and between-brood trade-offs in house wrens (*Troglodytes aedon*), we manipulated the clutch size that females incubated. We isolated effects of incubation by reversing the manipulation at hatching to allow all parents to provision their natural brood sizes. Females that incubated enlarged clutches had longer incubation periods than control females, both early and late in the season, suggesting that the experimental treatment increased incubation effort. Contrary to predictions, however, increased incubation effort did not adversely affect the allocation of effort to nestling provisioning. Rather, in the early season, but not in the late season, females that incubated enlarged clutches appeared to allocate more effort to nestling provisioning, producing heavier and larger fledglings than control females. Although females with enlarged early-season clutches consequently lost more mass than control females, this was likely an adaptive response to reduce wing loading in anticipation of high provisioning demands. There were no treatment-related differences in fledgling mass or size, or in female mass loss, in the late season. Thus, elevated incubation demands negatively affected a fitness-related trait (duration of incubation) that may constrain clutch size but not the allocation of resources to subsequent stages of the same breeding event or to subsequent breeding events. We suggest that environmental conditions may mediate clutch-size effects on trade-offs in allocation of resources between incubation and nestling provisioning.
Key words: clutch size, house wren, incubation, life history, trade-offs, *Troglodytes aedon*. *[Behav Ecol 17:849–856 (2006)]*

Contrary to the hypothesis of Lack (1947) that clutch size should be equal to the most productive brood size, birds tend to lay and incubate fewer eggs than they are successfully able to raise as offspring (Lindén and Møller 1989; Dijkstra et al. 1990; VanderWerf 1992). Understanding how trade-offs among life history traits influence this deviation from the "Lack clutch" has relied primarily on experimental manipulation of brood size and investigating the consequences for adult survival or future reproductive success (Lessells 1991; Stearns 1992). This focus, however, ignores possible constraints associated with other stages of reproduction (i.e., egg production, incubation) and trade-offs in the allocation of effort among stages within a reproductive event (Monaghan and Nager 1997; Visser and Lessells 2001).

The ability to incubate successfully clutches of different size has received little attention as a factor potentially limiting clutch size because it was long thought that physiological demands of incubation were much less than those of provisioning nestlings (e.g., King 1973; Walsberg 1983; Gill 1990). There is now evidence, however, that incubation in small terrestrial birds can require as much energy as nestling provisioning (Tatner and Bryant 1993; Williams 1996; Thomson et al. 1998; Reid, Monaghan, and Nager 2002; Tinbergen and Williams 2002). Furthermore, for species in which only the female parent incubates, foraging trips away from the nest and rewarming the eggs on returning to the nest create additional metabolic demands during incubation (Williams and Dwinnel 1990; Williams 1991).

In experimentally enlarged clutches, failure to cope effectively with increased demands of incubation may result in increased adult energy expenditure (Haftorn and Reinertsen 1985; Moreno et al. 1991; Moreno and Sanz 1994; Reid, Ruxton, et al. 2002), prolonged incubation period (Baltz and Thompson 1988; Moreno and Carlson 1989; Smith 1989; Siikamäki 1995; Engstrand and Bryant 2002; Larsen et al. 2003; Niizuma et al. 2005), decreased hatching success (Moreno et al. 1991; Siikamäki 1995; Reid et al. 2000b; Engstrand and Bryant 2002; Niizuma et al. 2005), and decreased adult body condition (Jones 1987; Moreno and Carlson 1989) (for reviews see Thomson et al. 1998; Reid, Monaghan, and Nager 2002).

Despite accumulating evidence that incubation is costly, the role of incubation costs per se in limiting clutch size remains unclear, in part because fitness-related consequences of incubation demands are generally confounded with those of subsequent nestling provisioning demands (Monaghan and Nager 1997). Only recently have workers begun to assess consequences of incubation demand by separating incubation effort from nestling provisioning effort. By manipulating clutch size during incubation only (i.e., by reversing clutch manipulations immediately before or after hatching), recent experiments have demonstrated trade-offs between incubation costs and subsequent brood-rearing success. Adult common terns (*Sterna hirundo*) that incubated enlarged clutches were less able than adults with control clutches to provision their natural brood size, raising young with lower growth rates and fledging mass (Heaney and Monaghan 1996). Similarly, when natural brood sizes were restored just before or after hatching, female collared (*Ficedula albicollis*) and pied (*Ficedula hypoleuca*) flycatchers that incubated enlarged clutches produced fledglings of lower mass and had lower fledging success, respectively, than females that incubated reduced clutches (Cichoń 2000; Ilmonen et al. 2002). European starlings (*Sturnus vulgaris*) that incubated enlarged clutches

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but experienced natural provisioning demands, fledged fewer young than controls (Reid et al. 2000b). Using a different approach, Reid et al. (2000a) heated starling nests during incubation, thereby allowing parents to invest less energy in incubation. Starlings with heated nests produced nestlings in better condition at the time of fledging. These results suggest a trade-off between the number and quality of offspring that is directly related to the number of eggs that females can incubate.

