

EGG MASS INFLUENCES NESTLING QUALITY IN TREE SWALLOWS, BUT THERE IS NO DIFFERENTIAL ALLOCATION IN RELATION TO LAYING ORDER OR SEX

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Abstract. Maternal allocation of resources to eggs and nestlings can potentially have a profound influence on offspring phenotype and fitness. However, it is often unclear how much of the variation in offspring quality is due to maternal or environmental effects. We examined the influence of maternal and environmental effects on egg mass and nestling quality (growth and immune function) in Tree Swallows (*Tachycineta bicolor*). There was no evidence that the allocation of maternal resources to eggs varied with laying order or offspring sex. Thus, there was no evidence of adaptive maternal allocation to eggs in terms of mass. Instead, egg mass in Tree Swallows appeared to be influenced primarily by ambient temperature during egg formation and differences among females. Nonetheless, female Tree Swallows were likely to realize substantial benefits from producing larger eggs because those young were larger at hatching and grew faster. Furthermore, nestlings that grew faster had a stronger immune function at 11 days of age. Our results suggest that the positive relationship between nestling growth and survival may be due, in part, to the effects of an enhanced immune response.

Key words: ambient temperature, egg mass, immune function, maternal effects, nestling growth, offspring sex, *Tachycineta bicolor*.

La Masa de los Huevos Influencia la Calidad de los Polluelos en *Tachycineta bicolor*, pero no Existe Asignación Diferencial con Relación al Orden de Puesta ni al Sexo

Resumen. La asignación maternal de recursos a los huevos y los polluelos puede tener una fuerte influencia sobre el fenotipo y la adecuación biológica de la progenie. Sin embargo, en muchos casos no está claro cuánto de la variación en la calidad de la progenie se debe a un efecto maternal o ambiental. Examinamos la influencia de los efectos maternos y ambientales sobre la masa de los huevos y la calidad de los polluelos en el nido de *Tachycineta bicolor*. No hubo evidencia de que la asignación de recursos maternos a los huevos variara con el orden de puesta o el sexo de la progenie. Por lo tanto, no hubo evidencia de una asignación maternal adaptativa para los huevos en términos de su masa. Por el contrario, la masa de los huevos en *T. bicolor* pareció estar afectada principalmente por la temperatura ambiental durante la formación del huevo y por diferencias entre las hembras. Sin embargo, es probable que las hembras de esta especie fueran capaces de percibir un beneficio substancial al producir huevos mayores, ya que los polluelos provenientes de huevos grandes fueron mayores al eclosionar y también crecieron más rápido. Además, los polluelos que crecieron más rápido en el nido tuvieron una mejor función inmunológica a los 11 días de edad. Nuestros resultados sugieren que la relación positiva entre el crecimiento de los polluelos y su supervivencia puede deberse en parte al efecto de una mejor respuesta inmune.

INTRODUCTION

Maternal effects can have a profound influence on offspring phenotype and fitness. In birds, maternal allocation of resources to eggs can have a substantial impact on nestling development and growth (Styrsky et al. 1999, Magrath et al. 2003, Krist et al. 2004) and, subsequent-

ly, their survival and recruitment (Hochachka and Smith 1991, McCarty 2001, Legge 2002). Egg mass may have a particularly strong influence on offspring phenotype (Williams 1994). There has been considerable recent interest in whether resource allocation to eggs is adaptive or influenced primarily by proximate mechanisms such as the female's body condition (Styrsky et al. 2002, Lifjeld et al. 2005) or environmental factors, such as temperature and food availability, during egg formation (Hargitai et al. 2005). However,

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few studies have examined all of these factors simultaneously.

In some circumstances, females may benefit from adaptive allocation of resources to eggs in relation to laying order or offspring sex. For example, increasing resources to eggs in relation to laying order may allow females to enhance the survival of later-hatching chicks, which may compensate to some extent for hatching asynchrony (Slagsvold et al. 1984, Rosivall et al. 2005). Females may also benefit by investing more in eggs that will produce males in sexually dimorphic species (Blanco et al. 2003, Magrath et al. 2003, Müller et al. 2005), or when male offspring are likely to have greater reproductive success than female offspring (Sheldon et al. 1999). The allocation of maternal resources to eggs in relation to offspring sex has received little empirical study, in part because it is often difficult to match particular nestlings with the egg from which they hatched.

