

## Producing extra eggs does not deplete macronutrient reserves in European Starlings *Sturnus vulgaris*

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Christians, J. K. 2000. Producing extra eggs does not deplete macronutrient reserves in European Starlings *Sturnus vulgaris*. – J. Avian Biol. 31: 312–318.

Recent studies have suggested that the size of avian clutches may be determined in part by the costs of egg production, which are thought to involve the depletion of endogenous nutrient reserves. I tested the hypothesis that producing extra eggs would deplete protein and lipid reserves in a passerine species, the European Starling *Sturnus vulgaris*. The first three eggs of clutches were removed on the days they were laid to induce females to lay extra eggs. Females responded to this manipulation by laying, on average, 1.3 eggs more than controls. However, based on the distribution of clutch sizes, it appears that some females responded to the removal of three eggs by producing more than one extra egg, whereas others responded by laying no extra eggs or by deserting. In unmanipulated females, the lean dry mass of the flight muscles and total body lipid were found to decline throughout breeding, an observation which is often interpreted as evidence that egg production depletes endogenous reserves. However, females experimentally induced to produce an extra egg did not have reduced stores of protein or lipid at clutch completion. No effect was observed in starlings even though this study had substantial (80%) power to detect a change in protein reserves of the same magnitude as that observed in a previous study of gulls. The decrease in flight muscle mass that has frequently been observed during breeding in passerines may not be due simply to mobilization of endogenous protein reserves.

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A major assumption of life-history theory is that reproduction is both nutritionally and energetically costly (Clutton-Brock 1991). Reproductive costs are believed to result in trade-offs between breeding attempts (through effects of current reproductive effort on survival and future fecundity) and/or within reproductive bouts (Stearns 1992). Although there has been some empirical evidence of reproductive trade-offs (Gustafsson and Sutherland 1988, Nur 1988, Deerenberg et al. 1996), little is known of their physiological basis (Sheldon and Verhulst 1996, Monaghan and Nager 1997). Reproductive trade-offs could occur through a variety of mechanisms, and recent work has implicated immune function (Gustafsson et al. 1994, Ots and Hörak 1996, Sheldon and Verhulst 1996, Deerenberg et al. 1997) and feather moult (Nilsson and Svensson 1996, Svensson and Nilsson 1997).

A number of recent experiments have shed light on potential costs of increased expenditure during egg production in birds, specifically costs of producing extra eggs. For example, female Great Tits *Parus major* induced to lay an extra egg had a higher prevalence of a malarial parasite than controls (Oppliger et al. 1996). Experimentally increasing the number of eggs produced has also been found to adversely affect the female's ability to provision offspring, resulting in reduced growth and survival of the young in non-passerines such as Common Terns *Sterna hirundo* (Heaney and Monaghan 1995) and Lesser Black-Backed Gulls *Larus fuscus* (Monaghan et al. 1998). Such costs may play an important role in limiting the size of avian broods (Monaghan and Nager 1997). However, Milonoff and Paananen (1993) found no effect of experimentally increasing the number of eggs laid by Common Goldeneyes *Bucephala clangula*.

A potential cost of producing extra eggs is the depletion of protein reserves from the pectoral (flight) muscles (Monaghan and Nager 1997). For example, Monaghan et al. (1998) found that pectoral muscle mass was reduced at clutch completion, as judged by pectoral profiling, in females which had laid an extra egg. Pectoral mass has been observed to decrease throughout laying in unmanipulated females in a number of species (e.g., Houston et al. 1995a, b) and it has been suggested that the flight muscles may contain a reserve of specific amino acids for egg production (Houston et al. 1995b). Depletion of protein reserves from flight muscles could potentially have a number of effects including reduced flight performance and foraging ability (Monaghan and Nager 1997).

