CLUTCH SIZE AND PARENTAL EFFORT IN THE GREAT TIT PARUS MAJOR

S. VERHULST[1,2] & J.M. TINBERGEN[2]

We experimentally reduced clutch size of Great Tits *Parus major* to investigate the effects on parental care (including Daily Energy Expenditure, *DEE*, measured with doubly labelled water), and the relationship between *DEE* and the residual reproductive value. The length of a working day was not affected, but the nestling phase was 0.7 days (4%) shorter in experimental clutches. Males fed reduced broods at a lower rate, but *DEE* and mass (loss) were not significantly affected by the experiment. However, male *DEE* was positively correlated with feeding frequency, and this relationship did not differ between control and experimental ♂ ♂. This suggests that the experiment resulted in a slight reduction in male *DEE*, and lack of statistical significance can be attributed to the power of the test applied. Females fed reduced broods at a lower rate, but female *DEE* and mass (loss) were not affected by the clutch size manipulation. Female *DEE* was not correlated with feeding frequency, the original number of eggs in the nest or the actual number of young. The division of labour (feeding rate, *DEE*) was not affected by the clutch size manipulation. Female *DEE* was significantly higher than male *DEE*, and this could be caused by the fact that only the ♀ roosts with the young. There was no consistent relationship between *DEE* and parental survival or future reproductive success. Since the experimental reduction in clutch size led to an increase in residual reproductive value (more second clutches), but not to a decrease in *DEE*, this costs of reproduction is apparently not contingent on increased rate of energy turnover. It is suggested that birds with equal *DEE* may differ in their residual reproductive value as a consequence of variation in energy allocation between reproductive effort and e.g. aspects of physiological maintenance and repair.

Key words: *Parus major* - clutch size - parental effort - daily energy expenditure

[1]Netherlands Institute for Ecology, Centre for Terrestrial Ecology, P.O.Box 40, 6666 ZG Heteren, The Netherlands; Present address: [2]Zoological Laboratory, University of Groningen, P.O.Box 14, 9750 AA Haren, The Netherlands; E-mail: s.verhulst@biol.rug.nl.
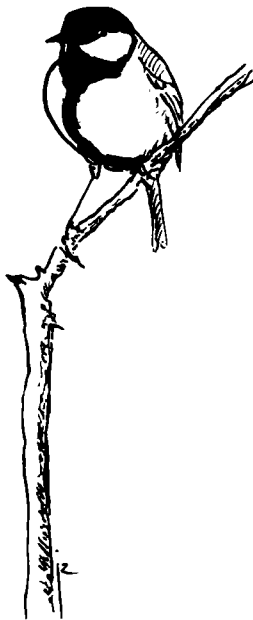
INTRODUCTION

Raising offspring is associated with both costs and benefits (Clutton-Brock 1991), and hence parental effort can be expected to be optimized (Williams 1966, Charnov & Krebs 1974, Houston & Davies 1985, Lazarus & Inglis 1986, Winkler 1987, Daan *et al.* 1990, Kacelnik & Cuthill 1990, Lessells 1993). Parental effort is often assumed to be proportional to clutch size (e.g. Charnov & Krebs 1974), but this relationship may be more complex (Winkler & Wallin 1987). Then the contribution of the clutch to the parents' fitness is a function of both clutch size and parental effort, while the residual reproductive value (the contribution to the parents' fitness of future reproduc-

tive attempts) is a function of only parental effort. Therefore, to understand the relationship between clutch size and residual reproductive value, it is necessary to have knowledge of the relationship between clutch size and parental effort, as well as the relationship between parental effort and the residual reproductive value.

Feeding rate is often used as a measure of parental effort, and feeding rate usually increases with manipulated brood size (Lessells 1993). However, feeding is only one aspect of parental care, and an estimate of parental effort should also include resources allocated to other forms of parental care such as brooding, deterring predators and territorial behaviour. This is important because there may be trade-offs between different forms of parental care (Verhulst 1995). Furthermore, parents may change their foraging behaviour in response to brood size manipulation (e.g. Tinbergen 1981), in which case the effort per feed is no longer comparable between brood sizes. Energy expenditure, which can be measured in free-living birds using the doubly labelled water technique (Lifson *et al.* 1955), integrates various aspects of parental care such as feeding and brooding, and thereby provides a potential currency for parental effort. To our knowledge there are only three studies of the relationship between (manipulated) brood size and parental energy expenditure (Williams 1987, Deerenberg *et al.* 1995, Moreno *et al.* 1995).

We manipulated clutch size in Great Tits *Parus major* and measured daily energy expenditure (*DEE*) of both parents during brood rearing. Manipulations are required because natural variation in brood size may be confounded with variation in individual and/or environmental quality (Grafen 1988, Gustafsson & Sutherland 1988, Tinbergen & Daan 1990). Clutch size manipulations solve this problem, as long as parents respond similarly as to a natural intra-individual change in clutch size. The effect of the clutch size manipulation on feeding frequency and time spent brooding young was also studied, because these activities can potentially play a role in the explanation of the experimental effects on *DEE*. Furthermore,

with respect to the investment of time, the number of hours per day that parents fed their young was recorded, as well as the total duration of the nestling period.

