

Effects of experimentally increased costs of activity during reproduction on parental investment and self-maintenance in tropical house wrens

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Life-history theory assumes that organisms trade-off current against future reproduction to maximize fitness. Experimental explorations of the costs of reproduction have not yielded a clear understanding of the nature of these costs but rather point to a complex set of allocation possibilities among several physiological functions and behaviors. We investigated how experimentally increased flight costs affected the trade-off between parental investment and self-maintenance in tropical house wrens, which have relatively high annual survival and multiple breeding opportunities per year. We predicted that handicapped wrens would not increase their energy expenditure but instead decrease their effort to rear young in order to maintain their own body condition. Our results largely supported these predictions: handicapped parents decreased their nestling feeding frequency but did neither alter their field metabolic rate (FMR) nor compromise their body condition as measured by basal metabolic rate (BMR) and several measures of innate immune function. Reduced feeding rates did not affect nestling body mass growth but resulted in decreased structural growth (length of tarsus). The latter result can be explained if parents shifted the type of prey brought to offspring or altered the amount of food brought per trip. The experiment-wide positive correlations among FMR, BMR, and feeding frequency are in agreement with the hypothesis that hard work requires elevated levels of BMR. These correlations, in combination with the absence of a handicap treatment effect on FMR or BMR, do not lend support for predictions from studies in the laboratory that birds compensate hard work during the day by lowering their BMR at night. Considering a complex set of allocation possibilities among several physiological functions and behaviors, we conclude that tropical wrens take out the costs of a handicap largely on their offspring quality not on self-maintenance processes. *Key words*: cost of reproduction, handicap experiment, life-history evolution, physiological trade-offs. [*Behav Ecol* 19:949–959 (2008)]

Life-history theory assumes that organisms trade-off current against future reproduction to maximize fitness (Williams 1966; Trivers 1972; Stearns 1992). This trade-off is based on the existence of costs that organisms incur in order to reproduce and that diminish survival and/or future reproductive output. These costs are often thought to be physiological in nature (Drent and Daan 1980; Daan et al. 1996; Deerenberg et al. 1996; Hauber 2002). If life-history variation is constrained by physiological processes, then understanding the mechanistic basis of the trade-offs underlying life-history evolution becomes crucial. Relatively high survival, for example, in tropical compared with temperate zone songbirds (Ricklefs 1997), is ostensibly associated with large investments in self-maintenance, such as robust immune function and low metabolic rate resulting in low oxidative damage (e.g., Martin

et al. 2004; Ardia 2005; Tieleman et al. 2005). Such large investments in self-maintenance are thought to be coupled with small investments per reproductive bout. Life-history theory predicts that shifts in the balance between survival and reproduction lead to different reactions in response to experimentally increased costs of reproduction, with long-lived birds favoring their own survival and short-lived birds favoring current offspring (Williams 1966; Mauck and Grubb 1995).

The cost of reproduction is a complex concept, often difficult to quantify in nature (Partridge and Harvey 1985). Originally, parental effort was measured as the number of feeding trips that parents made to the nest and/or body mass loss of adults during the nesting period (e.g., Hails and Bryant 1979; Nur 1984; Bryant 1988). With the advent of the doubly labeled water (DLW) technique (Lifson and McClintock 1966), field metabolic rate (FMR) of adults feeding young became a metric for parental effort (e.g., Hails and Bryant 1979; Williams 1987; Bryant 1988; Mock 1991). Surprisingly, feeding frequency, brood size, and FMR were often uncorrelated (Bryant 1988). This finding was later explained, at least partially, with the demonstration that parents can alter the amount of food brought per trip and the food quality without incurring

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energetic costs (Tinbergen 1981; Wright et al. 1998; but see Williams and Vézina 2001). In addition, in the laboratory, birds with high energy expenditure during the day compensated by lowering their basal metabolic rate (BMR) at night, keeping overall energy expenditure constant (Bautista et al. 1998; Deerenberg et al. 1998). Reduced BMR was thought to reflect decreased investment in self-maintenance and molecular repair processes. Hard work was linked to other physiological costs, including compromised self-immune function and oxidative damage (Deerenberg et al. 1997; von Schantz et al. 1999; Wiersma et al. 2004). If or how these physiological functions are connected through reallocation of energy or other resources is unresolved. The alternative possibilities for compensation among physiological and behavioral processes emphasize the need for experimental studies that measure attributes of both simultaneously on the same individuals, in order to gain insights into trade-offs and allocation patterns among birds.

Geographic variation in life-history strategies is thought to reflect differences in the optimal balance of energy allocation to survival and reproduction in different environments. Widespread and common species, such as the house wren (*Troglodytes aedon*), are ideal candidates for investigations of this trade-off because they live in both temperate and tropical environments. House wrens occur from Canada, where they are migratory, to the tropics, where they are year-round residents, and even to southern South America (Johnson 1998). Ornithologists have variously considered the continental forms to belong to as few as one species to perhaps 3 or more, but currently they remain officially recognized as a single species (Johnson 1998). Compared with north temperate zone conspecifics, tropical wrens have relatively high annual survival, small clutch sizes, fewer clutches per year, longer incubation, nestling and postfledging care periods, and extended reproductive periods (Freed 1987; Young 1994, 1996; Johnson 1998; Tieleman et al. 2006). These traits of tropical wrens are associated with lower rates of parental energy expenditure and water influx during the nestling phase and a lower nestling feeding frequency compared with temperate wrens in Ohio (Tieleman et al. 2006). Based on these patterns, life-history theory would predict that, when faced with increased costs for reproduction, tropical wrens should allocate resources to self-maintenance to assure long-term survival, whereas temperate zone wrens should invest in current offspring even at the expense of their own body condition. Experimental evidence provides ambiguous support for this prediction. Two brood manipulation studies in temperate zone populations in Ohio and Illinois found that enlarged broods fledged more chicks but that the increase in reproductive output had no negative effect on female body mass (Finke et al. 1987; Robinson and Rotenberry 1991). A 3-year brood size manipulation experiment in tropical Costa Rica found no effect on male and female survival and weak effects on future fecundity and offspring survival (Young 1996). Overall, it appeared that Costa Rican wrens successfully reared more young without a reduction in survival. Whether the decrease in future fecundity was the result of a compromised body condition that did not affect survival remained unresolved.

Most experimental studies of the costs of reproduction have manipulated reproduction directly by enlarging or reducing brood size. However, brood size manipulations do not consistently alter the energy expenditure of the parents (Bryant 1988; Moreno et al. 1995; Tinbergen and Verhulst 2000). In addition, brood size manipulations alter the proximate stimulus for parent birds, but, as it is unclear how proximate and ultimate measures are connected, brood size manipulations may not alter the parents' perceived fitness benefits (Moreno et al. 1999). Therefore, it may be complementarily profitable

to manipulate energy expenditure of adults directly to measure effects on reproduction, rather than vice versa (Moreno et al. 1999; Williams and Vézina 2001; Spencer and Bryant 2002). Handicapping birds, for example, by reducing their wingspan (Mauck and Grubb 1995; Winkler and Allen 1995), causes an unavoidable increase in cost for flight and provides the opportunity to study reallocation within the total energy budget.

