

University of Groningen

Parental energy and fitness costs in birds

Deerenberg, Charlotte Maria

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

1996

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Deerenberg, C. M. (1996). *Parental energy and fitness costs in birds*. s.n.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

CHAPTER 5

PARENTAL EFFORT AND COSTS OF REPRODUCTION OF ZEBRA FINCHES

Charlotte Deerenberg, Gerard J.F. Overkamp

Zoological Laboratory, University of Groningen, Haren NL

Submitted to: Auk

ABSTRACT

Brood size was experimentally manipulated in Zebra Finches *Taeniopygia guttata* to assess effects of brood size on various aspects of parental effort, to reveal interrelationships between these variables, and to study their consequences for the timing of the subsequent clutch. Parental effort as assessed by foraging time increased with brood size in both sexes, but feeding rates to the brood were not adjusted to brood size. DEE, assessed by turnover rates of doubly labeled water, and mass changes of male parents were not significantly affected by manipulation of brood size. On average, male DEE was $53.7 \text{ kJ}\cdot\text{day}^{-1}$ ($\text{sd}=15.8$, $n=16$). Variation in DEE of males was closely related to body mass. In female parents, both mass loss and DEE increased with experimental brood size. Data on body mass and DEE both indicated an increase in female parental effort with brood size. Female mass was clearly reduced in large broods. Average female DEE was $56.6 \text{ kJ}\cdot\text{day}^{-1}$ ($\text{sd}=12.3$, $n=18$), and showed an increase of $4.7 \text{ kJ}\cdot\text{day}^{-1}$ ($\text{se}=1.8$) per extra young in the nest. DEE of females was not related to body mass.

Correlations between behavioural and physiological aspects of parental effort were only apparent in feeding frequency to the brood and water flux rates, in males and in females. In turn, water flux rates, which were large ($12.0 \text{ ml}\cdot\text{day}^{-1}$, $\text{sd}=3.6$, $n=34$), were closely associated with DEE in both sexes. It is suggested that high water flux may be a consequence of soaking the food in the crop of the parent before feeding it to the young.

Each of the factors, time allocation, body mass, and DEE have been suggested previously to cause fitness costs. Timing of the subsequent clutch was related to water flux rates in both sexes, and to female DEE. Experimental brood size affected behavioural and physiological aspects of parental effort, and physiological effort in turn affected costs of reproduction, all of which was most consistent in female parents.

INTRODUCTION

Field experiments with manipulated brood sizes of altricial birds have repeatedly demonstrated a cost of reproduction for the parents. Parents raising an enlarged brood often face a reduction in residual fitness, as a result of reduced survival rates or negative effects on later reproduction (reviews in: Dijkstra *et al.* 1990; Stearns 1992). Several mechanisms have been proposed, by which costs of reproduction arise. They can be divided, according to their way of action, into ecological (external) and physiological (internal) mechanisms (Partridge 1992). Ecological mechanisms concern the temporal pattern of time allocation to mutually exclusive behaviours (*e.g.* duration of parental post-fledging care and initiation of a subsequent clutch; Tinbergen 1987; Smith *et al.* 1989, Verhulst 1995), or a change in time budget instantaneously affecting exposure to risks (Calow 1979; Magnhagen 1991; Partridge 1992).

Physiological mechanisms may exert their effects through negative influences of reproduction on body condition, either temporarily ('exhaustion' *sensu* Cavé 1968; Drent & Daan 1980), or permanently ('senescence' *sensu* Partridge 1987; Gustafsson & Pärt 1990). Often, a reduction in body mass is observed during parental care (review in Moreno 1989). This may be the result of reduced self-feeding (Martins & Wright 1993; Kacelnik 1984), allowing parents to economize on the energy for self-maintenance and food provisioning (Freed 1981; Norberg 1981; Houston 1993). Alternatively, parental work rates may temporarily exceed the sustainable level (Peterson *et al.* 1990), at the expense of body reserves, or physiological processes such as defence mechanisms (Chapter 7), according to the 'principle of allocation' (Sibly & Calow 1986b).

Another physiological mechanism has been suggested by Daan *et al.* (1990), who hypothesised that the rate of energy expenditure of parents plays a key role in the trade-off between current and future reproduction. Parent birds have been shown to adjust their daily energy expenditure (DEE), assessed either by time-energy budgets (Dijkstra *et al.* 1990) or by turnover rates of stable isotopes (Williams 1987; Deerenberg *et al.* 1995; Moreno *et al.* 1995; but see Verhulst 1995), to the experimental brood size. However, evidence for a causal relationship between rates of energy expenditure and future fitness parameters mainly has been indirect. Many studies have concentrated on assessing either parental rates of energy expenditure, or costs of reproduction in relation to manipulated brood size. Therefore, the association of DEE and fitness costs is mainly qualitative, *i.e.* when both factors are affected by brood size. Quantitative associations have been assessed in Kestrels (*Falco tinnunculus*) where local survival rates of individuals have been related to DEE during reproduction (Deerenberg *et al.* 1995), and in Great Tits (*Parus major*), where a relation has been shown between male DEE and size of the second clutch (Verhulst 1995). In Zebra Finches (*Taeniopygia guttata*), increased (parental) work rates have been shown to bear a fitness cost, due a reduced frequency of reproduction (Lemon 1993, Chapters 5 and 6), or reduced longevity (Lemon 1991).

It is as yet unclear whether parental fitness costs associated with brood size are the consequence of high rates of energy expenditure *per se*, or whether fitness costs are the result of secondary effects on, *e.g.*, body condition, due to an unbalanced energy budget or a reduced allocation of available energy to self-maintenance during the manipulated phase of reproduction. Deterioration of parental body condition may be one step in a chain of effects (*e.g.* reproductive behaviour → DEE → body condition) causing fitness costs of reproduction. Bryant (1988) has therefore suggested that rates of energy expenditure should be examined not only in conjunction with activity, but also with body mass.

In the life history problem of optimal brood size, each of the suggested mechanisms

(time, energy, body condition) may be instrumental in the trade-off between current and future reproduction. The exploration of potential mechanisms through which fitness costs of reproduction come about may benefit from studying parental behaviour in a constant and controlled environment. Such circumstances may rule out part of the variation between individuals resulting from constraints forced upon them by the individual's environment, such as territory quality and food availability. In a laboratory situation with food available *ad libitum*, we expect parental care not to be restricted by ecological factors, such as the availability of food, thus intake of energy. Physical constraints, however, such as the rate of energy expenditure during the phase of parental care, and parental body condition may still operate in a laboratory situation. I therefore studied behaviour (time budgets), changes in body mass, and daily energy expenditure (DEE) of parent Zebra Finches (*Taeniopygia guttata*) raising experimentally manipulated brood sizes. I aimed at identifying potential causes of variation in DEE, and at assessing an association between brood size and DEE, and of DEE and the main cost of reproduction, previously assessed as delayed timing of the subsequent clutch (Chapter 4). This analysis may help us to understand whether there is scope for a causal relationship between either time allocation, body condition or energy and costs of reproduction.