We examined whether the allocation of maternal resources varies among eggs within a clutch and whether females adaptively allocate resources to eggs in Tree Swallows (*Tachycineta bicolor*). Females could benefit from producing larger eggs if they result in young that grow faster, because nestling growth is positively related to survival and recruitment (McCarty 2001). Differential allocation of maternal resources to eggs laid later in the clutch could also compensate for asynchronous hatching, if late-hatching, smaller young are disadvantaged in competition for food (Zach 1982, Clotfelter et al. 2000). Finally, female Tree Swallows in better condition produce more male offspring (Whittingham and Dunn 2000, Whittingham et al. 2005) and the variance in reproductive success is much greater for males than females (Whittingham and Dunn 2005), suggesting that increasing investment in male offspring could provide greater fitness benefits to females. In this study, we examined whether resources for embryos (e.g., egg mass) were allocated adaptively in relation to laying order and offspring sex and whether female allocation of resources to eggs influenced offspring quality, specifically growth rate and immune function. These are likely to be important aspects of nestling quality, because nestlings that grow faster have better survival (McCarty 2001), and better immune function is an indicator of disease resistance and the

survival of nestlings in altricial birds (Lochmiller et al. 1993). This is one of the most comprehensive analyses of the effects of egg mass on offspring quality to date, as we have data on nestlings from known eggs and the environmental conditions when they were laid (temperature and food abundance), as well as detailed measures of nestling growth rate and immune function.

METHODS

STUDY AREA AND SPECIES

We studied Tree Swallows in 2000 at the University of Wisconsin–Milwaukee Field Station near Saukville, Wisconsin (43°23'N, 88°01'W). Our study area contained 88 nest boxes with predator guards, located in two grids spaced 600 m apart (Whittingham and Dunn 2000). In our population, Tree Swallows are predominantly socially monogamous and single brooded. Females build a grass cup nest inside the nest box during late April and early May, and begin laying eggs when the nest cup is 4–6 cm deep. They lay one egg per day and begin incubation (14–15 days) with the penultimate egg. The young remain in the nest for 18–22 days after hatching (Robertson et al. 1992).

All adults were caught inside nest boxes and marked with a U.S. Fish and Wildlife Service band on the right leg and a colored plastic band on the left leg. For each bird we measured wing chord with a wing chord ruler (to the nearest mm), tarsus length using digital calipers (± 0.01 mm) and body mass with an electronic balance (± 0.1 g). Sex of adults was determined by the presence of a brood patch or cloacal protuberance and females were classified as second-year (SY) or after-second-year (ASY) on the basis of plumage coloration (Hussell 1983).

Nest boxes were inspected daily to determine the start of egg laying and clutch size. Each egg was measured using digital calipers (length and width; ± 0.01 mm), weighed on an electronic balance (± 0.01 g), and numbered on the morning it was laid. We calculated egg volume (V) according to the formula $V = LW^2/6$, where L = egg length and W = egg width (Hoyt 1979). We checked nests every 1.5–2 hr on the expected day of hatching to determine the time of hatching of each egg. Thus, we knew the

exact age of each nestling to the nearest 1.5–2 hr. At hatching all nestlings were individually marked by clipping a toenail so that we could follow each individual throughout the nestling period. Nestlings were weighed individually at hatching (day 0) and on days 2, 4, 6, 10, and 12 posthatching, using an electronic balance (± 0.01 g). The growth rate for each nestling was calculated as the mass increase divided by the actual time span (in hours) between measurements (range: 40.8–52.8 hr). Growth rate is presented as g day^{-1} (24 hr). Young remain in the nest until 18–22 days of age; however, we only measured growth from day 2 to day 12. During this period of time nestling growth was essentially linear, as 96% of the variation in growth was explained by a linear regression (see also McCarty 2001:fig. 1). In addition, nestlings reach the asymptote of their adult size at 12 days posthatching (Zach 1982). On day 12 we weighed and measured nestlings (tarsus and ninth primary length as above for adults) and collected a small (50 μl) blood sample for molecular analysis of nestling sex. Blood samples were stored in lysis buffer at 4°C. All unhatched eggs and dead nestlings were collected and tissues were frozen at -20°C . We determined the sex of Tree Swallow young using primers (P2 and P8) that amplify an intron of the CHD1 gene on the avian sex chromosomes (Griffiths et al. 1998). The details of our protocol for sexing Tree Swallows are described by Whittingham and Dunn (2000).