Most experimental work on the costs of producing extra eggs has been performed in non-passerines. The goal of this study was to determine whether experimentally increased expenditure in egg production would reduce protein reserves in a passerine species, the European Starling *Sturnus vulgaris*. As in previous studies, eggs were removed as they were laid to induce females to produce more eggs. The clutch size in European Starlings ranges from 3 to 7 (mode 5; J. K. Christians, unpubl. data), making it possible to investigate how females of different quality respond to egg removal, in contrast to the study of Monaghan et al. (1998), where all females would have laid the same number of eggs. In addition to measuring protein reserves, I also quantified lipid reserves as these have also been observed to decrease during laying in passerines (Jones and Ward 1976, Fogden and Fogden 1979, Houston et al. 1995a). Data on unmanipulated females were collected to determine whether the dynamics of protein and lipid reserves in European Starlings are similar to those in other species.

## Methods

### General

Fieldwork was performed during the breeding seasons of 1996 and 1997 at the Pacific Agri-food Research Centre (PARC) in Agassiz, British Columbia (49°14' N, 121°46' W), using a nest-box population of European Starlings. The experimental protocol followed the guidelines of the Canadian Committee on Animal Care (Simon Fraser University Animal Care Committee Project # 442B; PARC ACC Experiment # 9702). Boxes were checked each morning to determine the timing of clutch initiation and completion. Eggs were measured (length and breadth,  $\pm 0.01$  mm) at clutch completion and fresh egg mass was calculated from egg dimensions using an empirical formula ( $\text{mass} = 0.0009159 \times \text{length}^{0.954} \times \text{breadth}^{1.877}$ ) derived

from 175 eggs measured within 12 hours of laying ( $r^2 = 0.98$ ). If no new egg was found on two consecutive days, a clutch was assumed to be complete.

### Experimental manipulation

In 1996, egg removal was used to stimulate females to lay extra eggs. The first three eggs of the clutch were removed on the days they were laid from 33 nests selected at random. All unmanipulated nests ( $N = 38$ ) with clutch initiation dates within the range of the experimental nests were used as controls.

### Body composition

A subsample of females was collected upon clutch completion from control ( $N = 11$ ) and experimental ( $N = 9$ ) nests. As part of another study (Christians and Williams 1999), unmanipulated females were also collected at various stages of breeding: (a) *non-breeding* ( $N = 14$  in 1996;  $N = 5$  in 1997): approximately six weeks before egg laying began; (b) *pre-breeding* ( $N = 14$  in 1997 only): after the oviduct had begun to develop, but before an oviducal egg was present; (c) *1-Egg* ( $N = 17$  in 1996;  $N = 26$  in 1997): between the laying of the first and second eggs; (d) *clutch completion* ( $N = 11$  in 1996, described above;  $N = 30$  in 1997): the second day after the last egg of the clutch was laid; and (e) *chick rearing* ( $N = 17$  in 1997 only): eighteen days after the first chick had hatched. The nestlings of females collected during chick rearing were euthanized since they would otherwise have starved to death.

Birds were captured in mist-nets prior to breeding or at their nest-box during breeding, killed by exsanguination under anesthesia (mixture of ketamine and xylazine at doses of 20 mg/kg and 4 mg/kg, respectively), usually within 15 min of capture, and frozen until further analysis. Birds were later thawed and the *pectoralis* and *supracoracoideus* muscles (hereafter flight muscles) were dissected from the carcass. The lengths of the tarsus, keel and the coracoid were measured ( $\pm 0.1$  mm) at the time of dissection. The rest of the carcass was autoclaved for 9 h, homogenized and a subsample was used in subsequent analyses. The lean dry masses of the flight muscles and of the carcass were obtained by drying to constant mass in a drying oven at 60°C (flight muscles) or in a freeze-drier (carcass) followed by Soxhlet extraction for 8 h, with petroleum ether as the solvent (Dobush et al. 1985). Total body lipid was calculated from the difference between the dry mass and the lean dry mass of the flight muscles and carcass.

Table 1. Distribution of clutch sizes in experimental and control females.

Treatment		N	Clutch size					
			3	4	5	6	7	8
Experimental	Actual	24	0	0	8	8	3	5
	Hypothesized <sup>1</sup>	9	1	4	4	0	0	0
Control	Total	33	1	4	12	8	3	5
		38	1	7	24	5	1	0

<sup>1</sup> Hypothetical intended clutch sizes for the 9 females that deserted (see text).