METHODS

Housing and experimental manipulation of brood size

Pairs of male and female zebra finches, originating from domestic stock, were kept in climate rooms at 25°C, and relative humidity 60%, in a LD cycle of 14:10 (lights on at 0800 h). Each pair was held in a cage of 40·80·40 cm (h·w·d) with two perches, and equipped with food and drinking water *ad libitum*. The food was a mixture of dry seeds. Each cage contained a nest box, and nesting material was available throughout. Daily checks of the nest box were carried out to determine start of reproduction, clutch size, hatching date and hatching success.

Broods of 2, 4 and 6 young were created to represent small, intermediate and large broods respectively. A total of 56 pairs was manipulated, of which 18 pairs were given large broods, 17 pairs intermediate broods, and 21 pairs small broods. Nests of similar hatching dates were assigned randomly to one of the three categories. Original clutch size was on average 4.3 eggs (sd=0.79, n=56). Clutch size did not differ significantly between subsequent brood size categories ($F_2=0.36$, ns) Manipulation of brood size was carried out at about three days after the first chick hatched (= day 0).

Experiment 1: Behaviour of parent birds

Parental behaviour was observed in 7 pairs with an intermediate brood size, 8 pairs with a small brood, and 7 pairs with a large brood. Observations were carried out at various times during the period of parental care (days after hatching), both during the nestling phase and after fledging of the young: on day 8 and 16 (nestling phase), on day 21, 25 (fledgling phase), and on day 35 (start of independence). All pairs were observed for 2 hours in the first four hours after lights-on. Observations of the pairs within each experimental category were spread randomly over the morning hours at every phase of parental care.

Of these 22 pairs, 8 (2 pairs with intermediate, 3 pairs with small and 3 pairs with large brood sizes) were also observed in the afternoon. Within pairs, overall behaviour did not vary significantly between morning and afternoon ($F_{5,4}=1.29$, ns), and therefore the analysis was restricted to observations carried out during the morning hours (14 pairs).

Behaviour of both parents was recorded simultaneously every 60 s. The following categories of behaviour (see also Ten Cate 1982) were considered:

<i>Foraging</i>	Pecking at seeds, handling and peeling of seed, and swallowing of seed.
<i>Locomotion</i>	Movements on the ground that were not part of foraging, and flying (mainly between perches).
<i>Sitting</i>	Sitting on a perch or on the ground.
<i>Preening</i>	Selfpreening or allopreening.
<i>At the nest</i>	Being present at the entrance or inside the nestbox.

These 60 s measurements were used to calculate time spent on various behaviours (time budget). In addition, feedings to nestlings (= number of beak contacts with young of parent regurgitating food) were recorded. These measurements were used for calculating frequencies.

Experiment 2: Body mass of parent birds

The birds were weighed, and body reserves were assessed using a fat index (Pettersson & Hasselquist 1985) at preset intervals. Body mass and fat reserves were assessed at the start of the experiment, thus prior to initiation of reproduction, once during incubation, and after manipulation of their brood size according to the following pattern: on day 8 and 16 after hatching of the young (nestling phase: 0-19 days of age), on day 21 and 25 (fledgling phase of post-fledging care: 20-35 days of age), and on day 35 and 50 (independence). In the analysis of body mass and fat reserves of parents, I used only complete records (98 cases). Indices of fat reserves at the start of the experiment were available for 44 individuals.

Experiment 3: Daily energy expenditure and water flux

Rates of energy expenditure of zebra finch parents were estimated with the Doubly Labelled Water (DLW) technique (Lifson & McClintock 1966, Nagy 1980). The measurements were obtained from a subsample of 6 pairs in each brood size category on day 14 to 16 of the nestling period.

Parent birds were captured between 9 and 12 am. Upon capture, they were weighed to the nearest 0.1 gram on a Mettler balance (type P1200). The birds were injected intraperitoneally with approximately 0.15 ml of a mixture of H_2^{18}O (90.0 atom%) and H_2O (99.9 atom%). The volume of water injected was determined by weighing the syringe to the nearest $0.1 \cdot 10^{-3}$ g on a Mettler balance (type H54), before and after injection. 'Initial' blood samples were taken 1 hour after injection to allow complete equilibration of the injected isotopes with the body water. During the interval between injection and initial blood sampling, the zebra finches were released into their cages to keep interruption of parental care to a minimum. After 48h, body mass and 'final' blood samples were taken. Blood samples were obtained by puncturing the jugular or the brachial vein. The blood was collected in 4 to 6 glass microcapillaries of 10 μl each, which were subsequently flame-sealed.

The isotopic enrichments of the blood samples were determined at the Centre of Isotope Research, University of Groningen, by means of mass spectrometry. Natural isotopic abundance (background) in the body water, determined from blood samples of three zebra finches of the same stock and in the same housing conditions, was 1.70 (sd=1.4) ^{18}O and -11.3 (sd=4.7) ^2H relative to Standard Mean Ocean Water (Ehleringer & Rundel 1988). Total body water volume was estimated from the dilution space of ^{18}O (N_o) (Schoeller *et al.* 1980). I used the extrapolation method that takes into account turnover of isotopes in between injection of isotopes and initial blood sampling, because the birds were released in their cage in between injection of isotopes and initial blood sampling, and are expected to have metabolic rates similar

to those in between initial and final blood sampling. Two birds were excluded from the analysis, because not all labeled water was injected, and estimates of total body water were therefore unreliable.

The dilution space of hydrogen (N_d) appeared relatively large ($N_d/N_t = 1.08$). Other than body water (first H pool), also protein (second H pool) contains a relatively large fraction of exchangeable H (Culebras & Moore 1977). The exchangeability of protein H is increased by exercise (Culebras & Moore 1977), and, assuming equal turnover rates of ^2H in body water and in protein, there was no need to correct for the observed difference in H dilution space and total body water volume (Speakman 1987). This difference, however, would yield underestimations of CO_2 production of about 20% (Roberts 1989), based on the assumption of an evaporative water loss of 50%, and given a ratio of ^{18}O to ^2H turnover rates of 1.36 (sd=0.09).

Water flux rate and carbon dioxide production were calculated according to equations 4 and 34, respectively, in Lifson & McClintock (1966). Water fluxes in this experiment were large and highly variable (5-20 ml·day⁻¹, that is 47-176% of body mass). Calder (1964) measured water relations in zebra finches and assessed evaporative water loss at 39% of body mass, or, when expressed as a fraction of total water efflux, at c. 80%. In the calculation of CO_2 production, I therefore assumed a fixed amount of evaporative water loss, thus a variable fraction (23-83%) that varied in accordance with water flux rate, while Lifson & McClintock (1966) assumed a fixed fraction of 50%. Increased evaporative water loss at higher work rates was not accounted for, but this extra fraction was expected to be relatively small in view of the high total water flux. DEE was then estimated using an RQ of 0.83, and an energetic equivalent of CO_2 of 24.4 kJ per liter, based on nutrient catabolism of a typical granivorous diet with 15% protein (Gessaman & Nagy 1988). The seed mixture contained 14.4% of dry weight protein (Kjeldahl analysis).

Data analysis

Of each pair, both male and female parents were measured. All data were analysed for males and females separately. Within-pair differences were tested with the sign test, and using a binomial table when sample sizes were smaller than 20 (Hays 1988).