IMMUNOASSAY

We estimated nestling immune function by measuring the swelling caused by a subcutaneous injection of a nonpathogenic antigen, phytohaemagglutinin (PHA; Goto et al. 1978, Cheng and Lamont 1988). An injection of PHA causes a local swelling that is a meaningful indicator of immune activity in ecological contexts (Goto et al. 1978, Cheng and Lamont 1988, Martin et al. 2006). This measure of immune function is correlated with nestling survival (Christe et al. 1998) and disease resistance (Lochmiller et al. 1993); thus, it provides an index of offspring quality. We tested 230 nestlings from 60 broods with a subcutaneous injection of 0.2 mg PHA dissolved in 40 μl of saline into the right wing. The injection site was located dorsally on the metacarpus. A control of 40 μl of saline was

injected into same location on the left wing. Wing thickness was measured to the nearest 0.01 mm before injection on day 10 posthatching, and 24 ± 1 hr after injection, using a micrometer with a rounded tip (model SM-112, Teclock, Nagano, Japan). As the soft tissue on the wing can easily be deflated during measurement, especially when the wing is swollen, we took great care to measure wing thickness without decompressing the tissue. The outer section of the wing was placed horizontally on the lower plate of the micrometer, and the rounded measuring tip was lowered dorsally onto the wing until it barely touched the skin. There was no increase in thickness of the left wing as a result of the control (saline) injection (mean change = -0.03 mm \pm 0.15 SD; paired *t*-test: $t_{261} = 3.52$, $P < 0.001$). The repeatability (Lessells and Boag 1987) of the two measurements of the control site on day 10 and day 11 was $r = 0.53$ ($F_{261,262} = 3.2$, $P < 0.001$). There was a strong correlation between estimates of the swelling using just the right wing (PHA) and estimates based on the difference in swelling between the right and left wings (PHA-control; $r = 0.98$, $P < 0.001$). Thus, for simplicity, we used the increase in wing thickness at the PHA injection site (right wing) as our measure of immune function. This one-wing technique is well documented as an appropriate technique for characterizing PHA-induced wing web swelling in birds (Smits et al. 1999, Lifjeld et al. 2002, Martin et al. 2006). The mean increase in wing thickness at the right wing was 0.93 mm \pm 0.27 SD (*t*-test: $t_{262} = 55.7$, $P < 0.001$). All measurements of immune response were performed by JTL. Previous studies of swallows (Christe et al. 2001, Lifjeld et al. 2002) indicate that the immune response to PHA is lower during cold, wet weather that reduces food abundance, so we also measured air temperature, precipitation, and food abundance (flying insects).

WEATHER AND FOOD ABUNDANCE

We obtained air temperature and precipitation from an automated weather station located halfway between our two nest-box grids and averaged the mean temperature (sampled every 30 min) over the 24 hr period between measurements of the wing web swelling. We also measured food abundance using a suction trap, 46 cm in diameter, which collected flying insects

into a bottle with 70% ethanol (Southwood 1978:fig 4.2). The suction trap was placed in an open area between the two nest-box grids and operated from 06:00 to 19:00 CST each day. Food abundance was expressed as the total dry biomass (± 0.1 mg) of insects collected per day (Lifjeld et al. 2002). The suction trap collected all of the taxa and size classes of insects fed to nestlings, although not in the same proportion, so it provided a relative index of food abundance (Dunn and Hannon 1992). As shown in this study and others, this index of food abundance is correlated with laying date, egg mass, and nestling growth (Nooker et al. 2005, Ardia et al. 2006). Tree Swallow eggs are formed over 5–6 days; however, egg mass is primarily influenced by insect availability 1–3 days prior to egg laying (Ardia et al. 2006). Therefore, we averaged insect biomass and ambient temperature over the three days before the laying of each egg to examine the influence of environmental conditions on egg mass.

