Plasticity in Body Composition in Breeding Birds: What Drives the Metabolic Costs of Egg Production?

François Vézina*
Tony D. Williams
Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

Accepted 4/1/03

ABSTRACT

Body composition in vertebrates is known to show phenotypic plasticity, and changes in organ masses are usually rapid and reversible. One of the most rapid and reversible changes is the transformation of the female avian reproductive organs before breeding. This provides an excellent system to investigate the effects of plasticity in organ size on basal metabolic rate (BMR) through relationships between organ masses and BMR. We compared body composition of female European starlings (*Sturnus vulgaris*) during various reproductive stages over 3 yr and investigated the pattern of changes in reproductive and nonreproductive organ mass during follicular development and ovulation. Furthermore, we analyzed the relationship between organ mass and resting metabolic rate (RMR) in nonbreeding, laying, and chick-rearing females. Our analysis revealed marked variation in organ masses between breeding stages but no consistent pattern among years except for kidney and pectoralis muscle. Furthermore, changes in nonreproductive organs did not parallel the cycle of growth and regression of the reproductive organs. The oviduct gained 62% of its 22-fold increase in mass in only 3 d, and oviduct regression was just as rapid and began even before the final egg of the clutch was laid, with 42% of the oviduct mass lost before laying of the final egg. In laying females, 18% of variation in mass-corrected RMR was explained by the mass of the oviduct ($r^2 = 0.18$, $n = 80$, $P < 0.0005$), while pectoralis muscle mass in nonbreeding individuals and liver and gizzard mass in chick-rearing females were the only organs significantly related to RMR ($r^2 = 0.31$–0.44). We suggest that the nonreproductive organs are affected more by changes in local ecological conditions than the reproductive state itself and that the activity and maintenance cost of the oviduct is high enough that selection has led to a very tight size-function relationship for this organ.

* Corresponding author; e-mail: fvezina@sfu.ca.

Physiological and Biochemical Zoology 76(5):716–730. 2003. 1522-2152/2003/7605-2163$15.00

Introduction

Several recent studies have demonstrated marked phenotypic plasticity in vertebrate anatomy in response to changes in ecological conditions or physiological state. For example, organs vary in size and function in response to such things as diet and food intake (Dykstra and Karasov 1992; Piersma et al. 1993; Geluso and Hayes 1999), migration (Piersma et al. 1996; Biebach 1998; Karasov and Pinshow 1998; Piersma et al. 1999*b*; Battley et al. 2000, 2001), altitude (Hammond et al. 1999, 2001), or stress (Rogers et al. 1993). Moreover, these changes can occur over a short timescale (Gaunt et al. 1990; Secor et al. 1994; Secor and Diamond 1995; Jehl 1997; Piersma et al. 1999*b*) and are reversible (Piersma and Lindström 1997; Piersma et al. 1999a).

Interindividual variation in body composition is generally believed to influence basal metabolic rate (BMR) through maintenance costs of organs and tissues (Kersten and Piersma 1987; Daan et al. 1990; Hammond and Diamond 1997; Piersma and Lindström 1997). Indeed, a common approach to investigating the basis of variation in metabolic rate is to examine the relationship between the mass of various body constituents and BMR (Konarzewski and Diamond 1995; Meerlo et al. 1997; Bech and Ostnes 1999; Chappell et al. 1999; Hammond et al. 2000; Piersma 2002). However, such studies have produced very inconsistent results in terms of which organs relate to metabolic rate even within a particular physiological state. For example, Chappell et al. (1999) found that BMR was related to the dry mass of liver, heart, lung, and pectoralis muscle in reproductive adult house sparrows (*Passer domesticus*) and to the dry mass of gut, liver, heart, and pectoralis muscle in juvenile individuals. However, in juvenile European shags (*Phalacrocorax aristotelis*), Bech and Ostnes (1999) found that RMR was only related to lean dry liver mass and intestine length, while another study by Burness et al. (1998) showed that daytime resting $\dot{V}O_2$ in adult reproductive tree swallows (*Tachycineta bicolor*) was related only to fresh kidney and intestinal mass. Conflicting results between studies are not necessarily surprising if (1) the physiological state (e.g., wintering, chick rearing, etc.) of the species under investigation is not the primary determinant of organ plasticity or if (2) changes in organ mass related to the

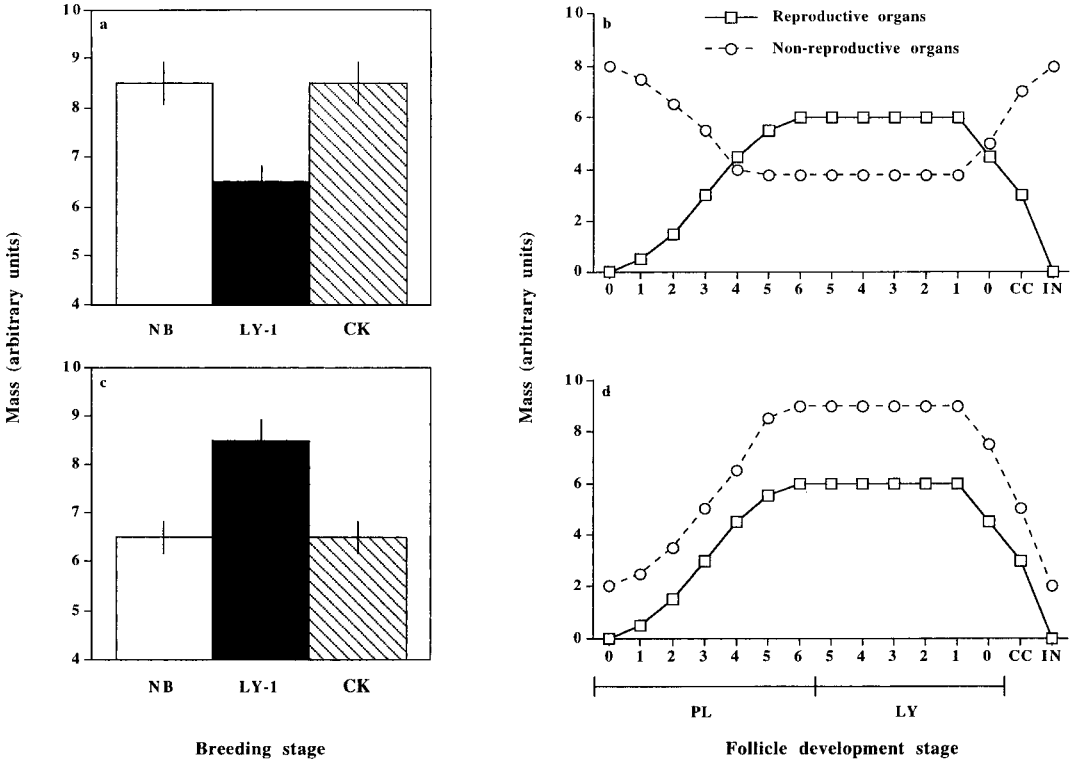

Figure 1: Examples of organ mass changes that would most strongly support the stated hypothesis. In the first case (*a*), a decrease in nonreproductive organ mass in birds at the one-egg stage (*LY-1*) compensates for the added cost of egg production to the overall energy budget through resource reallocation, resulting in bigger organs in nonbreeding (*NB*) and chick-rearing (*CK*) individuals. Within egg-producing birds (*b*), nonreproductive organ mass (*circles*) shows a pattern of change inverse to that of the reproductive organs (*squares*). In the second case (*c*), nonreproductive organs accommodate the increase in energy demand by an increase in their mass. This results in one-egg birds exhibiting heavier nonreproductive organs relative to nonbreeding and chick-rearing individuals. In this case, nonreproductive organs show a pattern of mass change that parallels that of reproductive organs (*d*). Any other pattern of mass change would be inconsistent with our hypothesis. *PL* = prelaying, follicular growth phase; *LY* = laying, follicle ovulation phase; *IN* = incubation; *CC* = clutch completion.

physiological state are of relatively small magnitude. One of the largest and most rapid reversible changes in anatomy is the seasonal recrudescence and regression of the avian reproductive system during breeding. This is most marked in females, where the ovary and oviduct gain a tremendous amount of mass, growing to full functional size generally in a few days. Given this large change in organ size (Christians and Williams 1999) and the increase in RMR associated with egg formation (Nilsson and Raberg 2001; Vézina and Williams 2002), egg-producing birds represent an excellent model system to investigate relationships between plasticity of organ mass and metabolic rate.