We used the house wren (*Troglodytes aedon*) to test the hypothesis that incubation costs limit clutch size through trade-offs in the allocation of effort within breeding events. Females assigned to an experimental treatment group incubated enlarged clutches but cared for broods of similar size to those of females incubating control clutches. We predicted that if incubation effort limits clutch size through within-breeding event trade-offs, then females incubating enlarged clutches would produce fewer fledglings or produce fledglings of lower mass than would control females. Because brood-manipulation experiments have shown that costs of reproduction may occur through between-breeding event trade-offs, we also tested for treatment effects in subsequent breeding attempts.

METHODS

Study area and species

We used the 108-ha Mackinaw (40°40′N, 88°53′W) and the 20-ha East Bay (40°39′N, 88°55′W) study areas in McLean Co., Illinois, United States (see Drilling and Thompson 1988), which consist of floodplain and adjacent upland forest along the Mackinaw River, where essentially identical nest-boxes have been in place since 1980. Nest-boxes were mounted on 1.5-m greased, metal poles spaced 30 m apart on north–south lines that were 60 m apart, resulting in a density of 5.4 nest-boxes/ha.

House wrens are small (10–12 g), altricial, secondary-cavity nesting migratory passerines that are typically double brooded in central Illinois, with egg-laying peaks in mid-May (early season) and early July (late season). In this study, we termed the date the first egg of a clutch was laid as the egg-1 date and classified nests as either early-season nests (egg-1 date before the median egg-1 date of all the nests that year) or late-season nests (egg-1 date on or after the median egg-1 date of the year). Female house wrens lay one egg per day during the early morning (Johnson 1998) until the clutch is complete. In our study population, modal clutch size declines from 7 eggs during the early season to 6 eggs during the late season (Finke et al. 1987). Females alone incubate the eggs and are rarely fed by their mates (Johnson and Kermott 1992). After the first egg hatches (brood-day 0), both parents feed the nestlings, which attain maximum (asymptotic) mass and size by brood-day 11 (Finke et al. 1987) and leave the nest at brood-days 15–17. As in many passerine species (e.g., Hochachka and Smith 1991; Magrath 1991), asymptotic mass of nestling house wrens in this population is positively correlated with juvenile survival and recruitment to subsequent breeding populations (CF Thompson, unpublished data).

General field procedures

Throughout the breeding seasons of 1998 and 1999, we inspected contents of all nest-boxes twice weekly to document nest-building activity. When nests were lined, we checked nest-boxes daily to determine egg-1 dates. During 1998 (only), we visited nests daily during egg laying and marked each egg with nontoxic, indelible ink and weighed each to the nearest 0.001 g with a portable electronic balance (Acculab PocketPro 2060D) generally before noon on the day that it was laid. In both years, we checked all nests daily late in the incubation period to determine brood-day 0. We weighed nestlings to the nearest 0.1 g with a portable electronic balance (Acculab PocketPro 150 or 250) on brood-days 4 and 12. Also on brood-day 12, we measured nestling tarsometatarsus (hereafter tarsus) length to the nearest 0.1 mm with dial calipers and banded each nestling with one US Fish and Wildlife Service numbered aluminum leg band. After brood-day 12, we monitored nest-boxes every 2–3 days to determine nestling survival from hatching to nest-leaving.

In both years, we captured adult females during the middle of their incubation periods and, if possible, also during the middle of their nestling provisioning periods. On capture, either in their nest-box or in a mist net just outside their nest-box, we banded adult females with one US Fish and Wildlife Service aluminum leg band (if not already banded) and weighed each to the nearest 0.1 g with a portable electronic balance. We also measured flattened wing and tarsus to the nearest 0.1 mm with a stopped metal ruler and dial calipers, respectively. Summary sample sizes for nests, adult females, and nestlings used in this study are presented by treatment group, season, and year in Tables 1 and 2.

