

Estimating expenditure on male and female offspring in a sexually size-dimorphic bird: a comparison of different methods

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Summary

1. The parents of sexually size-dimorphic offspring are often assumed to invest more resources producing individuals of the larger sex. A range of different methods have been employed to estimate relative expenditure on the sexes, including quantifying sex-specific offspring growth, food intake, energy expenditure and energy intake, in addition to measures of parental food provisioning and energy expenditure. These methods all have the potential to provide useful estimates of relative investment, but each has particular problems of interpretation, and few studies have compared the estimates derived concurrently from more than two of these measures.

2. In this study we compared these surrogate measures of parental investment in the brown songlark *Cinclorhamphus cruralis*, which exhibits one of the most extreme cases of sexual size dimorphism among birds.

3. At 10 days of age we found that male chicks, on average, were 49% heavier, received 42% more prey items, expended 44% more energy and ingested 50% more metabolizable energy than their sisters.

4. Furthermore, we created, experimentally, both all-male and all-female broods of 10-day-old chicks and found that mothers delivered 43% more prey items and expended 27% more energy when provisioning all-male broods, providing the first direct evidence for a change in parental energy expenditure in relation to brood sex ratio.

5. These data reveal remarkable agreement between these estimates of investment and suggest that all may provide quantitatively useful information on sex allocation. However, the lower variance associated with estimates of relative mass and energy intake suggest that these methods may be of greater utility, although this may primarily reflect the shorter period over which our provisioning data were collected.

Key-words: daily energy expenditure, metabolizable energy intake, nestling growth, parental care, sexual size dimorphism.

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Introduction

Patterns of sex allocation at both the individual and population levels should depend, at least in part, on the relative cost of producing sons and daughters (Fisher 1930; Bull & Charnov 1988; Frank 1990). In many organisms the cost of producing males and females is likely to be very similar. However, in species that

exhibit strong sexual size dimorphism, it is often assumed that offspring of the larger sex will generally require greater investment (Charnov 1982; Richner 1991; Hardy 1997), although this assumption has periodically been queried (Newton 1978; Maynard Smith 1980; Richter 1983).

Assessing the relative cost of producing one sex over the other is notoriously difficult as the 'cost' actually refers to the reduction in residual reproductive value of the parent (Williams 1966; Trivers 1972; Charnov 1982; Bull & Charnov 1988; Pen & Weissing 2002). Only a very few studies, mostly on mammals, have revealed differential costs of producing sons vs. daughters in such terms as reduced maternal condition, future reproductive output or survival probability of subsequent offspring (Clutton-Brock 1991; Hewison & Gaillard 1999), although even in these studies causality is uncertain because the relationships were correlational. Consequently, a range of surrogate measures, with different currencies, has been used that are predicted to correlate with actual reproductive cost.

Generally, the most easily acquired estimate is the difference between the sexes in body mass, and this measure has been used in a range of empirical and comparative studies to estimate the relative cost (e.g. Nonacs 1986; Slagsvold, Røskaft & Engen 1986; Pen 2000). This estimate first assumes that differences in mass closely reflect differences in the amount of food received from the parent(s) (Krijgsveld, Dijkstra & Daan 1998). However, concordance between mass and food intake may be limited if (1) the smaller sex expends more energy in competition with the larger sex, or (2) the sexes differ in their allocation of energy to different components of growth and development (Newton 1978; Fiala & Congdon 1983; Richter 1983; Stamps 1990; Teather & Weatherhead 1994; but see Richner 1991; Badyaev 2002).

One approach to evaluating the relationship between relative mass and food intake has been to document the relative amount of food consumed by the sexes, either in free-living (e.g. Newton 1978; Teather & Weatherhead 1988; McDonald, Olsen & Cockburn 2005) or captive young (e.g. Teather 1987; Krijgsveld *et al.* 1998; Martins 2004). Support for this association has been somewhat variable. Members of the larger sex have been shown to consume more food in a range of birds (e.g. Fiala & Congdon 1983; Teather 1987; Anderson *et al.* 1993; Krijgsveld *et al.* 1998; Boulet *et al.* 2001), some mammals (Hewison & Gaillard 1999), and several hymenoptera (e.g. Molumby 1997). However, several other studies on birds (e.g. Newton 1978; Torres & Drummond 1999; McDonald *et al.* 2005) and dimorphic mammals (e.g. Ono & Boness 1996; Guinet, Goldsworthy & Robinson 1999; Hewison & Gaillard 1999; Arnould & Hindell 2002) have failed to reveal a difference in consumption.

Alternatively, food intake has been estimated by deriving the total metabolizable energy (TME) intake for offspring from their energetic expenditure and mass

gain (e.g. Teather & Weatherhead 1988; Riedstra, Dijkstra & Daan 1998; Visser & Schekkerman 1999; Vedder *et al.* 2005). These studies have generally revealed closer agreement between relative food intake and mass differences, at least among birds (Fiala & Congdon 1983; Slagsvold *et al.* 1986; Teather & Weatherhead 1988; Anderson *et al.* 1993; Krijgsveld *et al.* 1998; Riedstra *et al.* 1998). Furthermore, comparative studies reveal a positive correlation between the degree of size dimorphism and the disparity in the amount of food (or energy) received by the sexes (Anderson *et al.* 1993; Krijgsveld *et al.* 1998). The study by Krijgsveld *et al.* (1998), however, indicates that this relationship is not proportional, as extreme body mass ratios overestimate the ratio of food intake.