The experimental reduction in clutch size enhanced the residual reproductive value of the parents: experimental pairs were more likely to start a second clutch, and experimental pairs were more successful in fledging young from second clutches (Verhulst 1995). To investigate the role of *DEE* in the mechanisms mediating the costs of reproduction, the relationship between *DEE* and components of the residual reproductive value was evaluated.

The relationship between parental effort and residual reproductive value may differ between the sexes, which could result in a differential response of the sexes to the clutch size manipulation (Moreno *et al.* 1995). Therefore the effect of the clutch size manipulation on the division of labour will also be evaluated.

METHODS

Study area and standard methods

Field work was carried out on Vlieland, an island in the Dutch Wadden Sea. There is one large woodland on Vlieland (the village wood), and four smaller woodlands. Woodlands consist mainly of pine, with patches of oak. See Verhulst & van Eck (1996) for more information. Experiments were carried out in all woodlands, but *DEE* measurements and behavioural observations were restricted to the village wood. Nest boxes were checked at least once a week during the breeding season. Laying date and clutch size were recorded. Nests were checked daily around the expected day of hatching to establish the hatching date. Adults were captured with spring traps when the young were 7-14 days old, and subsequently identified, weighed and measured. When the young were 15 days old they were ringed. At the same time mass, tarsus and wing length were measured. Broods were visited daily in late afternoon from day 16 onwards to establish fledging

date. Ages are in days after hatching (day of hatching = 0).

Clutch size manipulations

The experiments were carried out during three years (1989-1991). Dyads of clutches with approximately the same laying date (maximum difference of two days) and clutch size (maximum difference of one egg) were selected. Taking laying date, clutch size and distribution of the clutches over the different woods into account, in each dyad one randomly chosen clutch was manipulated. Fifty percent of the eggs were replaced by artificial eggs, approximately six days after the last egg was laid. There were no clutch enlargements. Prior to egg-replacement the nest-box was observed from a distance until the ♀ was seen to leave the nestbox. Then the eggs were replaced as quickly as possible to avoid detection by the breeding pair. The artificial eggs were removed from the nest with the same procedure one day after the eggs hatched. The effect of the experiment on components of fitness is described elsewhere (Verhulst 1995). In addition to the clutch size manipulations experiments were carried out with second clutches in 1990 and 1991. Pairs with manipulated second clutch were omitted from the analysis of the relationship between *DEE* and components of the residual reproductive value after the second clutch was laid.

Recording parental behaviour

The number of hours between the first and the daily last visit of the nest box was recorded using a threshold attached to a microswitch placed in the nest entrance. Visits to the nestbox were marked on a paper sheet strapped around a rotating cylinder (see Kluyver 1933 for a drawing). Initially, the threshold would be below the nest entrance and one or two days after the threshold was installed it would be placed a little higher, in such a way that the tits would activate the switch when going through the nest entrance. Nest boxes were observed to record parental behaviour from as great a distance as vegetation permitted (usually 50-100 m). As a rule, each observation period las-

ted two hours. Each visit of the nest box, and the time of visit, was recorded for both sexes. Feeding visits usually take only a few seconds, but the ♀ (never the ♂), sometimes remained in the nest box for a longer period, presumably brooding the young. Variation in time spent brooding is discussed elsewhere (Verhulst 1995).

Data analysis

Analysis of effects of different parameters on variation in parental behaviour was carried out with means per nest box to avoid pseudo-replication. Averages of observations were taken per nest for different age-categories and analysed separately for different age categories. Male and female feeding frequency were also analysed separately. The experimental effect on feeding and brooding was evaluated in multiple regression models, including year and age of the young, regardless of the significance of these parameters. Before calculation of the means, it was investigated if feeding frequency depended on time of day and if the effect of nestling age on feeding frequency was curvilinear. All observations on first broods were used except those which were carried out to see if ♂ ♂ resumed work after being injected with doubly labelled water (see below). These tests were carried out by adding variables to a model containing year (as a factor), manipulation category and age of the young. Age squared was not significantly correlated with feeding rate (♂ ♂ : $F_{1,264} = 0.2$, N.S.; ♀ ♀ : $F_{1,264} = 0.8$, N.S.). Time of day half-way the observation was taken as time, but this did not correlate significantly with feeding rate (♂ ♂ : $F_{1,264} = 0.4$, N.S.; ♀ ♀ : $F_{1,264} = 0.3$, N.S.). Feeding rate may for example be high early morning and/or late afternoon, but time of day squared when added to the model containing time of day did not explain a significant part of the variance (♂ ♂ : $F_{1,263} = 0.5$, N.S.; ♀ ♀ : $F_{1,263} = 0.2$, N.S.). Thus we conclude that feeding rate was linearly related to nestling age, and independent of time of day.

