Metabolic Costs of Egg Production in the European Starling (*Sturnus vulgaris*)

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ABSTRACT

The energy cost of egg production in passerine birds has typically been estimated to be 45%–60% of basal metabolic rate (BMR), but this is based on theoretical models using data on energy content of eggs and reproductive tissue; there are still very few empirical data on egg production costs. In this study, we directly measured resting metabolic rate (RMR) in egg-laying female European starlings (*Sturnus vulgaris*) over 3 yr. We compared these data with RMR of nonbreeding and chick-rearing birds and with estimated energy expenditure generated from a typical energy content model by using empirically derived data from body composition analysis for this species. We found marked variation in RMR between years and between reproductive stages, which complicates comparisons among breeding stages for the assessment of relative egg production costs. On the basis of this method, RMR during egg laying varied from +74% to −13% of nonbreeding RMR and from +20% to −7% of chick-rearing RMR. We therefore used an alternate approach: measuring changes in RMR through the complete cycle of follicle development and ovulation. The increase in RMR from the beginning of prelaying to the six-follicle stage (before first ovulation) when birds have a complete developing follicle hierarchy was 22.4%. This value is still much lower than that estimated from our energy content model. We discuss conceptual problems associated with the theoretical energy content approach but also suggest, on the basis of earlier work done in our lab, that the measured increase in RMR might still underestimate the actual cost of egg production if birds reallocate energy between different physiological systems.

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Introduction

Several recent studies have highlighted the importance of processes involved in actual production of young—that is, egg formation (cf. post–hatch rearing of young)—to our understanding of the evolution of clutch size and fitness costs of reproduction in birds (e.g., Heaney and Monaghan 1995; Monaghan et al. 1998; Nager et al. 2000; see also Partridge and Harvey 1985). These studies suggest that egg production can be costly in an evolutionary sense, with increased demand during egg production being associated with decreased chick-rearing performance (Monaghan et al. 1998) or a decrease in the female's future fitness (Nager et al. 2001; Visser and Lessells 2001). Ward (1996) and Stevenson and Bryant (2000) reported daily energy expenditure (DEE) measurements using the doubly labeled technique during egg production. They showed that DEE during egg laying can be comparable to that during incubation and chick rearing (Ward 1996) or close to 4 × basal metabolic rate (BMR) in birds laying large eggs (Stevenson and Bryant 2000). However, these DEE measurements represent the total energy budget for egg production, including foraging activities and thermoregulation costs (see Stevenson and Bryant 2000). Thus, these studies provide very little information about the actual energy expended in the physiological process(es) of producing eggs (e.g., yolk precursor production, ovary and oviduct growth and maintenance). These "direct" energy costs that an egg-producing female incurs during egg formation remain obscure and are still under debate (Carey 1996; Monaghan and Nager 1997; Nilsson and Raberg 2001).

One of the main reasons for this is that there are very few empirical measurements of metabolic rate during egg formation. Most estimates of egg production costs in birds have been based on theoretical models using chemical energy content of egg components and reproductive organs and information on the pattern and duration of yolk and albumen formation. For passerine birds, these models typically predict energy investment values during laying of about 45%–60% of BMR (King 1973; Ricklefs 1974; Ojanen 1983; Walsberg 1983; Rahn et al. 1985; Krementz and Ankney 1986; Carey 1996; Perrins 1996; Monaghan and Nager 1997; but see "Discussion"). However, these models make numerous assumptions, for example, that energy costs are added to routine maintenance costs; that they do not include costs of physiological processes such as yolk precursor production, transport, and uptake; and that they often use values for egg and organ energy content and BMR derived from allometric equations rather than empirically de-

rived values. Recently, Nilsson and Raberg (2001) measured resting metabolic rates (RMR) of great tits (*Parus major*) at different breeding stages and observed a 27% increase over wintering RMR in egg-laying females, a value that is much lower than that predicted by most energy content models.

In this article, we report on variation in RMR associated with egg production in free-living female European starlings (*Sturnus vulgaris*). In particular, we compare RMR at different breeding stages—including nonbreeding, egg laying, and chick rearing—over 3 yr and highlight high levels of variation both between years and between breeding stages. Second, in 2 yr we investigated changes in RMR during the egg production cycle in relation to specific stages of reproductive development (ovarian follicle size and number), from prelaying to clutch completion. Finally, we generate a typical "energy content" model of energy expenditure during egg formation for this species, but one based on empirically derived data from body composition analysis, and compare our measured RMR values with predicted RMR generated by the model.