Using either food or energy intake to measure parental investment, in turn, assumes that intake is a reliable indication of cost to the parent. However, the relative rates of food/energy intake by the sexes need not necessarily correlate closely with relative parental effort. For example, individuals of the larger sex could be provisioned with larger or more nutritious prey items without an increase to the parent in either search time or feeding rate (Teather 1987; Teather & Weatherhead 1988; Magrath *et al.* 2004). Alternatively, the provisioning of additional food to individuals of the larger sex may represent a negligible increase in cost to the parent, especially if food is plentiful (Stearns 1992). Empirical studies evaluating the link between energy expenditure of the parent in relation to offspring sex are lacking although clearly required, as they are likely to provide better measures of parental cost. Nevertheless, this measure may still be misleading as the relation between parental energy expenditure and loss of future reproductive value may not be linear (e.g. Daan, Deerenberg & Dijkstra 1996). Moreover, parents may offset the energy cost of increased foraging activity by foraging in a more risk prone manner or reducing nocturnal energy expenditure (Wiersma & Tinbergen 2003).

Considering these potential difficulties with interpreting any single one of these surrogate estimates of parental expenditure, the most informative approach to evaluating the relative cost of producing size-dimorphic young may be to estimate each of these measures concurrently. In this study we investigate the relative cost of producing sons and daughters in a population of brown songlarks *Cinchorhamphus cruralis* (Vigors & Horsfield). This species is a member of the Sylviidae (Old World warblers) family, endemic to Australia, and one of the most sexually size dimorphic of all birds (Andersson 1994). In our population, adult males were about 2.3 times heavier than adult females and the mating system was strongly polygynous, with females providing almost all parental care (Magrath *et al.* 2003a). Here, we report on the relative rates of growth, food acquisition and energy expenditure of male and female songlark chicks, coupled with data on parental provisioning effort and

energy expenditure at broods with manipulated sex ratio. This represents one of the first studies to provide estimates of sex allocation derived from both parents and offspring, and also allows us to directly compare and evaluate a range of different estimates of parental expenditure.

Materials and methods

STUDY SITES

The study was conducted in the semiarid grass- and shrub-lands of south-western New South Wales, Australia. Data were collected between September and December in the 3 years from 1998 to 2000. Local population densities across the region varied widely between years (Magrath *et al.* 2003a), so a different study site was selected each year based on an apparent high density of adults in early September. The location and area of these study sites was 33°21'S 144°58'E (60 ha) in 1998, 33°72'S 145°30'E (120 ha) in 1999, and 34°22'S 145°02'E (120 ha) in 2000.

NESTLING GROWTH

Following discovery, most nests were checked daily in the afternoon until either the brood fledged or failed. If the nest was found before or during laying, eggs were numbered with a permanent marker in the order in which they were laid, otherwise they were numbered randomly. Hatchlings were individually marked on the tarsus with a permanent pen, weighed (to the nearest 0.1 g) and had their wing (ninth primary feather), bill and tarsus length measured (to the nearest 0.1 mm) by MM. Hatchlings measured in the field were regarded as 1 day old. The chicks of broods found after hatching were also marked and measured in the same manner, but not included in the estimation of sex-specific growth curves. The characteristics of all known-aged chicks were usually measured at 5 and 10 days after hatching, and often on a number of other days during the nestling period (measurements per chick = 4.74 ± 2.45 SD, range 1–16, $n = 1485$ measurement-days for 180 males and 134 females in 122 broods). Few of these morphometric measurements ($n = 57$ for 30 males and 11 females) were collected after chicks left the nest at about day 12 because they became very difficult to locate.

SEX DETERMINATION

From most hatchlings, a blood sample of between 10 and 25 μ L was taken, suspended in 500 μ L of lysis buffer (0.1 M Tris-HCl, pH 8.0; 0.1 M EDTA, pH 7.4; 10 mM NaCl; 0.5% SDS) and stored at 5 °C. Unhatched eggs were collected and the embryos frozen. DNA was extracted from blood and tissue samples using a standard NaCl/ethanol extraction method (Lahiri *et al.* 1992). Molecular sexing was performed by

polymerase chain reaction amplification of introns in two homologous genes (CHD-W and CHD-Z) using the P2/P8 primer couple (Griffith *et al.* 1998). Products were resolved on 2% agarose gels stained with ethidium bromide, that revealed either one (male) or two (female) distinct bands. This technique was verified as reliable by the correct sexing of six male and six female adults.

PARENTAL PROVISIONING AND NESTLING FOOD INTAKE

Parental visits to broods were documented using a miniature CCD camera (width 35 mm, height 35 mm and depth 27 mm) and a Sony Handycam (TR840) as a video recorder. The mini camera was placed within 15 cm of the nest, providing a view of the chicks and feeding parent. The Handycam was hidden in vegetation 3–8 m from the nest. A replica mini camera was placed at the nest at least 24 h before most recordings to allow parents to habituate to the presence of the camera.

A total of 76 broods were video-taped on one or more occasion (mean = 1.34 ± 0.62 SD; range 1–4, $n = 102$), over the three study years (25, 29 and 22 in 1998, 1999 and 2000, respectively). Each video session was for a period of 3 h. Recordings started between 08.00 and 11.00 h ($n = 42$) or between 14.00 and 16.30 h ($n = 60$), at broods that varied between 5 and 12 days in age ($n = 8, 8, 15, 16, 17, 20, 5$ and 7 on days 5–12, respectively). For each parental visit to the nest, we recorded the sex of the parent and the number of prey items received by each chick in the nest (multiple prey items were commonly delivered during a single visit). Most prey items were identified at least to the level of order. The size of each prey item was also estimated relative to the bill length of the provisioning female and divided into three categories (1 = less than bill length; 2 = similar to bill length; 3 = greater than bill length). For analysis, the number of visits to the brood, number of prey items delivered, and number of prey items received by individual chicks during the 3-h recording sessions were all converted to a rate per hour.