Data on body mass, fat reserves, and behaviour consisted of repeated measurements on the same individuals during various stages of parental care. Data from successive sampling occasions were therefore not independent. Both behaviour and physical properties of parents were expected to change during consecutive phases of parental care (incubation, nestlings and fledglings), but were subsidiary to differences between categories. Differences on the first measurement occasion (body mass and fat: prior to the experiment; behaviour: on age 8 days of the brood) were assessed with one-way anova. Subsequent changes with age of the brood, and differences between the brood sizes - controlled for changes with age - , were tested using multivariate analysis of variance for repeated measures with the first measurement as the reference category.

Behavioural categories were analysed separately. Time allocated to behavioural categories was expressed as a fraction, which were arcsine transformed prior to analysis of behaviour. Each of the five behavioural categories was considered separately.

RESULTS

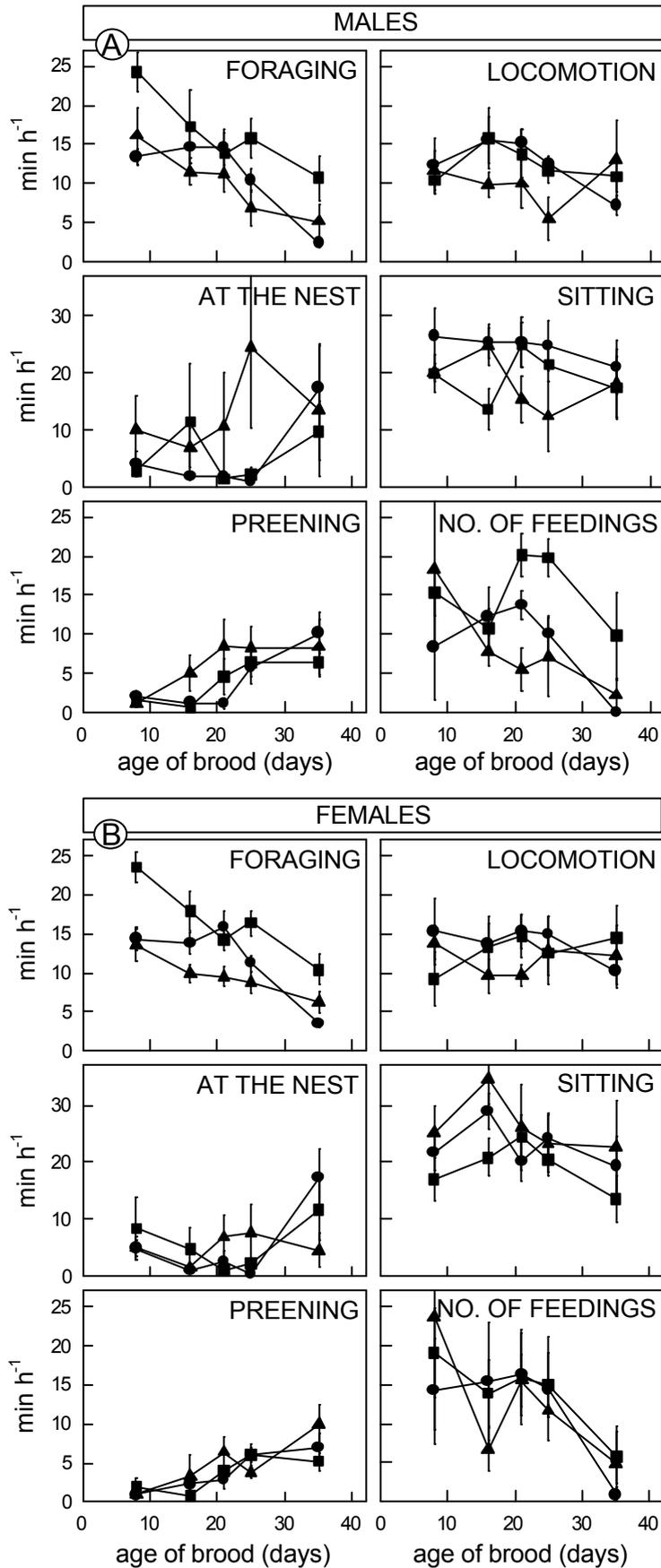
Time budgets and brood size

Six main aspects of adult behaviour were investigated: sitting, locomotion, foraging, at the nest, preening, and feeding frequency. Two of these aspects are related to parental care. Foraging time reflects amount of food that is collected to sustain the brood, while feeding frequency indicates the actual transfer of food to the young. With young nestlings, time at the nest is indicative of brooding of young. Preening may be an indicator of maintenance. Both parents were always observed simultaneously. Average time allocation of male and female parents to all five behavioural categories, and to feeding frequency is depicted in Figure 1 in relation to age of the brood.

MALES. Time budgets of individual male parents (Figure 1A) changed with age of the brood, with an increase in time allocated to preening ($F_4=8.53$, $P<.001$), and a decrease in foraging time ($F_4=12.74$, $P<.001$). Other aspects of male behaviour did not vary with age. However, the main issue was to reveal effects of experimental brood size on the time budgets. Foraging time was affected significantly by brood size. Foraging time differed between brood sizes on day 8 of the brood ($F_2=4.62$, $P=.04$), while subsequent changes in foraging time did not differ between brood size categories ($F_8=1.63$, ns). Otherwise, no relation of time allocation and brood size could be assessed.

Feeding of the fledglings ceased on day 35 (=start of independence). Observed feeding frequencies decreased significantly with age of the brood ($F_4=3.18$, $P=.02$). Number of feedings by the male tended to vary with brood size on day 8 ($F_2=2.98$, $P=.09$). The subsequent decrease in feeding frequencies with age did not differ between brood sizes.

FEMALES. Female time budgets also changed with age of the brood (Figure 1B). As in males, time allocated to preening increased with age ($F_4=15.20$, $P<.001$), and foraging time decreased with age of the brood ($F_4=9.63$, $P<.001$). In addition, time spent sitting



($F_4=5.10$, $P<.01$), and time at the nest ($F_4=3.59$, $P=.01$) varied significantly with age. Foraging time of females on day 8 differed significantly between brood size categories ($F_2=4.73$, $P=.03$), while changes within individuals in foraging time after day 8 were not related to brood size ($F_8=1.62$, ns). Other behaviours did not vary with either brood size or age of the brood.

As in the males, feeding frequencies of females decreased significantly with age of the brood ($F_{12}=3.20$, $P<.01$). Brood size did not affect differentially female feeding frequency on day 8 of the brood ($F_2=0.19$, ns), or on subsequent ages ($F_8=0.49$, ns). Average total number of feedings to the brood by male and female parents during the nestling phase and the early fledgling phase (day 8-25) was 24.1 h^{-1} (sd=25.6, n=16) for small broods, 26.2 h^{-1} (sd=19.2, n=20) for intermediate broods, and 33.3 h^{-1} (sd=12.0, n=19) for large broods. In small broods individual young received on average $12.0 \text{ feedings h}^{-1}$, in intermediate broods $6.6 \text{ feedings h}^{-1}$, and in large broods $6.4 \text{ feedings h}^{-1}$.

Body mass, body reserves and brood size

MALES. In Figure 2A, the overall pattern of change in body mass of male parents (n=49) during reproduction is depicted. Average body mass prior to brood size manipulation, *i.e.* at the start of the experiment, did not differ significantly between experimental groups ($F_2=1.90$, ns). Within-individual variation in body mass was analysed in a repeated measures design as changes relative to initial mass. Body mass of individual males varied with age of the brood ($F_5=59.94$, $P<.001$). Experimental brood size affected differentially the changes in body mass after manipulation ($F_{10}=2.45$, $P<.01$).