## Statistical analyses

Egg mass was highly repeatable within clutches. Therefore, when comparing the mass of the last egg of the clutch between treatments, the mass of the first egg of the clutch (which was laid prior to manipulation) was included as a covariate in a general linear model (proc GLM, SAS Institute 1988). This was done to remove variation due to differences between females, and so increase the statistical power to detect an effect of treatment. The lengths of the tarsus, keel, and coracoid were combined into a single index of body size using the first principal component of a principal components analysis (proc PRINCOMP, SAS Institute 1988; Freeman and Jackson 1990). This index of size was included as a covariate in analyses of flight muscle mass (proc GLM, SAS Institute 1988). Size was not correlated with total body lipid and so was not used as a covariate in these analyses. Analyses comparing experimental and control birds use data from 1996 only, whereas analyses of unmanipulated females pool data from 1996 and 1997; preliminary analyses indicated that year effects were not significant. Values are presented as least-squares means (which are estimates of what the means would have been had the sample sizes been equal)  $\pm$  standard errors (SAS Institute 1988). All statistical analyses were carried out using SAS (SAS Institute 1988), except power analyses, which were performed using PASS (Hintze 1996).

## Results

### Reproductive performance

Experimental females did not replace all of the eggs that were removed. In the following discussion, "clutch size" will refer to the number of eggs that the female laid, not to the number of eggs in the nest at clutch completion. The number of "extra eggs" will refer to the difference between the number of eggs that an experimental female actually laid and the number of eggs that she would have laid in the absence of experimental manipulation (this value can only be inferred from the distribution of clutch sizes, and cannot be measured directly for individual females).

Experimental females laid, on average, 1.3 eggs more than controls (6.2 vs. 4.9; Wilcoxon two-sample test  $Z = 4.4$ ,  $P < 0.0001$ ). However, nine females (out of 33) deserted experimental nests during laying, whereas none of the 38 control females deserted, and this difference in desertion rate was highly significant ( $\chi^2_1 = 11.87$ ,  $P < 0.01$ ). Given that some experimental birds deserted, it is possible that the difference in clutch size between treatments is an effect of selection, i.e., the manipulation caused lower-quality, small-clutch females to desert. I therefore hypothesized clutch sizes of 3, 4 or 5 for the deserted females such that the relative frequency of these clutch sizes in the experimental group was similar to that of controls (Table 1, Fig. 1). Using this hypothetical distribution, the difference in clutch size between experimental and control females, 0.8 eggs (5.7 vs. 4.9), was still significant ( $Z = 2.6$ ,  $P < 0.01$ ). However, based on the distribution of clutch sizes (Fig. 1), it does not appear that all experimental females laid one egg more than their intended clutch. The frequency of

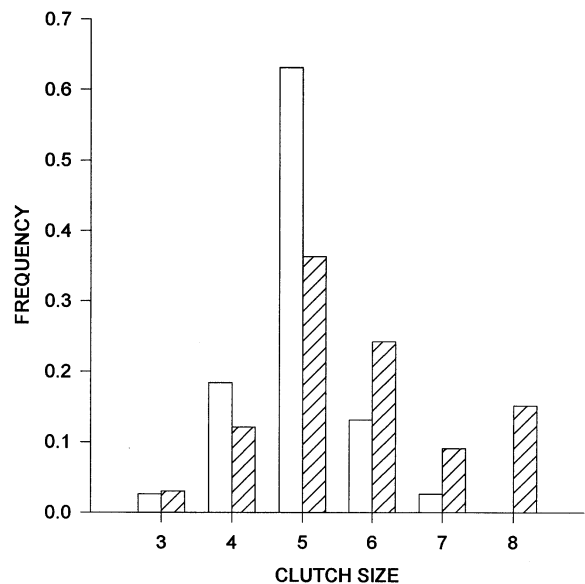


Fig. 1. Frequency distribution of clutch sizes in experimental (hatched bars) and control (open bars) females. The distribution for experimental females includes hypothesized clutch sizes for females that deserted (see Table 1).