Clutch-size manipulation experiment

In both years, we collected completed clutches of house wren eggs from the East Bay study area for use in experiments on the Mackinaw study area. We stored collected eggs in a refrigerator until needed. We randomly assigned nests on the Mackinaw study area to either experimental or control treatment groups. Within the first 3 days of incubation, we added 3

Table 1

Sample sizes for house wren nests used in experiments and summary statistics for unmanipulated clutch size and number of eggs incubated by house wrens in different treatment groups in early and late seasons of 1998 and 1999

				Mean ± SD	
Year	Season	Treatment	*n* nests	Clutch size	Eggs incubated
1998	Early	Control	33	6.7 ± 0.89	6.7 ± 0.89
		Enlarged clutch	28	7.0 ± 0.61	10.0 ± 0.61
	Late	Control	23	5.7 ± 0.82	5.6 ± 0.89
		Enlarged clutch	23	5.5 ± 0.51	8.4 ± 0.51
1999	Early	Control	21	7.0 ± 0.63	7.0 ± 0.63
		Enlarged clutch	18	7.0 ± 0.49	9.9 ± 0.54
	Late	Control	14	5.3 ± 1.08	5.2 ± 0.97
		Enlarged clutch	14	5.2 ± 0.61	8.1 ± 0.66

Table 2

Sample sizes for adult females and nestlings used in analyses of female mass loss and nestling mass and tarsus length, respectively, in treatment groups in early and late seasons of 1998 and 1999

				Nestlings		
				Brood-day 4	Brood-day 12	
Year	Season	Treatment	Adult females	Mass	Mass	Tarsus
1998	Early	Control	12	24	24	24
		Enlarged clutch	11	22	22	22
	Late	Control	12	22	22	22
		Enlarged clutch	18	22	22	22
1999	Early	Control	15	20	14	8
		Enlarged clutch	14	15	12	10
	Late	Control	10	12	11	11
		Enlarged clutch	8	13	13	10

marked eggs (foster eggs) to the natural clutch at experimental nests and visited but did not manipulate control nests. This resulted in early-season experimental clutch sizes of 8–11 eggs and late-season experimental clutch sizes of 7–10 eggs. Thus, experimental clutch sizes were near the upper limit but within the range of natural house wren clutch sizes (Bent 1948; Johnson 1998). Because added eggs had been stored at cold temperatures, they did not hatch and were removed and discarded when the first original egg of the natural clutch hatched.

There was no difference in natural clutch size between the 2 treatment groups in the early season (years combined; Wilcoxon 2-sample test: $Z = 0.69$, $P = 0.488$) or in the late season ($Z = -0.60$, $P = 0.546$). Mean natural clutch size and number of eggs incubated (experimental clutch size) in each treatment group in each year are presented in Table 1.

Statistical analyses

We analyzed early- and late-season data separately because natural clutch size declined significantly between early- and late-season nests (Finke et al. 1987) and clutch size was the variable manipulated. During this study, mean natural clutch size was 6.9 ± 0.7 eggs ($n = 100$ clutches) in early-season nests and 5.5 ± 0.8 eggs ($n = 74$ clutches) in late-season nests (years combined; Wilcoxon 2-sample test: $Z = -9.37$, $P = 0.0001$).

One measure of incubation effort is the duration of incubation, the time (days) between laying the last egg of the clutch and brood-day 0. Because natural clutch size and clutch-initiation date may influence incubation period, we conducted a multiple regression (REG procedure; SAS Institute Inc. 1990) with the duration of the incubation period as the dependent variable and natural clutch size and egg-1 date as independent variables. Because neither of the independent variables was significantly related to the duration of the incubation period, we removed them and used 2-way analysis of variance (ANOVA) (GLM procedure; SAS Institute Inc. 1990) to test for effects of treatment, year, and their interaction.

Hatching success is the proportion of original eggs in the natural clutch that successfully hatched. Nestling survival is the proportion of nestlings originating from the natural clutch that survived to leave the nest. We analyzed hatching success and nestling survival using generalized linear models with a binomial distribution and initial clutch size or brood size at brood-day 4 as denominators, respectively, and a clog-log link function (GENMOD procedure; SAS Institute Inc. 1990; following Styrsky et al. 2000; see also Allison 1995). To test for effects of treatment, year, and their interaction on hatching success, we included natural clutch size as a covariate because it may independently influence hatching success. In analyses of nestling survival, we included natural clutch size, brood-day 0, and average brood size as covariates because each may independently influence the number of nestlings surviving to nest-leaving. Data for hatching success and nestling survival, while satisfying the parametric assumption of equal variances, were not normally distributed and could not be normalized through transformation.

In analyses of nestling mass and tarsus length, we used mean values for each brood because each nest represented a sample unit. Again, because factors unrelated to the experimental treatment may affect nestling mass and tarsus length (e.g., brood-day 0), we attempted to control for these potentially confounding factors. Thus, we conducted multiple regressions with mean nestling mass (or tarsus length) as the dependent factor and natural clutch size, brood-day 0, hour of measurement, and average brood size as independent factors. We removed nonsignificant independent factors from regression models in a stepwise backward fashion to produce final models and used the residuals as the dependent factor in 2-way ANOVAs on mean nestling mass and tarsus length.

