

Rapid loss of fat but not lean mass prior to chick provisioning supports the flight efficiency hypothesis in tree swallows

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Summary

1. Birds often lose body mass during nesting. Determining whether this mass loss represents an energetic cost of reproduction (energetic stress hypothesis), serves an adaptive function (flight efficiency hypothesis), or results from physiological processes that are neutral with respect to fitness (e.g. gonadal regression hypothesis) is important to interpreting variation in body mass and energy stores in the context of life-history theory.

2. New quantitative magnetic resonance technology enables precise, repeated measurements of body composition (fat, lean, and water masses) on the same individuals, and we used this method to test a series of predictions to distinguish among competing hypotheses explaining mass loss in female tree swallows (*Tachycineta bicolor*, Vieillot, 1808).

3. Tree swallows lost mass abruptly prior to the peak foraging demands of feeding chicks. Lean mass and fat mass losses varied independently, with small and gradual losses in lean mass during incubation and dramatic losses of fat immediately prior to and following hatching. Females lost some body water early in incubation, but did not lose more water when brood patches would be expected to atrophy. The period of greatest parental foraging costs was not associated with any significant changes in total body mass, lean mass, fat mass or water. Net change in body mass from early incubation until mid-way through chick rearing was associated strongly with initial body mass and to a lesser degree, brood size.

4. These findings are consistent with the flight efficiency hypothesis. Females appear to facultatively modulate their endogenous energy stores to maximize insurance against bad weather and poor foraging opportunities during incubation, but then lower their body mass to maximize efficiency once chicks hatch, thereby reducing the costs of feeding nestlings.

5. This study corroborates results of a growing number of studies of small, insectivorous birds (especially cavity-nesting species), suggesting that increases in mass prior to reproduction (and the subsequent loss of that mass) are likely best viewed as part of an adaptive suite of interrelated reproductive decisions made by females each year.

Key-words: body composition, condition, energetic stress, flight efficiency, gonadal regression, incubation, mass loss, quantitative magnetic resonance, *Tachycineta bicolor*

Introduction

Reproduction is among the most energetically costly phases of an animal's life cycle (Karasov & Martínez del Río 2007). Parents must resolve conflicting selective pressures influenc-

ing the allocation of energy to their own survival and their offspring's development (Ricklefs 1974). Birds, as flying endotherms, must balance this allocation within tight constraints on the size of energy stores (accumulated nutrients exceeding those needed for normal function) they can carry while maintaining flight performance. Therefore, understanding the factors that affect levels of parental investment in current reproduction is central to studies of avian life history (Martin 1995).

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One potentially informative measure of parental investment is the amount of mass lost by parents during reproduction (Ricklefs 1974). Parents require energy buffers to fuel themselves during both incubation and during chick rearing phases of nesting. Incubation involves periodic fasting while incurring increased energetic demands of heating eggs (Conway & Martin 2000), and feeding young requires large energy expenditures to forage for rapidly growing nestlings (Moreno, Potti & Merino 1997; Parejo & Danchin 2006). However, while heavier birds (for a given body size) have more resources to allocate to young, they also incur greater energetic costs of flight, reducing their flight efficiency (Norberg 1981). In species requiring lengthy foraging trips (Niizuma *et al.* 2001) or agility to capture prey (Weimerskirch *et al.* 2006), large energy stores may especially compromise foraging abilities while exposing them to greater predation risk (Witter & Cuthill 1993; Gosler, Greenwood & Perrins 1995).

In species that lose mass during nesting (i.e. from egg laying until chick fledging), the temporal patterns of mass loss vary among species (Moreno 1989). In several small-bodied species (e.g. house wrens *Troglodytes aedon*, Freed 1981; collared flycatchers *Ficedula albicollis*, Cichón 2001; prothonotary warblers *Protonotaria citrea*, Blem & Blem 2006), the most marked declines in body mass occur soon after or about the time that eggs hatch. Three hypotheses have been proposed to explain this temporal pattern of body mass loss. For several decades, mass loss during reproduction was interpreted as evidence of reproductive or energetic stress (Ricklefs 1974). This hypothesis postulates that mass loss is caused by reduced food intake because of reductions in foraging during incubation and brooding, increased metabolic costs of heating eggs and altricial young, and especially, increased foraging costs of feeding developing chicks mid-way through the nestling phase when growth rates are the highest. This hypothesis is 'reactive' in the sense that energy stores are hypothesized to be depleted in much the same way a car burns fuel, thus representing a true fitness cost to the parent. The flight efficiency hypothesis (Blem 1976; Freed 1981; Norberg 1981; aka the 'adaptive mass loss', Curlee & Beissinger 1995; 'flight adaptation', Merkle & Barclay 1996; 'adaptive adjustment', Merilä & Wiggins 1997; 'programmed anorexia', Jones 1994; or 'wing-loading' hypothesis, Nagy, Stanculescu & Holmes 2007) postulates that incubating parents actively modulate body mass strategically according to the changing costs and benefits of carrying energy stores at different stages of reproduction. During incubation, birds maximize energy stores as a buffer against unforeseen food shortages or bad weather (risks that decline over time). However, prior to the period of peak food delivery to nestlings, birds are hypothesized to facultatively reduce energy stores to increase the effectiveness and reduce the costs of foraging. This hypothesis is 'proactive' because mass change occurs prior to the time when the costs of energy stores are greatest, integrating the nonlinear changes in costs and benefits of energy stores over time. A third explanation is the gonadal regression hypothesis (Peteresen 1955) which postulates that changes in mass result from

