



THE CONDOR

A JOURNAL OF AVIAN BIOLOGY

Volume 93

Number 4

November 1991

The Condor 93:793-798
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SEX-BIASED PROVISIONING: A TEST FOR DIFFERENCES IN FIELD METABOLIC RATES OF NESTLING EASTERN BLUEBIRDS¹

DALE L. DROGE AND PATRICIA ADAIR GOWATY

Department of Biological Sciences, Clemson University, Clemson, SC 29634-1903

WESLEY W. WEATHERS

Department of Avian Sciences, University of California, Davis, CA 95616

Abstract. Male Eastern Bluebirds (*Sialia sialis*) feed their daughters more frequently than their sons. One hypothesis which accounts for this pattern of paternal care is that daughters require more food because they have a higher energy expenditure than their male siblings. We measured the field metabolic rate of one male and one female nestling from 14 different nests using the doubly labeled water technique. Male and female CO₂ production did not differ over a 24-hr period (mean ± SD: 3.77 ± 0.59 ml/g hr and 3.51 ± 0.47, respectively). In 7 of the 14 broods male CO₂ production was more than 5% higher than female production. The lack of a significant difference in male and female field metabolic rates allows us to eliminate two potential explanations for sex-biased provisioning in Eastern Bluebirds: (1) one sex has a higher daily energy expenditure; and (2) one sex requires more energy to compete with the other sex for food. Sex-biased provisioning may be unrelated to the energetic demands of the offspring.

Key words: Eastern Bluebird; *Sialia sialis*; doubly labeled water; nestling; sex-biased provisioning; metabolic rate.

INTRODUCTION

Eastern Bluebird (*Sialia sialis*) fathers feed their daughters more frequently than their sons. Two types of evidence support this conclusion. First, males make more feeding visits to nests with daughter-biased brood sex ratios than to nests with equal or male-biased sex ratios (Gowaty and Droge, MS). Second, videotapes of food delivery to individual nestlings show that males fed daughters more frequently than sons (Gowaty and Droge, in press; Droge and Gowaty, MS). Budgerigar (*Melopsittacus undulatus*) fathers exhibit the same pattern, feeding daughter-biased broods more frequently than son-biased broods (Stamps et al. 1987). Stamps (1990) calls this behavior sex-biased provisioning: one sex of offspring receiving more food from a parent than do offspring of the opposite sex. In both Eastern

Bluebirds and Budgerigars, mothers do not show sex-biased provisioning.

The occurrence of sex-biased provisioning in bluebirds leads to two questions of primary interest: (1) why do daughters receive more feedings than sons? and (2) why do fathers, but not mothers, feed in a sex-biased fashion? In this study we address only the first question. Stamps (1990) developed several hypotheses to explain why one sex of offspring may need more investment than the other. All these hypotheses assume that one sex *requires* more energy because of asymmetries between the sexes in growth, maturation, competition or survival. We measured field metabolic rates (FMR) of male and female nestling bluebirds to determine if there was a difference in energy expenditure between the sexes. If sex-specific differences in offspring energy requirements select for sex-biased provisioning by male Eastern Bluebirds, we predict that female nestlings should have a higher field metabolic rate.

¹ Received 14 February 1991. Final acceptance 29 July 1991.

METHODS

STUDY SPECIES AND AREAS

Eastern Bluebirds are secondary cavity nesters and readily breed in nest boxes placed in suitable habitat. In northwestern South Carolina nest building begins in early March and pairs may produce as many as three broods by August. The modal brood size is four with most broods containing three to five nestlings (Gowaty 1980). Both male and female Eastern Bluebirds provision offspring during the nestling phase, which lasts 16–18 days after hatching (Pinkowski 1978, Belser 1981).

We studied Eastern Bluebirds in southwestern Pickens County, South Carolina from March 19–August 12, 1990. We used nest boxes that were in place at least two years before the beginning of the study.

FIELD PROCEDURES

We checked 62 nest boxes twice a week until the nest was completed. Daily nest checks established the date of clutch initiation. We visited nests daily around the time of expected hatching to record the exact hatching date. At most of the nests used in this study, we weighed nestlings every day until 13 days after hatching. Handling of nestlings beyond this age may lead to premature fledging. We captured the adult birds associated with a nesting attempt with a nest box trap and banded them with a USDI aluminum band and a unique combination of plastic color bands (three per bird). We banded nestlings in the same way when they were 9 or 10 days old, and sexed nestlings at 13 days post-hatch by differences in plumage characteristics (Pinkowski 1974).