Here we report on a comprehensive set of physiological and behavioral measurements in the field, taken simultaneously on the same individuals, to investigate how increased flight costs affected the trade-off between parental investment in offspring and adult self-maintenance in tropical house wrens. To shed light on the allocation of resources to various physiological functions and behaviors, we compared multiple indices of parental effort (feeding frequency, FMR, and nestling growth) and self-maintenance (BMR and immune function). We predicted that tropical house wrens when handicapped would decrease their effort to raise offspring, maintaining overall energy expenditure and the amount of resources allocated to self-maintenance constant, thereby keeping their own body condition at optimal levels while compromising nestling growth and development.

MATERIALS AND METHODS

Study area

We conducted our study during March to July 2004 in Gamboa and Summit Botanical Gardens, Republic of Panama (9°N, 79°W). The area has a humid lowland tropical environment with constant average annual temperatures of about 25 °C and a rainy season from late April until December. Year-to-year variation and month-to-month variation in temperature and rainfall is small, and breeding conditions (thermal environment and food availability) are mostly benign. Of the 22 nests that we used in this study, 6 were located in nest-boxes and 16 in other sites, varying from streetlamps and cracks in walls to ridges under elevated buildings. House wrens breed year-round in Panama, with a peak in breeding activity during May, at the beginning of the rainy season (Freed 1987). Average clutch size of the population is 3.6 eggs, and annual survival is assumed to be around 50%, resembling that of other tropical house wren populations and earlier measures in this population (Freed 1987; Tieleman et al. 2006).

Experimental protocol and time line of measurements

We monitored nests daily toward the end of the incubation period to determine hatch date (day 0). When nestlings were 4, 7, and 11 days of age, we counted feeding rates of the parents. On day 6, we captured both parents for injection with DLW (see below) and outfitted them with color bands for individual recognition. At the same time, we measured the wingspan of each parent and assigned the pair to experimental or control groups in an alternating fashion. Our experimental treatment consisted of reducing wingspan by clipping primary feathers such that work effort for flapping flight would be increased by at least 7.5%. This level of handicap falls within the range of previous handicapping experiments of 5–10% (Wright and Cuthill 1990; Saether et al. 1993; Mauck and Grubb 1995) and reflects naturally possible variation in body mass. The length of feather tips that we clipped depended on an individual's wingspan but averaged 7.6 ± 0.60 mm (average \pm standard deviation [SD], $n = 18$) per wing for all handicapped birds (average wingspan 180.2 ± 5.77 mm, $n = 33$). The minimum cost increase of 7.5% was estimated using Pennycuick's Flight program 1.14 (Pennycuick 2004). Pennycuick's equation

relating wingspan to energy expenditure during flight assumes that wings are aerodynamically similar. Because we clipped wing feathers straight across rather than maintaining the curvature of each primary, we recognize that aerodynamics of the wing changed. As a result, the increase in flight costs in reality was likely somewhat higher than 7.5%. Handicapped wrens clearly labored to fly. Control birds were subjected to the same measurement and banding protocol, but we did not clip their wing tips.

We recaptured parents on day 8, around 48 h after injection of DLW, to take a final blood sample for measurement of FMR. Parents were recaptured again on day 12, bled (50–70 μ L blood) within 5 min after capture for immune function assays (bacteria killing, phagocytosis, and blood smear), and transported to the laboratory for BMR measurements the same night. When we captured birds more than 3 h before the start of BMR measurements, we fed them mealworms. In addition, we fed nestlings with mealworms or crickets after we caught parents. We released the parents at their nest site after completing BMR measurements, usually between 0100 and 0300 h; all birds resumed feeding their nestlings early the following morning. The time line was shifted by 1 day for 2 control and 1 treatment nest and by 4 days for 1 control and 1 treatment nest that we found when the chicks were older. Therefore, we included nestling age in analyses where appropriate.

Nestling mass, wing, and tarsus were measured on days 6, 7, 8, and 11. We measured body mass (± 0.1 g) with a Pesola scale, tarsus (± 0.1 mm) using calipers, and wing length (millimeters) with a wing ruler.

Sample size and fate of the nests: response of parents and broods to treatment

We started with 11 nests for each treatment at day 0 but lost some nests or chicks during the nestling period. We abandoned 2 control nests before day 6, one because of a botfly infestation and the other because chicks died. We lost 4 handicapped nests at different times during the nestling period before the nestlings fledged: in one nest the chicks disappeared on day 7, in another nest the female did not return after injecting her with DLW, and in 2 nests the chicks disappeared between days 8 and 12. In addition to these complete brood losses, 1 control and 2 handicapped broods lost 1 chick during the nestling phase. These events explain the different sample sizes at different moments during the time line.

Behavioral observations

We observed the number of feeding trips that adults made by observing the nest with binoculars from a distance of at least 25 m for 4–6 h between 0600 and 1200 h (control: 4.53 ± 1.43 h, $n = 19$; treatment: 4.75 ± 1.18 h, $n = 16$). Because adults were not color banded until day 6, determination of sex of visiting adults was only possible on days 7 and 11. We compared feeding frequency between control and treatment groups and between sexes.

Doubly labeled water

FMR and water intake rate (WIR) were measured using the DLW technique, in which the rate of decline of ^2H in the body water pool provides a measure of WIR (Nagy and Costa 1980) and the loss rates of both ^2H and ^{18}O yield an estimate of CO_2 production (Lifson and McClintock 1966; Nagy 1980; Speakman 1997). WIR is the sum of water ingested from food and drinking and oxidative water generated by the processing of food. Assuming that the amount of drinking water ingested is the same, changes in the relative proportions of WIR and

FMR can indicate changes in diet (i.e., water content of food stuffs or efficiency of metabolism). We caught birds and injected them with 7 $\mu\text{L/g}$ mass of a 1:2 mixture of 99.9 atom percent ^2H and 95.5 atom percent ^{18}O using a 100 μL Hamilton syringe. We weighed birds with a Pesola spring balance. We kept females for a 1-h equilibration period (Williams and Nagy 1984) and thereafter took a 80- to 100- μL sample of blood (initial) from the brachial vein and then banded and released them. Males were released directly after injection and banding, and estimates of CO_2 production were obtained with the single-sample method (Ricklefs and Williams 1984; Speakman 1997). Capture, injection, and equilibration did not appear to disturb the behavior of the wrens; birds returned to feed nestlings within 1–4 h after we released them. After about 48 h (average: 47.2 ± 1.54 h, $n = 35$, range: 45.1–53.6 h; 22.9 ± 0.80 h, $n = 2$, range: 22.8–23.0 h), we recaptured birds, took a second blood sample (final), measured body mass, and released them. We took blood samples of 4 uninjected individuals to determine background levels of isotopes.