Brood sex ratio manipulations were also conducted in 2000 to provide further information on feeding rate and maternal energy expenditure in relation to brood sex ratio. In that year, most broods (57.0%) contained three chicks (mean = 2.55 ± 0.69 SD; range 1–4; $n = 121$), so we selected pairs of 9-day-old mixed sex broods that contained in total three female and three male chicks. Chicks were then swapped between nests to create one all-female and one all-male brood of three chicks. The following day, these broods were video-taped for 3 h and these tapes analysed as described above. The mean visit rate and total number and size of prey items were then compared between these all-male and all-female broods. These manipulations were possible because chick sex could be determined reliably by 8 days of age based on our morphological measurements. Nevertheless,

the sex of all chicks used in brood sex ratio manipulations was subsequently confirmed using the molecular sexing procedure (see above).

ENERGETIC EXPENDITURE OF NESTLINGS AND PROVISIONING FEMALE

Field procedure

During the 1999 season, 10 mixed-sex broods of three chicks were selected from a total of 31 such broods to study nestling energy expenditure and intake using doubly labelled water (DLW; Speakman 1997). When these broods were 9 days of age, a male and female from each brood was injected intraperitoneally with 100 μL of a DLW mixture (^{18}O enrichment 62.1%, ^2H enrichment 31.1%) at between 16.30 and 18.30 h. After an equilibration period of between 1 and 2 h (Speakman 1997), the two chicks were weighed and an initial blood sample (three samples of at least 15 μL from each chick) collected from the brachial vein. Approximately 24 h later, the chicks were again weighed and a final blood sample collected in a similar manner. Background samples were collected from six similar aged chicks (three males and three females) from other broods to measure natural abundances of ^2H and ^{18}O . Samples were collected in glass microcapillary tubes that were immediately flame sealed at each end using a propane torch and then stored at 5 $^{\circ}\text{C}$ until analysis.

During the 2000 season, we also applied the DLW technique to assess the energy expenditure of mothers that were provisioning 10-day-old broods that were manipulated to comprise either three male or three female chicks ($n = 10$ broods; see above). Selected mothers with 9-day-old broods were mist-netted adjacent to their nest in the early evening (18.00–19.00 h) and injected with 150 μL of the same DLW mixture. They were then held in cotton bags for 1 h before being blood sampled as described above, weighted and released. The following evening we recaptured these females, recorded their weight, and collected another set of blood samples.

Laboratory procedure

The samples were analysed in triplicate using isotope ratio mass spectrometry at the Biomedical Division of the Centre for Isotope Research of the University of Groningen (see Visser & Schekkerman 1999 and Visser, Boon & Meijer 2000a for more detail). For nestlings, daily energy expenditure (DEE) was calculated with Speakman's (1997) equation 7.17 using a factor of 27.3 $\text{kJ L}^{-1} \text{CO}_2$ to convert carbon dioxide production to energy expenditure, based on a diet consisting mainly of proteins (Gessaman & Nagy 1988). The size of the body water pool for each nestling was calculated using the principle of ^{18}O dilution ('plateau-method'; Visser *et al.* 2000b). Daily metabolizable energy (DME) intake was calculated using the following model:

$$\text{DME} = \text{DEE} + (\Delta M \times E_d) \quad (\text{Ricklefs 1974}) \quad \text{eqn 1}$$

where ΔM represents growth in g day^{-1} , E_d represents the energy density of fresh tissue in kJ per gram and DME is in kJ day^{-1} . E_d was estimated with:

$$E_d = 3.51 + 4.82 \times M/A \quad (\text{Weathers 1996}) \quad \text{eqn 2}$$

where M represents the actual body mass of the chick (g), and A the sex-specific asymptotic body mass (g).

For the adult females provisioning broods the DEE was calculated using Speakman's equation 7.17 and a gas-exchange conversion factor of 27.3 kJ L^{-1} .

DATA ANALYSES

Nestling growth characteristics were described for each sex by fitting standard Gompertz growth curves by means of the Statistica least-squares nonlinear estimation procedure. The Gompertz growth function can be written in the form

$$m(t) = A \exp[-\exp(-B(t - C))] \quad \text{eqn 3}$$

where $m(t)$ is body mass at age t , A is the asymptotic body mass, B is a rate constant and C is the inflection time. In this study we were interested primarily in describing generalized growth curves for the sexes, so the measurements ($n = 1485$) for all individuals of each sex were pooled. In this case, biases associated with the unequal contribution of data from individuals (one to 10 measurements) and broods (one to five chicks) would be minimal because of the comparatively large sample of brood ($n = 122$) and individuals ($n = 314$), and also unrelated to sex.

For analyses comparing the sexes either across broods or within broods, we first calculated the average value (e.g. mass, feeding rate) for the members of each sex within each brood to avoid pseudo-replication. For some within brood between sex comparisons, we also derived the proportion of the average male value relative to the average for his sisters. For example, the proportion of feeds to males was calculated as the average feeding rate to males divided by the sum of the average feeding rate to males plus the average feeding rate to female chicks.

All our measures of production cost (nestling mass, food intake, energy expenditure and energy intake and maternal nest visit rate, prey delivery rate and energy expenditure) were assessed when broods were 10 days of age to allow for a direct comparison. These comparisons were performed using ANOVA or paired t -tests. Additionally, some analyses on nest visitation and prey delivery rates were performed using data collected over a range of other ages during the nestling period, often including several observations at different ages for the same brood. For these analyses we used the multilevel modelling procedure in MlwiN 1.10 (Rasbash *et al.* 2000) to account for the hierarchical structure of our

data. In these cases, brood identity was specified as a random term at level 2 with each observation for that brood at level 1. When the dependent variable was nest visit/prey delivery rate, we used a normal response model as these data were distributed approximately normally. When the proportion of feeds to males was analysed, we used a binomial response model with logit-link function.