In a recent study (Vézina and Williams 2002), we investigated changes in resting metabolic rate (RMR; see "Material and Methods") in female European starlings (*Sturnus vulgaris*) through the complete cycle of follicular development and ovulation during three consecutive breeding seasons. We showed that RMR increases by 22.4% from the beginning of prelaying to the one-egg stage of laying, when birds have a complete developing follicle hierarchy and an egg in the oviduct. This estimate must reflect the additive energy costs of all the different physiological processes involved in egg formation: yolk precursor production in the liver (vitellogenin [VTG] and very low density lipoprotein [VLDL]), follicular growth in the ovary, and albumen and shell deposition in the oviduct. Here, we investigate the mechanistic basis of this increase in metabolic rate. We predicted that there would be consistent breeding-stage-related variation in nonreproductive body composition if reproductive state was the prime determinate of organ size and metabolic rate. This would occur either (1) to compensate for the added cost of egg formation to the overall energy budget through resource reallocation, for example, decrease in size of some organs resulting in energy savings through lower maintenance costs (Geluso and Hayes 1999; Vézina and Williams 2002), or (2) to accommodate the increase in energy demand resulting from egg production, for example, increase in size of the food-processing organs (Speakman and McQueenie 1996; Hammond and Diamond 1997; Piersma and Lindström 1997). We report data for two levels of analysis by (*a*) comparing three

different breeding stages—nonbreeding, one-egg, and chick rearing—and by (*b*) presenting a more detailed analysis of organ mass changes through the complete cycle of follicular development during egg production. Our hypothesis therefore was that organs specifically adjusted to the demands of egg production should be smallest (or biggest) in one-egg birds compared with nonbreeders and chick-rearing individuals and that within egg-producing birds, nonreproductive organ mass should show the inverse (or a parallel) pattern of mass change for gonadal development and regression through the follicle development and ovulation cycle (see Fig. 1).

Material and Methods

Field Site and Collection of Birds

Fieldwork was carried out at the Pacific Agri-Food Research Center (PARC) in Agassiz, British Columbia, Canada (49°14′N, 121°46′W) under Simon Fraser University animal care permit (499B), following guidelines of the Canadian Council on Animal Care. The site consists of ca. 175 nest boxes on farm buildings and telephone poles that were used each year by breeding starlings. Each year nest 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 an hour before sunset). Nonbreeding and prelaying females were mist-netted at two barns that were used as roosting sites. Eggs were collected for mass and size measurements at the time females were caught.

Reproductive Stages

In all 3 yr (1999–2001), 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 the one-egg stage of laying 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; but see Vézina and Williams 2002) and patterns of yolk precursor production (Challenger et al. 2001). At this point, all birds have laid an egg, the second egg of the clutch is in the oviduct, and all the remaining follicles are sequentially developing in the ovary.

In 2000 and 2001, we also measured RMR in random-caught females during the 10 d before the appearance of the first egg in the colony (prelayers, PL). 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 began measuring RMR in birds at all stages of egg laying (eggs 1–6) through to clutch completion. For all birds, we measured RMR and completed dissections for body composition analysis as described below. Sample sizes for RMR measurements are presented in Table 1.

Measurement of Resting Metabolic Rate (RMR)

We define RMR as the energy consumed 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 basal metabolic rate (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 induces elevated levels of energy consumption (Vézina and Williams 2002). Therefore, we consider the term "resting" metabolic rate more appropriate in the present case. RMR ($\dot{V}O_2$) was measured by flow through respirometry (Sable Systems International) following Vézina and Williams (2002). After capture, birds were brought to the laboratory, body mass was measured (± 0.1 g), and birds were then placed in metabolic chambers (3.5 L) for 1 h before the beginning of the measurements. All birds received about 500 mL/min of dry CO_2-free air and were kept in the dark at 25°C, which is within the thermoneutral zone for this species (Lustick and Adams 1977). Each chamber was sampled for oxygen and CO_2 analysis one at a time separated by 10 min of ambient baseline air readings (starting with baseline). RMR measurements were always carried out between 2300 hours and 0500 hours. Our setup allowed us to collect RMR data for four birds a night. On average, the birds stayed in their chambers for approximately 5.5 h. Preliminary data showed that sequentially measuring RMR in this way did not generate a chamber effect (see Hayes et al. 1992). Thus, having 1 or 4 h to rest

Table 1: Sample sizes for body composition and RMR data collected from 1999 to 2001

	1999	2000	2001
Body composition:			
NB	18	19	17
PL	…	10	25
LY-1	20	20	15
Rest of LY	…	38	63
CK	20	12	20
RMR:			
NB	17	19	17
PL	…	9	24
LY-1	12	20	14
Rest of LY	…	37	41
CK	5	12	19

Note. NB = nonbreeding, PL = prelaying, LY = laying, LY-1 = laying birds at the one-egg stage, CK = chick rearing.

did not affect RMR of the birds (F. Vézina, unpublished data). Following RMR measurements, the birds were reweighed, 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 $\dot{V}O_2$ taken as RMR. In some cases (less than 6%), females laid an egg in the chamber before the end of the recordings; RMR data collected from these birds were therefore discarded.

Body Composition Analysis

After RMR measurements, birds were killed by exsanguination under anesthesia (ketamine : xylasine at doses of 20 mg/kg 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 post-ovulatory follicles allowed us to confirm the breeding status of every bird. We also dissected out the following organs: pectoralis muscle (left and right reported here together as pectoralis muscle, not including supracoracoideus), heart, kidney, liver, gizzard, small intestine (from the gizzard to the caecae), and pancreas. 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, to avoid any dilution effect when investigating body mass or organ mass versus RMR relationships, all organs and carcasses were freeze-dried (Virtis-Freezemobile model 8ES) and fat-extracted in a Soxhlet apparatus using petroleum ether. 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). Final sample sizes for body composition data are presented in Table 1.

Yolk Precursor Analysis

In order to measure plasma levels of VTG and VLDL, blood samples were centrifugated at 5,000 rpm for 10 min, and the plasma portion of each sample was assayed for yolk precursors using vitellogenin zinc (Zinc kit, Wako Chemicals) and total triglycerides (Triglyceride E kit, Wako Chemicals) as indices of VTG and VLDL, respectively (Mitchell and Carlisle 1991; Williams and Christians 1997; Williams and Martiniuk 2000). The overall interassay coefficient of variation for the vitellogenic zinc and triglyceride assays (calculated from repeated analyses of the same sample) were 16.3% and 15.3%, respectively.