Validation studies on adult birds have shown that estimates of WIR using isotopes of hydrogen are usually within $\pm 10\%$ of values obtained by standard laboratory methods (Nagy and Costa 1980) and average estimates of CO_2 production are within 8–10% while individual measurements of the latter can deviate up to 22% in small birds (Nagy 1980; Williams and Nagy 1984; Speakman 1997). With one exception, all broods increased mass during measurements of FMR.

Isotope ratios of $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ in body water were determined in duplicate (initial) or triplicate (final) for each sample at the Center for Isotope Research, University of Groningen (Visser and Schekkerman 1999). We calculated water influx with equation 3 of Nagy and Costa (1980) and corrected for isotope fractionation effects assuming an evaporative water loss of 25% and a fractionation factor of 0.941 (equation 7.6; Speakman 1997; Visser et al. 2000). Total body water was estimated from isotope dilution. For single-sample measurements, we estimated initial isotope concentrations based on mass, injectate enrichment, and injection volume. Rates of CO_2 production were calculated with equation 7.17 of Speakman (1997). CO_2 production can be converted to energy expenditure using 24.39 kJ/L CO_2 for animals with an insectivorous diet (Gessaman and Nagy 1988; Weathers and Sullivan 1989).

Bactericidal activity assay

We assessed the bactericidal activity of fresh whole blood and fresh plasma in vitro (Tieleman et al. 2005; Matson et al. 2006; Millet et al. 2007). We collected sterile blood samples in the field within 5 min of capture and transported them to the laboratory, where the blood was processed within an hour after obtaining the sample.

A laminar flow hood (AC600, AirClean Systems, Raleigh, NC) provided a sterile working environment in the laboratory. We diluted blood by adding 10 μL blood to 190 μL CO_2 -independent media (#18045, Gibco Invitrogen, Carlsbad, CA) enriched with 5% heat-inactivated fetal calf serum and 4 mM L-glutamine. To this solution, we added 20 μL of a suspension of living *Escherichia coli* (ATCC #8739), reconstituted from lyophilized pellets (E^{POWER} Microorganisms #0483E7, MicroBioLogics, St Cloud, MN). We adjusted the concentration of the *E. coli* suspension to yield about 150–200 colonies per 75 μL of diluted blood–bacteria mixture. The mixture of diluted blood and *E. coli* was incubated for 30 min at 41 °C before we plated 75 μL on plates with Tryptic Soy Agar (Difco soybean casein digest agar, USP, Fisher catalog #DF0369176). Each sample was plated in duplicate. Control dilutions consisted of 200 μL CO_2 -independent media mixed with 20 μL

bacteria suspension. We made 1–5 control dilutions per session and used the average when $n > 1$. Plates were incubated upside down at ambient temperature (25–30 °C), and the number of colony-forming units was counted the following day.

We tested the variation among control plates by making 8 control dilutions from the same bacteria suspension (20 μ L bacteria suspension in 200 μ L media) and plating each dilution in duplicate. Following our normal procedure, we calculated the average of each pair of control plates. The coefficient of variation among our 8 dilutions was 3.8% (average number of colonies \pm SD: 120.8 ± 4.60 , $n = 8$). The bactericidal activity of blood was expressed as number of colony-forming units remaining after incubation of the blood–bacteria mixture relative to the number inoculated (control plates).

Phagocytosis assay

Phagocytic activity of macrophages and thrombocytes was measured by adding nonliving but fluorescent bacteria to fresh whole blood *in vitro* and counting the number of ingested bacteria in the phagocytes using a fluorescent microscope (Millet et al. 2007). We made a dilution of 30 μ L blood in 570 μ L CO₂-independent media enriched with 4 mM L-glutamine, 5% fetal calf serum, and 1% PenStrep (Gibco Invitrogen) and added 66 μ L of this dilution to each of 8 wells of a chamber slide (*154534, Lab-tek II Chamber Slide with Cover, Nalge Nunc International, Rochester, NY). In the first 4 wells, we added 250 μ L of a suspension of fluorescent dead *E. coli* bacteria (E-2864, Molecular Probes, Leiden, The Netherlands), and in the second 4 wells, we mixed in a similar suspension of *Staphylococcus aureus* particles (S-2854, Molecular Probes). The concentration of the suspension was modified to yield a good resolution of about 25–50% phagocytosis in the samples (i.e., for *E. coli*, a 1:100 white blood cell to particle ratio and for *S. aureus* a 1:200 white blood cell to particle ratio). The chamber slides were incubated for 15 min at 41 °C, washed twice with media, and fixed with methanol before storage in a dark box to maintain fluorescence for later reading. We used a fluorescent microscope (BODIPY, excitation/absorption spectrum 505/513 nm) to determine the proportion of white blood cells containing phagocytosed particles in a total number of at least 100 white blood cells.

Blood smears

We made blood smears immediately after capturing birds, fixed them with methanol within a day, and stained them with Giemsa stain 2–3 months later before counting leukocytes (Campbell 1995).

BMR and total evaporative water loss

We measured rates of oxygen consumption (VO₂) and total evaporative water loss for postabsorptive birds during their nocturnal phase by standard flow-through respirometry and hygrometry methods (Tieleman et al. 2002). Birds were placed in stainless steel metabolism chambers with Lexan lids, rendered airtight with rubber gaskets. Birds perched on a wire mesh platform over a layer of mineral oil that trapped excrement in order to exclude it as a source of water in the measurements. Chambers sat in a large insulated cool box with a Peltier device affixed in the lid (Sable Systems, Pelt-4, Las Vegas, NV) to control ambient temperature (T_a) at 31–32 °C, a temperature within the thermoneutral zone of wrens (Williams JB, unpublished data). Compressed air coursed through columns of Drierite to remove water through previously calibrated (Levy 1964) Mikrolis mass flow controllers (FC-2900; 2 standard liters per minute) set at 500 mL/min (standard temperature and pressure) and then into the

chamber. Exiting air passed through a dew point hygrometer (Dewprime II, Edgetech, Marlborough, MA) that had been recalibrated by the factory in August 2003. In addition, we checked the calibration of the dew point hygrometer before experiments using a dew point generator and found <2% difference. A subsample was then routed through silica gel, Ascarite, and silica gel to remove water and CO₂ before measuring the fractional concentration of O₂ with an oxygen analyzer (S3A-II, Applied Electrochemistry, Pittsburgh, PA). We validated our ability to measure O₂ consumption by infusing pure O₂ into our chamber using a syringe pump with a precisely determined flow rate while simultaneously pushing room air through another port into the chamber via our flow controller. Comparisons of known O₂ influx via the syringe pump with calculated influx showed a mean difference of $\pm 2\%$ for our 10 trials.