Material and Methods

Field Site

This research was carried out at the Pacific Agri-Food Research Center (PARC) in Agassiz, British Columbia, Canada (49°14′N, 121°46′W), under a Simon Fraser University animal care permit (499B), following guidelines of the Canadian Council on Animal Care. The site consists of approximately 175 nest boxes on farm buildings and telephone poles that were used each year by breeding starlings. In each year, all boxes were checked daily to determine dates of clutch initiation and clutch completion and the laying sequence of eggs. During laying and early chick rearing, females were taken from their nest boxes during nighttime (generally between 2000 hours and 2400 hours); during late chick rearing, provisioning females were trap caught (always within 1 h before sunset). Nonbreeding and prelaying females were mist netted at two barns that were used as roosting sites. Eggs were collected when present at the time females were collected, for mass and size measurements.

Collection of Birds

In all 3 yr from 1999 to 2001, we measured RMR in birds at the end of the wintering period (nonbreeding [NB]), at the one-egg stage of laying (LY-1), and during chick provisioning (CK). We measured birds at LY-1 since this represents the day of peak energy investment in egg formation based on theoretical models (e.g., Ojanen 1983; Krementz and Ankney 1986; Williams and Ternan 1999) and patterns of yolk precursor production (Challenger et al. 2001). At that point, all birds had laid an egg, the second egg of the clutch is in the oviduct, and all the remaining follicles are simultaneously developing in the ovary. Final sample sizes were as follows: 1999, NB = 17, LY-1 = 12, CK = 5; 2000, NB = 19, LY-1 = 20, CK = 12; 2001, NB = 17, LY-1 = 14, CK = 19.

In 2000 and 2001, we also measured RMR in random-caught females during the 10 d before the first egg appeared in the colony (prelayers [PL]; $n = 9$ in 2000 and $n = 25$ in 2001). This prelaying group contained individuals at different levels of ovarian follicle development and oviduct growth but included no females that had laid their first egg. After recording the first clutch initiation in the colony, we started measuring RMR in birds at all stages of egg laying (one to six eggs) through to clutch completion ($n = 57$ in 2000 and $n = 55$ in 2001). For all birds, we measured RMR and completed dissections as described in "Body Composition Analysis."

Measurement of RMR

We define RMR as the energy consumed (measured as $\dot{V}O_2$) by a postabsorptive bird during the resting phase of the circadian cycle at a temperature within the thermoneutral range for the animal. Note that this is what is usually defined as BMR (Blem 2000). By definition, BMR is the lowest measurable $\dot{V}O_2$, and because laying birds in this study are producing eggs, they have to be considered in an "active physiological state" that should induce elevated levels of energy consumption. Therefore, we consider the term "resting metabolic rate" more appropriate in this case. All RMR measurements were completed using a flow-through respirometry system (Sable Systems International). After capture, birds were brought to the laboratory, body mass was measured (± 0.1 g), and then birds were placed in metabolic chambers (3.5 L) for 1 h before the beginning of RMR measurements. In some cases, females laid an egg in the chamber before the end of the recordings; data collected from these birds were discarded. All birds received about 500 mL/min of dry CO_2-free air (using Drierite and Ascarite as scrubbers) and were kept in the dark at 25°C, which is within the thermoneutral zone for this species (Lustick and Adams 1977). RMR measurements were always carried out between 2300 hours and 0500 hours. Our setup consisted of four metabolic chambers fitted with a perch and connected to a divided air line with a valve multiplexer that allowed us to sample air coming either from ambient baseline air (scrubbed for water and CO_2) or from one metabolic chamber at a time. The air then passed through a mass-flow valve (Sierra Instruments) for proper air-flow reading (STP corrected) and through CO_2 (model CA-1, Sable Systems) and oxygen analyzers (model FC-1, Sable Systems; air water scrubbed before CO_2 analyzer and water and CO_2 scrubbed before O_2 analyzer). All measurement sequences started by recording 10 min of baseline air. After baseline recording, the multiplexer switched, and the outcoming air from the first chamber was sampled for 55 min. Then the system switched back to baseline for 10 min before changing again to the second, third, and fourth chambers. On average, the birds stayed in their chambers for about 5.5 h. Preliminary