Statistical Analysis

Variations in organ masses between NB, LY-1, and CK groups were investigated on a per year and per organ basis using ANCOVA models. In this particular case, we wanted to control for the effect of body mass on organ mass. Because LY-1 birds have fully developed reproductive organs, we used nonreproductive LDBM (NRLDBM) as a covariate, that is, the total LDBM minus the mass of the reproductive organs. Also, in order to avoid part-whole correlations (Christians 1999) we subtracted the mass of the organ used as the dependent variable from the covariate. Because this procedure generated a substantial amount of post hoc comparisons (three reproductive stages and 3 yr per organ) we used the Bonferroni procedure (Rice 1989) to correct the P level of significance. Organs were compared as functional groups (heart, kidney, and muscles being the "metabolic machinery" organs [Daan et al. 1990; Christians and Williams 1999] and liver, small intestine, pancreas, and gizzard being the "food-processing" organs). Therefore, Bonferroni-corrected P values were 0.002 and 0.001 for metabolic machinery and food-processing organs, respectively.

Organ mass variation in relation to follicular growth and ovulation was also analyzed for years 2000 and 2001. In this case, we used an ANCOVA model, including year and follicle development stage as independent variables and NRLDBM (corrected for part-whole correlation) as a covariate. Because we were interested in the pattern of change of nonreproductive organs in relation to growth and regression of the reproductive organs, we subsequently compared differences in organ masses from early rapid yolk development (RYD; no yolky follicles) to the peak of development (six follicles before first ovulation) and then from the peak to clutch completion. Analysis of the relationships between RMR and body composition was performed for the NB and CK birds and on a subsample of the LY group (see "Results"). In this case, ANCOVA models with year as an independent variable and NRLDBM (LY birds) or LDBM (NB and CK birds), corrected for part-whole correlation, as a covariate were used to generate residual RMR and residual organ masses when significant. Residual RMR was then compared with residual organ mass in a multiple stepwise regression model. Results are presented as least squares means $\pm$ SE.

Results

Variation in Organ Masses between Breeding Stages and Years

There was marked variation in body-mass-corrected organ masses both between reproductive stages (NB, LY-1, and CK) and between years (Figs. 2, 3). In two cases, the breeding stage × NRLDBM interaction term in the ANCOVA model was significant: for lean dry intestine in 1999 ($F_{2,52} = 3.28$, $P < 0.05$) and lean dry pancreas in 2001

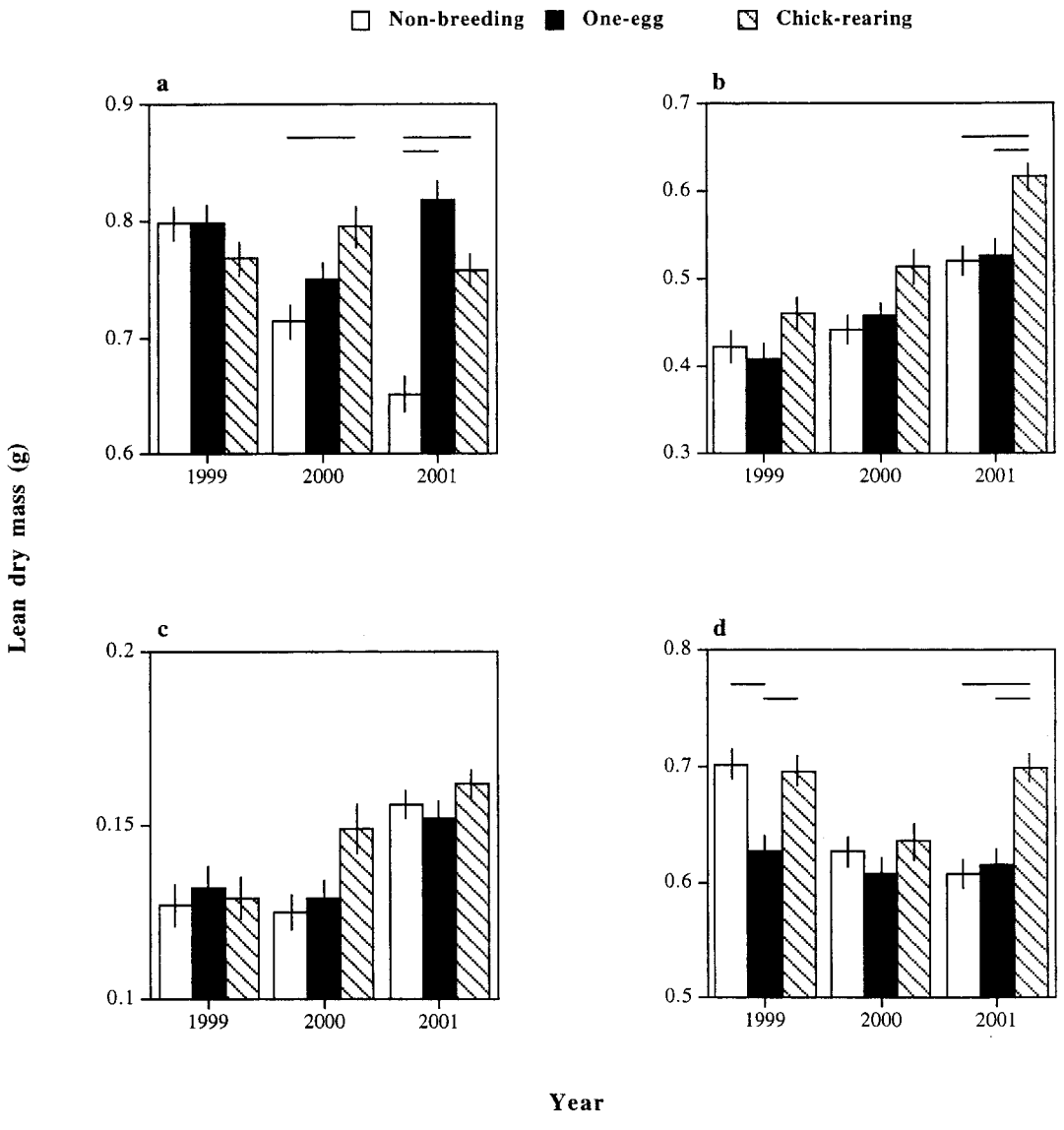

Figure 2: Interannual and interstage variation in lean dry mass of liver (*a*), small intestine (*b*), pancreas (*c*), and gizzard (*d*) in European starlings in 1999–2001. Values are least squares means ± SE controlling for NRLDBM (corrected for part-whole correlation). Bars over the columns indicate within-year significant differences between breeding stage. Level of significance is 0.001.

($F_{2,46} = 4.01$, $P < 0.05$). For these two cases, least squares means presented in Figure 2 were calculated with the interaction left in the model. In all other cases least squares means were calculated with only the covariate (NRLDBM corrected for part-whole correlation) left in the model.

Food-Processing Organs

For the food-processing organs, variation in organ mass by breeding stage was not consistent between years (Fig. 2). Lean dry liver mass tended to vary significantly between reproductive stages, but the pattern differed from year to year, with liver being 11.3% heavier in CK compared with NB birds in 2000 (Bonferroni-corrected post hoc *t*-test, $P < 0.0005$) and 25.4% heavier in LY-1 compared with NB birds in 2001 ($P < 0.0005$; Fig. 2*a*). In all years, lean dry small intestine tended to be heavier in CK birds, but this was significant only in 2001 ($P < 0.001$ in all cases), with a maximum mass difference of 18.2% (Fig. 2*b*). Lean dry gizzard mass also showed significant differences between reproductive stages in 1999 ($P < 0.0005$ in