After a 3-h equilibration period, we recorded O₂ concentration and dew point of inlet and outlet air, the temperature of the dew point hygrometer, and T_a in the chamber using a data logger (CR23X, Campbell Scientific, Logan, UT). When, during the fourth hour of measurements, the traces for O₂ consumption and dew point were stable for at least 10 min, we noted these times and used these data for calculations. The relative humidity of outlet air was always between 10% and 40% (Lasiewski et al. 1966). After completing the metabolism measurements, we immediately measured the body temperature of birds with a Physiotemp thermometer (Batt-12) and a 36-gauge copper constantan thermocouple. Oxygen consumption was calculated with equation 4 of Hill (1972). We used 20.08 J/mL O₂ to convert oxygen consumption to heat production (Schmidt-Nielsen 1997).

Statistical analysis

To analyze the nestling growth and development data, we used hierarchical mixed models with nest fitted as random effect to test the significance of fixed effects at the appropriate level. Individual nestling (nested within nest) was included as random effect for those tests where repeated measures of the same individual were taken (biometric data on days 7 and 11). We included handicap treatment and chick age as fixed effects in the model. We used the Wald statistic to evaluate statistical significance.

To test for differences in the bactericidal activity, we used a modified arcsine square root transformation for analysis of fractions when the actual counts are known (Zar 1996). Two-way interaction terms were always included in general linear model (GLM) models and removed when they were not significant before further testing the effects of main factors and covariates. We used MLwiN version 2.02 for the mixed model analyses and SPSS version 13.0 for all other statistical analyses. Averages are reported \pm 1 SD.

RESULTS

Brood size

We found no significant difference in brood size between nests assigned to control and handicap treatments on the day of the initial measurement (control: 3.6 ± 0.72 chicks, $n = 9$; handicap: 3.6 ± 0.74 chicks, $n = 8$; $t = -0.2$, $P = 0.85$).

Nestling growth and development

We compared nestling body mass, tarsus, and wing length at the first day of the experiment to assure that control and treatment groups did not initially differ. We used a mixed model with nestlings nested within nest and verified that none of the biometric measures differed significantly between the

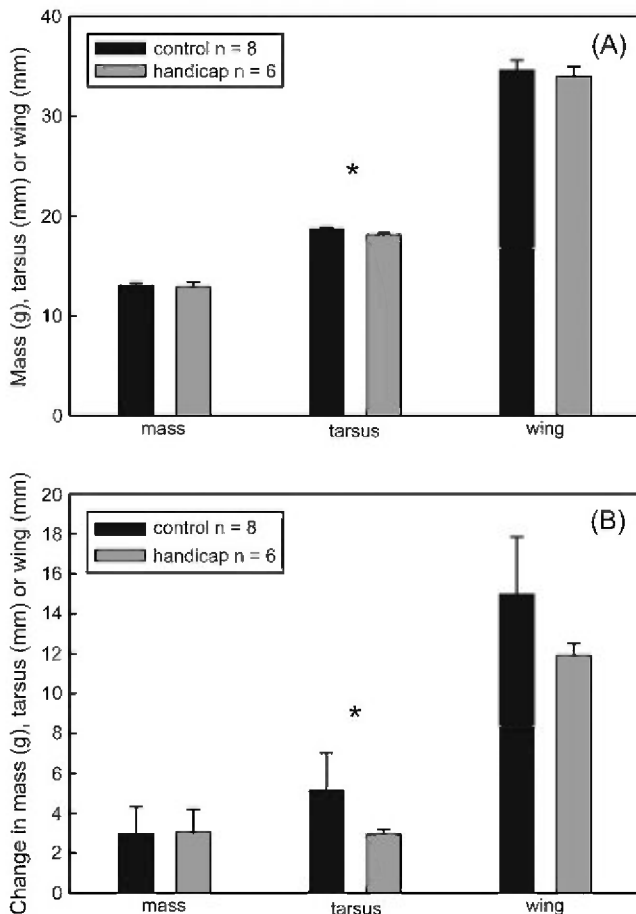


Figure 1
(A) Average nestling body mass, tarsus length, and wing length per nest at day 7 for nests with control and handicap treatments. (B) Growth in body mass, tarsus length, and wing length between days 7 and 11 of nestlings with control and handicapped parents.

2 groups (control–handicap: mass difference, average \pm standard error [SE]: -0.27 ± 0.54 , $\chi_1^2 = 0.26$, $P = 0.61$; tarsus difference, average \pm SE: -0.49 ± 0.54 , $\chi_1^2 = 0.84$, $P = 0.84$; wing difference, average \pm SE: -0.33 ± 1.37 , $\chi_1^2 = 0.06$, $P = 0.81$).

To test for an effect of treatment on nestling growth and development, we included body mass, tarsus, and wing at days 7 and 11 and tested the interaction between nestling age (day 7 or 11) and treatment while including age and treatment as fixed effects in the model. We found no significant differences in growth of body mass or wing length between treatments (mass: age \times treatment effect = -0.147 , 95% confidence interval [CI]: -0.329 to 0.035 , $\chi_1^2 = 2.48$, $P = 0.12$; wing: age \times treatment effect = -0.113 , 95% CI: -0.432 to 0.206 , $\chi_1^2 = 0.48$, $P = 0.49$), but tarsus grew significantly less in nestlings with handicapped parents (age \times treatment effect = -0.132 , 95% CI: -0.255 to -0.009 , $\chi_1^2 = 4.47$, $P = 0.03$). Confirming these growth results, at day 11, tarsus was significantly shorter in the nestlings of the handicapped parents ($\chi_1^2 = 4.13$, $P = 0.04$), but nestling mass and wing length did not differ significantly between treatment groups (mass: $\chi_1^2 = 0.07$, $P = 0.79$; wing: $\chi_1^2 = 0.12$, $P = 0.73$; Figure 1). We did not include nestling age in the latter analyses because older nestlings (day 14) were not measured to avoid premature fledging or because they had already fledged.