Energy expenditure

DEE was measured simultaneously in a con-

trol bird and an experimental bird that hatched their young on the same day. The original dyads in the experiment could not always be used because the control and experimental pair could breed in different woodlands, while *DEE* was measured in the village wood only. New dyads were formed of a control and an experimental pair with the same hatching date and approximately equal laying date and clutch size. In 1989, with one exception, *DEE* was measured in only one bird of each breeding pair. In later years we attempted to measure *DEE* of both members of a pair simultaneously.

Males were captured during the day when the young were eleven days old with a radio-activated trapping device that was installed in the nest box one or two days before capture. The birds were weighed, injected with doubly labelled water, and kept in a cloth bag for one hour for equilibration of the isotopes in the body fluid (Williams & Nagy 1984, Williams 1985). After equilibration, the first blood sample was taken and the ♂ ♂ were released. The ♂ ♂ were recaptured to collect the second blood sample 48 hours later. The whole brood was weighed and the number of young was counted when blood samples were taken. Approximately 1-2 hours after the initial blood sample of the ♂ was taken we returned to the nest box to check if the ♂ ♂ had resumed feeding. Feeding rates could be compared with similar observations that were done in the morning before the ♂ was captured. The ♂ ♂ resumed feeding in most cases, although they usually appeared cautious at first. However, some ♂ ♂ did not resume feeding at a normal rate during these observations, and were obviously reluctant to enter the nest box. When this happened (2 ♂ ♂ in 1989, 2 ♂ ♂ in 1990, 0 ♂ ♂ in 1991), we abandoned that measurement, and did not measure *DEE* of the ♀.

In addition to the observations of feeding rate before and after the initial blood sample was collected, data on feeding rate were also collected on the next day, and on the third day, prior to collection of the final blood sample. The observations that were carried out to check if the ♂ had re-

sumed feeding were not used in any of the analyses.

Females roost with the young. On the night between the 11th and the 12th day after hatching the ♀ was taken from the nest. The ♀ ♀ were weighed, and doubly labelled water was injected. After injection, the whole brood was weighed, the number of young was counted, and the ♀ was returned to the nest. We returned an hour after the injection to collect the first blood sample. After 24 hours we returned, again weighed the bird and the brood, and collected the second blood sample. Thus, energy expenditure of the ♂ ♂ was measured over a period of 48 hours, and energy expenditure of the ♀ ♀ was measured over a period of 24 hours, which fell completely within the period that male energy expenditure was measured. Data on feeding rate were collected during the day between the collection of the first and second blood sample. Some ♀ ♀ did not sleep in the nest box during the night that the second blood sample would otherwise be taken. In these cases the ♀ was captured the next morning to collect the second blood sample. The proportion of ♀ ♀ that did not sleep in the nestbox during the second night did not differ between control (3 out of 15) and experimental (3 out of 16) ♀ ♀.

Blood was drawn from the brachial vein on the wing, or from a vein on the leg, and collected in heparinized glass capillaries that were sealed directly after sampling. Concentration of [18]O and Deuterium was determined by mass spectrometry (Masman & Klaassen 1987) at the Centre for Isotope Physics of the University of Groningen. Calculation of CO_2 production was done according to Lifson & McClintock (1966), using an RQ of 0.75, and an energy equivalence of 19.9 kJ(L O_2)[-1]. The water content of individuals was assumed to be 66% of the body mass (Mertens 1987). The time elapsed between the collection of the initial and the final sample was rarely an exact multiple of 24 hours. For ♀ ♀, the ratio of night to day hours increased as the period of measurement increases, while for ♂ ♂ this ratio decreased. Because a systematic difference in energy expenditure between day and night can be expected this

needs to be corrected for. For $\female\female$, measurements were adjusted following the procedure of Tinbergen & Dietz (1994). The absolute change in *DEE* caused by this correction was only 1.4% of uncorrected *DEE* (SD = 2.7%, n = 31). This method could not be applied to $\male\male$ because no independent estimate of day-time metabolism is available. However, energy expenditure per 24 hours was independent of the duration of the measurement-period (R^2 = 0.01, n = 35, $F_{1,33}$ = 0.2, N.S.), possibly because variation in the measurement-period was small (Table 3A). We conclude that a possible bias is sufficiently small to be ignored.