FIELD METABOLIC RATE

We used the doubly labeled water (DLW) technique to measure the rate of CO₂ production of 37 nestlings (19 males, 18 females) at 18 different nest boxes. We sampled broods from May 13–August 4, 1990. We selected one male and one female nestling from each brood (two males and one female at one box), matching the nestlings for size when possible. Nestlings were 12–14 days old corresponding to the ages when we observed sex-biased provisioning in other studies (Gowaty and Droge, in press). Nestlings achieve asymptotic weight at 10 days after hatching (Pinkowski 1975, Gowaty 1980). After weighing nestlings to the nearest 0.05 g (K-Tron model DS-10 bal-

ance), we injected 60 µl of doubly labeled water intramuscularly. The water contained 97 atoms-percent ¹⁸O and ca. 12 MBq ³H per ml. We returned nestlings to the nest to allow for isotopic equilibration. After one hour, we obtained duplicate 50 µl blood samples from a brachial or femoral vein and stored them at 4°C in flame-sealed microhematocrit tubes for later analysis. Twenty-four hours later we returned to the nest, reweighed the nestlings, and took another duplicate set of blood samples.

One of us (WWW) microdistilled the blood samples to obtain pure water. We assayed the water samples for tritium activity (Packard liquid scintillation counter, toluene-Triton X100-PPO scintillation cocktail) and for ¹⁸O content by cyclotron-generated proton activation of ¹⁸O to ¹⁸F with subsequent counting of positron-emitting ¹⁸F in a Packard Gamma-Rotomatic counting system (Wood et al. 1975; analyses performed at Crocker Nuclear Laboratory, University of California, Davis by WWW). Using the equations of Lifson and McClintock (1966) as modified by Nagy (1975), we calculated rates of water flux and CO₂ production from the isotope measurements.

POTENTIAL ERRORS

Errors in calculated rates of CO₂ production using the DLW technique may result from analytical errors in the isotope measurements or insufficient isotope turnover (Nagy 1980). Buttemer et al. (1986) validated the DLW technique for budgerigars, where DLW measurements of CO₂ production differed by less than 6% from values determined simultaneously by the Haldane method (mean difference = -0.04%).

Because we measured FMR in nestlings within a cavity, two potential sources of error may occur with the DLW technique. First, measurements may be in error for rapidly growing animals due to irreversible and disproportional incorporation of isotopes into body tissue (Nagy 1980, Williams and Nagy 1985). Klaassen, et al. (1989) validated use of DLW for rapidly growing Arctic Tern chicks (*Sterna paradisaea*). They found that the DLW technique underestimated CO₂ production (measured by indirect calorimetry) by 4% during the first 24-hr period. Because the bluebird nestlings we measured had already reached asymptotic weight, and because we used 24-hr samples for the DLW measurements, the error attributable to incorporation of isotopes into body tissue is probably small. The second

TABLE 1. Mean (\pm SD) values of mass and field metabolic rate variables for 14 male and 14 female Eastern Bluebird nestlings. The range is given in parentheses below each value, and t statistics are from paired comparisons.

Sex	Mass (g)	% BW change	Water flux (ml/kg d)		CO ₂ (ml/g hr)
			In	Out	
Male	26.1 \pm 1.4 (23.4–28.6)	-0.10 \pm 3.7 (-8.0–8.5)	489 \pm 67 (386–647)	490 \pm 54 (435–586)	3.77 \pm 0.59 (3.06–4.86)
Female	25.7 \pm 1.3 (22.6–28.7)	-0.70 \pm 3.4 (-7.2–6.3)	464 \pm 76 (409–603)	469 \pm 78 (411–596)	3.51 \pm 0.47 (2.61–4.58)
	$t = 0.78$ $P > 0.40$	$t = 1.68$ $P > 0.10$	$t = 0.28$ $P > 0.75$	$t = 1.05$ $P > 0.30$	$t = 1.67$ $P > 0.10$

potential error could occur if nestlings breathed CO₂ exhaled by unlabeled siblings or adults in the nest box (Nagy 1980). Although we are unable to directly discount this source of error in our measurements, it seems unlikely to be significant. Howe et al. (1987) found a mean CO₂ level of only 0.37% in Northern Flicker (*Colaptes auratus*) nest cavities, a level unlikely to cause errors in the DLW method. The nest boxes we used were well-ventilated, having one-half inch gaps at the lid-side juncture, drainage holes in the bottom, and gaps where the sides could be removed for other experiments. Consequently, it is doubtful that CO₂ build-up reached even the modest levels observed in flicker nests. Overall, we believe our DLW measurements of individual nestling CO₂ production to be within $\pm 8\%$ of actual values.