Each multilevel model was derived using backward elimination of possible explanatory variables and their interaction terms. For the binomial response models, the Wald test was applied to determine the significance of explanatory variables. For normal response models, the significance of explanatory variables was determined by calculating the change in model deviance (which approximates the χ^2 distribution) as each term was eliminated from the final model. Final models included a constant together with any statistically significant ($P < 0.05$) explanatory variables. Nonsignificant interaction terms were not included in the model summary tables unless they were of specific interest.

Total metabolizable energy (TME) intake during the nestling period up until 10 days of age (for comparison with other estimates) and fledging (day 12) were estimated by integrating eqn 1, assuming a constant relationship between mass and DEE, estimated to be $DEE = 3.36m^{0.91}$. Rough standard errors for TME were obtained by constructing, for each sex, a thousand random growth curves, with parameters drawn from a multivariate normal distribution with covariance matrix that we obtained in fitting the Gompertz growth curves. The diagonal elements of the covariance matrix were rescaled to reflect a sample size of 30 in order to avoid pseudo-replication. For each simulated growth curve, eqn 1 was integrated to obtain a simulated TME value. The standard deviation of the resulting TME distribution was used to estimate the standard error. Percentage-wise standard errors were estimated conservatively as

$$100(T_m/T_f)\sqrt{(s_{T_f}^2/T_f^2 + s_{T_m}^2/T_m^2)}$$

where T_i is the TME of sex i and s_{T_i} its corresponding standard error. The calculations were performed with Maple (files available upon request from I.P.).

Results

NESTLING GROWTH

After hatching, chicks remained in the nest for an average period of 11.50 days (SD = 1.12; range 10–14; $n = 90$ chicks). In general, females left the nest marginally earlier than males (11.15 ± 0.19 vs. 11.70 ± 0.14 (SE) days; $F_{1,88} = 5.38$, $P = 0.02$), and this pattern was also apparent within mixed-sex broods, females leaving 0.40 ± 0.10 (SE) days before their brothers (one sample t -test; $t = 4.02$, $n = 81$ broods, $P < 0.001$).

Across broods ($n = 34$), there was no difference between male and female hatching mass (2.58 ± 0.08

vs. 2.61 ± 0.08 (SE) g; $F_{1,49} = 0.08$, $P = 0.78$) or structural size (tarsus length; 7.19 ± 0.09 vs. 7.09 ± 0.10 mm; $F_{1,49} = 0.57$, $P = 0.46$). Comparing within mixed-sex broods, however, female hatchlings were on average 3.5 ± 1.8 (SE)% heavier than their brothers ($t = 1.92$, $P = 0.07$, $n = 18$ mixed sex broods), a difference that was significant after controlling for the effects of laying sequence on hatching mass (Magrath, Brouwer & Komdeur 2003b).

During the nestling period, males became increasingly larger than females and were, on average, 47.8% heavier by day 10 (35.08 ± 0.39 (SE) vs. 23.73 ± 0.58 g; $F_{1,79} = 218.4$, $P < 0.001$, $n = 57$ broods) and about 60% heavier by the time they fledged (Fig. 1). This disparity in mass was also apparent between brothers and sisters in mixed-sex broods, with males on average 49.1 ± 3.1 (SE)% heavier than their sisters by 10 days of age (paired t -test; $t = 16.7$, $P < 0.001$, $n = 34$ broods). The Gompertz growth curves indicated that peak growth rate for males was 51% higher, on average (5.3 vs. 3.5 g day⁻¹), and occurred almost 2 days later than for females (age at curve inflection; 5.61 vs. 3.86 days) (Fig. 1).

When they left the nest, males were still only 53% of the mean adult male body mass (74.8 ± 4.0 g (SD); $n = 21$), while females were 77% of the mean adult female body mass (32.4 ± 1.9 g; $n = 141$). The Gompertz growth curves predicted an asymptotic body mass for males and females of 51.3 and 27.6 g, respectively. This underestimated mean adult mass, particular for males, probably because only limited morphometric data were collected after chicks left the nest. Nevertheless, these data indicate that offspring of both sexes continued to grow at a diminishing rate after leaving the nest (Fig. 1).

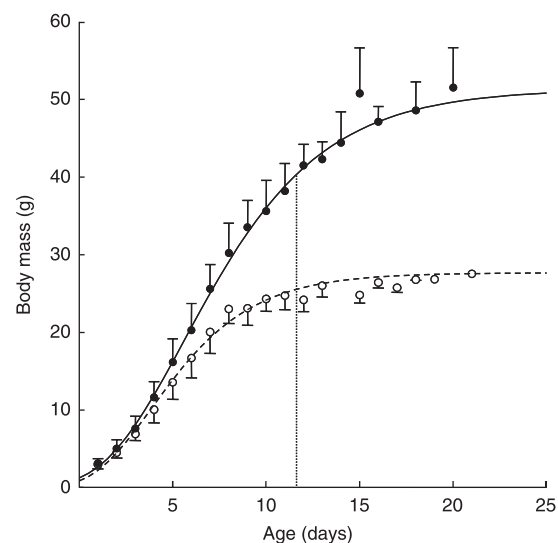


Fig. 1. Body mass of male and female brown songlark chicks from hatching to 20 days of age. The fitted curves show standard Gompertz growth functions for males (solid line) and females (broken line). Daily means with standard deviations from 1485 measurements of 180 male and 134 female nestlings of known age from 122 broods. The vertical broken line indicates the average time of chick departure from the nest.