One might question if the variance in body mass growth among nests was sufficient to detect possible differences be-

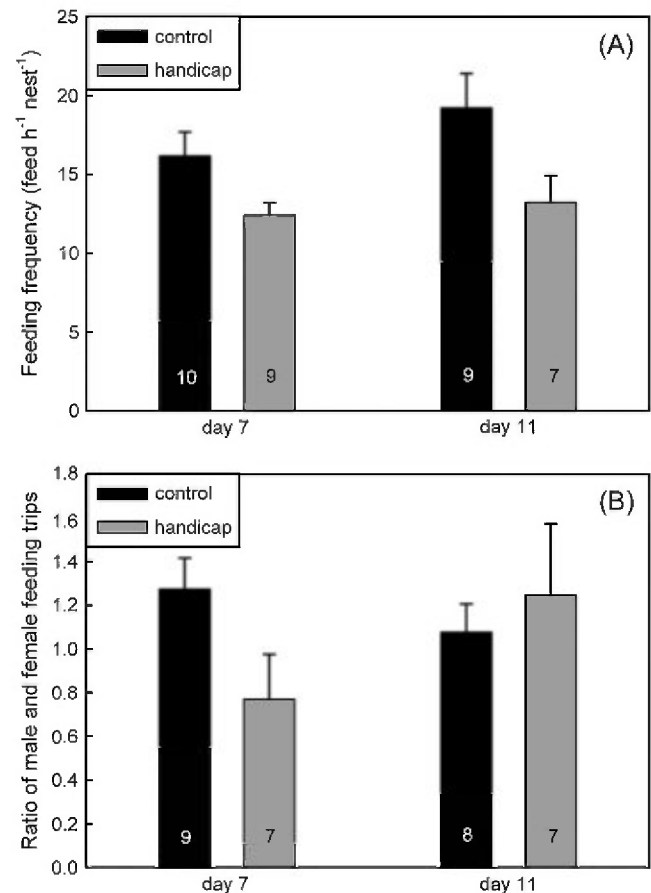


Figure 2
(A) Total number of feeding trips per hour by male and female combined to nests of control and handicapped parents on days 7 and 11. (B) The contribution of male and female to the total number of feeding trips to the nest, calculated as number of trips by male divided by number of trips by female, for control and handicap treatment on days 7 and 11.

tween treatments. Using a generalized linear mixed model with random slopes (interaction of nestling age \times nest), we found significant variation in nestling mass growth among nests (variance in within-nest slopes \pm SE = 0.062 ± 0.029 , $\chi_1^2 = 4.67$, $P = 0.03$). The handicap treatment explained only 3.2% of this variation in nestling growth among nests.

Parental feeding behavior

Handicapped parents made 23% and 31% fewer trips per hour to feed their nestlings than control birds on days 7 and 11, respectively (Figure 2a). We analyzed feeding frequency with GLM including treatment and stage (day 7 or 11) as fixed factors and chick age and brood size as covariates. Feeding frequency was significantly affected by treatment ($F_{1,30} = 9.66$, $P = 0.004$) and brood size ($F_{1,30} = 10.38$, $P = 0.003$), but not by stage ($F_{1,30} = 0.05$, $P = 0.83$) or chick age ($F_{1,30} = 0.38$, $P = 0.54$).

The relative contribution of the male and the female to the total number of feeding trips to the nest appeared to depend on the experimental treatment and the time after treatment (Figure 2b). We included only those observations where more than 60% of the feeding trips could be assigned to male or female (average $88 \pm 14\%$, $n = 31$). On day 7, the day after handicapping the birds, the male contribution showed

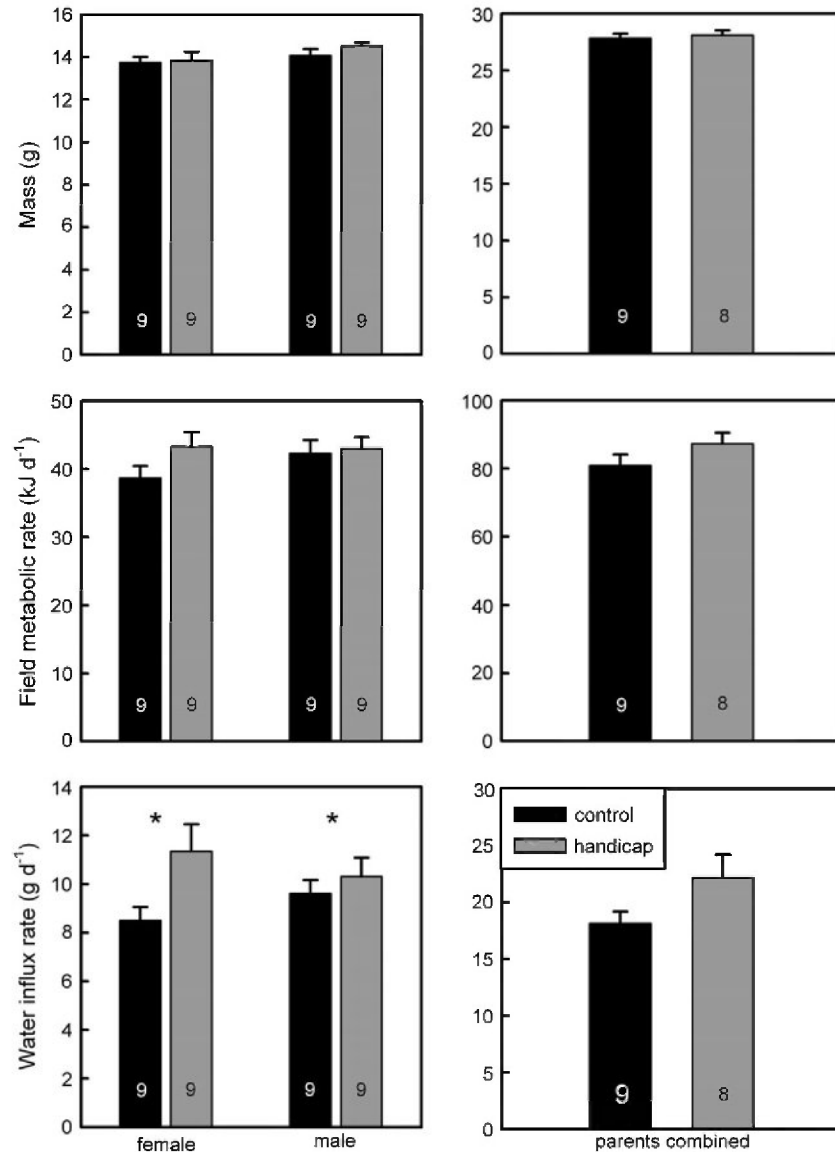


Figure 3

Body mass, FMR, and water influx rate (WIR) of female and male house wrens subject to control or handicap treatment. Values for both parents combined depict the sum of female and male body mass, FMR, or WIR per pair.

a nonsignificant trend to be lower in the handicapped pairs ($t = 2.08$, $P = 0.057$). On day 11, this apparent difference between male and female contribution had disappeared ($t = -0.51$, $P = 0.62$).