Of the 37 nestlings sampled, 15 had total body water volumes (TBW) that were much higher than the other birds (means: 0.978 versus 0.757), probably owing to an error in recording the volume of isotope injected. For these 15 nestlings we based the CO₂ and water flux calculations on the mean TBW for the other nestlings. Because a 1% change in TBW corresponds to a 1% change in CO₂ production and water flux, using mean TBW values should introduce only a small error in the calculations (Webster and Weathers 1989).

Data from five birds were suspect due to possible leaks during distillation or incomplete flame seals of the capillary tubes. Although the final values were within the range of the other samples, we chose to omit these samples from the analysis.

STATISTICS

We simultaneously sampled a male and female nestling from each nest to control for variables such as time, date, temperature, number of siblings, and parental visits. This design allowed the

use of a paired t -test, a relatively powerful test (Cohen 1988). All probability values we report are for two-tailed tests.

RESULTS

FIELD METABOLIC RATE

Male and female nestling bluebirds did not differ in mass, percent change in mass over 24 hr, water flux, or CO₂ production for the ages we sampled (Table 1). In 7 of the 14 broods male CO₂ production was more than 5% higher than the female value. CO₂ production by females exceeded male production by 5% in only one case and in six broods there was less than a 5% difference in the CO₂ production of male and female nestlings (Fig. 1).

POWER ANALYSIS

Because we could not reject the null hypothesis of no difference between male and female nestling CO₂ production, we performed a power analysis (Cohen 1988) to calculate the probability of accepting a false null hypothesis (Type II error). A power analysis of a paired t -test requires estimates of the difference between the populations and the degree of similarity (correlation) between the members of a pair. We used the difference between the mean CO₂ production of males and females for the estimate of the population difference. We estimated the degree of correlation indirectly. The advantage of a paired test is that almost all variables are perfectly matched. For example, the temperature, humidity, parents, number of feeding visits to the nest, and number of siblings were exactly the same for both members of each pair. The only variables that we could not match were nestling weight and the number of times each nestling was fed. There was a low correlation between the weight of male and female siblings ($r = 0.48$, $P < 0.09$, $n = 14$),

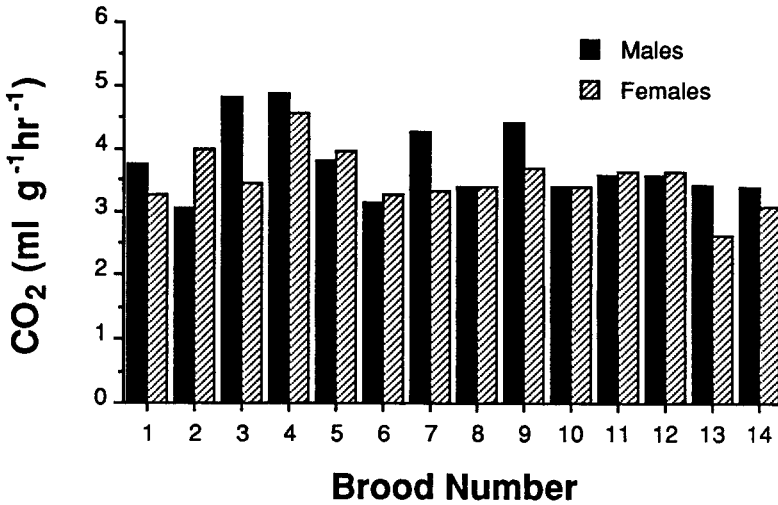


FIGURE 1. The difference in CO₂ production between male and female nestlings by brood. Brood number identifies individual broods.

but the marginal significance of the correlation indicated that the weights were matched more closely than if we had chosen the nestlings at random. Using a degree of similarity of 0.9, we calculated the power of the test as 95% using power tables in Cohen (1988). Thus, the probability of a Type II error was $1.0 - 0.95 = 0.05$. If the degree of similarity was actually 0.8, the power of the test was 75%. If these power estimates are accurate, we can be reasonably certain that there is no difference between the CO₂ production of male and female nestlings.