atrophy of the oviduct. While this hypothesis is commonly viewed as neither costly nor beneficial to adults, by regressing gonads, birds do save on the metabolic costs of maintaining organs throughout the year which likely more than compensates for the costs of rebuilding reproductive machinery each year; Vézina & Salvante 2010). Few studies have tested the gonadal regression hypothesis (but see Curlee & Beissinger 1995), perhaps because the magnitude of mass loss frequently exceeds estimates of change in mass of female reproductive organs (Slagsvold & Johansen 1998). However, one component of the reproductive machinery that has been ignored in this context is atrophy of the brood patch. Incubating parents greatly increase the vascularization of the skin on their lower breast and belly to facilitate heat transfer to eggs and newly hatched chicks. It is not known whether development of brood patches involves an increase in total body water or lean tissue. If either component of body composition substantially contributes to the development of brood patches, subsequent atrophy might account for some mass loss via reductions in lean mass and/or body water.

Despite several studies reporting evidence in support of both the energetic stress and flight efficiency hypotheses, the general conditions under which mass loss may be viewed as a cost of reproduction are still unclear. This is partly because of the fact that many predictions tested are incapable of distinguishing among hypotheses. For instance, birds would be predicted to lose more mass during bad weather due to energetic stress because they experience higher thermogenic costs. However, lower mass will also lower foraging costs and increase agility, thus also improving flight efficiency at times (i.e. during bad weather) when they most require it (e.g. in blue tits *Cyanistes caeruleus*; Merilä & Wiggins 1997). Likewise, both hypotheses would predict less mass loss in response to supplemental feeding (e.g. black-throated blue warblers *Setophaga caeruleus*; Nagy, Stanculescu & Holmes 2007) because feeding would help both maintain female condition under adversity as well as lower the costs of feeding chicks. Furthermore, many studies have failed to make robust conclusions because either (i) their study design lacked temporal precision because of difficulties obtaining repeated measures on the same individual at key phases of the reproductive cycle (e.g. Winkler & Allen 1995) or (ii) when repeated measures were obtained through remote monitoring devices, lack of control over the timing of measurements lead to large amounts of unexplained variation in mass because of diurnal foraging cycles (e.g. Nagy, Stanculescu & Holmes 2007). Lastly, destructive sampling has been the primary method used to examine how components of mass vary during reproduction (e.g. Elliott *et al.* 2008), a method which obviously precludes making repeated measures on the same individual.

Tree swallows (Fig. 1) incur high foraging costs (relative to birds foraging on other substrates) because of their aerobic mode of foraging (Williams 1988). Greater mass losses in female tree swallows with experimentally enlarged broods (DeSteven 1980) and experimentally impaired flight abilities (Winkler & Allen 1995) have been interpreted as evidence of increased energetic stress. However, the flight