Table 1. Model summaries examining the effect of brood characteristics on (a) parental brood visit rate, (b) prey delivery rate, and (c) mean prey size. Summaries derived from normal response mixed-modelling procedure in MLwiN with two levels (brood identity and observation session). All significant ($P < 0.05$) explanatory terms were included in the final models and are shown in bold

Dependent variable	Explanatory terms	Coefficient	χ^2	d.f.	P
(1a) Visit rate (h^{-1}) ($n = 76$ broods; 102 observations)	Brood size	2.22	29.9	1	< 0.001
	Brood age	0.50	18.9	1	< 0.001
	Brood sex ratio	1.83	6.47	1	0.01
	Year		20.8	2	< 0.001
	Day of the season	0.00	0.16	1	0.69
	Brood age \times brood sex ratio		0.21	1	0.58
(1b) Prey delivery rate (h^{-1}) ($n = 76$ broods; 102 observations)	Brood size	3.03	26.6	1	< 0.001
	Brood sex ratio	3.06	8.30	1	0.004
	Year		9.00	2	0.01
	Day of the season	-0.01	0.07	1	0.79
	Brood age	0.21	1.69	1	0.19
	Brood age \times brood sex ratio		1.13	1	0.29
(1c) Mean prey size ($n = 76$ broods; 102 observations)	Year		22.0	2	< 0.001
	Day of the season	-0.004	5.89	1	0.02
	Brood size	-0.02	0.28	1	0.60
	Brood age	0.01	0.79	1	0.38
	Brood sex ratio	0.11	2.38	1	0.12
	Brood age \times brood sex ratio		1.04	1	0.31

PARENTAL FEEDING RATE AND ENERGY EXPENDITURE

Nest visit and prey delivery rates

During the nestling period, broods received a range of invertebrate prey items that were provided almost exclusively by the female. Male adults were observed feeding at just four of 66 broods (6%), and at these four nests contributed an average of only 18% of feeds (Magrath *et al.* 2003a). Mothers were observed feeding young of both sexes for up to 3 weeks after they left the nest (Magrath *et al.* 2003a), but the data were too few to determine if the time to independence differed between the sexes.

The rate of feeding visits increased with both brood size and age (over the range 5–12 days), while the number of prey items delivered per hour increased with brood size but not brood age (Table 1). Feeding visit and prey delivery rates also increased with brood sex ratio (Table 1; Fig. 2). Adding ‘mean chick mass’ as an explanatory term to model 1a (Table 1) improved the overall model fit (for the term mean chick mass, $\chi^2 = 23.4$, d.f. = 1, $P < 0.001$), but both brood sex ratio and brood age became nonsignificant. This suggests that the increase in visit rate with brood sex ratio resulted primarily from the greater size of male chicks, especially late in the nestling period, rather than the sex ratio *per se*. The mean size of prey items delivered to broods varied between years and decreased over the season, but was not apparently related to brood size, age, sex ratio or mean chick mass (Table 1).

Among 10-day-old broods manipulated to contain either three male ($n = 5$) or three female nestlings

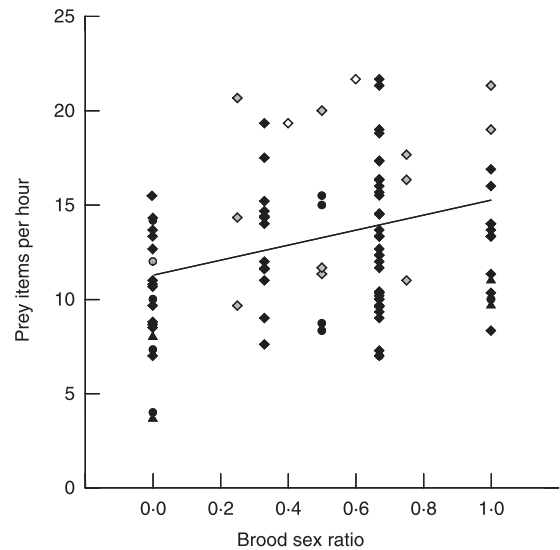


Fig. 2. Prey delivery rate by brown songlark parents in relation to brood sex ratio. The line shows the predicted association between delivery rate and brood sex ratio ($n = 76$ broods), as derived from the normal response mixed modelling procedure in MLwiN, after controlling for the significant effects of brood size and year (see Table 1). Points show actual values coded in relation to brood size: \blacktriangle , 1; \bullet , 2; \blacklozenge , 3; \blacklozenge , 4; \diamond , 5.

($n = 5$), the all-male broods were visited 42.7% more frequently (9.40 ± 0.81 (SE) vs. 6.59 ± 0.81 (visits h^{-1}); $F_{1,8} = 5.96$, $P = 0.04$) and provided with 47.6% more prey items (14.7 ± 0.87 vs. 9.97 ± 0.87 (prey h^{-1}); $F_{1,8} = 14.76$, $P = 0.005$), though the mean size of delivered prey was similar ($F_{1,8} = 0.35$, $P = 0.57$). Only females were observed provisioning at these manipulated broods.

Table 2. Model summaries examining the effect of brood characteristics on (a) the relative number and (b) relative size of prey items provided to male versus female chicks in mixed-sex broods. Summaries derived from the binomial response mixed-modelling procedure in MLwiN with two levels (brood identity and observation session). All significant ($P < 0.05$) explanatory terms were included in final models and are shown in bold

Dependent variable	Explanatory variable	Coefficient	χ^2	d.f.	P
(2a) Proportion of prey delivered to males ($n = 56$ broods; 67 observations)	Relative mass	3.99	8.41	1	0.004
	Day of the season	0.00	0.05	1	0.94
	Brood sex ratio	0.11	0.11	1	0.74
	Brood size	0.03	0.10	1	0.75
	Year		0.33	2	0.57
(2b) Relative size of prey delivered to males ($n = 56$ broods; 67 observations)	Relative mass	0.031	0.001	1	0.97
	Day of the season	0.002	0.281	1	0.59
	Brood sex ratio	0.021	0.005	1	0.94
	Brood size	-0.009	0.015	1	0.90
	Year		0.592	2	0.74

Maternal energy expenditure

Among a subset of the manipulated broods, the DEE of mothers provisioning all-male broods was found to be 27% higher than for mothers feeding all-female broods (153.7 ± 7.7 (SE) vs. 121.2 ± 3.5 kJ day⁻¹; $F_{1,4} = 7.67$, $P = 0.05$, $n = 6$ mothers). The DEE of these mothers was also positively related to total brood mass ($r = 0.85$, $t = 3.19$, $P = 0.03$, $n = 6$).