FMR and water influx rate of parents

FMR of parents did not differ significantly between sexes or treatments, whereas WIR did not differ between sexes but was significantly higher in handicapped birds when taking into account various covariates (Figure 3). We performed GLM analyses for FMR and WIR with sex and treatment as fixed factors and body mass, nestling age, and brood size as covariates. The interaction term between sex and treatment was not significant whether the dependent variable was FMR ($F_{1,29} = 3.26$, $P = 0.08$) or WIR ($F_{1,29} = 2.46$, $P = 0.13$). FMR was significantly related to body mass ($F_{1,30} = 17.1$, $P < 0.0001$) and nestling age ($F_{1,30} = 12.1$, $P = 0.002$), but not affected by

treatment ($F_{1,30} = 1.93$, $P = 0.18$), sex ($F_{1,30} = 0.02$, $P = 0.90$), or brood size ($F_{1,30} = 2.55$, $P = 0.12$). WIR was significantly influenced by treatment ($F_{1,30} = 4.89$, $P = 0.035$) and nestling age ($F_{1,30} = 6.41$, $P = 0.017$), but not by sex ($F_{1,30} = 0.06$, $P = 0.80$), mass ($F_{1,30} = 1.31$, $P = 0.26$), or brood size ($F_{1,30} = 0.06$, $P = 0.81$).

We also calculated the sum of male and female FMR and WIR per nest and compared total parental FMR and WIR between treatments (Figure 3) using a GLM that included treatment as fixed factor and total parental body mass, nestling age, and brood size as covariates. With FMR as dependent variable, nestling age ($F_{1,12} = 11.1$, $P = 0.006$) and total parental body mass ($F_{1,12} = 17.1$, $P = 0.001$) had significant effects in the model but treatment ($F_{1,12} = 2.74$, $P = 0.12$) and brood size ($F_{1,12} = 0.52$, $P = 0.48$) did not. With WIR as dependent variable, none of the above factors and covariates made significant contributions to the model (nestling age: $F_{1,12} = 2.82$, $P = 0.12$; mass: $F_{1,12} = 1.15$, $P = 0.31$; treatment: $F_{1,12} = 3.04$, $P = 0.11$; brood size: $F_{1,12} = 0.04$, $P = 0.84$).

The total parental FMR per nest increased with the number of feeding trips, and the energy expended per feeding trip was significantly higher for the handicapped birds than for the control group (treatment: $F_{1,13} = 10.6$, $P = 0.006$; feeding trips per hour: $F_{1,13} = 8.78$, $P = 0.011$; total parental body mass: $F_{1,13} = 14.1$, $P = 0.002$; Figure 4a). This was not the case for WIR (treatment: $F_{1,13} = 3.75$, $P = 0.075$; feeding trips per hour: $F_{1,13} = 1.02$, $P = 0.33$; total parental body mass: $F_{1,13} = 0.91$, $P = 0.36$; Figure 4b).

Body mass of parents

We measured body mass of parents on the initial day of the experiment and 6 days later, on the day of the BMR measurements (Table 1). Parental body mass initially did not differ between sexes or treatments (sex: $F_{1,34} = 1.59$, $P = 0.22$; treatment: $F_{1,34} = 0.32$, $P = 0.58$). Six days later, when we weighed birds before and after BMR measurements, we still did not detect significant differences in mass between sexes or treatment groups (sex: $F_{1,25} = 0.42$, $P = 0.52$; treatment: $F_{1,25} = 0.75$, $P = 0.40$). Also the within-individual change in body mass between days 6 and 12 was not affected by sex or treatment (sex: $F_{1,25} = 0.09$, $P = 0.77$; treatment: $F_{1,25} = 2.16$, $P = 0.15$).

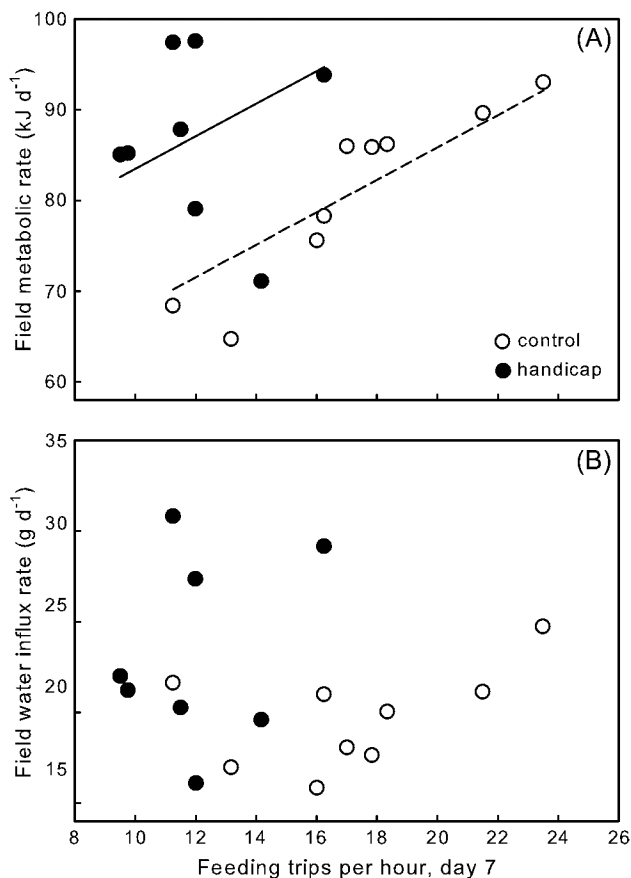


Figure 4
(A) The sum of male and female FMR, within a pair, as a function of the feeding frequency (total number of feeding trips per hour of paired male and female combined) on day 7 for control and handicap treatments. (B) The sum of male and female water influx rate, within a pair, as a function of feeding frequency on day 7 for control and handicap treatments.

BMR of parents on day 12

We measured BMR of parents on day 12 (Figure 5) and found that it was positively related to body mass but did not differ significantly between sexes or treatments (body mass: $F_{1,24} = 8.24$, $P = 0.008$; sex: $F_{1,24} = 0.21$, $P = 0.65$; treatment: $F_{1,24} = 0.03$, $P = 0.86$).

FMR versus BMR

The ratio between FMR and BMR averaged 2.20 ± 0.26 (SD, $n = 27$) and did not differ significantly between sexes and treatments (sex: $F_{1,24} = 0.21$, $P = 0.65$; treatment: $F_{1,24} = 1.67$, $P = 0.21$). Individuals with a higher BMR also had a higher FMR (Pearson $r = 0.51$, $P = 0.006$, $n = 27$). We then calculated residuals of BMR and FMR based on their respective linear regressions with body mass, to remove the effect of body mass, and found that these residuals were also positively correlated (Pearson $r = 0.45$, $P = 0.019$, $n = 27$). Therefore, individual birds with a relatively high BMR also had a relatively high FMR. Within a pair, FMR and BMR of males and females were not significantly correlated (FMR: Pearson $r = 0.42$, $P = 0.09$, $n = 17$; BMR: Pearson $r = -0.25$, $P = 0.42$, $n = 13$).