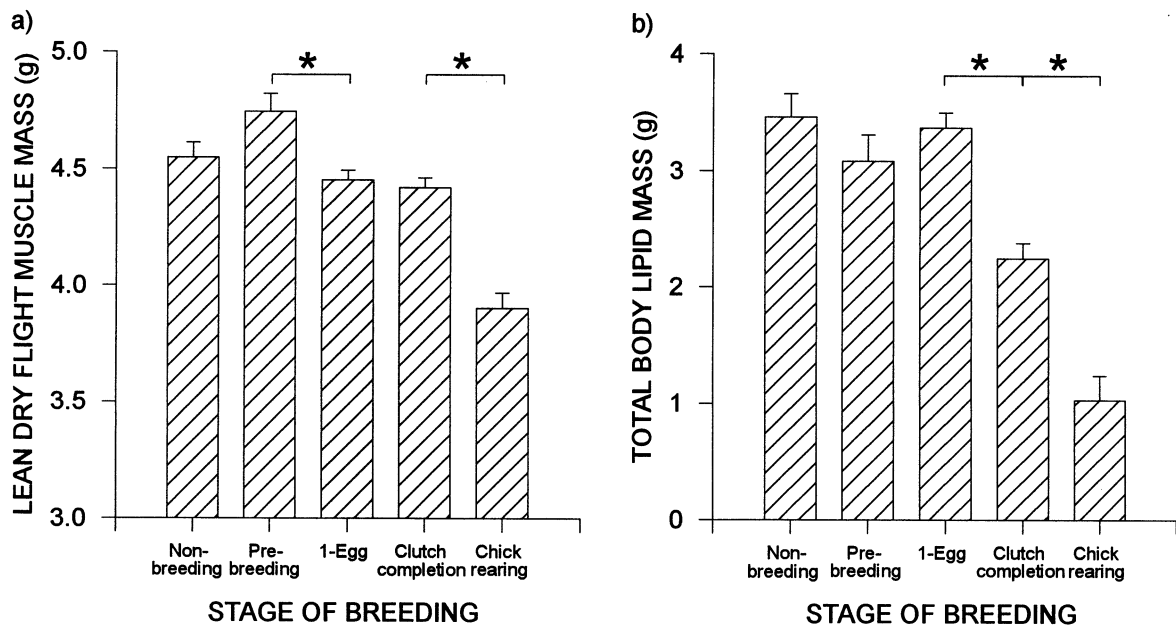


Fig. 2. Variation in the masses of (a) lean dry flight muscles, controlling for body size, and (b) total body lipid for unmanipulated females, in relation to stage of breeding. Only differences between adjacent columns were tested, and so 4 pairwise comparisons were made for each body component. The  $\alpha$ -level used for each comparison was therefore 0.05/4 (0.0125); an asterisk between adjacent columns indicates that the difference was significant. Values are least-squares means and standard errors.

8-egg clutches among experimental females (5/33, including deserted females) was higher than the frequency of 7-egg clutches among controls (1/38), although this difference was marginally nonsignificant ( $\chi^2_1 = 3.58$ ,  $P = 0.06$ ). Thus, some females appear to have responded to the removal of three eggs by producing more than one extra egg, whereas others appear to have responded by not laying any extra eggs or by deserting.

Producing extra eggs did not increase the proportion of females that skipped a day (i.e., did not lay an egg) during laying (4 of 24 experimental females vs. 5 of 38 controls;  $\chi^2_1 = 0.15$ ,  $P > 0.5$ ). The mass of the last (i.e., extra) egg of experimental birds ( $7.17 \pm 0.08$  g) was lighter than that of controls ( $7.34 \pm 0.06$  g), but this difference was marginally nonsignificant ( $F_{1,57} = 3.04$ ,  $P = 0.09$ ; including the mass of the first egg of the clutch as a covariate) and represented only 2.3% of the control value.