data showed that measuring RMR in a sequence like that did not generate "chamber" effect (see Hayes et al. 1992). Thus, having 1 or 4 h to rest did not affect RMR of the birds (F. Vézina and T. D. Williams, unpublished data). After RMR measurements, the birds were weighed for a second time, and the average of first and second mass was used in subsequent calculations. To calculate RMR, a running mean representing 10 min of recording was passed through the data for each bird, with the lowest average taken as RMR.

Body Composition Analysis

After RMR measurements, birds were killed by exsanguination under anesthesia (ketamine : xylasine at doses of 20 and 4 mg/kg, respectively), their feathers were plucked, and they were dissected. We recorded the fresh mass of the reproductive organs (oviduct and follicle-free ovary), the individual weights of all ovarian follicles, and the weight of the oviductal egg (± 0.001 g). The number of follicles and the presence of postovulatory follicles allowed us to confirm the breeding status of every bird. All samples were kept frozen at -20°C until the end of the field season for further processing. Adipose tissue in starlings is known to have a very low energy consumption (Scott and Evans 1992). Therefore, in order to avoid any dilution effect when investigating body mass versus RMR relationships, all organs and carcasses were freeze-dried (Virtis Freezemobile model 8ES) and fat extracted for 8 h in a Soxthlet apparatus using petroleum ether (Dobush et al. 1985; 8 h was enough in all cases to have several distillation cycles with samples soaking in completely clear ether, indicating complete fat extraction). Here we report lean dry body mass (LDBM) as being lean dry carcass mass plus lean dry organ mass (excluding the oviductal egg and feather mass).

Energy Investment Model

Energy investment models of egg production are typically constructed from the energetic content of the growing oviduct, which is assumed to grow at a constant rate, the developing follicles (calculated from the energy content of yolk taken from eggs), and albumen deposition (also calculated from egg content). This type of model generally divides investment over a species-specific period of rapid yolk development (RYD) and a 24-h period of albumen and shell deposition per egg. Body composition data collected in 2000 and 2001 allowed us to generate such a model based on empirical data (Fig. 1) for a European starling with a full six-follicle hierarchy. We present both the model and empirical data based on the pattern of follicular growth and ovulation (*X*-axis in Figs. 1 and 3); although this will broadly equate to number of eggs laid, they will not correspond exactly (e.g., because of follicular atresia; Challenger et al. 2001). Thus, the *X*-axis ranges from zero yolky follicles present (just before RYD, beginning of PL) to six yolky follicles (the maximum number of yolky follicles found in our birds) just before the first ovulation (PL part of *X*-axis; Figs. 1, 3). The number of yolky follicles then decreases as the bird ovulates one per day (LY part of *X*-axis; Figs. 1, 3) until all follicles are ovulated and the bird reaches the clutch completion stage (CC). Average clutch size in our colony was 5 ± 1, with clutches of 4, 5, 6, and >6 representing 17.6%, 56.5%, 17.6%, and 3.5% of all the clutches measured for the 3 yr. We used energy conversion values of 39 kJ/g for lipids, 18 kJ/g for tissue proteins (Blem 1990), and 23.5 kJ/g for follicle and egg proteins (Sotherland and Rahn 1987), and we evaluated the actual daily energy added to the reproductive system before and during egg production. Energy content of the oviduct, follicles, and follicle-free ovary was calculated based on their fat and lean dry content, and the average lean dry albumen content of the first egg laid in the clutch was used to determine energy transferred in that form. Egg biosynthesis efficiency in wild birds is poorly known (Perrins 1996). El-Wailly (1966) estimated a production efficiency of 42% for the zebra finch (*Taeniopygia guttata*), but estimates for domestic hens selected for increased level of pro-