To test for treatment effects on female mass, we weighed most (90%) adult females 4–6 days prior to hatching and again 5–8 days after hatching. Because female house wrens lose mass during incubation and nestling provisioning (Freed 1981; Cavitt and Thompson 1997), day of weighing may obscure any effects of treatment on female mass. Initial mass must also be taken into consideration because heavier birds tend to lose more mass than do lighter birds (Merilä and Wiggins 1997; Slagsvold and Johansen 1998). Thus, we conducted a multiple regression with female mass loss as the dependent factor and tarsus length, initial mass, natural clutch size, brood-day 0, day of weighing during incubation, and day of weighing during the nestling period as independent factors. The dependent factor in 2-way ANOVAs on female mass loss consisted of residuals from final regression models, within which we retained tarsus length, but removed, in a stepwise backward fashion, all other independent factors that were not significant. If treatment affected female mass during the early season, we performed an additional test to determine if it also affected female mass during incubation in the late season. Here, we performed a multiple regression with late-season incubation mass as the dependent factor and interbrood interval (the number of days between brood-day 12 of early-season nests and egg-1 day of late-season nests) and day of weighing during incubation as the independent factors and used the resulting residuals as the dependent factor in 2-way ANOVAs to evaluate hypotheses proposed to explain female mass loss during breeding.

For females that produced a first clutch in the early season and then a second clutch that was always in the late season, we used 2-way ANOVAs to test for effects of early-season treatment on the interbrood interval. The dependent factor in ANOVAs consisted of residuals from a linear regression with early-season natural clutch size as an independent factor. We used a similar approach to test for effect of early-season treatment on late-season clutch size. Means ± 1 SD are reported in the text.

RESULTS

Incubation period and hatching success

Females incubating experimentally enlarged clutches had significantly longer incubation periods than did control females in both early and late seasons (Table 3 and Figure 1). In the early season, females incubated enlarged clutches for 13.2 ± 0.11 days ($n = 46$) and control clutches for 12.8 ± 0.10 days ($n = 54$), and in the late season, females incubated enlarged clutches for 12.6 ± 0.14 days ($n = 37$) and control clutches for 12.2 ± 0.14 days ($n = 37$). There were no significant year or treatment × year interaction effects.

Hatching success of eggs in the natural clutch was not affected by clutch-size manipulation during the early season ($\chi^2_1 = 0.007$, $P = 0.94$) or late season ($\chi^2_1 = 0.01$, $P = 0.90$) or by year or treatment × year interaction. Of original eggs in early-season clutches, 92.8% in the enlarged ($n = 43$ clutches) and 92.4% in the control ($n = 53$ clutches) group hatched, and in late-season clutches, 92.7% in the enlarged ($n = 36$ clutches) and 92.9% in the control ($n = 36$ clutches) group hatched.

Nestling mass, tarsus length, and survival

Clutch-size manipulation did not affect mean brood-day 4 nestling mass in early- or late-season broods (Table 4). However, nestlings in early-season broods that had been assigned enlarged clutches weighed significantly more on brood-day 12 (10.3 ± 0.60 g, $n = 34$ broods) than did control nestlings (10.0 ± 0.61 g, $n = 38$ broods) (Table 4 and Figure 2a). Year also significantly affected mean brood-day 12 nestling mass, but there was not a significant treatment × year interaction. There were no significant treatment effects on mean brood-day 12 nestling mass during the late season (experimental: 9.57 ± 0.75 g, $n = 35$ broods; control: 9.56 ± 0.81 g, $n = 33$ broods) (Table 4 and Figure 2a).

In parallel with treatment differences in mean nestling mass on brood-day 12 in early-season broods, nestlings from enlarged clutches were also larger structurally, as measured by tarsus length (19.1 ± 0.48 mm, $n = 32$ broods), than nestlings in control nests (18.8 ± 0.48 mm, $n = 32$ broods), although statistical significance was marginal at $P = 0.06$ (Table 4 and Figure 2b). Treatment did not affect nestling tarsus length during the late season (experimental: 18.7 ± 0.56 mm, $n = 32$ broods; control: 18.9 ± 0.42 mm, $n = 33$ broods). There was a significant year effect on nestling tarsus length in the late season but no significant treatment × year interactions (Table 4).

Nestling survival from hatching to nest-leaving was not affected by clutch-size manipulation in the early season ($\chi^2_1 = 0.87$, $P = 0.35$), late season ($\chi^2_1 = 1.56$, $P = 0.28$), or by year or treatment × year interaction. In the early season, 95.9% ($n = 35$ broods) and 93.6% ($n = 38$ broods) of nestlings survived to leave the nest in the experimental and control groups, respectively. In the late season, 85.6% ($n = 33$ broods) and 90.3% ($n = 33$ broods) of nestlings survived to leave the nest in the experimental and control groups, respectively.