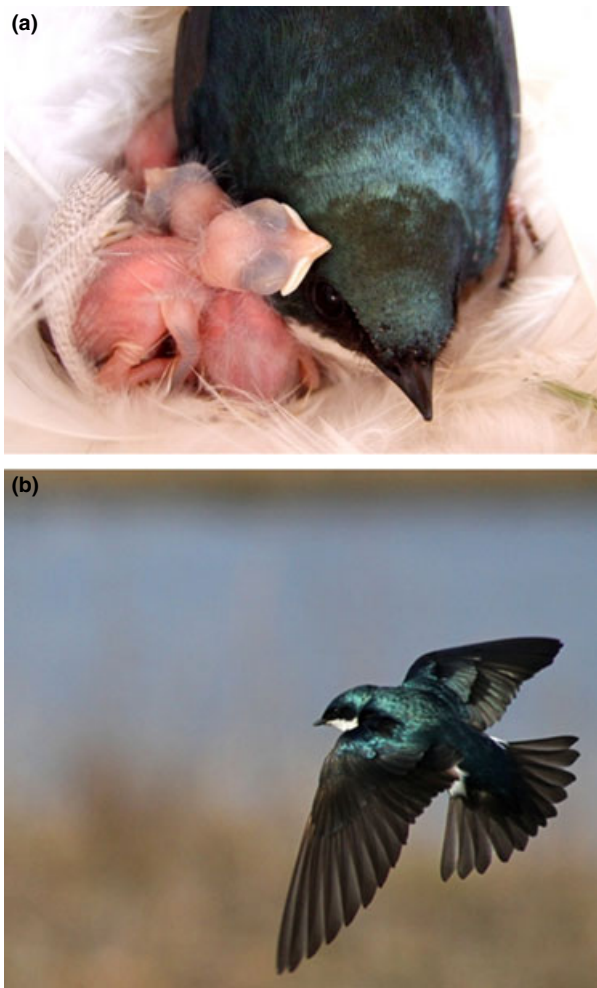


Fig. 1. (a) Female tree swallow brooding day 2 chicks. Photograph by Haruka Wada. (b) Tree swallow in flight, central New York state. Photograph by Don Miller.

efficiency hypothesis would also predict greater mass losses in manipulated vs. control birds in both studies because an increase in the number of nestlings to feed or flight abilities would increase the benefits to parents of increased efficiency via mass reductions. Other studies of tree swallows are also

consistent with the flight efficiency hypothesis; earlier-nesting females (who on average raise more young) are also better fliers (even accounting for differences in wing loading because of different amounts of mass loss among females), suggesting that the foraging costs of rearing young are modulated by parents via clutch size decisions (Bowlin & Winkler 2004).

We tested six predictions (Table 1) based on the timing, components and individual-level correlates of mass loss. We used a new technology (quantitative magnetic resonance, QMR) to make rapid, accurate, and repeated measurements of body composition with minimal disturbance to nesting birds (Guglielmo *et al.* 2011). The energetic stress hypothesis predicts that mass should decrease gradually over the whole nesting period with the greatest losses coinciding with the period of maximum daily growth rates of chicks, feeding rates, and energy expenditure of parents (i.e. between chick days 2 and 8). Furthermore, patterns of fat mass loss should parallel and explain much of the variation in total mass loss. Because depletion of lean mass also occurs during fasting and exercising birds (Lindstrom *et al.* 2000; Gerson & Guglielmo 2011), lean mass losses should also decline at roughly the same times that fat declines. Greater net mass loss over the whole nesting period should also be associated with larger clutches (de Heij *et al.* 2007), younger females (Winkler & Allen 1996) and worse weather, because of both increased energy expenditure (Reid, Monaghan & Ruxton 2000) and reduced food supply. The gonad regression hypothesis predicts that most lean mass loss should occur when ovaries and oviducts atrophy following egg laying (presumably early in incubation; Petersen 1955; Vézina & Williams 2003), that the total mass lost would be accounted for primarily by changes in lean mass, and that this amount would roughly equal the mass of enlarged female reproductive organs. Furthermore, if brood patch atrophy contributes to mass loss, then lean mass and/or body water should decline once chicks begin thermoregulating (roughly days 4–8 depending on brood size; Winkler *et al.* 2011). The flight efficiency hypothesis predicts that mass loss would occur prior to peak energetic demands of parents (i.e. prior to chick day 2), occur relatively abruptly, and be primarily attributable to changes in fat mass. Females should more or less maintain lean mass or possibly increase it via flight

Table 1. Summary of the hypotheses and the predictions tested in this study

	Energetic stress	Gonad regression	Flight efficiency
Timing of mass loss	Synchronous with increases in chick feeding costs (chick day 2–8)	Early in incubation	Prior to peak demand for food by chicks (prior to chick day 2)
Pattern of mass loss	Gradual	Abrupt (early incubation)	Abrupt (just prior to hatch)
Lean mass	Declines roughly in proportion to fat mass	Accounts for much of total mass loss	Maintenance or increase
Fat mass	Declines through whole nesting period, greatest declines when feeding rates highest (chick day 2–8)	n/a	Declines abruptly prior to chick day 2 to baseline level
Body water	n/a	Losses mid-chick rearing	n/a
Correlates of net mass loss	Greater with (i) larger clutches; (ii) young females; (iii) recent bad weather	None; loss approximately equal to max gonad mass	Greater in (i) initially heavier birds (ii) larger broods

muscle mass (van den Hout *et al.* 2010) to maximize flight performance. Finally, net mass loss should be greater in initially heavier birds, converging on some baseline (i.e. non-breeding) mass and proportion body fat, and should be more strongly related to brood size than clutch size because foraging demands would be proportional to the number of mouths parents have to feed, not the number of eggs they incubate.

Materials and methods

Tree swallows are migratory passerines that breed over most of the northern parts of North America and winter from coastal New York State and California southward to Central America and northern South America (Winkler *et al.* 2011). Tree swallows forage aerially for insect prey in flights requiring agility and speed. Females build nests in natural cavities or man-made nest boxes in open environments and assume sole responsibility for incubation of eggs. Males do not feed incubating females; females are intermittent incubators, interrupting incubation with foraging bouts. Both sexes feed nestlings (Winkler *et al.* 2011).