Parental body condition as measured by constitutive innate immune function

To assess whether increased energy costs influenced allocation of resources to the innate arm of the immune system, we measured 3 functional parameters of the immune system, quantified hematocrit, and counted white blood cell types of parents on day 12 (Figure 6). We tested each variable with a GLM including sex, treatment, and their interaction. The interaction term was never significant and therefore removed from models. Phagocytosis of *S. aureus* was significantly higher in males than in females, but neither sex nor treatment caused significant differences in any of the other immune function variables (Table 2).

DISCUSSION

Handicapped tropical house wrens confronted with an increase in the costs of activity during reproduction fed their young less frequently than control birds but did neither alter their energetic workload nor compromise their body condition as measured by FMR, BMR, and immune function. The reduced feeding frequency did not lead to an overall reduction in nestling body mass growth but caused a decrease in the growth of tarsus, a measure of structural size. Therefore, handicapped parents likely shifted the type of prey brought to their offspring, possibly affecting the quality of food more than the quantity (Tinbergen 1981; Wright et al. 1998). The increased WIR combined with the lack of change in FMR suggests that the parents' diet composition may have changed as well. An alternative explanation is that diet did not change but that the DLW measurements were too variable (Nagy 1980; Williams and Nagy 1984; Williams and Vézina 2001) to detect a significant increase in FMR in handicapped birds, concomitant with the increased WIR.

Comparison of our results with the only other experimental study of the costs of reproduction in a tropical passerine, also on house wrens, yields a clouded pattern. In response to brood size enlargement with 2 nestlings, tropical house wrens increased their feeding frequency (Young 1996), whereas in the current study, handicapped house wrens did not increase their parental effort as indicated by the reduced feeding frequency and unchanged FMR. Young did not measure physiological variables but suggested that wrens raising larger broods

Table 1

Average body mass \pm 1 SD (sample size) of female and male house wrens feeding nestlings at the start of the experiment and at the day of BMR measurements (6 days after applying the treatment)

Treatment	Female mass (g)		Male mass (g)	
	Initial day	Day of BMR measurement	Initial day	Day of BMR measurement
Control	13.8 \pm 0.81 (9)	13.2 \pm 0.65 (8)	14.1 \pm 0.95 (10)	13.1 \pm 0.65 (6)
Handicap	13.8 \pm 1.20 (9)	13.2 \pm 1.32 (7)	14.4 \pm 0.83 (9)	13.7 \pm 0.43 (7)

The body mass at the day of the BMR measurement is measured in postabsorptive birds, which explains the lower values compared with the measurements directly after capture in the field on the initial day of the experiment.

had a compromised body condition, which explained their reduced future fecundity. In contrast, our measurements on BMR and immune function do not provide evidence for a compromised body condition in handicapped house wrens, but we did not measure subsequent clutches or survival. One difference that may explain the conflicting results between these studies is the fact that Young did not manipulate workload independently from the perceived current offspring fitness benefits. The prospect of rearing 2 extra chicks, as in Young's experiment, could make a difference in whether or not parents increase their effort. Young suggested that the compromised body condition did not affect survivorship in his study because, without pressing needs for migration or molt and a long reproductive period, birds in the tropics have sufficient time to recover from a period of hard work.

Comparing the experiments on tropical house wrens with other birds is complicated because of the unique combination of demographic traits, characteristic for many tropical passerines: The annual survival of Panamanian house wrens (48%; Freed 1987) is high relative to their temperate zone conspecifics (29–30.7%; Kendeigh and Baldwin 1937; Drilling and Thompson 1988), although similar to survival rates found in other temperate zone species. In addition, their reproductive window is relatively long (5–8 months per year; Freed 1987; Young 1996) offering opportunities for reneating. Temperate zone passerines with survival rates similar to the tropical wrens have shorter breeding seasons but when handicapped do not fully opt for shifting their fitness balance toward maximizing

short-term reproductive output, as predicted based on life-history theory. Instead, in most handicap experiments on relatively short-lived temperate zone passerines, both parents and offspring assumed part of the costs (Mauck and Grubb 1995; Winkler and Allen 1995), and one study even showed that all costs were assumed by the offspring leaving parental condition unaffected (Moreno et al. 1999). Some long-lived birds on the other hand, including seabirds which usually have only one breeding attempt per year, shunted increased costs of reproduction fully to their single chick without compromising adult survival, a finding consistent with life-history theory (Saether et al. 1993; Mauck and Grubb 1995). A problem in attempting to understand the ambiguous results from these studies is the lack of insights into the manifold permutations for reallocation between physiological and behavioral processes.

Possibilities for reallocation of resources among parental workload and physiological functions involve variables such as foraging behavior, FMR and WIR, BMR, and immune function. The main potential for shifts between foraging behavior on the one hand and FMR and WIR on the other hand are feeding frequency and diet selection, for both the parents and the young. Faced with increased costs for flight, our house wrens opted for increasing feeding efficiency without incurring energetic costs. The resulting decreased feeding frequency did only affect nestling structural growth, not nestling body mass growth, which suggests that the parents brought more or different prey per feeding trip. We did not measure diet composition, but other studies have reported significant shifts, presumably in favor of food quantity over quality (Tinbergen 1981; Wright et al. 1998). That diet composition changed for the parents themselves can be concluded from the increase in WIR while FMR was unaffected (Nagy and Peterson 1988). Possibly, the change in diet affected nestling body condition independent of body mass, tarsus, and wing length, but we did not measure physiological performance of the nestlings.

Understanding metabolic consequences of work requires insights into the allocation of energy between maintenance and activity. Studies on captive birds have shown that increased activity, and concomitant energy expenditure, leads to reduction in nighttime BMR (Bautista et al. 1998; Deerenberg et al. 1998; Nudds and Bryant 2001). Only 2 previous studies have measured BMR and FMR on the same individuals in the field, and their results differ from the laboratory findings. In the first study, female marsh tits, induced to work harder by brood size enlargement, increased parental feeding rate and BMR (Nilsson 2002). In addition, in a subset of 6 birds, FMR was positively related with BMR and feeding frequency. In the second study, female great tits, again induced to increase work effort by brood size enlargement, increased parental feeding rate and FMR but did not alter nighttime metabolic rate (Wiersma and Tinbergen 2003). Unfortunately, nighttime