NESTLING FOOD INTAKE AND ENERGY EXPENDITURE

Nestling prey intake

By 10 days of age, males were receiving, on average, 41.2% more prey items than females (5.22 ± 0.21 (SE) vs. 3.70 ± 0.21 (prey h⁻¹); ANCOVA; for sex; $F_{1,51} = 25.1$, $P < 0.001$, $n = 36$ broods), after controlling for significant brood size and years effects. A similar disparity was also apparent within the subset of 10-day-old mixed-sex broods, where males received on average 42.2 ± 9.4 (SE)% more items than their sisters (paired t -test; $t = 4.50$, $P < 0.001$, $n = 26$ broods). However, there was no detectable difference in the relative size of prey provided to male chicks compared with their sisters at this age (paired t -test; $t = 0.97$, $P = 0.34$; $n = 26$).

In mixed-sex broods of between 5 and 12 days of age, the rate at which males received prey items relative to their sisters increased almost directly in proportion to the disparity in mass between the sexes (proportional mass) (Table 2; Fig. 3). In comparison, brood age was a poor predictor of the proportion of prey received by males when substituted into model 2a instead of proportional mass ($\chi^2 = 1.69$, d.f. = 1, $P = 0.19$). There was no evidence that the relative prey size changed with the degree of size dimorphism (Table 2) or age ($\chi^2 = 0.58$, d.f. = 1, $P = 0.76$).

Within broods of between 5 and 12 days of age, the feeding rate of individual chicks was related to their mass ($\chi^2 = 24.9$, d.f. = 1, $P < 0.001$, $n = 66$ broods, 205 chicks) but not sex ($\chi^2 = 0.03$, d.f. = 1, $P = 0.87$; sex \times mass interaction, $\chi^2 = 0.17$, d.f. = 1, $P = 0.68$),

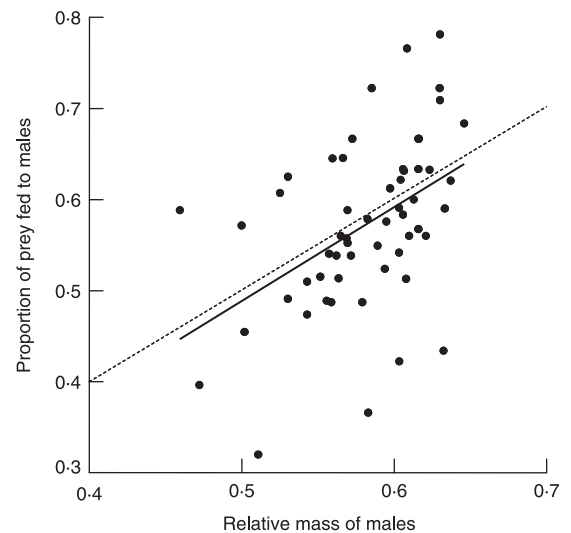


Fig. 3. Feeding rate of male brown songlark chicks relative to their sisters in mixed-sex broods of between 5 and 12 days of age in relation to their proportional mass. The relative feeding rate is expressed as the proportion of feeds received by the average male compared with the average female within the brood, and points indicate actual values. The solid line shows the predicted relationship as derived from the binomial mixed-modelling procedure in MLwiN (see Table 2). The broken diagonal represents the line where relative feeding rate is directly proportional to relative mass.

again suggesting that chick size was a better predictor than sex of prey distribution within the brood. However, this conclusion should be regarded with some caution because there was no overlap between the mass of male and female siblings in older broods. Moreover, within single-sex broods, the distribution of prey was not explained by chick mass ($\chi^2 = 0.60$, d.f. = 1, $P = 0.44$), although the sample of these broods for which mass data were collected was small ($n = 10$ broods, 34 chicks).

Nestling energy expenditure

The mean DEE of 10-day-old chicks was 76.5 ± 20.4 (SD) kJ day⁻¹ ($n = 10$ males and 10 females). Comparing male and female siblings we found that sons

Table 3. Summary of mass, mass change, energy expenditure and total metabolizable energy intake of 10-day-old male and female siblings ($n = 10$ pairs). Values shown are means with their SD in brackets. Difference between the sexes expressed as a percentage of the female value. t -values derived from paired t -tests comparing values for the 10 siblings pairs

Measurement	Male	Female	Difference (%)	t -value	P -value
Mass (g)	36.8 (2.6)	24.0 (1.7)	+53.3 (9.8)	17.1	< 0.001
Mass gain (g day ⁻¹)	3.3 (1.4)	1.5 (1.2)	+125 (102)	3.9	0.004
Daily energy expenditure (kJ day ⁻¹)	90.3 (17.7)	62.7 (11.8)	+44.1 (14.9)	9.4	< 0.001
Mass-specific daily energy expenditure (kJ day ⁻¹ g ⁻¹)	2.4 (0.4)	2.6 (0.5)	-7.7 (8.1)	2.5	0.034
Daily metabolizable energy intake (kJ day ⁻¹)	109.9 (18.9)	73.0 (14.6)	+50.4 (16.6)	9.6	< 0.001
Mass-specific daily metabolizable energy (kJ day ⁻¹ g ⁻¹)	2.98 (0.54)	3.06 (0.54)	-2.5 (14.9)	0.52	0.62