STATISTICAL ANALYSIS

For analyses of eggs and nestlings, we used mixed model ANOVAs that included individual nest identity as a random effect to control for the nonindependence of eggs or young from the same nest. Interactions among predictor variables were not significant unless stated otherwise. In this study each pair had only one nest; thus, nests were independent. Overall, our study included 263 nestlings from 60 broods, for which we knew the hatching mass of each nestling. This is the dataset we used for most analyses. We also had a more restricted dataset, of 146 nestlings from 33 broods, for which we knew exactly which nestling hatched from which egg. This data set was used to examine the relationship between egg mass and other variables. Sample sizes vary because it was not possible to collect data on all variables from each individual. Statistical analyses were performed with JMP® (SAS Institute 2003). All tests were two-tailed and means are reported \pm SE unless noted otherwise.

RESULTS

Both egg size and egg mass are often used as measures of initial maternal investment in young. In a mixed model analysis (with nest as a random factor), fresh egg mass was strongly correlated with egg volume (range:

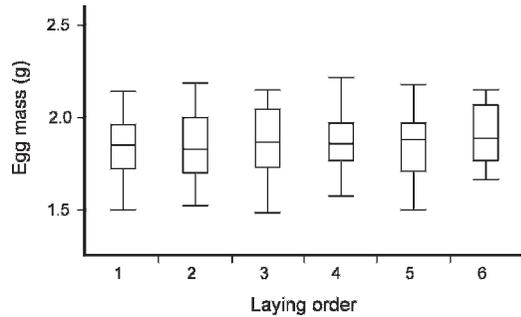


FIGURE 1. Fresh egg mass (g) of Tree Swallows in Wisconsin was not related to laying order. Each box plot displays the median (horizontal line inside box), 25th and 75th percentiles (ends of the box), and 10th and 90th percentiles (ends of lines outside each box) of egg mass.

1.07–2.65 g; $n = 329$, $r^2 = 0.94$, $F_{1,264} = 1897.1$, $P < 0.001$). In further analyses we used egg mass as an indicator of maternal investment in eggs. Egg mass was not related to female age (SY or ASY; $F_{1,222} = 0.2$, $P = 0.64$), female breeding experience in our population (0–3 years; $F_{3,222} = 0.6$, $P = 0.64$), female body mass ($F_{1,222} < 0.1$, $P = 0.87$), female size (tarsus length: $F_{1,222} = 0.4$, $P = 0.51$), clutch size ($F_{1,222} < 0.1$, $P = 0.89$), laying date ($F_{1,222} = 0.1$, $P = 0.73$), or laying order ($F_{1,222} = 0.4$, $P = 0.52$; Fig. 1) in a mixed model with all seven variables ($R^2 = 0.72$, $F_{62,222} = 11.9$, $P < 0.001$). Thus, we did not consider these variables further. In this mixed model, 74% of the variation in egg mass was explained by female identity (variance component estimate from restricted maximum likelihood; $n = 305$ eggs from 58 nests).

EGG MASS AND HATCHING MASS

Egg mass was a strong predictor of hatching mass (range: 0.94–1.74 g; $n = 146$, $r^2 = 0.73$, $F_{1,113} = 231.0$, $P < 0.001$; Fig. 2) in a mixed model analysis (nest was a random effect) of 146 young in 33 broods for which we were able to mark nestlings at hatching (toenail clip) and match them with a particular egg. Using the larger dataset of all nestlings ($n = 263$) for which only hatching mass was known, we found that hatching mass was a strong predictor of nestling mass throughout the growth phase of the nestling period (Table 1), especially in the early portion of the nestling period (days 2–6). In separate analyses of egg mass and

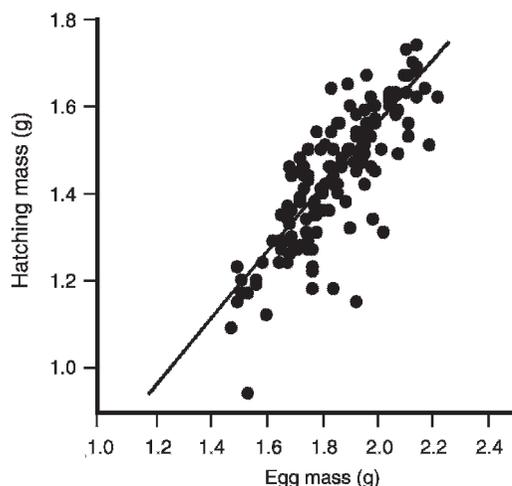


FIGURE 2. Fresh egg mass of Tree Swallows in Wisconsin was a strong predictor of nestling hatching mass.