Female mass and subsequent reproductive effort

An analysis correcting for initial mass and structural size showed that adult females that had incubated enlarged early-season clutches lost more mass than control females by brood-days 5–8 of the nestling period, but statistical significance was marginal at $P = 0.07$ (Table 3 and Figure 3). Females that incubated enlarged clutches lost 1.38 ± 0.37 g ($n = 25$ females), whereas control females lost 1.23 ± 0.56 g ($n = 27$ females) between midincubation and midnestling stages. A nonsignificant trend in the same direction occurred during the late season (Table 3 and Figure 3), with experimental and control females losing 1.14 ± 0.36 g ($n = 26$ females) and 1.01 ± 0.39 g ($n = 22$ females), respectively.

Mass of females during incubation in the late season was not affected by early-season treatment ($F_{1,32} = 1.51$, $P = 0.47$). Females that incubated enlarged early-season clutches weighed 12.07 ± 0.47 g ($n = 19$ females) during the late season, whereas early-season control females weighed 11.96 ± 0.71 g ($n = 17$ females) during the late season.

Early-season treatment did not affect the likelihood that females that successfully raised a brood to nest-leaving during the early season would attempt a late-season nest. Twenty-two of 45 (49%) females that successfully incubated early-season

Table 3

ANOVA results for effects of clutch-size manipulation on (a) duration of incubation and (b) female mass in house wrens in early and late seasons of 1998 and 1999 (years combined)

	Early season			Late season		
Source	*F*	df	*P*	*F*	df	*P*
(a) Incubation length						
Model	3.00	3,96	0.03	2.15	3,70	0.10
Treatment	5.49	1,96	0.02	5.63	1,70	0.02
Year	0.89	1,96	0.34	0.81	1,70	0.37
Treatment × year	1.03	1,96	0.31	0.50	1,70	0.48
(b) Female mass loss						
Model	1.52	3,48	0.22	0.91	3,44	0.44
Treatment	3.26	1,48	0.07	2.43	1,44	0.12
Year	0.01	1,48	0.92	0.29	1,44	0.59
Treatment × year	1.79	1,48	0.18	0.65	1,44	0.42

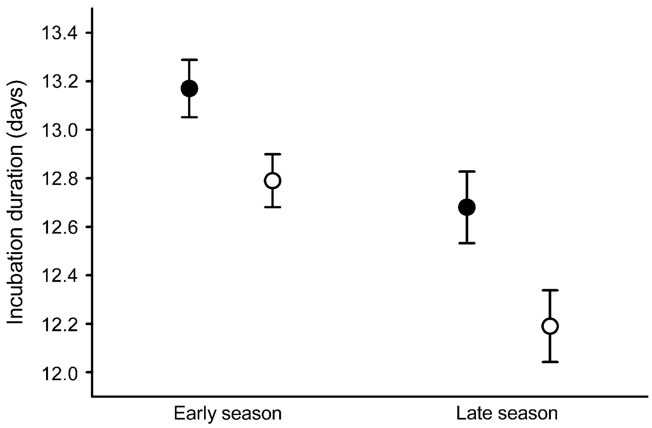

Figure 1
Mean (±1 SE) duration of the incubation period (days) in 1998 and 1999 (years combined) early- and late-season nests in the enlarged clutch group (closed circles) and control clutch group (open circles) of house wrens.

enlarged clutches and 24 of 48 (50%) females that successfully incubated early-season control clutches attempted late-season nests. Early-season treatment did not affect the interbrood interval of females attempting a late-season second brood ($F_{1,39} = 1.05$, $P = 0.31$), with early-season experimental females requiring 15.9 ± 5.2 days ($n = 22$ females) between brood-day 12 of their first brood and egg-1 day of their second brood, compared with 14.5 ± 4.2 days ($n = 21$ females) for early-season control females. Similarly, early-season treatment did not affect late-season clutch size ($F_{1,40} = 1.12$, $P = 0.29$) of females attempting a late-season second brood, with early-season enlarged treatment females laying 5.6 ± 0.6 eggs ($n = 22$ clutches) and controls 5.8 ± 0.8 eggs ($n = 22$ clutches). Further, in both 1998 and 1999, clutch-size manipulation did not affect the likelihood that females would return to breed in the next breeding season (1999 and 2000, respectively; $n = 138$ females; $\chi^2_1 = 0.74$, $P = 0.38$).