We studied tree swallows breeding at Unit 1 of the Cornell Experimental Ponds near Ithaca, NY (42°30'N, 76°27'W) in May and June 2010 (described in McCarty & Winkler 1999a). Mean clutch size at this site in 2010 was 5.4 eggs (\pm SE 0.1, $n = 112$), and young typically hatch after *c.* 14 days of incubation. After hatching, females brood young overnight into the second week of nestling development. The nestling phase lasts > 16 day, and in 2010, a mean of 3.6 (\pm SE 0.2, $n = 111$) chicks fledged from successful nests at this site. Tree Swallow chick mass gain follows a sigmoidal pattern with growth rates increasing during the first 3 days following hatching and chick mass approaching that at fledging by around day 10–11 (McCarty 2001). Maximal growth rates occur between days 4 and 7 when chicks gain 2.5 g day⁻¹. During this time, mean nestling mass increases from 3.8 to 17.1 g (McCarty 1995). Parental feeding rates (g of food per hour) are expected to increase in parallel with these growth rates. Field metabolic rates of incubating female tree swallows are *c.* 4 times basal metabolic rate (BMR) (Williams 1988). Despite the fact that both parents feed chicks, metabolic rates increase in females to 5.0–5.6 times BMR when feeding nestlings, values that are among the highest reported in birds. Mean energy expenditure of parents feeding days 8–9 chicks is 101.0 \pm 18.8 kJ day⁻¹ (Burness, Ydenberg & Hochachka 2001). Therefore, the increased foraging demands of feeding nestlings would be expected to place strong selective pressures on the most energy-efficient flight during this time.

We visited nests every other day during egg laying, incubation and nestling phases to determine the date that females initiated incubation and eggs hatched. Female tree swallows generally lay one egg per day early in the morning, but may skip a day between successive eggs. Likewise, broods of chicks hatch over a period from several hours to up to 2 days. For these reasons, and because we did not check nests every day (to minimize disturbance at nests), our accuracy in determining both incubation and hatch day is subject to an error of ± 1 day.

We selected 20 females at random nesting in boxes mounted over water. Females in this study were not part of any other experiments in 2010. Nine of the 20 females were less than a full year old (based on plumage; Hussell 1983) and known to be nesting for the first time. The 20 females completed clutches of 3–6 eggs (mean 5.3), initiated incubation between 20 and 30 May, and we made our last measurements on 21 June. Because of partial losses of some clutches and broods, mean brood size was 4.8 chicks at day 8 of chick development.

Weather during the study was on average a little warmer and drier than the long-term average. Mean daily temperatures were 2.7 °C warmer and received 52 mm less rain than during the same period in 1971–2000 (D. W. Winkler unpublished data and NOAA). However, birds in this study experienced one bout of heavy rain (8.6 mm on 6 June) followed by 4 days of below-normal temperatures (mean 13.3 °C, 3.8 °C colder than normal). This period of 6–10 June coincided with a range of nest stages from incubation day 8 to chick day 6. Chick growth rates in 2010 (W. A. Boyle, D. W. Winkler & C. G. Guglielmo, unpublished data) closely matched growth rates at this site in 1990–1993 until *c.* day 4, after which mean masses lagged 2–4 g behind the 1990–1993 masses. This lag likely reflects the effects of the mid-June period of cold weather (McCarty & Winkler 1999b).

We measured females on days 4, 9 and 13 of incubation and on days 3 and 9 of chick development (incubation day 1 estimated as the first day after which no new eggs were laid and chick day 0 = hatch day). We captured females in nest boxes just before dawn (*c.* 05.00). This allowed us to standardize the time of capture and reduce the variation in body mass attributable to feeding state. We transferred birds in individual cloth bags to a mobile laboratory trailer at the study site (< 500 m from all nests in the study) where we fitted birds with numbered leg bands, then weighed them on an Acculab Vicon VIC-123 electronic scale (precision \pm 0.001 g).

We measured wet lean mass, fat and total body water using QMR (Echo MRI-B; Echo-Medical Systems, Houston, TX, USA; Guglielmo *et al.* 2011). We placed individual tree swallows in a 33 mm diameter clear plastic tube to gently restrain them. Measurements were made in duplicate using the two accumulation and 'small bird' settings of the QMR (total scan duration 2 min). We immediately released birds following scanning. Instrument readings are unaffected by aluminium leg bands (C.G. Guglielmo, unpublished data).