nestling size at day 12, we found that egg mass was not related to nestling tarsus length ($F_{1,97} = 1.7$, $P = 0.19$) or length of the ninth primary ($F_{1,97} = 0.7$, $P = 0.39$) at 12 days of age. However, mass at hatching was positively related to tarsus length on day 12 ($F_{1,198} = 4.8$, $P = 0.03$) and nestlings with longer ninth primaries also tended to be heavier on day 12 ($F_{1,198} = 3.0$, $P = 0.09$). Using the restricted data set, egg mass was not related to nestling mass on days 2, 4, 6, 10, or 12 posthatching (all $F < 0.1$, all $P > 0.50$).

NESTLING QUALITY

In a mixed model analysis (with nest as a random factor) using our dataset of all nestlings ($n = 263$), we found that growth rate during the early phase of nestling growth (days 2–6; Zach 1982) was positively related to insect

TABLE 1. Relationships between mass at hatching (day 0) and mass at 2 to 12 days of age of Tree Swallows in Wisconsin (fledging occurs at 18–20 days), based on mixed models with nest as a random effect.

Age (days)	<i>n</i>	<i>r</i> ²	df	<i>F</i>	<i>P</i>
2	258	0.49	1, 201	12.1	< 0.001
4	263	0.50	1, 205	13.5	< 0.001
6	261	0.53	1, 203	12.5	< 0.001
10	252	0.41	1, 195	9.3	< 0.01
12	251	0.41	1, 194	7.2	< 0.01

abundance at this time ($F_{1,192} = 16.6$, $P < 0.001$), and was greater for nestlings that weighed more at hatching ($F_{1,192} = 26.8$, $P < 0.001$) and that hatched earlier in the hatching sequence ($F_{1,192} = 137.2$, $P < 0.001$). However, some of these patterns reversed later in the nestling period. In a separate mixed model analysis, we found that nestling growth was also positively related to insect abundance during days 6–12 ($F_{1,184} = 12.6$, $P < 0.001$), but nestlings grew faster during days 6–12 if they hatched later in the hatching sequence ($F_{1,184} = 65.6$, $P < 0.001$) and weighed less at hatching ($F_{1,184} = 7.7$, $P < 0.01$). Furthermore, later-hatched nestlings weighed less on day 12 ($F_{1,199} = 7.5$, $P < 0.01$), and thus did not compensate for the effect of hatching later in the sequence. Overall, mean growth rate (averaged for each brood) was faster earlier (days 2–6) than later (days 6–12) in the nestling period (paired *t*-test; $t = 2.7$, $P < 0.01$, $n = 51$ broods).

Nestling immunocompetence was related to growth rate, but not directly to egg mass, mass at hatching, or overall size (tarsus length). In a mixed model analysis using our restricted data set with matches between egg and nestling, we found the immune response of nestlings (at 11 days of age; $n = 110$) was positively related to the rate of growth during days 2–12, but it was not related to egg mass, laying order, clutch size, tarsus length, ambient temperature during the immune assay, or date of the immune assay (Table 2). We also examined the immune response of all nestlings, which precluded us from analyzing egg mass and laying order, but allowed a larger sample size. In this mixed model analysis, we found that the immune response of nestlings (at 11 days of age; $n = 229$) was positively related to growth rate during days 2–12 and ambient temperature during the immune assay, but not to hatching mass, hatching order, brood size on day 11 when immune response was measured, nestling sex, tarsus length, or date of the immune assay (Table 2). When we redid the analysis of immune response with growth rate separated into early and late periods, we found that immune response was related to growth only during days 6–12 ($F_{1,176} = 8.9$, $P < 0.01$; Fig. 3) and not to growth during days 2–6 ($F_{1,171} = 0.2$, $P = 0.63$). The model was not improved by adding interactions between hatching mass and the two growth periods. Thus, it appears that

TABLE 2. Results of a mixed model analysis (with nest as a random factor) of Tree Swallow nestling immune response in relation to several predictor variables. The first model includes only nestlings for which we had a match between nestling and egg ($n = 110$, $df = 1, 78$). The second model includes all nestlings sampled ($n = 229$, $df = 1, 171$). Significant probability values are denoted by an asterisk.