Background concentrations of ^{18}O and Deuterium

In each year blood samples were collected from birds where no doubly labelled water was injected to measure the background concentration of ^{18}O and Deuterium. These values are used in the calculation of the CO_2-production. Due to an accident, background samples of 1990 were not available, and therefore all calculations were carried out with background levels as measured in 1989 (n = 4) and 1991 (n = 8). Background levels of Deuterium differed significantly between years (t-test, P < 0.01), but there was no significant difference between years in background level of ^{18}O (t-test, P = 0.2). The mean of the annual mean isotope enrichment (in ‰ relative to Standard Mean Ocean Water) was used for both isotopes in all calculations of CO_2-production (Deuterium: 7.108 ‰SMOW; ^{18}O: 2.0634 ‰SMOW). The absence of background samples for 1990 could result in biased estimates of CO_2 production. However, when year specific background values were

used in calculation of *DEE* for 1989 and 1991, instead of the mean of the annual means, the absolute changes in *DEE* were smaller than one per cent ($\male\male$: $\bar{x}$ = 0.9%, SD = 0.2, n = 19; $\female\female$: $\bar{x}$ = 0.3%, SD = 0.8, n = 20). This suggests that consequences of possible errors in the estimates of background levels can be ignored.

RESULTS

The number of hours between the first and the last recorded visit of the day was 15.3 h (SD = 0.49, n = 24 broods), and did not differ between years ($F_{2,21}$ = 0.3, P = 0.6). A working-day was slightly shorter for parents feeding an experimentally reduced brood but this difference was not significant (control pairs: $\bar{x}$ = 15.45 h, SD = 0.48, n = 13; experimental pairs: $\bar{x}$ = 15.14 h, SD = 0.47, n = 11, $F_{1,22}$ = 2.5, P = 0.1). Controlling for date did not change this result.

With some exceptions, all young of a brood fledged on the same day. The mean age at which broods fledged was 18.4 days (Table 1; range 15-25 days). Experimental broods fledged 0.7 days earlier than control broods (Table 1; $F_{1,109}$ = 4.1, P < 0.05, controlling for year: $F_{2,109}$ = 4.9, P < 0.01).

Feeding frequency in $\male\male$

Age-ranges used are I: 1-5 days (n = 25 nests, 98 hours of observation), II: 6-10 days (n = 37 nests, 105 h) and III: 11-14 days (n = 65 nests, 270 h). The number of young during the observations was significantly lower in experimental clutches in all three age-categories (Table 2; con-

Table 1. Fledging age (in days) of young in control and experimental clutches. At day of hatching age = 0.

	Control clutches			Experimental clutches		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n
1989	18.5	1.3	23	17.9	1.3	22
1990	18.5	1.3	16	17.6	2.1	18
1991	19.4	2.4	15	18.9	1.7	19

Table 2. Reproductive parameters, feeding frequency (feeds h⁻¹) and proportion of time spent brooding of control and experimental birds. (A) Age-category I, 1-5 days. (B) Age-category II, 6-10 days. (C) Age-category III, 11-14 days after hatching.

	1989 Control			1989 Reduced			1990 Control			1990 Reduced			1991 Control			1991 Reduced		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
(A) Age-category I																		
Age of nestlings (days)	-	-	-	-	-	-	3.6	1.1	5	3.6	0.9	6	3.2	0.6	7	3.2	0.6	7
Number of nestlings	-	-	-	-	-	-	8.4	1.1	5	3.4	1.0	6	7.4	1.2	7	3.6	1.1	7
Hours observed	-	-	-	-	-	-	3.2	1.8	5	3.9	2.1	6	3.9	1.2	7	4.4	1.6	7
Male feeding rate, ♂ h⁻¹	-	-	-	-	-	-	11.8	8.5	5	6.6	5.2	6	7.1	2.7	7	5.4	2.6	7
Female feeding rate, ♂ h⁻¹	-	-	-	-	-	-	6.5	4.4	5	3.3	1.0	6	4.7	2.2	7	3.9	3.5	7
Female brooding	-	-	-	-	-	-	0.52	0.24	5	0.45	0.35	6	0.44	0.08	7	0.56	0.19	7
(B) Age-category II																		
Age of nestlings, days	-	-	-	-	-	-	8.7	1.2	10	7.4	1.4	7	7.1	1.5	9	7.5	1.2	11
Nestlings, n	-	-	-	-	-	-	8.1	1.9	10	3.6	1.1	7	7.4	1.2	9	3.5	1.0	11
Hours observed	-	-	-	-	-	-	2.8	1.1	10	2.9	1.6	7	2.7	1.0	9	3.0	1.6	11
Male feeding rate, ♂ h⁻¹	-	-	-	-	-	-	24.6	7.5	10	10.7	5.4	7	15.1	9.3	9	8.4	5.4	11
Female feeding rate, ♂ h⁻¹	-	-	-	-	-	-	14.4	5.9	10	6.5	6.0	7	12.0	8.1	9	8.0	7.6	11
Female brooding	-	-	-	-	-	-	0.04	0.05	10	0.41	0.30	7	0.20	0.13	9	0.33	0.22	11
(C) Age-category III																		
Age of nestlings, days	12.0	0.6	12	12.1	0.4	13	11.8	0.5	12	11.8	5.9	10	12.2	0.4	7	12.2	0.3	11
Nestlings, n	6.3	1.7	12	3.8	0.7	13	8.4	1.0	12	4.1	1.0	10	6.0	1.3	7	3.9	0.7	11
Hours observed	3.5	1.9	12	3.6	1.4	13	4.7	2.1	12	4.0	1.8	10	4.8	0.9	7	4.7	1.3	11
Male feeding rate, ♂ h⁻¹	21.6	11.9	12	19.3	7.3	13	30.6	10.1	12	9.8	7.7	10	19.4	8.5	7	13.9	8.6	11
Female feeding rate, ♂ h⁻¹	24.9	14.2	12	14.9	10.0	13	25.8	8.3	12	14.8	5.9	10	14.5	10.0	7	16.7	8.0	11
Female brooding	0.06	0.06	12	0.07	0.08	13	0.01	0.02	12	0.06	0.11	10	0.01	0.02	7	0.03	0.03	11