### Body composition

There was no significant difference in lean dry flight muscle mass between experimental ( $4.46 \pm 0.06$  g) and control birds ( $4.55 \pm 0.06$  g;  $F_{1,17} = 1.17$ ,  $P > 0.2$ ; including body size as a covariate) at clutch completion. Similarly, egg removal did not affect total body lipid (experimental:  $2.61 \pm 0.19$  g; control:  $2.55 \pm 0.18$  g;  $F_{1,18} = 0.06$ ,  $P > 0.8$ ). The power to detect a 5.5% (0.25 g) difference in flight muscle mass was 80% with the

sample sizes and standard errors obtained in this experiment, using  $\alpha = 0.05$  and a two-sided test. To have 80% power to detect an effect on total lipid mass, the difference between treatments would have to be 29% of the control value (0.75 g). As in the analysis of clutch size, the desertion of experimental females could have eliminated low-quality birds, and thereby artificially inflated the mean masses of muscle and lipid in the experimental group. I therefore repeated the analyses after removing control birds with clutch sizes smaller than 6. Removing these potentially lower-quality control birds actually decreased lean dry flight muscle mass and total body lipid in the control group, and did not qualitatively change the comparisons with experimental females (data not shown). Thus, the desertion of low-quality females from the experimental group would not have inflated measures of macronutrient stores.

Fig. 2 illustrates the patterns of change in flight muscle mass and total body lipid throughout the breeding cycle. Of particular relevance to this study, flight muscle mass decreased by 6% between pre-breeding and the 1-egg stage, but did not change significantly between the 1-egg stage and the end of laying (see Fig. 2a). In contrast, total body lipid did not differ between pre-breeding and the 1-egg stage, but did decline by 34% between the 1-egg stage and clutch completion (see Fig. 2b).

Among unmanipulated females collected at clutch completion, females that had laid different numbers of

eggs did not differ in flight muscle mass ( $F_{3,33} = 0.11$ ,  $P > 0.5$ ; including body size as a covariate) or total body lipid ( $F_{3,34} = 0.78$ ,  $P > 0.5$ ; see Table 2). Clutch size, flight muscle mass and total body lipid all decline throughout the breeding season among clutch completion birds, and so the effect of clutch initiation date was controlled statistically by including this term as a covariate in these analyses.

## Discussion

Experimental females responded to the removal of their first three eggs by producing, on average, one egg more than controls. However, it appears that some females responded to egg removal by producing more than one extra egg, whereas others responded by laying no extra eggs or by deserting. Meijer (1993) made similar observations in another study of European Starlings: females laid one to three extra eggs in response to the removal of three eggs, but only when the first egg was removed on the first day of laying, or early in the morning of the second day. When the removal of eggs was initiated in the afternoon of the second day of laying, there was no response to egg removal (Meijer 1993; see also Kennedy and Power 1990). Partial replacement has been observed in Great Tits, which lay one extra egg in response to the removal of two eggs (Oppliger et al. 1996). That females respond to egg removal, but do not replace all of the removed eggs, suggests that producing extra eggs carries some cost(s). The delay in incubation and hatching that could result from laying extra eggs does not appear to be a deterrent to replacing eggs, since European Starlings in my study area do not attempt to "save time" by laying fewer eggs when replacing entire clutches; clutch size does not differ between first and replacement clutches (Williams et al. 1999).

If endogenous nutrient stores limit the production of eggs, the last-laid (i.e., extra) egg of experimental females might be expected to be smaller than that of controls. However, there was no effect of treatment on egg size in this study. Similarly, no decrease in egg size was observed in Common Terns (Heaney et al. 1998) or Lesser Black-Backed Gulls (Monaghan et al. 1995) induced to lay an extra egg. However, the last-laid eggs

of experimental Common Terns had thinner, lighter shells (Heaney et al. 1998), and in Lesser Black-Backed Gulls (Monaghan et al. 1995), the extra eggs produced lighter chicks which were less likely to survive until fledging.