Changes in fat index ($\times 2$) of male parents are depicted in Figure 2B. Initial body fat reserves, as indicated by the fat index, were not significantly different between experimental groups prior to manipulation ($F_2=1.87$, ns). Fat index, unlike body mass, did not change significantly with age of the brood ($F_5=2.13$, $P=.07$). Fat indices after manipulation were not significantly affected by experimental brood size ($F_{10}=1.12$, ns).

FEMALES. Females (15.27 g, sd. 1.94, n=49) initially had a higher body mass than males (14.49 g, sd. 2.34, n=49), but the difference was not significant ($F_1=3.27$, $P=.07$). Figure 2C shows the changes in body mass during parental care of females of the three experimental categories. Initial body mass of females, *i.e.*, prior to manipulation, did not differ significantly between the three groups ($F_2=4.23$, $P=.02$). Again, within-individual variation in body mass was analysed with a repeated measures design relative to initial mass. As in males, female body mass changed with age of the brood ($F_5=74.29$, $P<.001$), and body mass of females also varied with experimental brood size ($F_{10}=1.99$, $P=.04$).

← Figure 1. Time allocation ($\text{min}\cdot\text{h}^{-1}$) to foraging, at the nest, preening, sitting, and locomotion, and number of feedings (h^{-1}) to brood (beakcontacts) during various stages of parental care. A. Males. B. Females. Means and standard errors of 22 pairs with experimentally manipulated brood sizes are given. Behaviour of parents of small broods is indicated by (\blacktriangle), of intermediate broods by (\bullet), and of large broods by (\blacksquare).

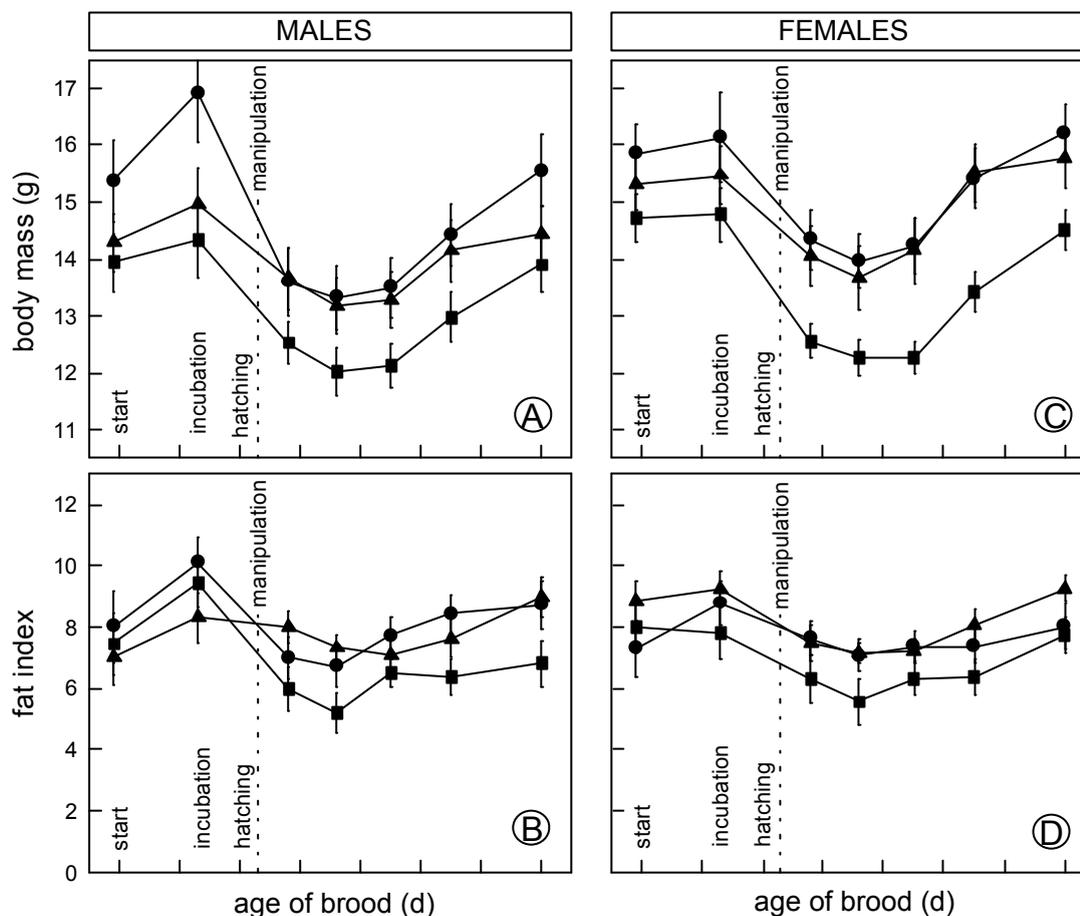


Figure 2. Body mass (a,c) and fat index (b,d) of parents of small (▲), intermediate (●), and large (■) broods. Sample sizes consisted of 19, 14 and 16 pairs respectively. Sample sizes during incubation were incomplete, 13, 9, and 9 pairs respectively. Means and standard errors are given. Fat indices were obtained by visual scores of breast and belly fat deposits. Samples of fat index were incomplete at the start of the experiment (7, 7, and 11 pairs respectively), and during incubation (14, 9, and 9 pairs respectively). A, B. Males. C, D. Females.

Variation in fat index ($\times 2$) of females of small, intermediate and large brood size is depicted in Figure 2D. Fat reserves of females at the start of the experiment did not differ significantly between experimental groups ($F_2=0.89$, ns). Unlike in males, fat index of females varied with age of the brood ($F_5=5.84$, $P<.001$). Experimental brood size did not significantly affect variation in female fat index with age of the brood ($F_{10}=0.51$, ns).

Parental daily energy expenditure (DEE) and brood size

MALES. Daily energy expenditure (DEE) of male parents on day 14 to 16 of the nestling phase is depicted in Figure 3A for each experimental group in relation to brood size during the DEE measurements. Energy expenditure of male parents was not related to brood size (Table 1A). Average manipulation (= number of nestlings added to the