trolling for year, all $P < 0.0001$). Laying date, original clutch size, hatching date and age of young during the observations did not differ significantly between control and experimental pairs in any age-category. Experimental ♂ ♂ fed their broods at a lower rate than control ♂ ♂ in all three age categories (Fig. 1A), and this effect was significant in the two oldest categories (age-category I: $b = -3.3$ feeds h⁻¹, $P < 0.09$; II: $b = -9.2$ feeds h⁻¹, $P < 0.001$; III: $b = -9.7$ feeds h⁻¹, $P < 0.001$). The experimental effect differed significantly between years in the oldest age category ($P < 0.001$), but not in the other two categories.

Daily energy expenditure in ♂ ♂

The individual ♂ ♂ whose *DEE* was measured were a subset of all birds used in the experiment. However, control and experimental ♂ ♂ did not differ significantly in the criteria used in the selection of the original dyads: laying date and clutch size (Table 3A), and there was no significant difference in hatching date between control and experimental broods (Table 3A). Male *DEE* was on average 85.2 kJ d⁻¹ (SD = 14.9, $n = 35$). Males did not significantly alter their *DEE* in response to the clutch size manipulation (Fig. 2A; $F_{1,31} = 2.2$, $P = 0.15$; controlling for year: $F_{2,31} = 3.5$, $P < 0.05$), and there was no significant interaction between year and the clutch size manipulation ($F_{2,29} = 0.2$, $P = 0.8$). Comparing *DEE* of control and experimental ♂ ♂ within dyads measured simultaneously with a paired *t*-test could provide a more sensitive test, since it accounts for day-to-day variation in *DEE*, but this
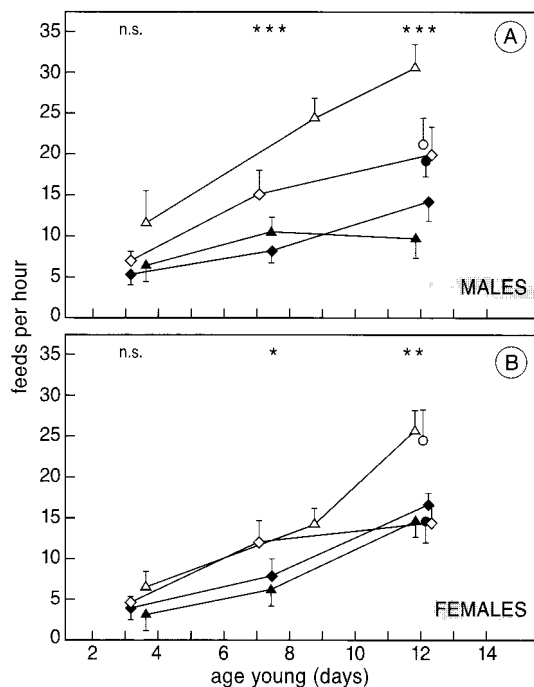
Fig. 1. Feeding rate (± SE) of (A) ♂ ♂ and (B) ♀ ♀ rearing control or experimentally reduced clutches at different ages. 1989: ○ control, ● reduced; 1990: △ control, ▲ reduced; 1991: ◊ control, ◆ reduced. N.S. not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

did not change the result ($n = 11$ dyads, $P = 0.8$). Furthermore, *DEE* was not correlated with the number of young in the nest at the time of measurement, with the clutch size before or after manipulation, or with the mass of the whole brood.

Experimental ♂ ♂ fed their broods at a lower rate, and feeding rate was significantly correlated with *DEE* (Fig. 3A; $b = 0.6$ kJ per visit h^{-1}, $P < 0.005$, controlling for year). This correlation contrasts with the absence of an experimental effect on *DEE*, because this relationship (Fig. 3A) did not differ in slope or elevation between control and experimental ♂ ♂. On the basis of the relationship between feeding rate and *DEE* among control ♂ ♂, and the experimental effect on feeding rate, an experimental effect on *DEE* of -9.8 kJ would have been expected, which is close to the

(non-significant) value of -6.9 kJ that was found. This suggests the absence of a significant experimental effect on male *DEE* was due to insufficient sample sizes, and not due to an inconsistency in the data. This is confirmed by a power-analysis (following Zar 1984), which showed that, given our sample sizes and variance, the probability of detecting a difference of 9.8 kJ was only 55%.