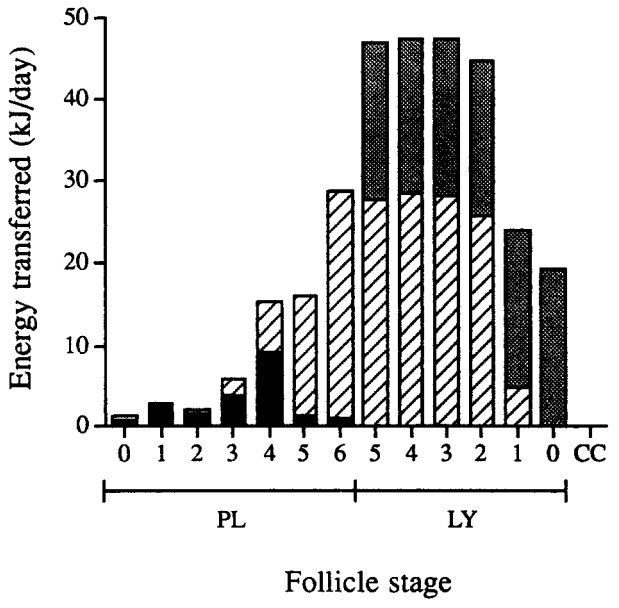

Figure 1. Pattern of energy transfer into eggs and reproductive tissue relative to the number of yolky follicles for European starlings with full six-follicle hierarchy (calculated from pooled data for 2000 and 2001). Prelaying (*PL*) stage extends from zero yolky follicles (just before rapid yolk development) to the maximum number of yolky follicles reported in our population (six). During laying (*LY*), the number of follicles decreases as they are ovulated until clutch completion (*CC*). Although the energy content of follicle-free ovaries is included in this model, it is not visible because it remained under 1 kJ in all cases. Energy content was transformed using 50% egg production efficiency (see "Material and Methods"). *Solid bars* = oviduct; *hatched bars* = follicles; *gray bars* = albumen.

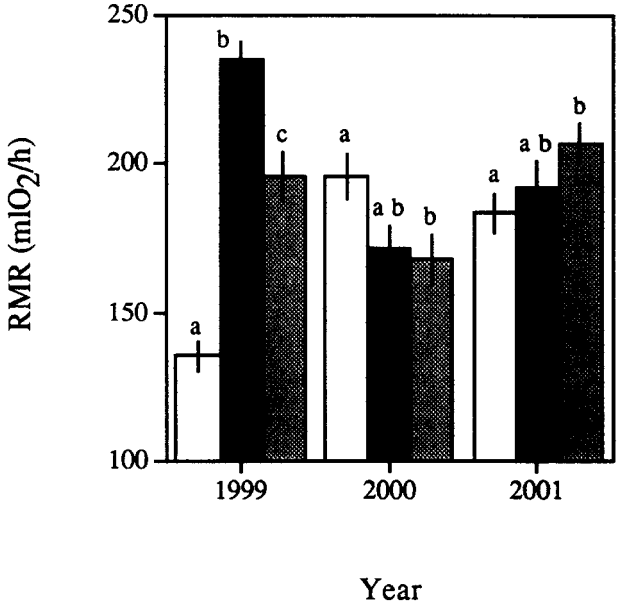

Figure 2. Interannual and breeding stage–related variation in resting metabolic rate (*RMR*) in European starlings. RMR values are least square means ± SE correcting for lean dry body mass. Different letters over the bars indicate significant difference between stages within a given year. *Open bars* = nonbreeding; *solid bars* = one-egg stage of laying; *gray bars* = chick provisioning.

duction were reported to be as low as 30%–37.5% (Van Es 1980) and as high as 65%–68% (Blaxter 1989). We therefore used a value of 50% for egg production efficiency; that is, all energy content values were multiplied by a factor of two (see "Discussion").