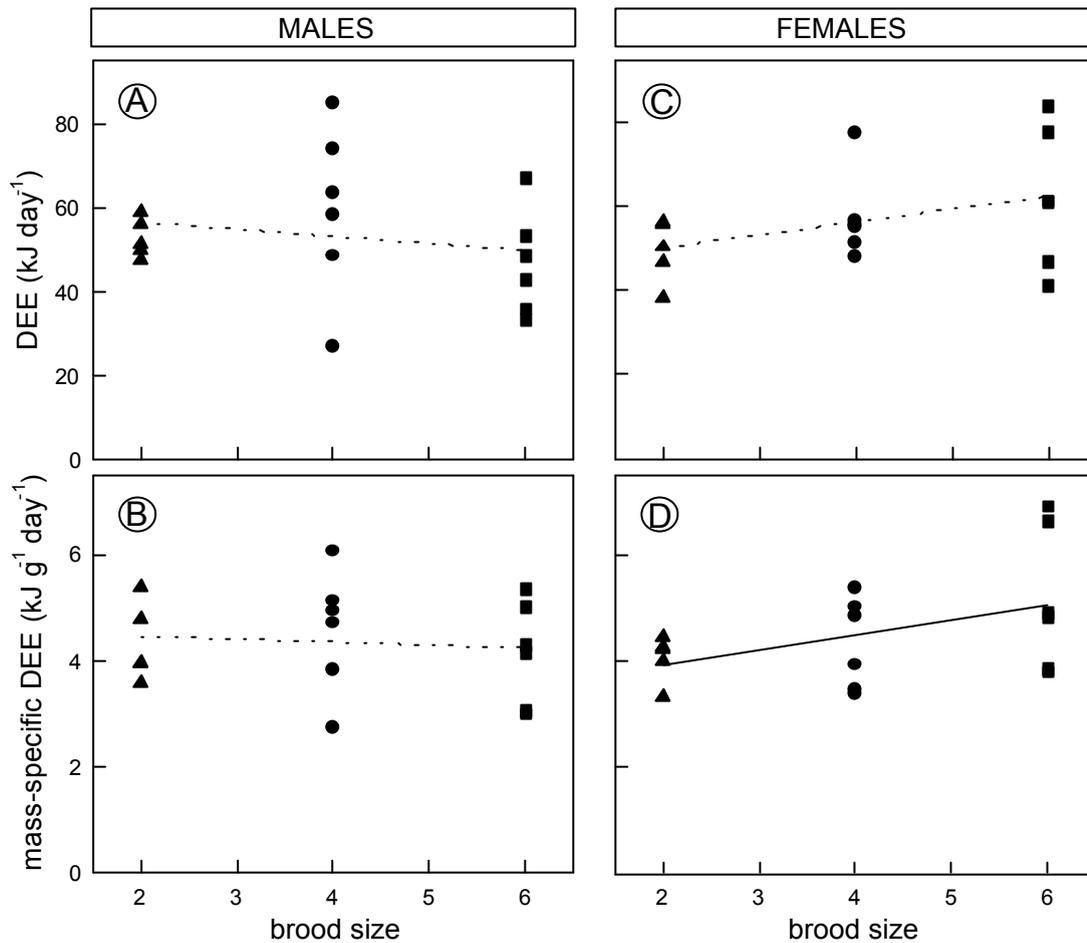


Figure 3. Daily energy expenditure (DEE), and mass-specific DEE of individual parents as a function of brood size (b). Regression lines are drawn. A, B. Males. Regressions ns. C, D. Females. $DEE = 39.5 + 4.66 b$, $r^2=0.31$, $n=18$, $P=.02$. $DEE_{ms} = 2.92 + 0.42 b$, $r^2=0.97$, $n=18$, $P<.01$. Symbols as in Figure 2.

original clutch size) of experimental brood size two was -2.5 nestlings ($sd=1.05$), of brood size four was -0.33 nestlings ($sd=0.52$), and of brood size six was $+1.67$ nestlings ($sd=1.21$). In three of six large broods, nestling mortality occurred just prior to or during the DEE measurements. One nestling died in one brood, in another brood two nestlings died, and in a third brood three nestlings died. The separate contributions of the three components of brood size during the measurements, *i.e.*, original clutch size, manipulation, and nestling mortality after manipulation were analysed in a multiple linear regression model (Table 1B). These components did not explain variation in male DEE.

Variation in DEE of males was affected by body mass ($\partial deviance=2364.6$, $P<.001$). To control for differences in mass between individuals, and to allow the comparison of rates not obscured by effects of body mass, rates of energy expenditure are often expressed as mass-specific rates, that is DEE_{ms} ($kJ \cdot g^{-1} \cdot day^{-1}$). Mass-specific DEE

Table 1. Linear regressions of Daily Energy Expenditure (DEE) and mass-specific DEE (DEE_{ms}) of male and female parents. P-values indicate significance of the contribution to the

explained variance of the model. A. Regressions on brood size during the DEE measurement (thus controlled for post-experimental nestling mortality). B. Regressions on components of brood size: original clutch size, manipulation (no. of nestlings added) and nestling mortality (no. of nestlings that died after experimental manipulation).

	(increase in) deviance	(increase in) df	P	(increase in) deviance	(increase in) df	P
A.						
Males:	--- DEE ---			--- DEEmS ---		
null model	3724.9	15	<.001	13.17	15	<.001
brood size	52.2	1	ns	0.01	1	ns
Females:						
null model	2564.3	17	<.001	18.49	17	<.001
final model	1781.2	16	<.011	12.04	16	<.001
brood size	783.1	1	=.02	6.45	1	<.01
B.						
Males:	--- DEE ---			--- DEEmS ---		
null model	3724.9	15	<.001	13.17	15	<.001
rejected terms:						
clutch size	370.1	1		0.54	1	
manipulation	11.6	1		0.02	1	
mortality	294.8	1		0.91	1	
Females:						
null model	2564.3	17	<.001	18.49	17	<.001
final model	1646.7	14	<.001	9.35	14	<.001
clutch size	453.7	1	=.07	7.08	1	<.01
manipulation	843.1	1	=.02	7.69	1	<.01
mortality	478.6	1	=.06	2.94	1	=.05

of males is shown in Figure 3B. Again, DEEmS was not related to brood size (Table 1A), or to the components thereof, *i.e.*, clutch size, manipulation, and nestling mortality (Table 1B).

FEMALES. Female energy expenditure is depicted in Figure 3C. DEE of females increased linearly with brood size (Table 1A). Accounting for the separate contributions of original clutch size, manipulation, and nestling mortality to DEE gave similar results. Both clutch size (coefficient=7.27, se=3.70) and manipulation (coefficient=4.90, se=1.83) increased DEE, while nestling mortality (coefficient=-7.43, se=3.86) reduced DEE. Interactions between the variables did not contribute to the explained variation (Table 1B). The variables were not related separately to DEE.

Unlike in males, energy expenditure of females was not related to body mass (Δ deviance=37.6, ns). Mass-specific rates of female energy expenditure are depicted in Figure 3d. DEE_{ms} of females increased significantly with brood size (Table 1A), and variation in DEE_{ms} could be contributed to all three components of brood size (Table 1B). Again, components of brood size were not related separately to DEE.

Variation in female energy expenditure was not due to intra-pair compensation (partial correlation of female DEE_{ms} and male DEE_{ms}, controlling for brood size: $r=0.19$, $n=16$, $P=.5$).

Gender-related differences in parental effort

Why did the response to experimental brood size in DEE differ between male and female parents? Within-pair differences in current (= day 16) and previous (= day 8) parental effort were investigated by comparing foraging and feeding behaviour (Experiment 1, Figure 1), and body mass (Experiment 2, Figure 2) with DEE (Experiment 3, Figure 3) of males and females. Although females increased their energy expenditure in response to the experimental brood size, while males did not, neither DEE ($n=16$, $P=.39$), nor DEE_{ms} ($n=16$, $P=.24$) differed consistently between partners. In observational data at this stage of the nestling phase, there was also no indication of behavioural differences between the sexes, neither in foraging time ($n=14$, $P=.37$), nor in feeding frequency ($n=13$, $P=.31$). There was also no indication of sex differences at an earlier stage (= day 8) in foraging time ($n=14$, $P=.37$) or feeding frequency ($n=11$, $P=.32$). Mass loss tended to be larger in males than in females early in the nestling phase (= day 8) ($n=48$, $P=.10$). This difference between the sexes in mass change since the start of the experiment was significant at day 16 ($n=49$, $P=.02$).