Mass and mass loss in ♂ ♂

Male mass ($n = 156$) was not significantly correlated with the time of day or nestling age at capture. More importantly, there was no significant effect of the experiment on male mass (Table 3A; $b = 0.03$ g, SE = 0.11, $n = 156$, controlling for year). In agreement with these results, the change in mass between the first and second blood sample in ♂ ♂ where *DEE* was measured was not significantly different from zero ($b = 0.09$ g, SE = 0.06, $n = 35$), and not significantly affected by the experiment.

Feeding frequency in ♀ ♀

Feeding rate of control ♀ ♀ was higher than feeding rate of experimental ♀ ♀ in all three age categories (Fig. 1B), and this effect was significant in the two oldest categories (age-category I: experimental effect -1.8 feeds h^{-1}, $P < 0.09$; II: experimental effect -5.2 feeds h^{-1}, $P < 0.03$; III: experimental effect -7.2 feeds h^{-1}, $P < 0.01$). The effect of the manipulation on feeding rate did not differ significantly between years in any age category (although the interaction was close to significance in the oldest age-category: $P < 0.08$).

Daily energy expenditure in ♀ ♀

Clutches of control and experimental ♀ ♀ did not significantly differ in laying date, clutch size or hatching date (Table 3B). *DEE* was on average 97.0 kJ d^{-1} (SD = 15.0, $n = 31$), and was not affected by the experiment (Fig. 2B; $F_{1,27} = 0.0, P > 0.9$, controlling for year $F_{2,27} = 0.3, P = 0.7$). The interaction between year and the experiment approached significance ($F_{2,25} = 2.9, P < 0.08$). *DEE* was also compared within experimental dyads with a paired-t-test, but this yielded the same re-

Table 3. Reproductive parameters, daily energy expenditure and mass of control and experimental birds. Hatching date is in April days, counting on from April 1st. (A) ♂♂, (B) ♀♀. Notes: [a] Adult mass at the start of measurement of *DEE*. [b] Mass of all birds captured when young were 7-14 days old.

|	1989 Control			1989 Reduced			1990 Control			1990 Reduced			1991 Control			1991 Reduced		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
(A) ♂♂																		
Laying date	32.0	4.1	4	29.8	3.9	5	18.8	6.0	9	20.8	4.8	6	21.2	3.1	6	20.0	3.1	5
Clutch size	9.0	0.8	4	8.6	0.5	5	9.0	0.9	9	9.2	1.3	6	8.7	1.0	6	9.2	0.4	5
Hatching date	53.0	3.5	4	50.8	4.8	5	40.3	5.3	9	42.0	4.2	6	43.3	4.1	6	42.2	3.3	5
Nestlings, n[a]	7.3	1.0	4	4.2	0.8	5	8.6	1.0	9	4.5	0.8	6	7.0	1.1	6	3.8	0.8	5
Mass[a], g	18.0	0.3	4	18.0	0.3	5	18.0	0.9	9	17.6	1.1	6	18.4	0.7	6	18.2	0.6	5
Mass[b], g	17.8	0.6	33	17.8	0.7	28	17.8	0.7	23	17.7	0.8	20	17.6	0.7	26	17.8	0.6	26
Hours between samples	49.0	0.9	4	49.3	1.0	5	48.8	0.8	9	48.1	1.7	6	48.3	0.8	6	48.0	0.9	5
DEE, kJ d^{-1}	92.4	18.2	4	90.0	8.3	5	82.4	18.3	9	72.6	16.1	6	92.7	5.5	6	85.8	10.9	5
(B) ♀♀																		
Laying date	25.5	6.8	4	27.2	6.9	5	19.3	6.8	7	22.0	3.4	5	19.5	1.9	4	21.5	4.6	6
Clutch size	7.8	0.5	4	8.0	0.7	5	9.0	0.8	7	9.4	1.5	5	9.0	0.8	4	9.2	0.4	6
Hatching date	45.8	5.3	4	47.8	6.5	5	40.7	6.0	7	43.2	2.5	5	41.3	3.0	4	43.5	4.4	6
Nestlings, n[a]	6.3	2.1	4	3.8	0.8	5	8.4	1.0	7	4.6	0.9	5	7.0	1.4	4	3.8	0.8	6
Mass[a], g	17.7	0.3	4	17.6	0.3	5	17.3	0.5	7	17.5	0.6	5	17.6	0.5	4	17.8	0.6	6
Mass[b], g	17.5	0.7	30	17.4	0.9	33	17.4	1.0	20	17.6	0.6	23	17.2	0.9	28	17.5	1.1	26
Hours between samples	26.6	4.7	4	24.4	0.4	5	26.9	4.7	7	30.8	6.0	5	24.4	0.6	4	24.2	0.1	6
DEE, kJ d^{-1}	105.6	13.2	4	86.3	14.2	5	97.5	16.6	7	103.1	15.4	5	88.6	7.1	4	100.2	16.2	6
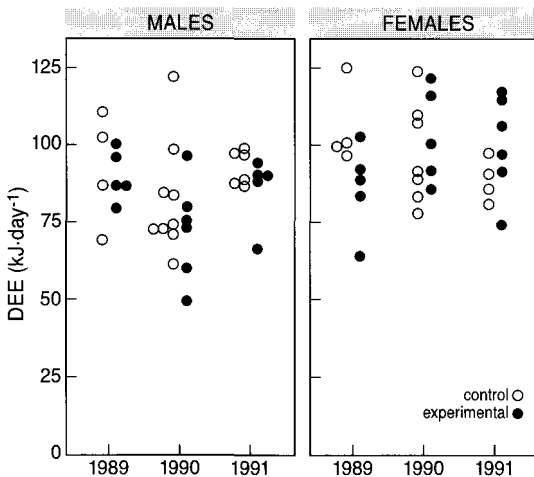
Fig. 2. Daily energy expenditure (*DEE*) of parents rearing control or experimental clutches in the three years of study. (A) ♂♂. (B) ♀♀.