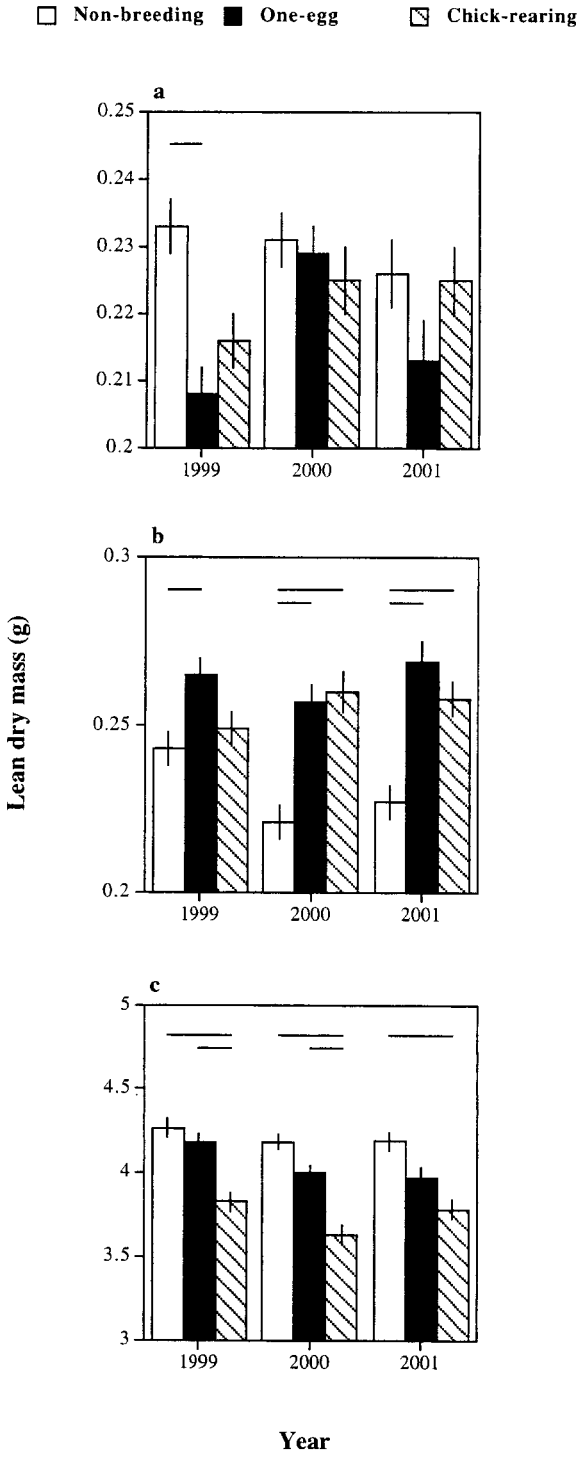

Figure 3: Interannual and interstage variation in lean dry mass of heart (*a*), kidney (*b*), and muscle (*c*) in European starlings in 1999–2001. Values are least squares means ± SE controlling for NRLDBM (corrected for part-whole correlation). Bars over the columns indicate within-year significant differences between breeding stage. Level of significance is 0.001.

all cases; maximum difference of 12.1%) and 2001 ($P<$ 0.0005 in all cases; maximum difference of 15.1%), but again the pattern differed between years (Fig. 2*d*). Lean dry pancreas showed no significant difference between stages in all years ($P>0.02$ in all years, Fig. 2*c*).

Metabolic Machinery

For the metabolic machinery organs, there were more consistent breeding-stage-related changes between years. Lean dry kidney mass showed a consistent pattern in 2000 and 2001, being on average 17.2% (Bonferroni-corrected post hoc *t*-test, $P<0.0005$) and 16.3% ($P<0.0005$) heavier in LY-1 and CK birds, respectively, compared with NB birds (Fig. 3*b*), while muscle mass decreased from NB to CK by 10.9%, 13.2%, and 9.6% in 1999, 2000, and 2001 ($P<0.0005$ in all cases; Fig. 3*c*), respectively. In contrast, heart mass did not show any consistent pattern between reproductive stages among year (Fig. 3*a*).

Organ Mass Variation and Yolk Precursors during Follicular Development

Combining data from prelaying and laying birds for 2000 and 2001 allowed us to look at variation in lean dry organ mass over the complete sequence of egg formation from the beginning of RYD—that is, no yolky follicles—to clutch completion. Figures 4–7 present changes in lean dry organ mass and plasma yolk precursors as the animal is growing the yolky follicles (PL group, follicles 0 to 6 on the *X*-axis), then through egg laying as one follicle is ovulated per day (LY group, follicles 5 to 0 on the *X*-axis) until the clutch is completed (CC).

Reproductive Organs

The reproductive organs varied according to the stage of follicle development (Fig. 4; lean dry follicle-free ovary: $F_{13,157} =$ 14.25, $P<0.0001$; lean dry follicles: $F_{13,144} = 129.00$, $P<$ 0.0005; lean dry oviduct: $F_{13,144} = 164.46$, $P<0.0005$). Although there was a significant year × follicle stage interaction both for lean dry follicle mass ($F_{12,144} = 2.76$, $P<0.005$) and oviduct mass ($F_{12,144} = 2.55$, $P<0.005$), both organs showed very similar patterns for 2000 and 2001 (Fig. 4*a*, 4*b*), with a very rapid growth at two yolky follicles and a similarly rapid loss of mass after the last ovulation. The oviduct increased in lean dry mass 22-fold from follicle 0 to follicle 6 (PL group; Fig. 4*b*), achieving 62% of its growth in 3 d (between the two- and four-follicle stages in PL), and lean dry follicle mass reached 72% of its maximal mass during the same time. The oviduct reached a peak mass just before the first ovulation in both years (follicle 6 in PL group; Fig. 4*b*), maintained a constant mass until the last follicle was ovulated, and then began to regress rapidly. In fact, the oviduct started to regress in mass as soon as there were no more yolky follicles in the ovary, that is, the oviduct

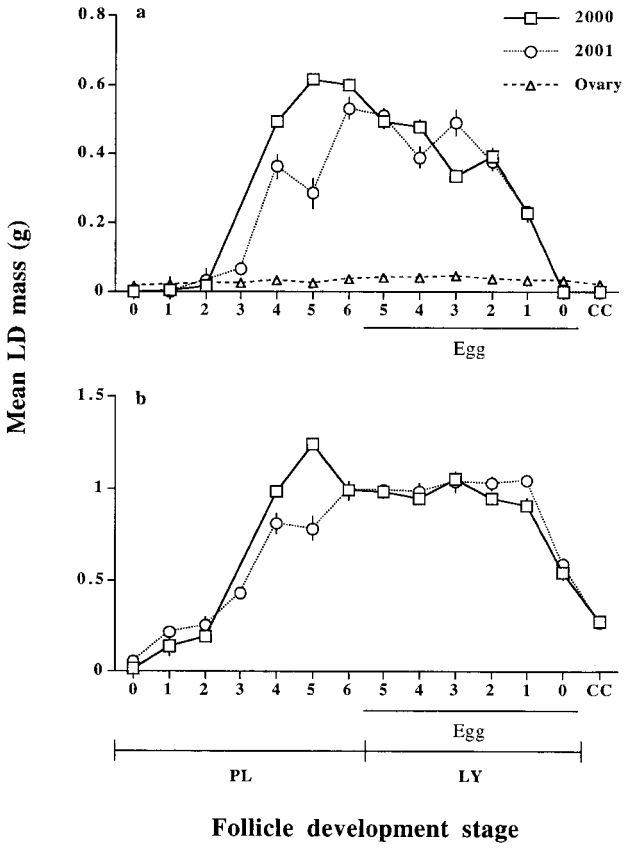

Figure 4: Changes in lean dry mass of ovary (*triangles*), total yolky follicles (*a*), and oviduct (*b*) relative to the number of yolky follicles for a European starling with a full six-follicle hierarchy in 2000 and 2001 controlling for NRLDBM and year. Prelaying (*PL*) stage extends from zero yolky follicles (just before RYD) 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*). There was a significant interaction between year and follicle stage in lean dry total follicle and oviduct mass. Therefore, both years are presented separately. *Squares* = 2000, *circles* = 2001. Values are least squares means ± SE.

lost mass while it was still producing an egg. Indeed, in LY birds at follicle stage 0 (no remaining follicles, with an oviductal egg), the oviduct had already regressed by an average of 42.5% of peak mass (both years, Fig. 4).