Predictor	Immune response of nestlings matched with eggs		Immune response of all nestlings	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Egg mass (g)	0.4	0.52		
Laying order	<0.1	0.83		
Clutch size	0.2	0.67		
Hatching mass (g)			0.4	0.53
Hatching order			1.7	0.20
Brood size			0.2	0.70
Sex			0.1	0.82
Growth rate (days 2–12)	9.4	< 0.01*	8.0	< 0.01*
Tarsus (mm)	1.2	0.28	1.5	0.22
Temperature (°C)	2.1	0.15	4.7	0.03*
Date	0.1	0.70	<0.1	0.84
Adjusted R^2	0.25		0.26	

egg mass was positively related to hatching mass and hatching mass was positively related to early growth of nestlings. However, nestling growth during days 6–12 was negatively related to hatching mass (i.e., smaller nestlings grew faster); thus, by 11 days of age, when immuno-competence was estimated, there was no significant effect of hatching mass.

NESTLING SEX

Over days 2–12 of the nestling period, male offspring grew faster (1.79 ± 0.17 g day⁻¹, $n =$

101) than female offspring (1.71 ± 0.16 g day⁻¹, $n = 99$; $F_{1,156} = 19.2$, $P < 0.001$; mixed model analysis using only broods with both sexes; Fig. 4). Next we examined nestling growth during the early (days 2–6) and middle (days 6–12) portions of the nestling period in separate mixed model analyses for each period that included nestling sex as a fixed factor and nest as a random factor. The difference in growth rate between male and female offspring was similar late (days 6–12: $n = 200$, $r^2 = 0.63$, $F_{1,156} = 9.8$, $P < 0.01$), and

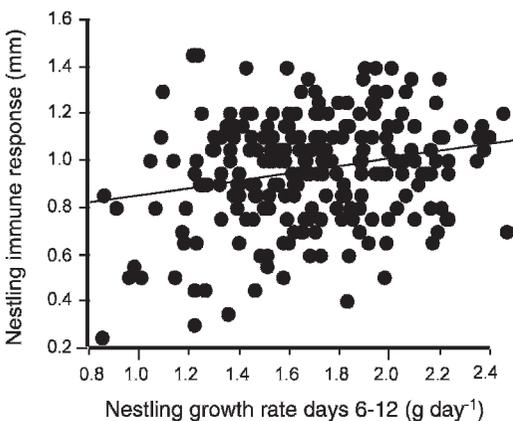


FIGURE 3. Nestling Tree Swallows that grew faster during days 6–12 (g day⁻¹) also had a greater immune response (mm swelling) to injection of phytohaemagglutinin. Plot shows the regression of predicted immune response (from the mixed model) on nestling growth rate.

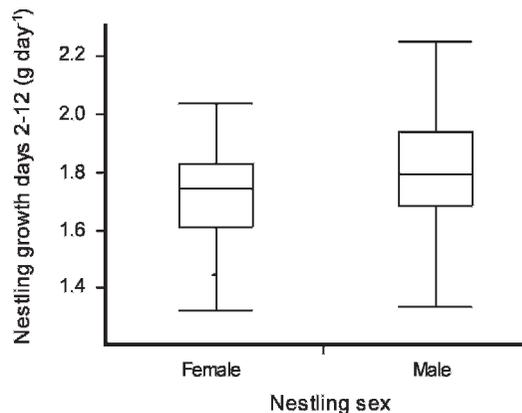


FIGURE 4. Male nestling Tree Swallows grew faster than female nestlings during days 2–12. Each box plot displays the median (horizontal line inside box), 25th and 75th percentiles (ends of the box), and 10th and 90th percentiles (ends of lines outside each box) of increase in body mass (g) per day.

early in the nestling period (days 2–6: $n = 227$, $r^2 = 0.65$, $F_{1,177} = 6.8$, $P = 0.01$). Overall, male offspring reached a heavier asymptotic weight at day 12 (21.2 ± 1.9 g) than female offspring (20.3 ± 2.1 g; $n = 261$, $F_{1,202} = 18.8$, $P < 0.001$). Although there was a difference in growth rate and asymptotic body mass between male and female young, they appeared to receive similar levels of maternal investment at laying because nestling sex was not related to egg mass ($n = 155$, $F_{1,122} < 0.1$, $P = 0.80$).