Prior to analyses, we made slight adjustments to the raw QMR estimates according to equations in Guglielmo *et al.* (2011) that were developed using a house sparrow *Passer domesticus* calibration sample. We checked the QMR results by correlating individual net changes in mass with changes in the corrected fat and lean mass values between the first and last scans. QMR wet lean mass largely reflects muscle and organs and includes a large proportion of total body water. Fat mass primarily represents energy stores available for use as fuel to a fasting bird as well as small amounts of lipids in membranes. Water mass includes all body water including water in blood, tissues and in the digestive tract. We analysed total mass, lean mass, fat mass and water mass using mixed models (individual as random effect and nesting stage as fixed effect) and conducted pair wise comparisons between measurements taken at all five times during nesting to determine the phases during which significant changes in body mass and body composition occurred. We used Tukey's HSD to determine which mass changes over time differed from one another at $P = 0.05$ (Sokal & Rohlf 1995).

Results

We weighed and obtained QMR scans of all 20 females in each of the three incubation stages. One female failed to hatch any eggs and abandoned her nest after 15 days, and all the chicks in two of the remaining 19 nests died in the cold snap between the 4th and 5th scan (chick day 2 and 8). Thus, our sample size for analyses of net mass change was 17. Changes in lean and fat mass were measured by QMR highly accurately, together explaining 96% of the variation in net change in total body mass measured separately with a balance with a

slope indistinguishable from one (1.09, 95% CI 0.97–1.23) and an intercept indistinguishable from zero (−0.15, 95% CI −0.61 to 0.31).

Body mass declined over the nesting cycle (nesting stage effect, $F_{4,70.45} = 73.9$, $P < 0.0001$). However, significant mass loss occurred only between incubation day 9 and day 13, and chick day 2 (Fig. 2a). Early in incubation and during most of chick rearing, body mass did not change significantly. The difference in mean mass of all females between early incubation and late chick rearing was 4.0 g which represents 17.6% of initial or 21.4% of final body mass. Of the 17 females with active nests on chick day 8, net change in body mass over the 17–21 days (since incubation day 4) ranged from −1.82 g to −7.82 g (8.9–30.1% of initial mass).

Lean mass declined over the nesting cycle (nesting stage effect, $F_{4,70.24} = 43.6$, $P < 0.0001$) and did so more gradually than did body mass, with significant changes between all phases of the nesting cycle except during the period of peak chick energetic demands (Fig. 2b). Mean net change in lean mass was −1.53 g (−0.29 to −3.44 g). Therefore, on average, changes in lean mass accounted for 38.3% of the change in total body mass. Within individuals, net changes in lean mass were not significantly associated with changes in fat mass ($P = 0.060$, $R^2 = 21.7$).

Fat mass varied substantially over the nesting period (nesting stage effect, $F_{4,71.25} = 51.4$, $P < 0.0001$) with a slight trend for increasing fat stores early during incubation, then a sharp decline late in incubation until soon after chicks

hatched, with no reduction later in chick rearing (Fig. 2c). Mean net change in fat mass was −1.83 g (−0.82 to −3.18 g). Therefore, on average, changes in fat mass accounted for 45.8% of the change in total body mass. Fat loads on chick day 8 were only 0.69 g \pm SE 0.23 g ($t = 2.6$, $P = 0.051$) lower than the fat loads on females captured at this site *c.* 1 month before breeding (W. A. Boyle, D. W. Winkler & C. G. Guglielmo, unpublished data). These fat values represent 6.0% of total mass on chick day 8 vs. 9.2% in pre-breeding females. Water mass declined from incubation day 4 to incubation day 9 (nesting stage effect, $F_{4,71.16} = 5.6$, $P = 0.0006$; Fig. 2d) and did not change significantly thereafter.

Neither the absolute amount of mass lost nor the proportion of initial mass lost was associated with clutch size (net mass loss, $F_{2,14} = 1.3$, $P = 0.311$, $R^2 = 0.15$; proportion mass lost, $F_{2,14} = 1.4$, $P = 0.280$, $R^2 = 0.17$). Likewise, young birds breeding for the first time lost similar amounts ($t_{15} = -0.2$, $P = 0.853$) and proportions ($t_{15} = 0.3$, $P = 0.764$) of their initial mass as older birds. Higher mean temperatures during the 3 days prior to final measurements (on chick day 8) were associated with greater mass losses over the entire nesting period ($F_{1,15} = 6.5$, $P = 0.022$, $R^2 = 0.30$). However, the weather during chick rearing, clutch initiation date, initial mass and brood size were all correlated with one another. When these variables were combined in a single model predicting mass change, only initial mass and brood size explained significant portions of the variation in mass loss (whole model, $F_{6,10} = 12.2$, $P < 0.001$;