DISCUSSION

Female house wrens that incubated experimentally enlarged clutches had significantly longer incubation periods than females that incubated their natural clutch size in both early and late seasons of the 2 years of this study. These results are consistent with the results of previous experimental studies, demonstrating that clutch size can directly influence incubation efficiency in passerines (Baltz and Thompson 1988; Moreno and Carlson 1989; Smith 1989; Møller 1993; Siikamäki 1995; Engstrand and Bryant 2002). Allocation of additional effort to incubation, however, did not negatively affect the allocation of resources to subsequent nestling provisioning. In fact, during the early season, females that incubated enlarged clutches unexpectedly appeared to allocate more effort to nestling provisioning, producing heavier and larger fledglings, than control females. These results are contrary to our predictions and to the results of other comparable experiments (e.g., Heaney and Monaghan 1996; Cichoń 2000; Reid et al. 2000b; Ilmonen et al. 2002).

Four previous clutch-size experiments have used comparable designs to manipulate reproductive effort only during incubation, using species that share multiple life-history traits with house wrens (e.g., uniparental incubation, altricial offspring, insectivorous diet). In collared and pied flycatchers, increased incubation effort induced by clutch-size manipulation did not affect the duration of incubation or hatching success but did negatively affect the allocation of resources to nestling provisioning, resulting in lower fledgling mass or lower fledging success (Cichoń 2000; Ilmonen et al. 2002). Conversely, female barn swallows (*Hirundo rustica*) that incubated enlarged clutches had longer incubation periods and lower hatching success than females that incubated reduced clutches, but increased incubation demands did not affect subsequent provisioning effort or parental body condition (Engstrand and Bryant 2002). As in barn swallows, house wrens with experimentally increased incubation effort took longer to hatch their natural clutches but subsequently were apparently able to allocate as much or more effort to provisioning nestlings as controls (the present study). In general, studies that have examined within-breeding event trade-offs show that costs of incubation may be manifest primarily during the incubation stage (e.g., increase in duration of incubation or decrease in hatching success; the present study; Engstrand and Bryant 2002) or during nestling provisioning (e.g., number or condition of fledglings; Heaney and Monaghan 1996; Cichoń 2000; Ilmonen et al. 2002). However, European starlings that incubated enlarged clutches and

Table 4

ANOVA results for effects of clutch-size manipulation on (a) brood-day 4 mean nestling mass, (b) brood-day 12 mean nestling mass, and (c) brood-day 12 mean nestling tarsus length in house wrens in early and late seasons of 1998 and 1999 (years combined)

	Early season			Late season		
Source	*F*	df	*P*	*F*	df	*P*
(a) Brood-day 4 mass						
Model	1.41	3,77	0.24	0.27	3,65	0.84
Treatment	1.83	1,77	0.17	0.03	1,65	0.85
Year	2.08	1,77	0.15	0.01	1,65	0.93
Treatment × year	0.17	1,77	0.67	0.63	1,65	0.43
(b) Brood-day 12 mass						
Model	6.14	3,68	<0.01	0.06	3,64	0.98
Treatment	5.89	1,68	0.01	0.03	1,64	0.85
Year	11.3	1,68	<0.01	0.14	1,64	0.71
Treatment × year	0.16	1,68	0.68	0.01	1,64	0.94
(c) Brood-day 12 tarsus						
Model	2.99	3,60	0.03	2.61	3,62	0.05
Treatment	3.56	1,60	0.06	0.22	1,62	0.63
Year	4.48	1,60	0.03	7.06	1,62	0.01
Treatment × year	0.21	1,60	0.64	0.28	1,62	0.59