Table 4. Estimates of the relative expenditure on male vs. female brown songlark offspring at 10 days of age derived from eight different measures of assessment

Measure of parental expenditure	Comparison	Percentage greater for males			
		Mean	SE	SD	N broods
Nestling mass	Across all broods	47.8	2.9	21.9	57
	Within mixed-sex broods	49.1	3.1	18.1	34
Nestling prey intake rate	Across all broods	41.2	9.9	59.4	36
	Within mixed-sex broods	42.2	9.8	50.0	26
Nestling energy expenditure	Within mixed-sex broods	44.1	5.4	17.1	10
Nestling metabolizable energy intake	Within mixed-sex broods	50.4	6.1	19.3	10
Maternal nest visit rate	Across manipulated single-sex broods	42.7	20.1	63.6	10
Maternal prey delivery rate	Across manipulated single-sex broods	47.6	14.6	46.2	10
Maternal energy expended on brood care	Across manipulated single-sex broods	26.9	27.0	66.1	6
Total metabolizable energy intake	Across all broods up until day 10	38.9	18.6	101.9	30

expended 44.1 ± 10.7 (SE)% more energy than their sisters, though female mass-specific expenditure was marginally greater (Table 3). For these 20 chicks, the total DME intake was 50.4 ± 10.4 (SE)% higher for males than their sisters, similar to their mean difference in mass (Table 3). Indeed, there was no mass-specific difference in DME intake between brothers and sisters (Table 3). Moreover, comparing across the same individuals, DME intake remained positively correlated with chick mass ($F_{1,17} = 4.54$, $P = 0.048$) after controlling for sex ($F_{1,17} = 1.12$, $P = 0.74$) and this relationship was similar for both sex (sex \times mass interaction $F_{1,16} = 0.95$, $P = 0.34$).

COMPARISON OF ESTIMATES AND PREDICTION OF RELATIVE INVESTMENT OVER THE NESTLING PERIOD

When nestlings were 10 days of age, all eight estimates of relative investment in male and female offspring were in close agreement (Table 4). All estimates of expenditure for males were significantly greater than for females (see previous sections), but the magnitude of the bias did not differ between estimates (all means fall within 1 SE around each of the estimates; Table 4), although the statistical power for some of these comparisons was low because of small sample sizes. However, the variance (SD) was considerably lower for estimates associated with relative mass and energy intake compared with those related to nestling prey

intake and maternal provisioning and energy expenditure (Table 4).

Based on the sex-specific growth curves and the predicted relation between DEE and mass (see Methods), we estimated the TME intake between hatching and nest departure to be 38.9% greater for males than females at day 10 (471 vs. 654 kJ; Table 4) and 47.0% higher at day 12 (609 vs. 895 kJ).

Discussion

NESTLING GROWTH

Between hatching and departing from the nest (about 12 days old), male songlark chicks grew, on average, about 60% heavier than their sisters. This represents one of the most extreme cases of nestling sexual size dimorphism in birds (Richner 1991). Moreover, at the time of nest departure, males were still about 25% below their predicted asymptotic body mass, and only just over half the mean mass of adult males in the population, which is likely to be a better estimate of male asymptotic weight. In most size-dimorphic passerine species, both sexes reach fledging weight at roughly the same time (Richner 1991). Brown songlarks, however, depart the nest at a relatively early stage of development, when incapable of even rudimentary flight. This early departure is likely to result from the high risk of nest predation in this ground nesting species (Magrath *et al.* 2003a). Females generally left

the nest about half a day earlier than their larger brothers. Earlier fledging by the smaller sex has been reported in a range of size-dimorphic species, including red-winged blackbirds *Agelaius phoeniceus* (Holcomb & Twiest 1970), yellow-headed blackbirds *Xanthocephalus xanthocephalus* (Richter 1983), and a number of raptors (Newton 1979). This may be attributed to the more advanced developmental state of the smaller sex, but also possibly their declining ability to compete for food. In this study, mothers concurrently fed chicks that had left the nest and those that remained, but it was unknown how the level of provisioning changed with nest departure.

FOOD CONSUMPTION AND ENERGY INTAKE

The higher rate of mass gain by males was facilitated by the consumption of more food. Our analysis of prey intake rate indicates that the proportion of prey received by males in mixed-sex broods of between 5 and 12 days of age increased almost in direct proportion to the difference in mass between the sexes. Comparing across 10-day-old broods only, males received 41% more prey items than females and, in the subset of mixed-sex broods, 42% more than their sisters. At this age, males weighed about 49% more than their sisters, suggesting that males received slightly fewer prey than in direct proportion to their relative mass. However, our energetic data indicate that the metabolizable energy intake of 10-day-old young was almost proportional to their mass, and that there was no sex difference in mass-specific metabolizable energy intake (Table 3).

One potential explanation for this discrepancy between the number of prey items received and our estimate of metabolizable energy intake is that males were receiving larger prey items. Our analysis of prey size failed to reveal such a difference; however, our rudimentary assessment of prey size may have been insufficiently accurate to detect a real difference. An asymmetry in the nutritional quality of the diet delivered to the sexes is another possible explanation. Elsewhere we show a difference between male and female siblings in diet composition, and we argue that this difference may equate to sons receiving a nutritionally superior diet (Magrath *et al.* 2004).