Results

Annual Variation in RMR with Reproductive Stage

Comparing RMR among NB, LY-1, and CK birds, we found a significant effect of year ($F_{2,125} = 13.0$, $P < 0.001$), breeding stage ($F_{2,125} = 10.6$, $P < 0.001$), and LDBM ($F_{1,125} = 15.4$, $P < 0.001$). However, there was also a significant interaction between breeding stage and year ($F_{4,125} = 30.1$, $P < 0.001$; see Fig. 2), so we reanalyzed variation in RMR for each year separately (no other interaction terms were significant in the full model). For each year, there was a significant effect of breeding stage on RMR (1999: $F_{2,30} = 75.0$, $P < 0.001$; 2000: $F_{2,47} = 4.1$, $P < 0.05$; 2001: $F_{2,46} = 3.8$, $P < 0.05$; controlling for LDBM in ANCOVA; no stage × LDBM interaction in any case). In 1999, LY-1 birds showed the highest RMR of the three groups, with a value 73.8% and 20.3% higher than NB and CK birds, respectively (Fig. 2). However, in 2000, the only significant difference in RMR was between NB and CK females, with NB individuals showing RMR values on average 16.6% higher than CK birds, and RMR in LY-1 birds was not significantly different from NB or CK birds. In 2001, NB individuals had the lowest RMR of the three groups (as in 1999). However, LY-1 and CK birds had mean RMR only 4.8% and 12.9%, respectively, higher than NB birds.

Variation in RMR during the Egg Production Cycle

RMR increased with the number of growing follicles in PL birds and then decreased in laying females as the number of follicles decreased ($F_{13,130} = 2.79$, $P < 0.005$; ANCOVA controlling for LDBM and year; no significant interaction terms; Fig. 3). At the six-follicle stage, peak RMR was 22.4% higher than at the prelaying zero-follicle stage (multiple contrast analysis, $P < 0.005$) and 13.9% higher than at clutch completion (multiple contrast analysis, $P < 0.05$; Fig. 3).

In order to investigate egg size effect on energy investment in LY-1 birds, we looked at the relationship between residual RMR (controlling for LDBM) and first-egg mass, oviductal egg mass, F1 follicle mass (subsequent follicle to be ovulated), and total follicle mass at the time of RMR measurement. Oviductal egg mass and first-egg mass were positively correlated ($r_{104} = 0.64$, $P < 0.001$). In 1999, residual RMR was negatively correlated with oviductal egg mass ($r_{12} = -0.59$, $P < 0.05$) and first-egg mass ($r_{12} = -0.78$, $P < 0.005$) but was independent of F1 and total follicle mass ($P > 0.05$ in both cases; Fig. 4). In 2000

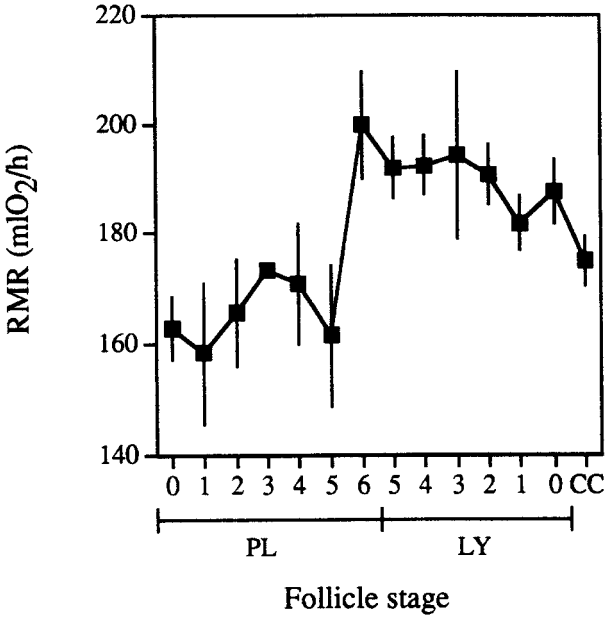

Figure 3. Pattern of variation in resting metabolic rate (*RMR*) relative to the number of yolky follicles in development during prelaying (*PL*) and laying (*LY*). RMR values are least square means ± SE correcting for lean dry body mass.