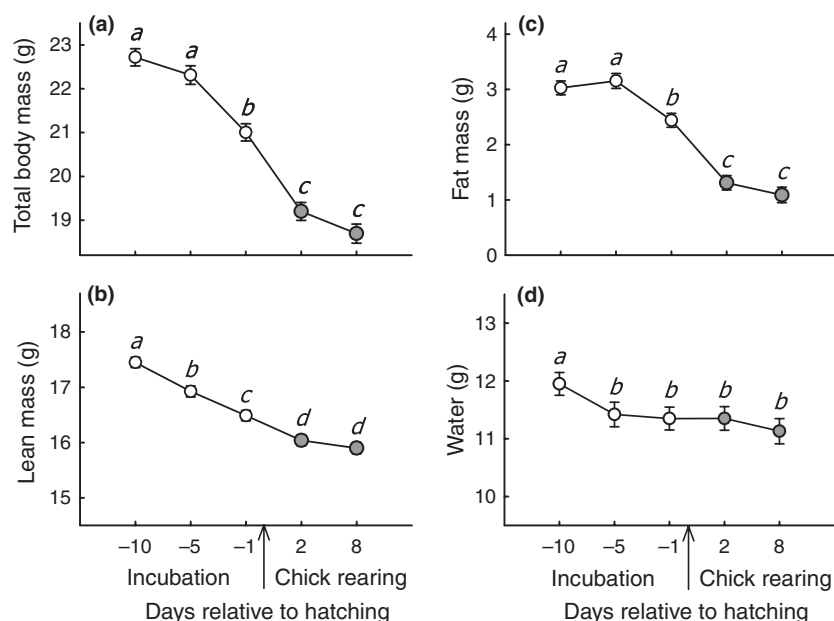


Fig. 2. Changes in female total body mass (a), wet lean mass (b), fat mass (c) and body water (d) on days 4, 9 and 13 of incubation (white circles), and days 2 and 8 of chick development (grey circles) scaled according to average number of days prior to or following hatch day (indicated by arrow). Values on the y-axes are least square means of mass (g) in mixed models (to account for repeated measures on randomly selected individual birds). Summed mass of each component of body mass does not equal total body mass because skeletal and keratinized tissues are not measured by quantitative magnetic resonance (QMR) (Guglielmo *et al.* 2011), and because lean wet mass includes some water. Total body water includes water bound up in tissues, in blood and in the digestive tract. Error bars represent 1 SE of the mean. Means sharing the same italicized letter do not differ from each other statistically at $P = 0.05$, whereas means with different letters do differ from one another. Note the different y-axes for each measure.

initial mass effect test, $F_{1,10} = 19.5$, $P = 0.001$; brood size effect test, $F_{3,10} = 5.4$, $P = 0.027$). Alone, initial mass and brood size explained 83% of the variation in net mass loss (whole model $F_{4,12} = 14.3$, $P < 0.001$; brood size effect test, $F_{3,12} = 5.0$, $P = 0.045$; Fig. 3), and the nature of these results was independent of skeletal body size measurements (relationship between head-bill length and mass loss, $P = 0.625$; head-bill effect test in previous model plus this variable; $P = 0.566$). On average, every increase of 1 g in initial mass was associated with 0.7 g lost between incubation day 4 and chick day 8 (based on differences among least square means). After accounting for variation in initial mass, a one-chick increase in brood size was associated with a decrease of 0.48 g (\pm SE 0.21 g) in maternal body mass. Clutch size explained less variation in mass loss than did brood size, even though these two variables are closely related. Substituting clutch size for brood size in the above model resulted in a lower R^2 (0.77 vs. 0.83) and non-significance of the clutch size variable (whole model $F_{3,13} = 15.2$, $P < 0.001$, $R^2 = 0.77$; clutch size effect test, $F_{2,13} = 3.2$, $P = 0.075$).

Discussion

The results of this study are consistent with the flight efficiency hypothesis while providing no support for the energetic stress or gonad regression hypotheses (Table 1). Our repeated measures design, standardization of capture time at the end of an overnight fast and QMR analysis of body composition allowed us to show that reproductive stage explains 86%, 83% and 77% of the variation in total, lean and fat mass, respectively, in our data set. Our approach permitted us to detect mass changes as small as 0.5 g (*c.* 2.5% of total body mass) and to identify which components of body mass are lost during various phases of incubation and nestling feeding. Consistent with the flight efficiency hypothesis, female tree

swallows lost most mass immediately prior to and following hatching. They lost negligible mass through most of incubation and during the period of greatest foraging demands while feeding chicks. Females lost mass relatively abruptly, not gradually through the whole nesting period. Even if the costs of incubation increase over time as the thermal impedance of eggs increases (Turner 1994), temporal patterns of mass loss are not consistent with the energetic stress hypothesis because we detected no change in mass until late in incubation. Fat mass losses primarily drove patterns in total body mass with strong declines in the days prior to chick day 2. Fat levels at our last measurement during chick development were similar to those measured in pre-breeding females. Body water declined slightly near the beginning of incubation, but there were no consistent changes in water thereafter. Furthermore, total mass loss during nesting (incubation day 4 to chick day 8) was greater in initially heavier birds and those raising larger broods of chicks.