Monaghan and Nager (1997) hypothesized that the costs of producing extra eggs might be mediated by the depletion of protein reserves, particularly those found in the flight muscles. The decrease in flight muscle mass at the onset of laying in unmanipulated European Starlings was consistent with the hypothesis that protein stores are mobilized for egg production (Houston et al. 1995a, b). The pattern of change in flight muscle mass was similar to that observed by Houston et al. (1995a), i.e., a drop around the day the first egg is laid followed by a plateau throughout laying, rather than a linear decline throughout laying (cf. the interpretation of Houston et al. 1995a). Among unmanipulated European Starlings collected at clutch completion, there was no difference in flight muscle mass between females that had laid different numbers of eggs. Such results have previously been interpreted to suggest that females limit the number of eggs they lay such that their nutrient reserves are not depleted below some threshold (Jones and Ward 1976, Ankney and MacInnes 1978). However, in this experiment, females which were experimentally induced to lay, on average, one extra egg did not have reduced protein stores. Similarly, total body lipid decreased dramatically between the 1-egg stage and clutch completion in unmanipulated females, but laying one extra egg did not reduce lipid stores in experimental females. Thus, the mass of macronutrient stores decreases in association with egg production, but the magnitude of this decrease is not related to the extent of reproductive expenditure. In contrast, in Lesser Black-Backed Gulls Monaghan et al. (1998) found that producing one extra egg reduced the lean dry weight of the pectoral muscles by 5.3%, as estimated by the profiling of pectoral muscle. The power to detect such a difference in flight muscle mass in my experiment was over 80% and thus there appears to be a real difference between gulls and starlings in the costs of producing an extra egg. This is not surprising given the differences between these two species. For instance, the difference in body size alone will influence the relative protein and energy costs of egg production

Table 2. Variation in the masses of lean dry flight muscles and total body lipid for unmanipulated females collected at clutch completion, in relation to the number of eggs laid. Values are least-squares means and standard errors.

Clutch size	N	Lean dry flight muscle mass (g)		Total body lipid (g)	
		Mean	S.E.	Mean	S.E.
4	2	4.50	0.15	1.84	0.35
5	30	4.45	0.04	2.32	0.09
6	5	4.45	0.09	2.12	0.22
7	2	4.52	0.15	2.34	0.35

(Meijer and Drent 1999). The cost of producing an extra egg will also depend upon the degree to which stored reserves and dietary intake are used in the production of eggs. Lesser Black-backed Gulls use both endogenous stores and dietary intake for egg production (Bolton et al. 1993) whereas European Starlings may rely entirely on daily food intake (Meijer and Drent 1999). It is possible that in European Starlings, and perhaps passerines in general, the decrease in the mass of the flight muscles during laying is not due to the depletion of macronutrient stores (e.g., Williams 1996, Williams and Martyniuk 2000). Instead, the mass of the flight muscles may be reduced because of a decrease in activity (Williams and Ternan 1999), or to lower the costs of tissue maintenance (Williams and Martyniuk 2000).

In conclusion, I found no evidence of depletion of macronutrient stores in European Starlings experimentally induced to lay an extra egg, and so it may not be possible to extend the results of Monaghan et al. (1998) to passerines. However, the observation that females did not replace all of the removed eggs suggests that there are substantial costs to producing extra eggs in this species. Interactions between egg production and immune function could provide an alternative mechanism for the costs of producing extra eggs (Oppliger et al. 1996, Sheldon and Verhulst 1996), although this hypothesis has not been supported in European Starlings (Williams et al. 1999).

*Acknowledgements* – T. D. Williams provided encouragement, helpful comments and financial support throughout this study. C. Redfern, D. J. Moore and one anonymous reviewer also offered a number of useful suggestions and constructive criticism of the manuscript. I thank the staff of the Pacific Agri-food Research Centre in Agassiz, British Columbia, for permission to work on their grounds, and J. J. Aiken and S. P. Ternan for help in the field and in the laboratory. This study was funded by an operating grant to T. D. Williams from the Natural Sciences and Engineering Research Council of Canada (NSERC), and by NSERC postgraduate scholarships to JKC.

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(Received 29 June 1999, revised 1 October 1999, accepted 13 October 1999.)