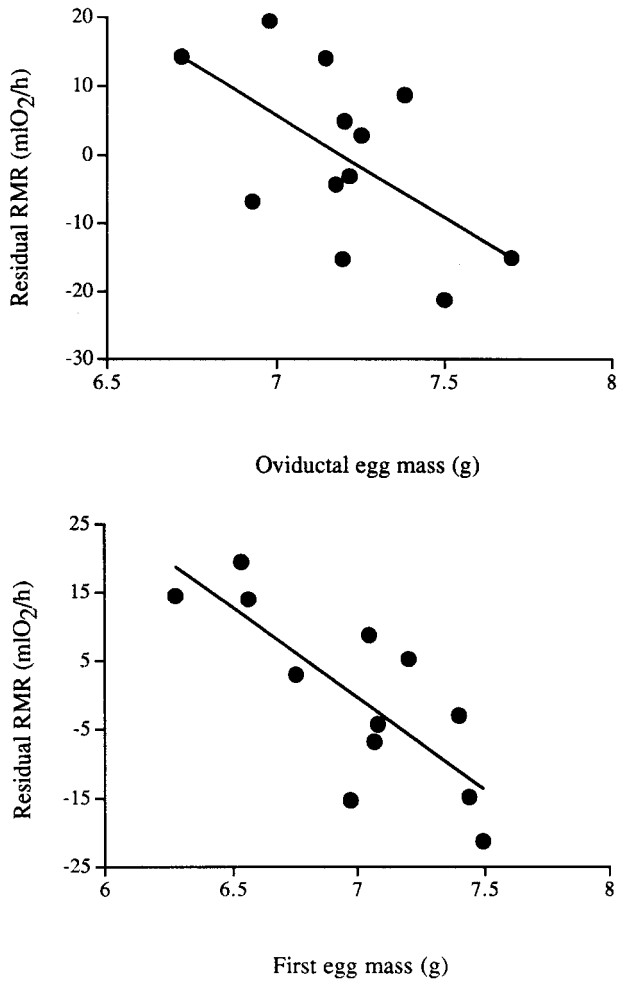

Figure 4. Relationships between residual resting metabolic rate (*RMR*; controlling for lean dry body mass) and oviductal egg mass (*top*) and first laid egg mass (*bottom*) in European starlings in 1999.

and 2001, there was no significant relationship between residual RMR and any measure of reproductive output ($P > 0.15$ in all cases).

Comparison of Measured versus Predicted RMR

Figure 1 shows the estimated energy transferred into reproductive organs and egg components in relation to the number of developing yolky follicles from prelaying to clutch completion. For this model, the energy transferred to the oviduct peaked at prelaying four-follicle stage and then started to decrease, while energy transferred in the follicle-free ovary was minimal (never more than 1 kJ/day). This model assumes that the turnover cost of maintaining these organs after the first ovulation is negligible—that is, that there was no new material added after the sixth follicle—and does not consider costs resulting from albumen and shell deposition by the oviduct or the specific costs of production of yolk precursors by the liver or their uptake by the follicles. However, on the basis of this model, the predicted energy transferred into eggs and reproductive organs in our starling population peaks at 47.7 kJ on the day of the second ovulation (at the four-follicle stage in the LY group). From Figure 3, measured RMR peaked at the six-follicle stage with a value of 199.1 ± 9.8 mL O_2/h compared with a value of 162.7 ± 5.5 mL O_2/h at the beginning of prelaying (zero-follicle stage; an increase of 36.4 mL O_2/h). The mean respiratory quotient (RQ) during laying in 2000 and 2001 was 0.77, which complicates energy conversion to kilojoules per day since an RQ of 0.77 might result from combustion of protein or a mixture of fat and carbohydrates (Schmidt-Nielsen 1990). Using the two extremes of RQ = 0.71 (fat only) and RQ = 1 (carbohydrate only), we calculated the energy equivalent for the 36.4-mL O_2/h increase in LY-1 as 17.2 kJ/day and 18.3 kJ/day, respectively, above PL levels (conversion factors taken from Schmidt-Nielsen 1990). These values represent only 36.1% and 38.4%, respectively, of the model predictions of peak energy transfer into eggs and reproductive tissues (Fig. 1).

Discussion

Female European starlings showed marked inter- and intra-annual variation in RMR over the 3 yr of this study, which has important implications for assessing the cost of egg production relative to other parts of the reproductive cycle. Nevertheless, in each of the 3 yr, absolute mass-corrected RMR was as high during egg laying as during the chick-rearing period (cf. Ward 1996), and in 1 yr, peak RMR occurred during egg laying. In birds undergoing egg formation, RMR changed systematically in relation to the number of developing follicles present in the ovary and was 22% higher in birds with a full follicle hierarchy (six-follicle stage) than in prelayers at the zero-follicle stage. However, controlling for stage of ovarian development, we found that measures of reproductive output (oviductal and first-egg mass) were significant predictors of RMR in only one of the 3 yr, and then the relationship was negative. Finally, the energy cost of egg production was overestimated by the model (peak RMR 47.7 kJ/d vs. a maximum of 18 kJ/d), even though this model was based on empirically derived data for the species.