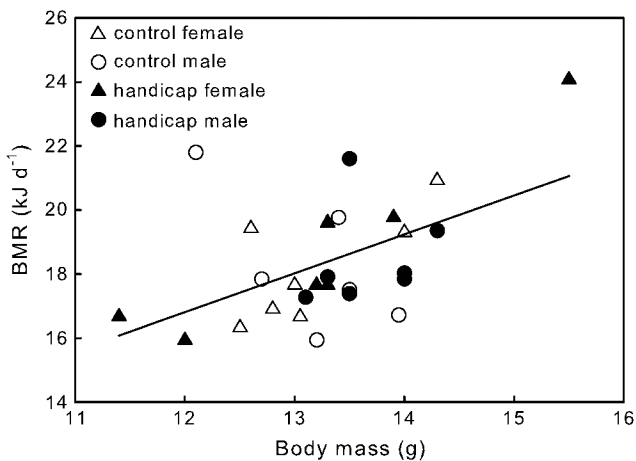
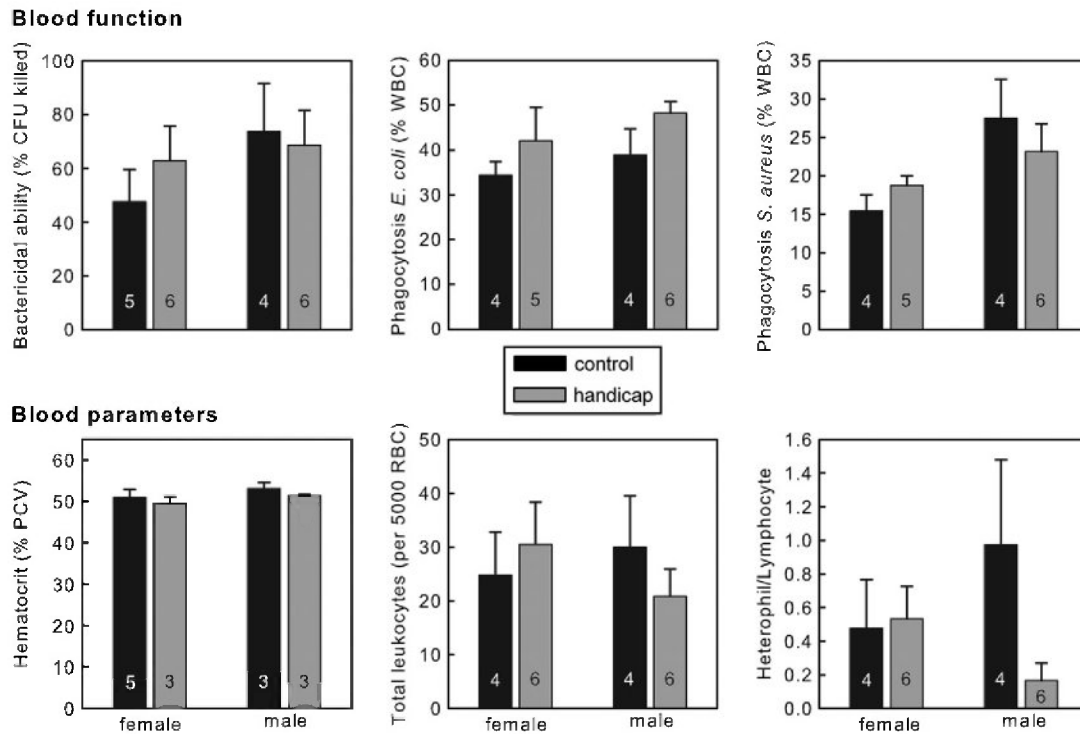


Figure 5
BMR of male and female house wrens subject to control and handicap treatments, measured on day 12 during the nestling period, as a function of body mass.

**Figure 6**

Three functional and 3 descriptive parameters of constitutive innate immune function in control and handicapped house wrens measured on day 12. The functional parameters include bactericidal ability of fresh whole blood exposed to *E. coli* and phagocytosis of *E. coli* and *S. aureus* by fresh whole blood. The descriptive parameters include hematocrit, total white blood cell count, and heterophil:lymphocyte ratio.

metabolic rate was not measured under thermoneutral conditions, but corrections for temperature effects did not alter the outcome. In contrast to Nilsson (2002), Wiersma and Tinbergen (2003) did not find a relationship between feeding rate and nighttime metabolic rate. In our study on house wrens, we found positive correlations among BMR, FMR, and feeding rate, but neither FMR nor BMR changed in response to the experimental treatment. Therefore, our results contribute neither correlative nor experimental evidence for the laboratory-based predictions of compensation for hard work during the day by lowering BMR at night. In addition, the positive correlations among BMR, FMR, and feeding frequency are in agreement with the idea that hard work requires elevated BMR, possibly mediated by food processing requirements and organ size (Daan et al. 1990; Nilsson 2002).

The final potential for reallocation of resources that we explored is among work effort, metabolic rate, and immune function. Previous studies have shown detrimental effects of

increased reproductive effort on various measures of immune function or vice versa (e.g., Norris et al. 1994; Deerenberg et al. 1997; Nordling et al. 1998; Hasselquist et al. 2001; Ardia et al. 2003). Our house wrens, however, adjusted their behavior in response to the handicap treatment and thereby maintained constant energetic work effort. It is therefore not surprising that their white blood cell counts and several functional measures of innate immunity were unaffected. In conclusion, tropical house wrens do not sacrifice their body condition when faced with increased costs for activity during the nestling phase.

This study provides a comprehensive data set on physiological, behavioral, and ecological variables in a field experiment on birds. Despite incorporating all these variables in one study, we do not find a clear indication of the mechanisms underlying the hypothesized cost of reproduction. Our results emphasize the flexibility and interdependence of the physiological, behavioral, and ecological variables involved. We conclude that

Table 2

Results of GLMs testing differences in immune function and blood parameter between sex and treatment in house wren parents on day 12 (Figure 6)

Variable	Sex		Treatment	
Bactericidal ability <i>E. coli</i>	$F_{1,18} = 1.11$	$P = 0.31$	$F_{1,18} = 0.17$	$P = 0.67$
Phagocytosis <i>E. coli</i>	$F_{1,16} = 1.23$	$P = 0.28$	$F_{1,16} = 2.97$	$P = 0.10$
Phagocytosis <i>S. aureus</i>	$F_{1,16} = 5.06$	$P = 0.04$	$F_{1,16} = 0.04$	$P = 0.85$
Hematocrit (% packed cell volume)	$F_{1,11} = 1.36$	$P = 0.27$	$F_{1,11} = 0.80$	$P = 0.39$
Total leukocytes (per 5000 RBC)	$F_{1,17} = 0.25$	$P = 0.63$	$F_{1,17} = 0.05$	$P = 0.83$
Heterophil/lymphocyte ratio	$F_{1,17} = 0.01$	$P = 0.94$	$F_{1,17} = 1.78$	$P = 0.20$

The interaction between sex and treatment did not have a significant effect in any of the models (all $P > 0.05$).

it may be time to critically reevaluate conventional wisdom regarding the trade-off between current reproduction and self-maintenance. The mechanisms underlying this trade-off are much more versatile than that current theory assumes.

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