Daily energy expenditure, water flux rates and behaviour

DEE and water flux Water flux rates of parents nourishing a brood were large, and variable ($12.0 \text{ ml}\cdot\text{day}^{-1}$, $sd=3.6$, $n=34$), and exceeded the species-specific estimate (*c.* $6 \text{ ml}\cdot\text{day}^{-1}$) given by Calder (1964), or estimates based on allometric equations for captive birds (*c.* $5 \text{ ml}\cdot\text{day}^{-1}$) from Nagy & Peterson (1988).

Water flux rates were associated closely with mass-specific DEE, both in males ($T_{14}=3.62$, $P<.01$), and in females₁₆ ($T=4.53$, $P<.001$). The slopes ($T=0.10$, $P=.9$) and intercepts ($T_{27}=0.93$, $P=.4$) of the regressions of water flux on DEE_{ms} did not differ between the sexes. Average water flux rate in relation to DEE ($0.22 \text{ ml}\cdot\text{kJ}^{-1}$, $sd=0.06$, $n=34$) was far higher than evaporative water loss related to energy expenditure ($0.12 \text{ ml}\cdot\text{kJ}^{-1}$, Calder 1964). Oxidative water production in relation to energetic yield from food ($0.05 \text{ ml}\cdot\text{kJ}^{-1}$) was estimated based on water content of seeds of 25 %, assuming nutritive composition of seeds *c.f.* Gessaman & Nagy (1988), oxidative water production *c.f.* Schmidt-Nielsen (1991), and could not meet water requirements. I observed the parents drinking frequently, often immediately after feeding the brood.

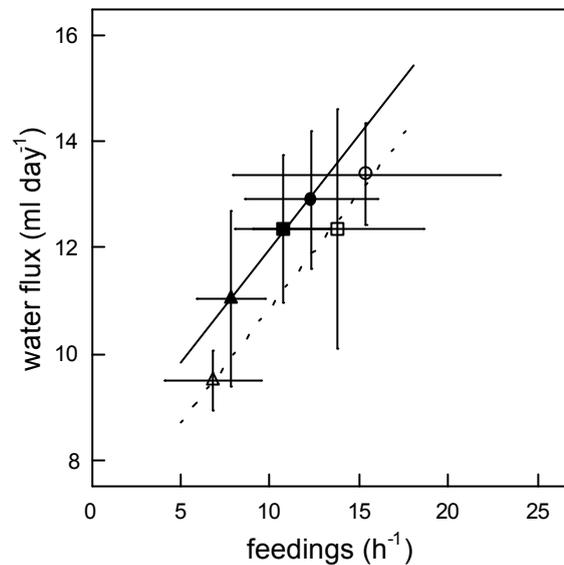


Figure 4. Water flux rates ($\text{g}\cdot\text{day}^{-1}$) of parents in relation to number of feedings (h^{-1}) to brood. Means and standard errors are given per group of male (filled symbols), or female (open symbols) parents of small, intermediate and large broods. Symbols as in Figure 2. Lines represent linear regression. Water flux = 0.49 feedings , male intercept = 8.33 , female intercept = 6.91 , $r^2=0.98$, $n=6$, $P<.01$.

DEE and foraging What may have caused the variation in DEE in response to brood size that we showed for female zebra finches? Foraging behaviour is a most likely factor, because it was the only aspect of behaviour that varied most obviously in accordance with experimental brood size. Data on energy expenditure and behaviour were obtained in two separate experiments with different birds. Mean values for each sex in each experimental group of foraging time on day 16 are derived from Experiment 2, and of DEEs from Experiment 3. Mass-specific DEE of males was not related to foraging time ($r^2=0.59$, $n=3$, $P=.4$). The data from the females of both experiments corresponded closely ($r^2=0.99$, $n=3$, $P=.03$). The slope of the association of female DEEs and foraging time was $0.010 \text{ kJ}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$.

Water flux and feeding rates An important aspect of parental care is feeding intensity of the brood. The mean number of feedings of male and female parents to broods of different sizes (Experiment 1), and average water flux rates as assessed in Experiment 3, are depicted in Figure 4. The average values of water flux and feeding frequencies showed a close correlation ($T_3=20.2$, $P<.001$), with a higher level for males than for females ($T_3=8.4$, $P<.01$).

Table 2. Reproductive interval (=days between hatching of experimental brood and laying date of first eggs of subsequent clutch) of parents of experimentally small, intermediate and large brood sizes. Means and standard deviation (between brackets) are given for pairs in Experiment 1 (behaviour), Experiment 2 (body mass), and Experiment 3 (DEE).

	Experimental groups			T	P
	Small	Intermediate	Large		
EXPERIMENT 1: behaviour					
sample size (pairs)	4	5	5		
reproductive interval (d)	31.5 (13.8)	34.2 (3.4)	39.0 (6.6)	1.38	ns
EXPERIMENT 2: body mass					
sample size (pairs)	11	11	14		
reproductive interval (d)	31.4 (10.4)	39.6 (6.8)	43.0 (11.5)	2.88	<.001
EXPERIMENT 3: DEE					
sample size (pairs)	6	5	6		
reproductive interval (d)	30.2 (9.7)	42.2 (3.6)	45.7 (9.2)	3.27	<.001

Reproductive interval

The main short-term cost of reproduction in zebra finches is the subsequent reproductive interval (Chapter 4). Having identified parental behavioural (foraging time, feeding frequency) and physiological factors (female body mass, DEEms and water flux rates of females) that are affected by brood size, these factors should in turn be examined for their effects on the reproductive interval. Reproductive intervals for each brood size category of the three experiments (body mass, behaviour, DEE) are listed in Table 2.

Reproductive interval of pairs in experiment 1 (n=14) did not show an association with brood size or time allocated to any of the behavioural categories.

In the sample of pairs subjected to experiment 2 (n=56), reproductive interval was associated negatively with body mass of female parents on most sampling occasions (day 8: $P=.03$; day 16, $P=.07$, day 25, $P=.01$, day 35, $P=.01$). However, female body mass did not add significantly to the explained variation in reproductive interval when included in a model with experimental brood size (b : $T_{34}=2.88$, $P<.01$).

Experiment 3 (n=16) comprised the most complete data set in terms of variables covered. Here, variation in reproductive interval could be tested in a model including experimental brood size, body mass, DEE and water flux. Mass-specific values of the latter two variables were used to account for their association with body mass. One pair was excluded from this analysis, because it had initiated a new clutch (on day 11) before the DEE measurements.