Variation in RMR between Years and Reproductive Stages

Variation in RMR among years for the same breeding stage was, in some cases, as large as within years when comparing among breeding stages (Fig. 2), but the pattern of variation was not consistent among years. For example, RMR of nonbreeders was 31% lower in 1999 than in 2000, whereas the reverse pattern occurred in LY-1 females, with RMR being 37% higher in 1999 than in 2000. In contrast, in 2001 the maximum difference in RMR among stages was only 13% and occurred

between nonbreeders and chick-rearing birds. This level of variation makes it very difficult to assess the cost of egg production relative to other stages of reproduction. By definition, the only difference between nonbreeders, one-egg birds, and chick-rearing birds in our study was the physiological or reproductive state of the birds. The cost of producing eggs should be reflected in the difference in RMR between birds at the peak of investment (one-egg stage) and birds not engaged in egg formation (the control), but which one is the appropriate "control," nonbreeders or chick-rearing birds, since both would have fully regressed ovaries? In a comparable study, Nilsson and Raberg (2001) chose to use wintering RMR in great tits as a control group and reported a 27% increase in RMR associated with egg-laying values compared with wintering values (note that these RMR values were not mass corrected, which might bias this result; Blem 1984; Packard and Boardman 1988, 1999; Hayes and Shonkwiler 1996; Hayes 2001). It appears from our results that using wintering or nonbreeding RMR as a "baseline" level to estimate relative costs of egg production can be misleading. In our study, the relative difference in RMR between nonbreeders and laying birds was +74%, −13%, and +4.8% in 1999, 2000, and 2001, respectively. Clearly, future studies will need to obtain data from more than 1 yr (cf. Nilsson and Raberg 2001) to control for annual variation, since data from any one year can lead to very different interpretations. It is well known that metabolism is variable between seasons (Aschoff and Pohl 1970; Daan et al. 1989; Dawson and Marsh 1989; Cooper and Swanson 1994; Piersma et al. 1995) and that wintering acclimatization in birds induces higher levels of RMR (Swanson 1990, 1991*a*, 1993; Cooper and Swanson 1994; Saarela et al. 1995); therefore, it seems most likely that annual variation in temperature and extent of seasonal acclimation might be responsible for the extreme differences in nonbreeding RMR in the 3 yr of our study. However, we also found substantial between-year differences in RMR during laying and chick rearing, and the reasons for this variation are not clear. One possibility is that birds make adjustments to the size of their "metabolic machinery" in relation to variable ecological conditions between years (Daan et al. 1990; Piersma and Lindstrom 1997), and this may cause variation in RMR. Indeed, Christians and Williams (1999) and Burness et al. (1998) have reported significant between-year differences in the mass-corrected size of various organs during laying and chick rearing, respectively (we are currently exploring this further in our laboratory). Bech et al. (1999) showed that BMR was repeatable in kittiwakes (*Rissa tridactyla*) provisioning chicks; thus, variation in which individuals breed in any given year (i.e., "high" vs. "low" RMR phenotypes) could also contribute to annual variation in mean metabolic rate.

Metabolic Cost of Egg Production

Given the problems of assessing metabolic adjustments for egg production by comparing RMR among breeding stages, we suggest that a better method is to look at changes in RMR throughout the ovarian cycle, as measured by changes in follicle number. In other words, the direct physiological costs of egg production are calculated as the difference (increase) in RMR comparing birds forming eggs and those not (these include production costs of eggs and production and maintenance costs of reproductive tissues). There was a 22% increase in RMR comparing prelayers (with no yolky follicles) to laying birds with a full six-follicle hierarchy (note that RMR values for laying birds with three to five follicles are also 190–200 mL O_2/h). Given the frequency of six-egg clutches in our population, clearly not all of these birds would have laid six eggs. Therefore, we do not think that the estimated 22% increase in RMR is driven solely by high quality birds. The increase in RMR in laying birds that we report is somewhat lower than the 27% increase reported by Nilsson and Raberg (2001) when comparing RMR of wintering and egg-laying great tits, but it is higher than the 15% difference in RMR between their nest-building birds (comparable to our prelayers) and laying individuals (calculated from data presented in their article).