Plasma Yolk Precursor Levels

The pattern of variation in plasma yolk precursor levels in relation to follicular development was similar to that for reproductive organ growth and regression. Plasma VTG increased rapidly during RYD and stayed high until the last ovulation ($F_{13,154} = 25.54$, $P < 0.0005$; Fig. 5*a*). The year × follicle stage interaction was significant for the VLDL analysis ($F_{12,140} = 3.00$, $P < 0.001$). However, plasma VLDL levels showed similar patterns and mirrored VTG with a sharp decrease after the last ovulation (Fig. 5*b*). This confirms the pattern of plasma yolk precursors production reported earlier by Challenger et al. (2001) for the same population of birds.

Food-Processing Organs

Lean dry liver, small intestine, and pancreas mass varied significantly with stage of follicle development when controlling for year and NRLDBM (liver: $F_{13,143} = 2.82$, $P < 0.005$; small intestine: $F_{13,155} = 2.94$, $P < 0.001$; pancreas: $F_{13,155} = 1.95$, $P < 0.05$; Fig. 6). The year × follicle stage interaction was significant for liver mass ($F_{12,143} = 2.17$, $P < 0.05$), so we present data for both years separately (Fig. 6*a*; no other significant interaction terms in any cases). In 2000, lean dry liver mass increased by 28.8% from the beginning of prelaying (follicle = 0 in the PL

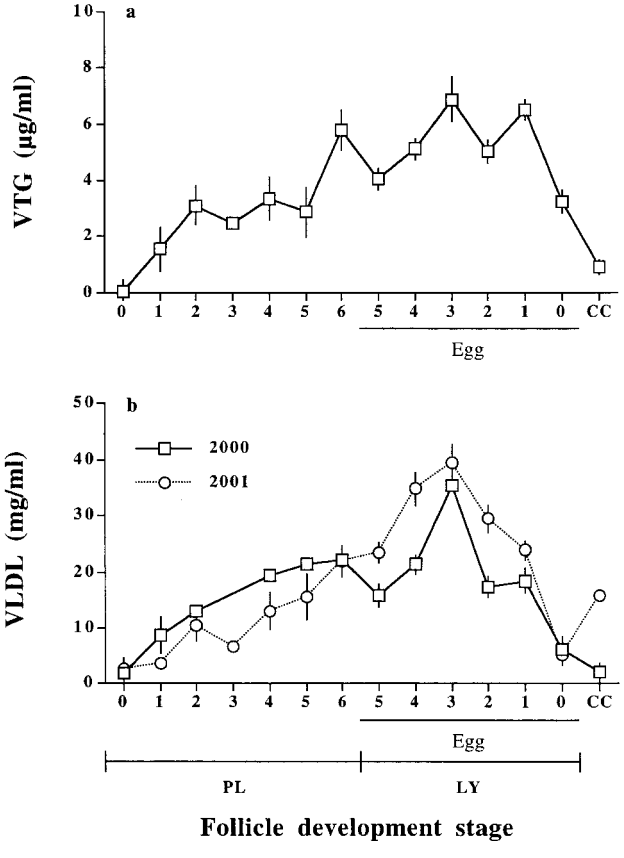

Figure 5: Changes in yolk precursors vitellogenin (*a*) and very low density lipoprotein (*b*) relative to the number of yolky follicles for a European starling with a full six-follicle hierarchy in 2000 and 2001 controlling for year. There was a significant interaction between year and follicle stage VLDL. Therefore both years are presented separately. *Squares* = 2000, *circles* = 2001. *PL* = prelaying, *LY* = laying, *CC* = clutch completion. Values are least squares means ± SE.

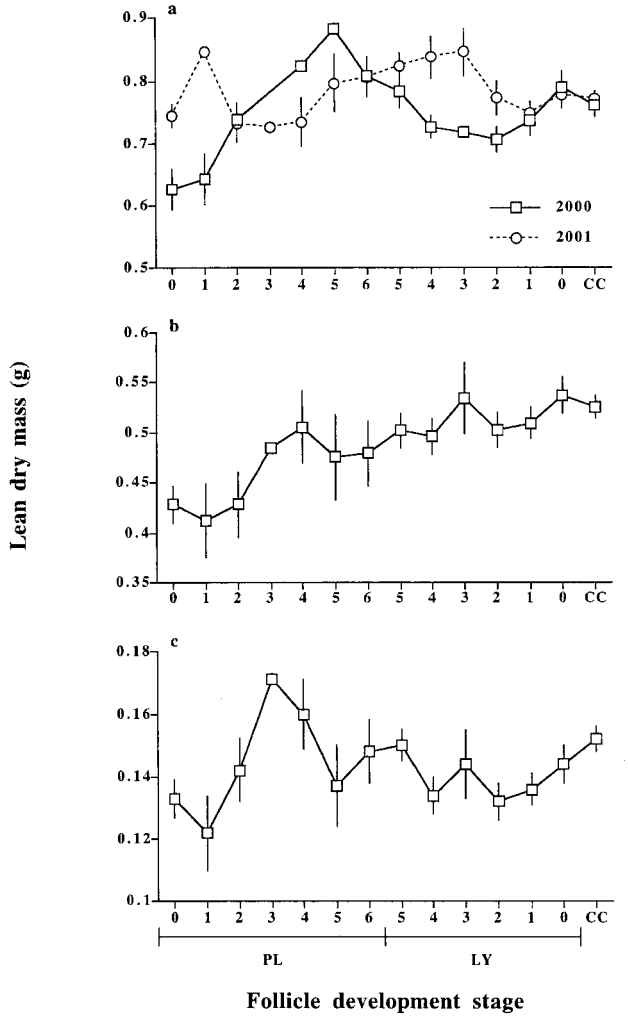

Figure 6: Changes in lean dry mass of liver (*a*), small intestine (*b*), and pancreas (*c*) relative to the number of yolky follicles for a European starling with a full six-follicle hierarchy in 2000 and 2001 controlling for NRLDBM and year. There was a significant interaction between year and follicle stage in lean dry liver mass. Therefore, liver mass is represented by year (*squares* = 2000, *circles* = 2001). *PL* = prelaying, *LY* = laying, *CC* = clutch completion. Values are least squares means ± SE.

group; Fig. 6*a*) to the day before the first egg was laid (follicle = 6 in the PL group; Fig. 6*a*; independent contrasts $P < 0.05$). It then remained constant until clutch completion (independent contrast $P = 0.5$). However, this pattern was not detected in 2001 (Fig. 6*a*). Lean dry small intestine mass did not peak at the six-follicle stage (independent contrast $P = 0.2$) but instead showed a gradual increase in mass (22.7%) throughout the egg-production cycle from the zero-follicle stage to clutch completion (independent contrast $P < 0.0005$; Fig. 6*b*). The pancreas showed a similar pattern (no peak at six follicles, independent contrast $P = 0.2$), with a total increase in mass of 14.3% between follicle stage 0 and clutch completion (independent contrasts $P < 0.01$; Fig. 6*c*). Lean dry gizzard mass did not vary in relation to follicle development stage.