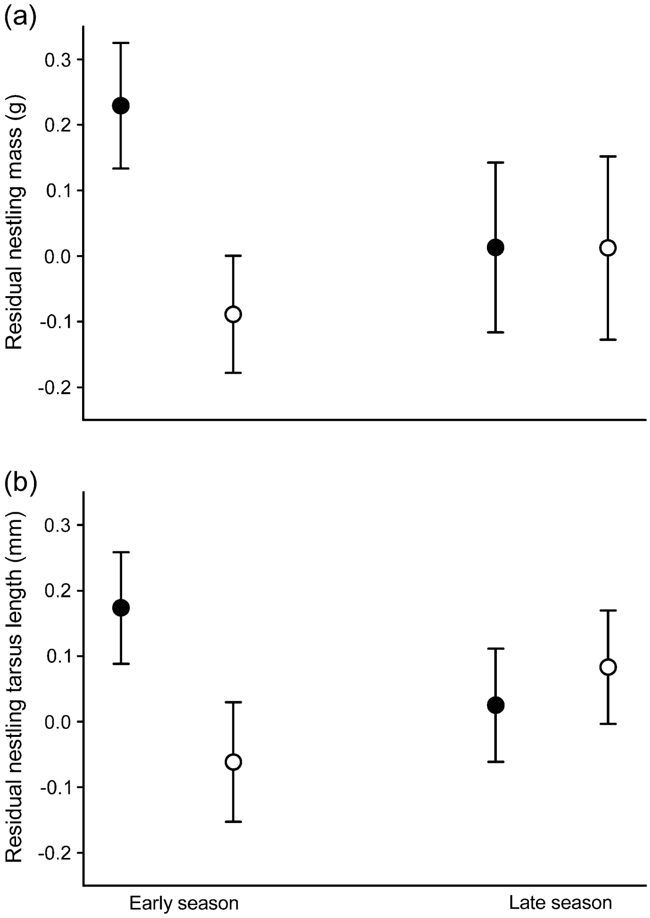

Figure 2
Mean (±1 SE) residual brood-day 12 (a) nestling mass and (b) nestling tarsus length in 1998 and 1999 (years combined) early- and late-season broods in the enlarged clutch group (closed circles) and control group (open circles) of house wrens. Vertical axes represent mean deviations in (a) mass (g) or (b) tarsus length (mm) from that expected based on natural clutch size, brood-day 0, mean brood size, and hour of weighing (where those factors were significantly related to mass or tarsus).

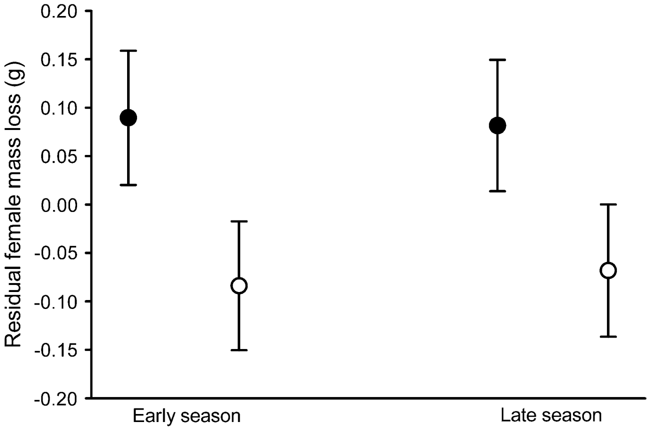

Figure 3
Mean (±1 SE) residual female mass loss between brood-days 5–8 in 1998 and 1999 (years combined) early and late seasons in the enlarged clutch group (closed circles) and control group (open circles) of house wrens. The vertical axis represents the mean deviation in female mass loss (g) from that expected based on initial mass, tarsus length, natural clutch size, egg-1 date, day of weighing during incubation, and day of weighing during the nestling stage (where those factors were significantly related to female mass loss).

provisioned their natural broods had both lower hatching success and lower fledging success than controls (Reid et al. 2000b). Viewed as a whole, these studies show that birds may allocate resources among phases within a breeding event in different ways in response to increased costs of incubation. The extent to which species-specific variation and variation in environmental conditions affect how and when costs of incubation are manifest is not known.

Unlike collared and pied flycatchers (Cichoń 2000; Ilmonen et al. 2002) and barn swallows (Engstrand and Bryant 2002), female house wrens that incubated enlarged clutches lost more mass than females that incubated unmanipulated clutches. The difference in mass loss between treatment groups was greatest when the treatment effect on nestling mass and structural size was greatest. That is, increased female mass loss was accompanied by increased nestling condition in early-season enlarged clutch groups. Female house wrens with high incubation demands thus appear at first to have traded-off body condition for the ability to allocate additional effort to provisioning. Although male provisioning was not measured, it is unlikely to explain the treatment-related differences in nestling condition. Male passerines typically increase provisioning effort when female provisioning effort is experimentally reduced, but male compensation is usually incomplete (Wright and Cuthill 1989; Markman et al. 1995). However, this does not seem to be the case in this population of house wrens, where male provisioning effort increases significantly with natural and experimentally increased brood sizes and female provisioning rates remain constant (KP Eckerle, unpublished data). Nonetheless, because clutch size, but not brood size, was manipulated, males were not confronted with additional begging nestlings in experimental nests. Thus, although we cannot say that males did not compensate for reduced female provisioning ability, we argue that male compensation is unlikely to explain our results.