Only patterns of lean mass change differed from those predicted by the flight efficiency hypothesis. Lean mass declined gradually throughout incubation and the first few days following hatching, remaining relatively constant thereafter. The amount of lean mass lost did not account for most of the total body mass lost, as predicted if gonad regression primarily explains changes in mass, nor did it decline during the most energetically costly phase of chick rearing as predicted if energetic stress explains changes in mass. However, the net change in lean mass was roughly equivalent to predicted masses of enlarged female reproductive organs. The mean wet mass of enlarged oviducts (excluding ovaries) immediately following egg laying in similarly sized barn swallows was 1.098 g (\pm SD 0.066 g, $n = 2$; Ward & Bryant 2006). This value closely matches an estimate of 1.067 g for tree swallows based on 4.7% of total body mass (derived from measurements of canaries; Steel & Hinde 1963). Thus, it is possible that a portion (*i.e.* *c.* 30–100%) of our observed lean mass change could be attributed to regression of reproductive organs. However, gonads are expected to decrease rapidly following egg laying (Vézina & Williams 2003). Thus, whether the decline in lean mass through incubation can be accounted for by gonad regression seems doubtful. Because our lean mass data cannot distinguish between changes in muscle mass vs. organ mass, the gradual decreases in lean mass among females in this study does not rule out the possibility that flight muscle mass remained constant or even increased prior to elevated foraging demands. However, previous studies using ultrasound reveal that in fact, breast muscle thickness declines along with body mass and fat score during reproduction in tree swallows (Winkler & Allen 1995). Thus, an alternative interpretation is that reductions in lean mass contribute to achieving an optimal wing loading. Fat levels in birds on chick day 8 of this study approached what may be a pre-breeding baseline proportion of fat. If the optimal relationship between power and wing loading cannot be achieved solely via reductions in fat, then lean mass declines may also function to improve flight efficiency in this context (Schwilch *et al.* 2002). In this sense, the dramatic and swift regression of reproductive organs

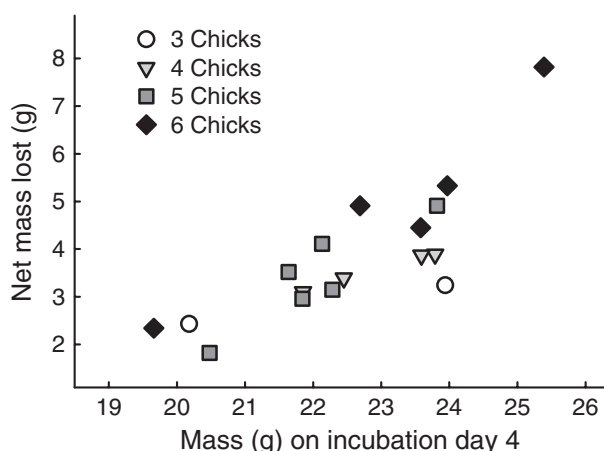


Fig. 3. Net mass lost between incubation day 4 and chick day 8 relative to initial body mass in 17 female tree swallows. Symbols indicate brood sizes; females represented by white circles raised three chicks, pale grey diamonds raised four chicks, dark grey squares raised five chicks and black diamonds raised six chicks.

following reproduction in flying endotherms (common to both birds and bats; Racey & Tam 1974) likely also ultimately reflects selection for flight efficiency.

Our study reinforces the view that lean mass is a dynamic component of body mass that cannot be inferred based on calculations from structural size (*contra* Peig & Green 2009) and accounts for why indices based on mass corrected for structural size perform poorly when compared with direct body composition measurements (Schamber, Esler & Flint 2009). Several studies have demonstrated how lean mass is actively modulated during migration (Lindstrom *et al.* 2000; Guglielmo & Williams 2003). Furthermore, experimental evidence demonstrates that substantial changes in lean mass and relative size of muscles and organs occur in response to what appear to be pre-programmed endogenous processes prior to migratory flights (Dietz, Piersma & Dekinga 1999). Likewise, programmed endogenous cycles underlie declines in total body mass, muscle mass and selective mobilization of bone minerals prior to and during moult in waterfowl (Brown & Saunders 1998; Portugal, Green & Butler 2007; Portugal *et al.* 2011). Here, we show that even outside migratory and moulting phases of a bird's life cycle, both fat and lean mass vary dynamically and nonlinearly over time-scales of a few days. Furthermore, as at other times of the life cycle, reproductive modulation of body mass also occurs prior to the time when such changes would benefit the individuals, thus acting in a proactive rather than reactive fashion.