Metabolic Machinery

Among the metabolic machinery organs, only lean dry kidney and pectoralis muscle varied significantly in relation to follicle development stage when controlling for year and NRLDBM (kidney: $F_{13,155} = 5.90$, $P < 0.0005$; muscle: $F_{13,155} = 2.21$, $P < 0.05$). Lean dry kidney mass increased by 17.3% from zero to six follicles in the prelaying group but then stayed constant until clutch completion (Fig. 7*a*; independent contrasts zero–six follicles $P < 0.005$; six follicles to clutch completion $P = 0.4$). Lean dry pectoralis muscle mass decreased gradually (3.9%) throughout the follicular cycle (Fig. 7*b*; zero follicles in the PL group to clutch completion; independent contrasts $P < 0.05$) and showed no distinctive pattern of mass loss (prelaying zero to six follicles or six follicles to clutch completion in the laying group; independent contrasts $P > 0.05$).

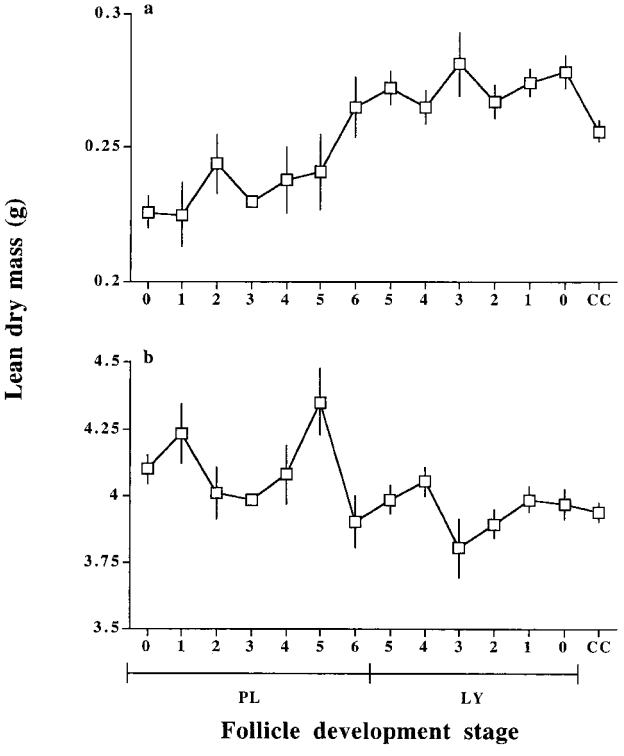

Figure 7: Changes in lean dry mass of kidney (*a*) and muscle (*b*) relative to the number of yolky follicles for a European starling with a full six-follicle hierarchy in 2000 and 2001 controlling for NRLDBM and year. *PL* = prelaying, *LY* = laying, *CC* = clutch completion. Values are least squares means ± SE.

Table 2: Intercorrelation matrix of residual organ masses for laying birds having five to one yolky follicle left to ovulate for 2000 and 2001

	Liver	Heart	Kidney	Ovary	Oviduct	Muscle	Intestine	Pancreas
Heart	.08							
Kidney	.41*	.09						
Ovary	.04	−.06	.04					
Oviduct	.08	.02	.16	.06				
Muscle	−.13	.12	.13	−.008	−.14			
Intestine	.23	.15	.21	.08	.02	−.17		
Pancreas	.15	−.02	.17	−.05	.16	−.16	.38*	
Gizzard	.08	.003	.11	−.05	.17	−.09	.10	.11

Note. Residuals correct for effect of follicular stage, year, and lean dry nonreproductive body mass.

* Significant correlation with a level of significance adjusted to $P < 0.0013$ using a Bonferroni correction.

What Drives the Metabolic Cost of Egg Production?

The pattern of development of the reproductive organs, in addition to the pattern of yolk precursors production in relation to follicle development stage, is very similar to changes in RMR in egg-producing females we reported in an earlier article (Vézina and Williams 2002). Therefore, in order to investigate the effect of body composition on metabolism in laying females, we analyzed the potential relationships between residual RMR and residual organ mass (correcting for follicular stage, year, and NRLDBM effect). Since prelaying birds were caught at different stages of reproductive development, we excluded them from the analysis because they would artificially increase the mass range of the reproductive organs. The same reasoning applies to birds with no remaining follicles (LY at zero follicles). We therefore restricted the analysis to LY birds having five to one follicle left to ovulate. At this point, all individuals had fully grown oviducts (Fig. 4*b*) and high plasma levels of VTG and VLDL (Fig. 5). All the variables to be included in the model were first checked for multicollinearity (Zar 1996; Table 2).

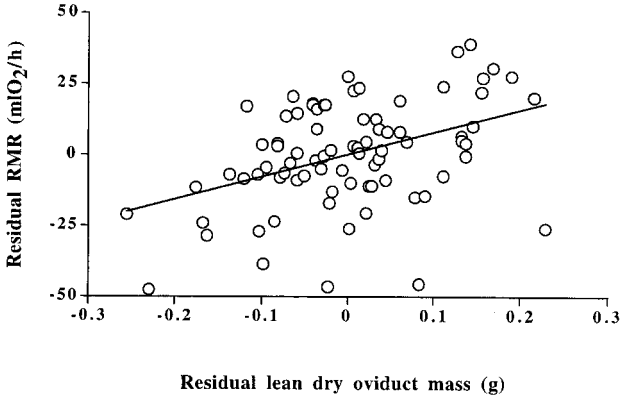

Figure 8: Relationships between residual RMR and residual oviduct mass. Residuals are correcting for the effect of follicular stage, year, and nonreproductive lean dry body mass.

Including all the organs in the model, stepwise multiple regression indicated that for the 3 yr of study, 17.6% of the variation in residual LY RMR was explained by residual lean dry oviduct ($r^2 = 0.18$, $n = 80$, $P < 0.0005$; Fig. 8). Residual RMR was independent of residual plasma level of VTG or VLDL (correcting for year effect), and residual precursor levels were not related to residual lean dry liver mass (correcting for year and NRLDBM).

RMR and Residual Organ Masses in Nonbreeders and Chick-Rearing Individuals

As for the laying birds, we performed stepwise multiple regressions relating residual RMR to residual organ masses (controlling for effects of year and LDBM) in nonbreeding and chick-rearing birds (see Table 3 for multicollinearity). In nonbreeders, residual pectoralis muscle mass was the only organ significantly related to residual RMR ($r^2 = 0.31$, $n = 53$, $P < 0.0001$). In chick-rearing birds, variation in residual RMR was explained by two organs: residual liver mass (34%) and residual gizzard mass (10%; overall model $R^2 = 0.44$, $n = 36$, $P < 0.0001$).

Discussion

In the present study we have confirmed that during egg production, female starlings undergo rapid and very large mass changes in reproductive organs (22-fold for the oviduct). This is accompanied by major changes in plasma protein and lipid (yolk precursors) levels and a 22% increase in RMR (Vézina and Williams 2002). This confirms that our egg-producing females were in a very different physiological state compared with nonbreeding and chick-rearing individuals. We have also shown that body-mass-independent nonreproductive organ masses varied markedly (9%–25%) between breeding stages; however, there was no consistent pattern among years in relation to specific breeding stages. Moreover, in egg-producing females, the pattern of nonreproductive organ mass change did not

Table 3: Intercorrelation matrix of residual organ masses for nonbreeding and chick-rearing birds for 1999, 2000, and 2001

	Liver	Heart	Kidney	Ovary	Oviduct	Muscle	Intestine	Pancreas	Gizzard
Liver		.06	.59*	−.12	.14	.2	.22	.17	.24
Heart	−.02		.02	−.22	.05	.08	−.01	.11	.12
Kidney	.26	−.01		−.1	.12	.09	.05	.31	.18
Ovary	.01	.18	−.06		.3	.15	−.13	−.22	−.18
Oviduct	−.08	−.16	−.03	.35		.05	−.22	.17	.08
Muscle	−.16	.42	.05	−.07	−.01		−.15	−.15	−.02
Intestine	.32	.08	.03	.26	−.02	−.13		.22	−.06
Pancreas	.37	−.06	.27	−.07	.04	−.19	.34		.12
Gizzard	.18	−.32	−.06	.05	.12	−.01	.18	.28	

Note. Values below the diagonal are for nonbreeding individuals; values above the diagonal are for chick-rearing individuals. Residuals correct for effect of year and lean dry body mass.