Our findings clearly do not support the idea that males and females have similar energy demands because of differences in growth dynamics (sex-specific growth hypothesis; Richter 1983) or energetic expenditure (competitive behaviour hypothesis; Stamps 1990). Instead, our data are consistent with the majority of previous studies that show energy intake is almost proportional to the relative mass of the sexes (Fiala & Congdon 1983; Teather 1987; Teather & Weatherhead 1988; Anderson *et al.* 1993; Krijgsveld *et al.* 1998; Riedstra *et al.* 1998; Vedder *et al.* 2005). In a comparative study of 16 size-dimorphic species, Krijgsveld *et al.* (1998) found that estimates of total food intake based on either food consumption or metabolizable energy

intake were strongly correlated with the extent of dimorphism. However, this analysis indicated that the relationship was not directly proportional and that the larger sex had a lower mass-specific food requirement. This was also apparent in the brown songlark, as males were about 60% heavier than females at day 12, but were estimated to have consumed only about 48% more metabolizable energy by this age. This disparity is likely to result from the marginally lower mass-specific metabolic rates observed in the larger males. The lower mass-specific energy expenditure of males is consistent with allometric scaling of metabolic rate to body size (Krijgsveld *et al.* 1998), but could also result, at least in part, if females have to beg more intensely than their larger brothers to compete for food.

PARENTAL PROVISIONING AND ENERGY EXPENDITURE

Consistent with the greater prey intake of males we also found that both maternal visit and prey delivery rates increased with natural brood sex ratio. Furthermore, manipulated all-male broods were visited 43% more often and received 47% more prey items than all-female broods, indicating that mothers were capable of responding to changes in brood demand. Surprisingly few previous studies have examined provisioning in response to manipulated brood sex ratio, and generally little change has been evident. In the size-dimorphic Eurasian kestrel *Falco tinnunculus*, no difference was observed in the delivery rate or biomass of prey fed to manipulated broods (Laaksonen *et al.* 2004), nor were differences apparent in the marginally dimorphic great tit *Parus major* (Lessells, Oddie & Mateman 1998) or monomorphic western bluebird *Sialia mexicana* (Leonard *et al.* 1994). In contrast, Green (2002) reports that females, but not males, provision more frequently at both natural and experimentally male-biased broods in the size-dimorphic brown thornbill *Acanthiza pusilla*. Several correlational studies have also reported increases in parental provisioning with brood sex ratio (e.g. Nishiumi *et al.* 1996), but associations between parental quality and brood sex ratio may contribute to these patterns.

In this study, the greater work rate of female songlarks also appeared to translate to elevated energy expenditure, as mothers expended more energy provisioning all-male than all-female broods. As far as we are aware, an association between parental energy expenditure and brood sex ratio has not previously been reported in birds. This may be the most important indication of a real difference in the fitness cost of raising sons vs. daughters, as experimentally elevated parental workloads in other birds have resulted in reduced survival (e.g. Deerenberg *et al.* 1995; Visser & Lessells 2001) and immune function (e.g. Saino *et al.* 2002).

We estimated that the difference in maternal energy expenditure between all-male and all-female broods was about 27%, though this estimate is very approximate

because of the small sample size. Furthermore, our estimate of maternal DEE includes energy the female expends on nonparental activities including her resting metabolic rate and self-maintenance activities. Consequently, the difference in energy expended provisioning the manipulated all-male and all-female broods, after subtracting the field metabolic rate, would have been greater, proportionally, than the disparity observed. Quantifying this difference, however, is not feasible because of the uncertainty in predicting field metabolic rates (Tieleman & Williams 2000). Furthermore, differences between energy expenditure may not reveal true costs because females may make behavioural and physiological adjustments to compensate for increased parental workload (Wright *et al.* 1998). Indeed, increased feeding rates of parents to enlarged broods do not usually correlate well with parental energy expenditure (Bryant 1988; Tinbergen & Verhulst 2000; Wiersma & Tinbergen 2003), although biparental provisioning may complicate the relationship in these studies.

ESTIMATES OF RELATIVE COST

All surrogate estimates of relative cost, as measured on day 10, indicated that sons were more expensive than daughters (Table 4). At this age, males were estimated to cost between 40 and 50% more in seven of these eight measures, and the somewhat lower estimate derived from maternal energy expenditure (27%) was known to be an underestimate of the real difference (see above). All of these measures therefore provide a highly consistent picture of relative expenditure in our study population. We suggest therefore that any of these measures can provide valid estimates of cost difference in this species and among sexually size-dimorphic birds more generally. However, estimates associated with relative mass and energy intake had considerably lower variance than those related to food provisioning, suggesting that these may be more reliable, especially in cases where the sample size is small. However, these differences in variance may simply reflect the fact that our provisioning data were collected only for 3 h per brood, while energy intake estimates were derived over 24 h, and mass differences had accumulated over the preceding 10 days.

How well these surrogate measures of investment correspond to the actual fitness cost to the parent, however, remains unclear, and it is unlikely this question can be explored in this species given their nomadic movements and low breeding site fidelity (Barrett *et al.* 2003; Magrath *et al.* 2003a). Moreover, our estimates of relative investment represent mean values for this population, some of which were derived over only a single season. Clearly, relative investment will not be a fixed ratio but should vary between parents within the population and also between different locations and in different years (Frank 1990). Each female is likely to adjust the distribution of resources in relation to such factors as her condition, age and the size and sex

composition of the brood. Similarly, in different seasons or locations the availability of resources will vary and may be expected to influence relative investment in the sexes. Generally, dimorphism is more pronounced when food is plentiful, especially in species where males are the larger sex (Badyaev 2002). However, the degree of dimorphism need not always be a good reflection of the difference in parental expenditure on the sexes. If food is plentiful, the reduced effort in collecting sufficient prey may mean that the relative cost of producing the larger sex is the same or less, even though size dimorphism is greater, than under poor conditions when food is limited. Consequently, the association between parental energy expenditure, offspring size dimorphism and food availability may be complex but would be amenable to experimental investigation.

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