Understanding why female house wrens lost more mass than controls when given enlarged clutches is critical to the interpretation of how incubation demands affect allocation of effort to subsequent nestling provisioning. Females of many species of passerines lose mass between egg laying and fledging (Moreno 1989). This mass loss has sometimes been attributed to a negative energy balance (the energy-deficit hypothesis) resulting from incubation or nestling provisioning effort (e.g., Askenmo 1977; Nur 1984; Martins and Wright 1993; Merilä and Wiggins 1997). However, breeding females of many species, including house wrens, are thought to lose mass primarily as an adaptive measure to increase flight efficiency during nestling provisioning (the wing-loading hypothesis) (Freed 1981; Merkle and Barclay 1996; Cavitt and Thompson 1997; Slagsvold and Johansen 1998). Although the issue can be decided only by directly measuring energy expenditure, we propose that adaptive mass loss to reduce wing loading is more likely to account for the results of this study. If females incubating enlarged clutches lost more mass than controls because of excess energy expenditure, they would likely have been in poorer condition when they began to incubate clutches in the late season than control females that incubated only their natural clutches during the early season. However, this was not the case. Early-season treatment did not affect female mass during the incubation stage of late-season nests, consistent with the wing-loading hypothesis. Thus, mass loss allowed females that expended extra effort during incubation to feed their natural broods more effectively, producing nestlings that were significantly heavier and larger than control nestlings in the early season. Females apparently made allocation decisions during incubation, based on elevated incubation demands, and did not readjust their provisioning effort based on the actual number of nestlings present. This did not occur during the late season, when females that incubated enlarged clutches may have simply been unable to produce heavier offspring because of reduced food availability.

Long-term data from central Illinois forests that are similar to and within 100 km of the Mackinaw study area show a seasonal decline in arthropod abundance (Kendeigh 1979). This decline in numbers of arthropods in the taxa that house wrens feed to their nestlings is accompanied by reduced growth rates and lower asymptotic mass characteristic of late-season nestlings compared with early-season nestlings on the Mackinaw study area (see Styrsky et al. 1999, 2000). If food availability was relatively low during the late season of the current study, late-season females may have simply not been able to find enough food for themselves, their nestlings, or both. Thus, female mass loss may have been adaptive during both early and late seasons, but because of lower food availability in the late season, experimental and control females produced late-season fledglings of similar mass and size. Alternatively, female mass loss may have been adaptive during the early season but not the late season. Some evidence suggests that mass loss during breeding may be adaptive up to a point, but that below some threshold, which likely varies with environmental conditions (e.g., food availability), mass loss may reflect a physiological cost (Hillström 1995; Cavitt and Thompson 1997).

An alternative explanation for why experimental females produced heavier and larger fledglings is that developing embryos received thermal benefits of enlarged clutches (e.g., reduced cooling rates of eggs during female's absence), which placed experimental nestlings on different growth trajectories from control nestlings. Although nestling mass did not differ between treatment groups on brood-day 4, we can not rule out this possibility. We point out, however, that foster eggs were stored in a refrigerator prior to being used in field experiments and, as a result, they did not contain developing embryos. Thus, experimental females did not incubate additional developing eggs but rather additional eggs that did not contain a developing embryo. Although we do not know if or how thermal characteristics of the added eggs differed from those of developing eggs, we doubt that their presence greatly reduced the cooling rate of the eggs when females were absent from the nest.

In conclusion, experimentally increasing incubation effort prolonged the duration of the incubation period, an important fitness-related trait. This is noteworthy because the probability of nest depredation, an important selective pressure influencing incubation patterns, increases with the time that nests are active (Bosque C and Bosque MT 1995; Conway and Martin 2000). Increased nest predation risk, then, is one possible cost of increased incubation duration, which, as our results show, is mediated in part by clutch size during incubation. Experimentally increasing incubation costs affected the allocation of effort to nestling provisioning but not in the direction predicted by the hypothesis (i.e., that incubation costs limit clutch size). Nevertheless, this result suggests that house wrens do vary how effort is allocated among phases of a breeding event in response to incubation costs. Fitness-related consequences of incubation costs may be, in part, condition dependent. There is evidence that temperature, which often varies greatly during the breeding season in temperate and higher latitudes, plays a major role in producing incubation constraints (Bryan and Bryant 1999; Reid et al. 1999, 2000a). Most studies that have demonstrated energetic costs of incubation have been conducted in relatively cold environments compared with that of this study (for review see Reid, Monaghan, and Nager 2002). Further, of the few studies to date demonstrating that incubation demands negatively affect the allocation of resources to nestling provisioning, almost all were conducted in high latitude (i.e., 55–61° N), cool, maritime climates (Heaney and Monaghan 1996; Cichoń 2000; Engstrand and Bryant 2002; Ilmonen et al. 2002). In contrast, we conducted our study during years of normal weather patterns at 41° N, in a warm, continental climate. We suggest that our results are consistent with condition dependence of incubation costs and that more experimental work on how environmental conditions influence trade-offs between incubation constraints and other life-history traits is necessary to understand the role of incubation in limiting clutch size in altricial birds.

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