DISCUSSION

Our study, the first to measure the sex-specific FMR of nestling birds with no sexual size dimorphism, poses an apparent paradox. Female nestling bluebirds may receive more feedings than males, yet the FMRs of male and female nestlings do not differ significantly. A similar enigma exists for the sexually size-dimorphic Red-winged Blackbird (*Agelaius phoeniceus*). Fiala and Congdon (1983) measured nestling blackbird FMR with the DLW method. They found no difference in the mass specific FMR of male and female nestlings, although males did have a greater total energy expenditure because of their larger size. All else being equal, the larger sex of nestling in sexually size dimorphic species should require more energy (Stamps 1990). Surprisingly, there is little evidence for sex-biased provisioning in Red-winged Blackbirds or any other species of

bird with sexual size dimorphism (reviewed by Stamps 1990, but see Yasukawa, et al. 1990). These discrepancies emphasize the difficulty of inferring energy intake from feeding rate alone (Hubbard 1978, Williams and Prints 1986). Furthermore, gross intake of food may vary, but metabolized energy can be equal, if the proportions of fats, carbohydrates and proteins in the diet differ (because of the different energy contents of these macromolecules). This could account for our finding that female nestling bluebirds were fed more often than males, yet had similar FMRs. For example, if fathers fed sons insects with a higher fat content, then daughters would have to be fed more insects to have the same FMR. Alternatively, the diets of male and female nestlings could be the same, but females might process food less efficiently than males, perhaps due to decreased gut passage time with a corresponding decrease in nutrient absorption. In the absence of data to the contrary, we assume that diets were equivalent, and hence rates of energy expenditure the same for male and female nestlings.

Stamps (1990) proposed several hypotheses concerning sex-biased provisioning in birds. Some of these hypotheses deal explicitly with sexually size-dimorphic species and will not be considered here. Using the FMRs of nestling bluebirds we can reject two explanations for sex-biased provisioning: (1) one sex has a higher daily energy expenditure; and (2) one sex requires more

energy to compete with the other sex for food (intra-brood food competition hypothesis, Stamps 1990). According to the latter hypothesis one sex uses more energy to attract the parent's attention. Although this sex needs more food, it may grow at the same rate as the other sex. The masses of male and female bluebird nestlings are not different at any age (Gowaty 1980, Table 1; unpubl. data), and the energy expenditure of male and female nestlings did not differ (at the ages measured). These data are inconsistent with the intra-brood food competition hypothesis.

There are other hypotheses of sex-biased provisioning that we cannot test with our metabolic rate data, and which thus remain viable explanations for bluebirds. One sex of offspring may mature at a different rate than the other. For example, motor skills may develop more rapidly in one sex of nestling (Richter 1983). In this case the sex with more rapid development would require more energy. We do not have metabolic expenditure information for younger Eastern Bluebird nestlings, but equal energy expenditure by older nestlings does not support this hypothesis. There is also no difference between male and female nestlings in the age at fledging or tarsus length (Gowaty, unpubl.), which suggests maturation rates are similar for the sexes.

Other hypotheses explaining sex-biased provisioning focus on the effects that parental provisioning may have on the behavior of offspring after independence. Stamps (1990) suggests that one sex may require more food reserves to compete with the dominant sex or because there is sex-biased dispersal. In the latter case, the dispersing sex would presumably benefit from a higher level of provisioning.

Finally, Gowaty and Droge (in press) suggest why sex-biased provisioning may be unrelated to the energetic demands of the offspring. Conflict over the sex ratio of the brood between the parents may lead to selection for differential investment in the two sexes of offspring by each parent. Because sons are more philopatric than daughters, sons may compete as adults with both their fathers and mothers for nest sites and food, but only fathers for mates. This asymmetry in local resource competition between sons and daughters may select for the sex-biased provisioning of daughters by male Eastern Bluebirds. This explanation does not require that daughters necessarily gain any immediate or measurable advantage from "extra" feedings received from

their fathers. However, it leaves open questions related to physiological processes associated with this additional food.

ACKNOWLEDGMENTS

This work was supported in part by a Research Scientist Development Award (5K02MH00706-02 to PAG) from the ADAMHA NIMH, and NSF grants BNS-8919822 to PAG, BSR 85-05490 and BSR 87-18195 to WWW.

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