Reproductive interval increased with brood size ($T_{14}=3.12$, $P<.01$). In males,

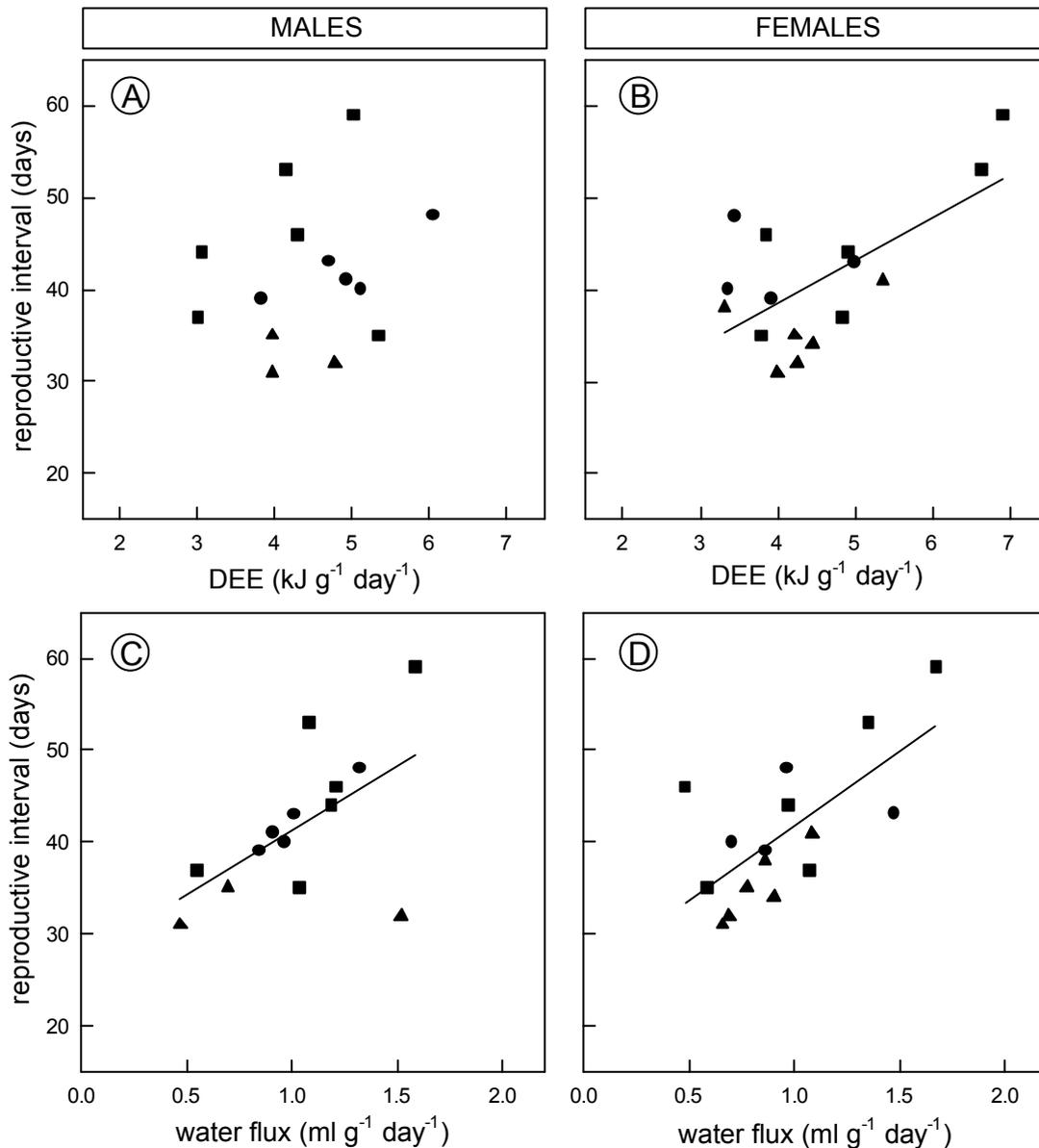


Figure 5. Reproductive interval (laying date of first egg of subsequent clutch in days since hatching of experimental brood) as a function of mass-specific DEE (A, C), or water flux (B, D) during the nestling phase of parents of small, intermediate and large broods. Symbols as in Figure 2. Linear regression lines are drawn. A, B. Males. DEEs: regression ns. Water flux: Interval = $27.3 + 14.0 \text{ flux ms}$, $r^2=0.33$, $n=14$, $P=.03$. C, D. Females. DEEs: Interval = $20.2 + 4.6 \text{ DEEs}$, $r^2=0.41$, $n=16$, $P<0.01$. Water flux: Interval = $25.8 + 16.0 \text{ flux ms}$, $r^2=0.46$, $n=16$, $P<.01$.

reproductive interval was not related to *DEEs* (Figure 5A), but increased with mass-specific water flux rate (Figure 5B). Only mass-specific water flux rate explained an additional amount of variation in reproductive interval ($T_{11}=2.18$, $P=.05$). In females, reproductive interval was

associated positively with both DEEMs (Figure 5C) and water flux rates (Figure 5D). Most of the variation in reproductive interval was explained by brood size together with mass-specific water flux rate ($T_{13}=3.18$, $P<.01$).

DISCUSSION

Parental effort: behavioural and physiological adjustments to brood size

Parent zebra finches showed behavioural and physiological responses to experimental variation in brood size. The observed increase in foraging time with experimental brood size may be viewed as a first order reaction to manipulation of a fitness parameter (= brood size). Second order reactions, *i.e.* physiological adjustments to experimental brood size, were mainly apparent in female parents. Female daily energy expenditure and water flux rates increased with brood size, whereas both male and female body mass was negatively affected by brood size. Length of the subsequent reproductive interval, a third order response to manipulation of brood size, was associated positively with the intensity of physiological processes in the females, *i.e.*, energy expenditure and water flux rates.

Growth of nestlings can only be accomplished when they are fed adequately by the parents. Consequently, behavioural adjustments to experimental brood size were most obvious in foraging activities. In Experiment 1, foraging time increased with experimental brood size. However, this did not result in an increase in feeding rates with brood size. This finding is inconsistent with most data on brood provisioning rates that have been obtained with clutch or brood size manipulations in field situations, and which show an increase with brood size (review in Lessells 1993). Feeding frequency of both parents combined was not proportional to the number of young in the brood, and the resulting feeding rates per young decreased with brood size. This was reflected in body mass of the young at the start of independence (=35 days of age), which also decreased with brood size (Chapter 4).

Although adjustment of feeding behaviour of the parents to the demands of the brood was incomplete, there was a shift in time allocation to foraging that was associated with brood size. This shift most likely was accomplished by a reduction of time allocated to behaviour aimed at self-maintenance (sitting, preening). Apparently, there was no time constraint: birds were inactive, *i.e.*, sitting, for *c.* 30% of the daylight hours. Potential functions of apparently inactive behaviour are a) (pre-)digestion of food for the young, and b) compensation for otherwise high rates of energy expenditure to reduce overall energy expenditure. The first suggestion does not seem to apply, because the brood was usually fed immediately following a bout of active foraging. Economizing on other energy intensive processes, *e.g.*, immunological defence, in reaction to high rates of energy expenditure during the day, such as manipulated parental effort, has been shown by Apanius *et al.* (1994; Chapter 7), and may also have been achieved by an adaptive reduction in body mass.

Effects of experimental brood size on physiological parameters were observed most consistently in female parents. In Experiment 2, mass changes in relation to brood size occurred in parents of both sexes. Loss of body mass may reflect previous effort, and may be indicative of a depletion of stored energetic or nutrient reserves. Or body mass may be adaptive to current effort (Freed 1981; Norberg 1981; Houston 1993). From the negative relationship of changes in body mass with brood size, it may be concluded that parental effort increased with brood size.