The general patterns we present here corroborate reproductive mass loss patterns in many other species (e.g. pied flycatcher *Ficedula hypoleuca*, Potti & Merino 1995; prothonotary warbler, Blem & Blem 2006; house wren, Freed 1981). However, our interpretation of the same results differs from those of some previous authors. In particular, we interpret greater mass losses in initially heavier birds as evidence supporting the flight efficiency hypothesis, while others have cited this as evidence that mass loss is condition dependent and therefore reflective of energetic stress (Potti & Merino 1995; Merilä & Wiggins 1997). Insight into the mechanisms underlying this relationship come from the interconnected associations between initial body mass, flight abilities, nest initiation date, climate and food variables, clutch size and female quality. Despite low food availability early in the breeding season, Winkler & Allen (1995, 1996) showed that clutch size is largely explained by laying date, with earlier-nesting females raising more young. Earlier-nesting females are also heavier (Winkler & Allen 1995), older and have longer wings (which decreases wing loading because those birds did not have larger skeletal size or more visible fat; Winkler & Allen 1996). Females raising larger broods do not appear to incur elevated energetic costs (Burness, Ydenberg & Hochachka 2001) which is consistent with the conclusions of Bowlin & Winkler (2004); earlier-nesting females are higher quality (see also Hasselquist, Wasson & Winkler 2001), possessing superior flying abilities (enabling egg laying during lower food availability). Taken together, these studies suggest that the most likely interpretation of our data is that

earlier-nesting, high-quality females accumulate greater endogenous resources than do later-nesting, lower quality females. In other words, earlier breeding females breed at a time when interruptions to foraging because of cold temperatures are more likely and put on more mass as insurance. This view essentially inverts the concept of mass loss during reproduction into mass *gain* prior to reproduction. Because females appear to end their reproductive bout in roughly 'baseline' condition, they must anticipate the costs of reproduction, adjusting their investment in endogenous reserves, clutch size, lay date, incubation and foraging behaviour in interrelated ways. In effect, this parallels recent findings from waterfowl suggesting that variation in mass loss during moult reflects variation in pre-moult accumulation of endogenous resources (Portugal *et al.* 2011).

Intriguingly, net change in body mass is associated with nest initiation date of black-throated blue warblers as well (Nagy, Stanculescu & Holmes 2007). If earlier-nesting warblers are also heavier (and higher quality), then potentially a similar mechanism could underlie individual variation in mass loss in this and other species. The interspecific relationship between initial mass and the proportion of mass lost parallels intraspecific patterns but may reflect a different mechanism. Interspecifically, this association has been explained by incubation duration (Moreno 1989) whereas intraspecifically, variation in incubation duration is negligible.

The fact that net mass change in our study was significantly associated with brood size but not clutch size is somewhat surprising (although consistent with the *a priori* predictions of the flight efficiency hypothesis). This finding suggests that either (i) partial egg losses are somehow under control of females via alteration of incubation behaviours or (ii) although mass loss begins prior to hatching, the amount of mass lost after hatching is adjusted to compensate for the fewer-than-expected number of chicks to feed, consistent with the observation that the amount of mass lost in tree swallows is related to chick feeding rates (Murphy *et al.* 2000). It is important to consider in this context the ways that females reduce mass. Although we still know little about the physiological processes by which fat and lean mass are regulated, it is clear that female tree swallows late in incubation are either reducing food intake or (less likely) increasing metabolic costs to effect the change in body mass that we observed (Portugal, Green & Butler 2007).

Our results suggest that interpreting mass losses of nesting female tree swallows as a reactive consequence of energetic stress would be misguided. The same seems to be true for many other passerines, but the literature is strongly biased toward cavity-nesting insectivores. Is the association between cavity-nesting and mass loss late in incubation an artefact of the ease of studying species that conveniently nest in boxes where they are easy to find, catch and manipulate? Or is it that cavity-nesting per se allows elevation of body mass to relatively high levels through incubation? Cavities are among the safest of nesting substrates (Martin 1995). Safety from predators could affect mass allocation decisions in incubating

cavity-nesting parents by permitting extra insurance during incubation (in the form of endogenous energy reserves) and reduced take-off speeds and agility. That they lack effective insurance later during chick rearing is evidenced by the fact that chick abandonment is frequent in this species when bad weather coincides with periods of greatest chick growth (Winkler *et al.* 2011).

Clearly, producing eggs and rearing chicks is costly to adults (Visser & Lessells 2001). Indeed, life-history theory rests on the assumption that investment in current reproduction is made at a cost to future survival and reproduction (Stearns 1976). However, determining which metrics accurately capture variation in investment and the costs of reproduction at the individual level is challenging. We suggest that in tree swallows, as in many other similar species in which mass loss appears to be facultatively controlled by females, the mass gained prior to nesting (then lost during incubation and chick rearing) may be a valid metric of parental investment in current reproduction and self maintenance. Mass loss likely represents the amount of insurance a female invests in surviving and successfully reproducing in a given year. Thus, contrary to the traditional view, the amount of mass gained and subsequently lost ought to be viewed as just one of a suite of related allocation decisions serving to maximize the chances of successfully fledging young.

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