sult ($P = 0.4$, $n = 10$ dyads). Furthermore, the number of young in the nest, the mass of the whole brood or clutch size before or after manipulation did not significantly correlate with female *DEE*. Considering sample sizes and variance in our study, an experimental effect of 15.7 kJ would have been detected in 80% of cases (calculated following Zar 1984). Thus it is reasonable to assume that if an (undetected) experimental effect exists it is probably smaller than 15.7 kJ. However, there was no significant correlation between feeding rate and *DEE* (Fig. 3B; $P > 0.6$, controlling for year), which is in agreement with the absence of an experimental effect on *DEE*.

Mass and mass loss in ♀♀

Controlling for year, ♀♀ increased in mass during the day ($b = 0.10$ g h^{-1}, SE = 0.02, $n = 160$, $P < 0.001$), and decreased in mass in the course of
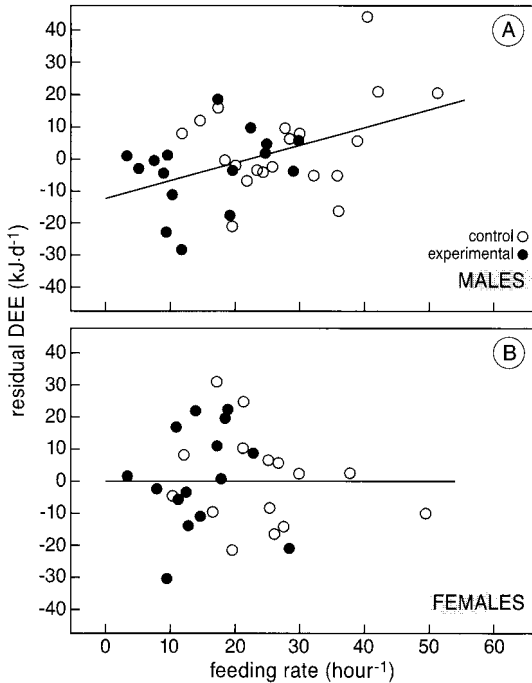
Fig. 3. Residual daily energy expenditure (*DEE*) (corrected for variation between years) and feeding frequency of (A) ♂♂ and (B) ♀♀ rearing control or experimental.

the nestling period (b = -0.14 g d^{-1}, SE = 0.05, P < 0.006). Controlling for these variables, there was no significant effect of the experiment on female mass (b = 0.19 g, SE = 0.13, N.S.). There was no significant interaction between the experiment and the age of the nestlings at the day of capture. The change in mass between the collection of the two blood samples was -0.22 g (SE = 0.06, n = 31), significantly smaller than zero (P < 0.01), and of the order expected on the basis of the analysis of all captures (see above). The change in mass was not significantly affected by the experiment, and not significantly correlated with the number of young, feeding rate or *DEE*.

Division of labour

Male feeding rate was higher than female feeding rate in the youngest age-category, (♂♂: $\bar{x}$ = 7.4, SD = 5.2; ♀♀: $\bar{x}$ = 4.5, SD = 3.0, n = 25

pairs; paired-t-test, t_{24} = 3.4, P < 0.003), and in the middle age-category (♂♂: $\bar{x}$ = 14.9, SD = 9.4; ♀♀: $\bar{x}$ = 10.4, SD = 7.4, n = 37 pairs; paired-t-test, t_{36} = 3.3, P < 0.003). In the oldest age-category male and female feeding rates were very similar (♂♂: $\bar{x}$ = 19.4, SD = 11.1; ♀♀: $\bar{x}$ = 19.0, SD = 10.7, n = 65 pairs, paired-t-test, t_{64} = 0.6, P = 0.6). The proportion of female feeds (of the total feeding rate) did not significantly differ between control and experimental pairs in any age-category (all P > 0.3). This suggests that the response to the experiment did not differ between the sexes.