Nevertheless, this 22% increase in measured energy consumption associated with egg formation is much lower than our model prediction (by 62%–64%). Estimated costs of egg production from previous studies, all using the energy content models, are confounded by the fact that these different studies used no or different corrections for biosynthesis efficiency (Perrins 1996; Monaghan and Nager 1997). For example, King (1973), Walsberg (1983), and Krementz and Ankney (1986) used biosynthesis efficiency coefficients of 70%, 75%, and 77%, respectively. Overall, their estimates of egg production costs range between 45% and 60% of BMR. Recalculating this with a coefficient of 50% gives costs of egg production ranging between 63% and 83% of BMR. In other words, these models appear to totally overestimate costs of egg production relative to our measured increase in RMR. This difference is unlikely to be due to errors in estimating the nutrient content (lipid or protein) of follicles or reproductive tissue, since in our study we used empirically derived data to construct our energy content model. Rather, we believe that this discrepancy arises from problems in converting energy content per se to energy expenditure relative to BMR. As described above, these models typically calculate the energy content of eggs and reproductive tissue and express that as a percentage of estimated BMR. First, as Nilsson and Raberg (2001) pointed out, the energy content of the nutrients that form the reproductive organs (follicle-free ovary and oviduct) and eggs (follicles and albumen) may be higher than the cost of forming them. In other words, the chemical energy content of the eggs is higher in absolute value than the energy expanded in physiological work to transfer the nutrients from the food to the eggs. Second, many studies have compared predicted peak energy investment with RMR or BMR values calculated from multispecies allometric relationships (e.g., King 1973; Ricklefs 1974; Ojanen 1983; Walsberg 1983;

Krementz and Ankney 1986). However, the relationship between metabolic rate and body mass differ between species; therefore, the use of allometric relationships, especially when based on multiple species, may lead to significant biases (Williams and Vézina 2001). In conclusion, we believe that energy content models do not provide accurate estimates of the metabolic costs of the processes of egg formation (they simply assess the result of this process). Thus, comparing estimates based on these models with measured metabolic rates is really invalid, with the latter providing the more accurate method for assessing egg production costs.

Does our empirically derived estimate of a 22% increase in RMR accurately reflect the metabolic costs of egg production? In fact, the cost may still be higher if females also reallocate energy between different demands during egg formation. It is well known that the size and physiological activity of different organs is plastic and can vary depending on such things like diet (Imondi and Bird 1967; Piersma et al. 1993; Hammond and Janes 1998; Geluso and Hayes 1999), migration (Biebach 1998; Piersma and Gill 1998; Piersma et al. 1999; Battley et al. 2000, 2001), or season (Swanson 1991*b*; O'Connor 1995, 1996). Several studies have reported, for example, that thermoregulation costs can be compensated for with heat generated from muscular activity (Webster and Weathers 1990; Bruinzeel and Piersma 1998) or digestion (Masman et al. 1989; Chappell et al. 1997). During egg formation, there is a more than 10-fold increase in the size of the reproductive organs, presumably with a concomitant increase in the energy cost of maintaining these organs (thus leading to an increase in RMR). However, Christians and Williams (1999) reported that laying female starlings have relatively small gizzard, intestine, heart, and pancreas mass compared with nonbreeding birds. Thus, the increase in RMR caused by maintenance costs of reproductive tissues (as well as costs of yolk precursor production, follicular development, albumen, and shell deposition) might be partly compensated for by a reduction in the size and energy maintenance costs of other organs (e.g., see Geluso and Hayes 1999; Williams and Vézina 2001). Clearly, future studies investigating the metabolic costs of egg production should include body composition (organ mass) analysis in order to highlight possible adjustments in the metabolic machinery associated with egg production and, thus, evidence of energy reallocation between different physiological systems. Thus, simply measuring absolute RMR (summation of all maintenance costs) might be misleading in informing about costs of specific physiological function (Williams and Vézina 2001).

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