The observations on body mass from Experiment 2 corresponded to data on parental effort of females from Experiment 3, which showed an increase in female daily energy

expenditure (DEE) with brood size. DEE of females was related to brood size during the measurement, and not specifically to manipulated change in brood size or nestling mortality. In *ad libitum* food availability, such as in this experiment, it seemed that brood size - as a representative of the actual situation - instead of clutch size - as a representative of parental ability or quality - was the major determinant of energy expenditure of female parent zebra finches. Strategic reproductive decisions such as clutch size may be of limited importance with respect to costs of reproduction due to the absence of (ecological) risks, *e.g.*, predation or starvation, in the laboratory.

It remains intriguing why male parents did not adjust their energy expenditure to brood size. In this respect, physiological measures of parental effort (mass and DEE) were inconsistent with the observations on parental behaviour: male and female time budgets did not differ. This apparent discrepancy may be due to differences between pairs, in combination with small sample sizes in Experiment 1. In addition, the setup of Experiment 1 may not have allowed identification of changes in the temporal pattern of behaviour, *e.g.*, changes in the time budget may have occurred early in the morning, or at the end of the day, at times that were not covered by the observations.

It is thus concluded from the physiological estimates of parental effort that female zebra finches adjusted their effort during the late nestling phase to experimentally manipulated brood size, but male parents did not. Measures of parental effort in terms of energy expenditure in response to experimental manipulation of brood size, have been reported from several other species. Gender differences in parental effort also have been reported for Pied Flycatchers (*Ficedula hypoleuca*), with males, but not females, adjusting their effort to the manipulated brood size (Moreno *et al.* 1995). In two studies, on the Great Tit (*Parus major*) (Verhulst 1995), and on the Starling (*Sturnus vulgaris*) (J.Wright pers. comm.), both male and female parents failed to show a physiological response, either in DEE or in body mass. Two other studies report an increase in effort of both sexes with experimental brood size (Savannah Sparrow *Passerculus sandwichensis*: Williams 1987; Kestrel *Falco tinnunculus*: Deerenberg *et al.* 1995). This variety of responses in terms of physiological effort may reflect different, or mixed strategies. Such strategies may be related to reproductive tactics of both parents, *e.g.*, dependent on effort of the partner (*e.g.* Winkler 1987), environmental possibilities, *e.g.*, food supply allowing a change in diet (*e.g.* Tinbergen 1981), species-specific characteristics, *e.g.*, foraging technique (Peterson, Nagy & Diamond 1990; Bryant & Tatner 1991), or individual abilities, *e.g.*, body condition or territory quality of the species and specimens involved.

Potential causes of high rates of energy expenditure

Parental effort in relation to experimental brood size was apparent in foraging behaviour, and in variation in DEE of female parents. Therefore, differences in DEE between groups are most likely related to aspects of feeding behaviour. Average female DEE of each experimental category measured in Experiment 3 was closely related to average foraging time assessed in Experiment 1. The marginal cost of foraging estimated as the slope of the regression of female DEEs on foraging time was $10.3 \text{ J}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$. This value far exceeds the cost of foraging of $2.7 \text{ J}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ estimated by Lemon (1993) in a similar situation with respect to body mass and food availability. Lemon (1993) measured the increase in oxygen consumption rate of birds that were foraging after overnight fasting. Apparently, parents feeding nestlings have additional energetic costs other than the costs of foraging activity alone.

What could have caused the extra increase in DEE above the costs of foraging? The slope of the association between DEE and water flux rates indicates intake and loss of water

beyond the effects of hard work, *i.e.*, metabolic water production. Obviously, nestlings need water for growth and for thermoregulation. In crop feeders like zebra finches, water is provided together with the food. Parents were seen drinking frequently, but the extent of water flux rates as assessed from turnover rates of labeled hydrogen indicates that water transferred to the young was drawn from the parental body water pool. The food on offer (dry seeds) had a low water content, and probably absorbs water while being collected and stored in the crop of the parent. Water flux rate and DEE were associated, but they are not necessarily causally linked (Lifson & McClintock 1966), because DEE is estimated from the difference in disappearance rate of labeled oxygen through water (expired, excreted, or regurgitated) and CO₂ (expired). It is mainly the fraction of water that is lost through expiration which is related to energy turnover. Thus, if large water fluxes are due to transfer of crop fluid to the young - relative to the amount of (dry!) seeds fed, and counteracted by high intake rates through drinking - high rates of water flux may occur while rates of energy expenditure remain largely unaffected.

Energy costs as a result of high water flux rates may arise for two reasons. Firstly, there may be costly osmotic activity associated with production and excretion of saliva (Vonk 1978) or crop fluid. Secondly, the food transferred to the young, while it was soaked in crop fluid, also may have absorbed enzymes (*e.g.* amylase of salivary origin, Ziswiler & Farner 1972), thus causing a 'protein drain'. If so, a mechanism of increased levels of protein turnover is suggested, in which proteins are mobilized from stored body reserves to supply enzymes lost to the young. This process requires water (hydrolysis) and energy. Other indications for this hypothesis are derived from a virtually absent reduction in parental mass during the nestling phase when birds were provided with a protein supplement to the standard seed diet (Deerenberg, unpublished data).

DEE instrumental in trade-off: the role of the female

Female parental effort thus manipulated by brood size negatively affected future reproduction by delaying the subsequent clutch. This study assessed a quantitative association between DEE and reproductive interval among individuals within each experimental group. Such a direct association between parental energy expenditure and costs of reproduction in individuals has only been shown previously in survival rates of European Kestrels (*Falco tinnunculus*) (Deerenberg *et al.* 1995), and, in male Great Tits (*Parus major*), in the size of second clutches (Verhulst 1995). In the latter study, DEE of female Great Tits, however, was associated positively with the success of the second brood, thus apparently contradicting a cost of reproduction in relation to previous energy expenditure (Verhulst 1995). It was not clear whether this effect was due to previous energy expenditure only, or confounded by effects of the experiment on, *e.g.*, timing of the second clutch, which in turn may have affected its fledging success. The observed associations between DEE and a residual fitness component are correlative, however, and not necessarily causal. To test for causality, additional experimentation is needed, *e.g.* in which rates of energy expenditure are manipulated independently of parental care (Chapter 6) to avoid the influence of the presence of first brood young on reproductive decisions of the parents.

Costs of reproduction in this study with captive zebra finches appeared in the delayed timing of subsequent reproduction (Chapter 4). Whether laying date in general is determined by the male or the female is difficult to assess due to significant behavioural interaction between the sexes, *e.g.* induction of reproductive activities in males by hormonal treatment of females (Moore 1982; Runfeldt & Wingfield 1985), or courtship feeding (Meijer *et al.* 1989). In multiple breeders, it seems likely that the female plays a crucial role in the timing or initiation of

second or subsequent clutches, in view of her (body) resources involved in the energetically and nutritionally demanding process of egg production (Houston *et al.* 1995a,c).

ACKNOWLEDGEMENTS

This study was supported by NWO-BION grant 436.912P to S.Daan, J.M.Tinbergen, and J.P.Kruijt. Permission for the animal experiment (nr. BG00192) was obtained from the Animal Experimentation Committee of the University of Groningen. We wish to thank Y.Boorsma for assistance with the experiments and especially the observations. We also thank A.Strijkstra for fruitful discussions, and J.Tinbergen and S.Daan for commenting on earlier drafts of the paper.