* Significant correlation with a level of significance adjusted to $P < 0.0013$ using a Bonferroni correction.

reflect the cycle of reproductive development and regression seen for the oviduct, ovary, and yolk precursors. Rather, organs changed either linearly through laying or not at all. However, the pattern of oviduct recrudescence and regression closely followed that of follicular development, with a very rapid mass gain at the two-follicle stage and a rapid loss of mass after the last ovulation (even though at this stage the oviduct was still processing an egg). In laying birds, 18% of the variation in residual RMR was explained by residual lean dry oviduct. In contrast, nonbreeding residual RMR was correlated to residual lean dry pectoralis muscle mass, while residual lean dry liver and gizzard were the organs that significantly predicted RMR in chick-rearing birds.

Adjustments of Nonreproductive Organs for Egg Production

We hypothesized that if reproductive state is a prime determinant of organ size, in order to adjust to the demand of egg production, nonreproductive organs should either (1) be consistently heavier or lighter in laying birds compared with nonbreeding and chick-rearing individuals and/or (2) show a pattern of change in mass mirroring the cycle of gonadal development and regression. We found little support for this hypothesis. In fact, only the gizzard showed one of the predicted patterns: a significant decrease in mass in laying individuals compared with nonbreeding and chick-rearing birds. However, this was not consistent among years. Conversely, kidney and pectoralis muscle mass did show a consistent pattern between years, but it appears that the laying stage per se was not driving this morphological change since the mass of these organs did not peak or dip at the one-egg stage compared with nonbreeding or chick-rearing individuals. Rather, mass tended to stay constant (kidney) or continued to decrease (pectoralis) after clutch completion through to chick rearing. Furthermore, our more detailed analysis based on the pattern of follicular development and ovulation showed that marked changes in reproductive physiology in terms of ovary, follicles, oviduct, and yolk precursors were not accompanied by similar changes in nonreproductive organs. That is, the only organs that showed significant changes in mass related to follicular stages (liver, small intestine, pancreas, kidney, and pectoralis muscle) did not vary in a pattern similar or inverse to that of reproductive organs. These results strongly suggest that the physiological state of a bird in itself (i.e., its breeding stage) does not determine the marked organ plasticity that we documented.

Christians and Williams (1999) investigated organ mass changes in breeding female starlings of the same population during 2 yr preceding this study. They also reported an increase in lean dry glycogen-free liver mass from nonbreeding to laying, but this was significant for only 1 yr. Similarly, our results for lean dry liver mass showed a significant increase from NB to LY-1 in 2001, but no significant differences were found in 1999 and 2000. Furthermore, liver mass variation showed completely different patterns between the three stages for the 3 yr, and the reasons for this between-year difference are not clear. Christians and Williams (1999) also found a correlation between plasma levels of yolk precursors and liver mass but in only one of two years in this population. We did not find such a relationship for the 3 yr of our study, and these combined results indicate that increased production of VTG and VLDL is rarely associated with liver hypertrophy.

If plasticity in nonreproductive organs is not directly related to breeding stage, what is responsible for the substantial changes in organ masses reported here? Part of the answer may come from the food-processing organs. Lean dry small intestine mass increased by 22.7% from the beginning of rapid yolk development to clutch completion and tended to gain even more mass later, as shown by a significantly heavier intestine in CK birds in 2001. Changes in small intestine function and mass associated with changes in diet have been reported before for this species (Levey and Karasov 1989; Geluso and Hayes 1999). We do not have information on diet composition for our pop-

ulation, but adjustments in small intestine mass may reflect a gradual seasonal change in diet. Indeed, lean dry kidney and pancreas mass both increased during the same period, and these organs are known to gain weight on protein-rich diets (Imondi and Bird 1967; Hammond and Janes 1998; but see Goldstein et al. 2001). It is possible that the diet of our experimental birds included an increasing proportion of protein, possibly coming from insects (Feare 1984).

We also documented a gradual loss in lean dry pectoralis muscle mass from nonbreeding to chick rearing (−10.9%, −13.2%, and −9.6% in 1999, 2000, and 2001, respectively) as well as throughout the cycle of follicle growth and egg laying. Many other studies have reported loss of muscle mass during breeding in a variety of avian species (Jones 1990; Houston et al. 1995*a*, 1995*b*, 1995*c*; Cottam et al. 2002). Lean dry muscle contains mostly proteins (Jones 1990), so muscle protein breakdown could also contribute to the reported increase in kidney mass in response to high protein levels in the blood. Some studies have suggested that muscle proteins could be transferred into egg material (Jones 1990; Houston et al. 1995*a*, 1995*b*, 1995*c*; Cottam et al. 2002). Although our experiment was not designed to study this phenomenon in particular, it is of interest to note that the loss of pectoralis muscle mass did not precede onset of follicular growth. Indeed, average NB lean dry pectoralis mass for 2000 and 2001 combined was virtually the same as early PL birds at follicle 0 (4.1 ± 0.05 g in NB and 4.2 ± 0.05 g in PL-0). At clutch completion their muscle weighed 3.9 ± 00.3 g (7.1% loss during egg production). However, the average CK lean dry muscle mass was 3.7 ± 0.1 g, indicating that pectoral muscle lost a further 5.1% mass during the incubation and chick-rearing periods. Thus, it appears that the loss of pectoralis muscle mass was not specifically associated with the egg-production phase but was a more general phenomenon associated with all breeding stages. An alternative explanation for muscle mass loss during LY and CK periods is a higher state of physical activity, since activity training has been reported to result in loss of muscle mass in this species (Swaddle and Biewener 2000). Overall, it appears that kidney and muscle mass changes, even though they show consistent patterns between years, are not strictly related to egg production but are affected by factors more or less independent of reproductive state.

Our results suggest that, in European starlings, egg formation has no consistent effect on plasticity of nonreproductive organ masses. Organ masses are dynamic, and evidence that they are simply adjusting to local ecological conditions is accumulating (Piersma and Lindström 1997; Summers et al. 1998; Hammond et al. 1999; Hilton et al. 2000). For example, Hilton et al. (2000) showed intraspecific variation in seabird organ masses in seabirds living in different geographic locations in Iceland and therefore facing different ecological conditions. It is reasonable to assume that varying conditions between years for a given geographic location might have similar effects on body composition as intraspecific differences between different locations. We therefore suggest that yearly differences in ecological conditions at the time of breeding may have more impact on nonreproductive organ mass variation in breeding starlings than the physiological changes associated with egg formation. However, organs may adjust their mass-specific metabolism (Kvist and Lindström 2001) and possibly reduce their energy expenditure without a change in mass, thus still allowing for energy reallocation in breeding females.

What Drives the Cost of Egg Production?