ENVIRONMENTAL EFFECTS

Environmental factors may have influenced egg mass in Tree Swallows. In a mixed model analysis using data from all eggs ($n = 296$; nest was a random factor), masses of individual eggs were positively related to ambient temperatures three days prior to the days they were laid ($F_{1,233} = 10.5$, $P = 0.001$), but not to insect abundance (ln-transformed) during the three days prior to laying ($F_{1,233} = 0.8$, $P = 0.37$) or precipitation three days prior to laying ($F_{1,233} = 2.2$, $P = 0.14$; overall model: $R^2 = 0.73$, $F_{62,233} = 12.5$, $P < 0.001$). Variation in temperature over the entire period of egg formation (i.e., five days prior to laying) also appeared to affect the average mass of the entire clutch (mean clutch mass). Overall, mean clutch mass was smaller when temperatures five days before the formation of each egg (averaged for each brood) were more variable (bivariate regression of mean clutch mass on the coefficient of variation [CV] of temperature five days before laying date; $r^2 = 0.21$, $df = 1, 55$, $P < 0.001$; Fig. 5).

DISCUSSION

We found that Tree Swallows that hatched from heavier eggs were larger and grew faster, at least early in the nestling period. Furthermore, nestlings that grew faster early in the nestling period had better immune function later in the nestling period, which may be unusual since previous studies (Soler et al. 2003, Brommer 2004) have found a trade-off between nestling growth and immunity. We also found that nestlings grew faster when there were more insects available, similar to other populations of Tree Swallows (Ardia 2006). In birds, both body mass and immune function can enhance offspring survival. Thus, it seems that young that hatch from larger eggs are likely to provide greater fitness benefits to female Tree Swallows

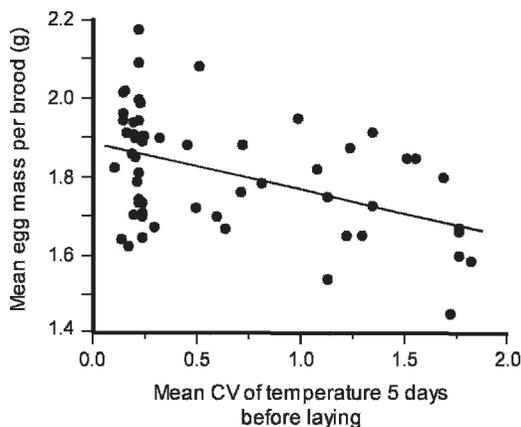


FIGURE 5. The mean egg mass per brood of Tree Swallows in Wisconsin was smaller when ambient temperatures were more variable.

than young that hatch from smaller eggs. However, our data do not support the idea that females allocate resources adaptively to eggs, or influence egg mass, in relation to either offspring sex or laying order. Instead, variation in egg mass, and its subsequent benefits, was due primarily to differences among females and a positive influence of warmer air temperatures.

As nestlings, males grew faster and reached a higher asymptotic body mass than females. Female Tree Swallows may eventually benefit from such differences because faster growth and greater body mass have a positive influence on nestling survival (McCarty 2001) and male offspring in good condition may realize greater reproductive success than female offspring in good condition (Trivers and Willard 1973). However, these differences between male and female offspring were not related to variation in egg mass and they are unlikely to be due to differential allocation of food from parents. Parents did not allocate more food to male nestlings during days 10–14 of the nestling period (Whittingham et al. 2003); however, food allocation earlier in the nestling period remains unknown. Adaptive variation of egg mass in relation to nestling sex has been studied in other species of birds that are sexually monomorphic in body size. In some studies egg mass was not related to nestling sex (Cichoń et al. 2003, Lifjeld et al. 2005), but in others, males hatched from larger eggs than females (Mead et al. 1987, Cordero et al. 2000). To date, there is no direct evidence in monomorphic

species that males hatching from larger eggs have greater reproductive success; however, further investigation is needed.

Allocating resources to eggs in relation to laying order could increase the fitness of females if it provides later-hatching nestlings with an advantage when competing for food with their earlier-hatched nest mates (Slagsvold et al. 1984, Budden and Beissinger 2005, Rosivall et al. 2005). In our study, egg mass did not vary in relation to laying order, but in other populations of Tree Swallows egg mass increased with laying order (Zach 1982, Ardia et al. 2006). However, even if later-laid eggs are larger, this is unlikely to provide a benefit to nestling Tree Swallows because nestlings that hatched later were still smaller than earlier-hatched nestlings at day 12 (Zach 1982), and smaller nestlings are severely disadvantaged in competition for food from parents (Whittingham et al. 2003).

It is clear that egg mass can have fitness-related consequences for female birds, due to its positive effect on nestling growth (this study), which has a strong influence on subsequent survival in many species, including Tree Swallows (McCarty 2001, Legge 2002). Nestling quality is also related to immune function, because immunity is an indicator of disease resistance and nestling survival in altricial birds (Lochmiller et al. 1993). In House Martins (*Delichon urbica*) and Zebra Finches (*Taeniopygia guttata*), nestling immune response to PHA was positively correlated with survival (Christe et al. 1998) and longevity (Birkhead et al. 1999). Our results suggest that Tree Swallow young benefit from both faster growth and an enhanced immune system. Overall, we found a positive relationship between nestling growth rate and immune response in Tree Swallow nestlings; however, this relationship varied across the nestling period. Early in the nestling period (days 2–6), when nestling growth rate was faster, we found no relationship with immune response, but later in the nestling period (days 6–12), when nestling growth rate was slower, we found a positive relationship with immune response. In contrast, studies on Blue Tits (*Parus caeruleus*; Brommer 2004) and Magpies (*Pica pica*; Soler et al. 2003) found an overall negative relationship between nestling growth and immune response. We also found that nestling immune response was lower when

ambient temperatures were lower, similar to a previous study of adult Tree Swallows (Lifjeld et al. 2002) and nestlings in other species (Christe et al. 2001, Garvin et al. 2006). Environmental factors, such as ambient temperature or food abundance, could influence whether or not nestlings face a trade-off between growth and immune function.

Weather and food availability are also likely to be important factors influencing egg mass, because they will influence the trade-off that females face between their own metabolic requirements and egg production. Similarly to Tree Swallows, females of other species laid larger and heavier eggs when temperatures prior to laying were warmer (Saino et al. 2004, Hargitai et al. 2005, Pendlebury and Bryant 2005), and similarly to Great Tits (*Parus major*; Pendlebury and Bryant 2005), female Tree Swallows produced smaller eggs when temperatures prior to laying were more variable. In general, warmer temperatures are thought to have a positive effect on egg mass because they enhance insect abundance, but insect abundance is rarely measured in studies of bird populations. In our study area, warmer temperatures were associated with greater abundance of flying insects in 2000 (Lifjeld et al. 2002) and greater egg mass when we analyzed individual eggs.

Despite the importance of weather during egg formation, much of the variation in egg size and mass occurred among clutches due to differences among females, but not due to typical female traits (e.g., female age, breeding experience, body mass, body size, clutch size, or laying date). Large variation in egg mass among females, and relatively less intraclutch variation, is generally attributed to high heritability of egg size, which has been documented in several different birds (Christians 2002, Styrsky et al. 2002, Budden and Beissinger 2005), including Tree Swallows (Wiggins 1990).

In summary, we found no evidence that female Tree Swallows adaptively allocated resources to eggs in relation to either laying order or nestling sex. Instead, most of the among-clutch variation in egg mass was due to differences among females, while the within-clutch variation in egg mass was influenced primarily by ambient temperature during egg formation. However, female Tree Swallows are likely to realize substantial benefits from pro-

ducing larger eggs, because the young from those eggs are larger and grow faster, and young that grow faster have better immune function, both of which can enhance offspring survival. Our results suggest that the positive relationship between nestling growth and survival in Tree Swallows may be due, in part, to the effects of an enhanced immune response.

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