We recently showed that RMR in breeding female starlings increases by 22.4% from early RYD (zero follicles in PL) to the day before the first ovulation (follicle 6 in PL; Vézina and Williams 2002). Results of the present study demonstrate that, during laying, when the reproductive organs are fully developed and plasma levels of yolk precursors are maximal, the mass of the active oviduct explains 18% of the variation in mass-corrected RMR. This is consistent with the hypothesis that the maintenance and activity costs of the oviduct are high enough to affect the overall energy consumption of the animal at rest. A positive relationship between metabolic rate and the fresh mass of the oviduct has also been observed in prelaying zebra finches in our laboratory (F. Vézina, unpublished results). Furthermore, Chappell et al. (1999) reported a correlation between BMR and dry combined ovary and oviduct mass in breeding house sparrows. However, the present study is the first to investigate the full development of the reproductive system in relation to follicular growth and to relate it to breeding metabolic expenditures. It appears that a breeding-size oviduct is consuming enough energy to have an effect on RMR in the only three species for which data are available. This suggests that this organ is probably responsible for part of the 22% increase in breeding RMR in starlings (Vézina and Williams 2002). The very rapid pattern of growth and regression of the oviduct supports the idea that this is an energetically expensive organ. However, an alternative explanation could be that a hypertrophied oviduct adds mass and reduces flight maneuverability, thus potentially increasing the cost of flight.

The pattern of recrudescence and regression of the oviduct occurs very rapidly, with most of the mass gain or loss occurring in 3 d or less. Our most important finding in regard to this organ was that the oviduct begins to lose mass before the last egg of the clutch is laid (LY birds at follicle 0). The day before clutch completion, while the last egg is being processed, the oviduct had already lost an average of 43% of its mass. This differs somewhat from the pattern of oviduct regression shown by Houston et al. (1995b) in the zebra finch (*Taeniopygia guttata*), where the oviduct apparently starts to regress after the first egg is laid. However, their oviduct data were presented relative to the number of days in the laying cycle, and since there is a lot of variation in clutch size in this species (Williams

1996), this may result in a significant bias if their sample contains several small clutch individuals.

Since the last egg of the clutch in starlings does not differ in size or quality (Ricklefs 1984; Greig-Smith et al. 1988) compared with previously laid eggs, it seems that a full grown oviduct may not be necessary for proper albumin and shell deposition. However, we think this assumption is unlikely. Why would birds maintain a large oviduct if it can be as efficient when reduced in mass by 42%? Ricklefs (1976) reported a positive correlation between oviduct mass and egg size in starlings, and we confirmed this observation by finding a positive relationship between lean dry oviduct mass and oviductal egg mass in our birds across years ($r = 0.49$, $n = 87$, $P < 0.001$; F. Vézina, unpublished data). Similarly, Christians and Williams (2001) found that the mass of the oviduct explains approximately 21% of the variation in albumen protein content in starlings. Therefore, because larger eggs generally tend to confer higher early chick survival (Williams 1994), it is reasonable to assume that producing a large oviduct is advantageous. However, there is a downside to having a large oviduct. Our results imply that this organ may be energetically expensive to maintain and that it is presumably preferable to shut it down as soon as it has accomplished its function (i.e., after the last ovulation). A possible mechanism explaining the regulation of oviduct recrudescence and regression could be that this organ is sensitive to the same hormonal controls that regulate follicle development and ovulation. Although we did not record data on the pattern of development and regression of the particular subsections within the oviduct itself, we know, from birds that expulsed eggs in the metabolic chambers, that our birds were in the phase of shell formation during measurements. It is therefore reasonable to assume that the oviduct is simply regressing top-down as the follicle moves down from the infundibulum to the shell gland. Assuming this is true, the shell gland would still be fully functional at measurement time even if the infundibulum and magnum were starting to regress.

If the oviduct is playing a role in the cost of egg production, its mass nevertheless explains only 18% of the variation in elevated laying RMR. Therefore, there must be other processes, which do not result in significant nonreproductive organ mass variation, that are involved in the increased energy consumption of egg-producing females. Yolk precursor production is estimated to triple the amount of proteins secreted by the liver in laying hens compared with nonlaying individuals (Gruber 1972). This apparently is not, or very rarely, associated with liver hypertrophy (Christians and Williams 1999; this study). Laying RMR was not related to VTG and VLDL plasma levels in our study. Nevertheless, because we only measured the amount of yolk precursor present in the blood and not liver metabolic activity, it is still possible that the liver-mass-specific metabolism affects RMR in laying females. Liver mass did not correlate with RMR in our birds, but a highly active liver could increase its energy consumption per unit mass without changing its total mass, resulting in no relationship between the mass of this organ and RMR. This could be investigated by comparing liver-mass-specific oxidative capacity in nonbreeding and laying birds. Clearly, studies investigating effects of organ-mass-specific energy consumption on RMR are needed.

Body Composition Effects on Nonbreeding and Chick-Rearing RMR

Comparing the relationship between organ masses and RMR for nonbreeding, laying, and chick-rearing groups allows us to highlight two important points about our understanding of the basis of variation in RMR or BMR. In our study, 31% of RMR variation was explained by lean dry pectoralis muscle mass in nonbreeding birds. These birds were caught at the end of the wintering season (mid-March), and we suggest that this relationship is driven by thermoregulatory demands associated with shivering thermogenesis (Swanson 1991; O'Connor 1995). This hypothesis is supported by the fact that nonbreeding birds had the heaviest pectoralis muscle mass relative to body size of all three breeding stages. In chick-rearing birds, 44% of variation in residual RMR was explained by the combined effects of liver and gizzard mass, but, in this case, it is unclear why these organs had the biggest effect in the model. These conflicting observations clearly illustrate that, within a single species, analysis relating body composition to RMR variation may yield very different results, depending on the animal's physiological state. Moreover, as we pointed out in the "Introduction," several studies have shown a relationship between RMR and very different organs even in birds with similar physiological states, for example, those functioning at presumed maximum sustained metabolic rates. It is noteworthy that although Daan et al.'s (1990) article reported an interspecific relationship between combined heart and kidney mass and BMR (but not other organs) in chick-provisioning individuals, very few articles have shown consistent results with regard to these organs and RMR or BMR. For example, Burness et al. (1998) showed that the mass of kidney and intestine was significantly related to resting $\dot{V}O_2$ in chick-rearing tree swallows, but in our study, RMR was related to liver and gizzard in chick-rearing starlings. Similarly, in a study on red junglefowl (*Gallus gallus*), Hammond et al. (2000) demonstrated differences between sexes, with males exhibiting a correlation between BMR and the mass of small intestine, proventriculus, large intestine, lung, and caecum, while in females only the mass of the spleen was related to BMR. Furthermore, there were no significant correlations between reproductive organ mass and metabolic rates, although according to their data (see Hammond et al. 2000, table 1) their birds were in a reproductive state. These data therefore conflict with Chappell et al.'s (1999) study as well as the results of our study, which showed a significant effect of oviduct mass on RMR. Another important point is the relatively low coefficient of determination generally ob-

tained in this type of analysis for all studies to date. In our study, independent organ masses explained 10%–34% of variation in RMR, which is comparable to other published results (5%–18%: Hammond et al. 2000; 30%–52%: Chappell et al. 1999; 39%–44%: Bech and Ostnes 1999). This highlights the fact that relative organ masses are often a relatively poor predictor of RMR or BMR variations (cf. Piersma 2002). Organs show plasticity in response to local ecological conditions, but the direction of the changes may differ between organs and systems, resulting in compensatory effects on overall resting energy consumption, which truly complicates comparisons between studies. There is no doubt that more research is needed to clarify this problem. However, future studies investigating the physiological basis of variation in BMR or RMR should investigate both the size and the metabolic intensity of specific organs.

Acknowledgments

We are grateful to Oliver Love, Wendell Challenger, Mylaine Lessard, Julian Christians, Kristin Cumming, and Mandy Hindle for countless hours of help on the field and in the laboratory. We also thank Tom Scott and Lee Struthers from Pacific Agri-Food Research Center for providing the field laboratory as well as two anonymous reviewers for constructive comments on an earlier version of this article. This research was funded by an operating Natural Sciences and Engineering Research Council (NSERC) grant to T.D.W. and postgraduate funding to F.V. from NSERC and Formation des Chercheurs et l'Aide à la Recherche.

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