DEE of ♂♂ and ♀♀ was compared within pairs (n = 21). Female *DEE* was higher than male *DEE* (within pair comparison: difference: 13.8 kJ d^{-1}, paired-t-test: t_{20} = 3.2, P < 0.005), despite the fact that ♂♂ had higher mass (difference: 0.6 g,
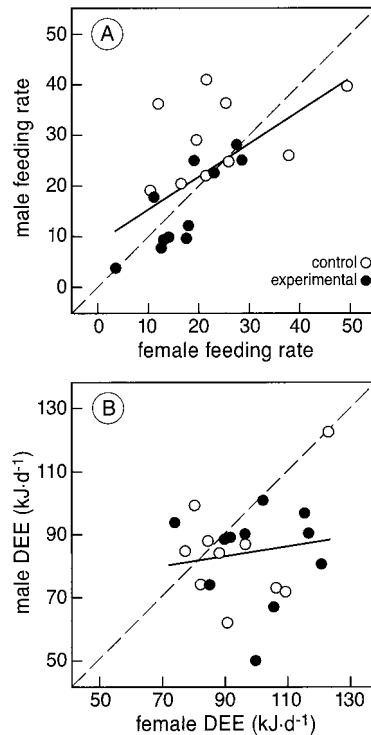

Fig. 4. Within-pair comparison of (A) feeding rate, (B) daily energy expenditure.

paired-t-test: $t_{20} = 3.1$, $P < 0.01$). Male and female feeding frequency were significantly correlated in this data-set (Fig. 4B; $R^2 = 0.38$, $n = 21$, $P < 0.003$), but there was no correlation between male and female DEE (Fig. 4B; $R^2 = 0.02$, $n = 21$, $P > 0.5$).

DEE and the residual reproductive value in ♂♂

The relationship between DEE and components of the residual reproductive value was evaluated. The fitness-components that were studied are: the occurrence of second clutches, clutch size and success of the second clutch, and parental survival until the next breeding season (survival was defined as being recaptured in the next breeding season). In 1989 only one ♂ of which DEE was measured started a second clutch, and therefore the analysis of the relationship between male DEE and multiple breeding was restricted to 1990

(60% with second clutch, $n = 15$) and 1991 (82% with second clutch, $n = 11$). Controlling for year, the occurrence of second clutches was not significantly correlated with male DEE (DEE of ♂♂ with second clutch was 0.2 kJ higher, SE = 6.5, $n = 26$, $P > 0.9$). However, male DEE was negatively correlated with clutch size of the second clutch (Fig. 5A; $b = -0.05$ egg kJ^{-1} d^{-1}, SE = 0.02, $n = 18$, $P < 0.02$, controlling for year, overall $R^2 = 0.38$). The number of young that fledged from the second clutch was not significantly correlated with male DEE ($b = -0.07$, SE = 0.05, $n = 11$, $P = 0.2$), although the slope of the regression line was approximately the same as in the regression of clutch size on male DEE. (Sample size is smaller than in the previous analysis, because second clutches that were manipulated were omitted). There was no difference in DEE between surviving and non-surviving ♂♂ ($t_{33} = 0.6$, $P = 0.6$).

DEE and the residual reproductive value in ♀♀

In 1989 no second clutch was started by a ♀ where DEE was measured, while in 1991 all ♀♀ started a second clutch, and this hampers the analysis of the relationship between DEE and multiple breeding. In 1990 multiple breeding was not correlated with female DEE (one clutch: $\bar{x} = 102.2$ kJ d^{-1}, SE = 7.0, $n = 5$; two clutches: $\bar{x} = 98.2$ kJ d^{-1}, SE = 6.3, $n = 7$, $t_{10} = 0.4$, $P = 0.7$), suggesting there is no relationship between DEE and multiple breeding. This is confirmed by two other comparisons. Firstly, DEE did not differ between 1989 and 1991, which differed greatly in the proportion of pairs that started a second clutch (1989, no second clutch: $\bar{x} = 94.9$ kJ d^{-1}, SE = 5.5, $n = 9$; 1991, all ♀♀ second clutch: $\bar{x} = 95.6$ kJ d^{-1}, SD = 4.5, $n = 10$, $t_{17} = 0.1$, $P = 0.9$). Secondly, hatching date and the clutch size manipulation, two factors that are strongly related to the occurrence of second clutches (Verhulst 1995, Verboven & Verhulst 1996), were not related to female DEE (multiple regression analysis, controlling for year, both $P > 0.4$).

DEE was not significantly correlated with clutch size of the second clutch ($b = -0.02$ egg kJ$^-$
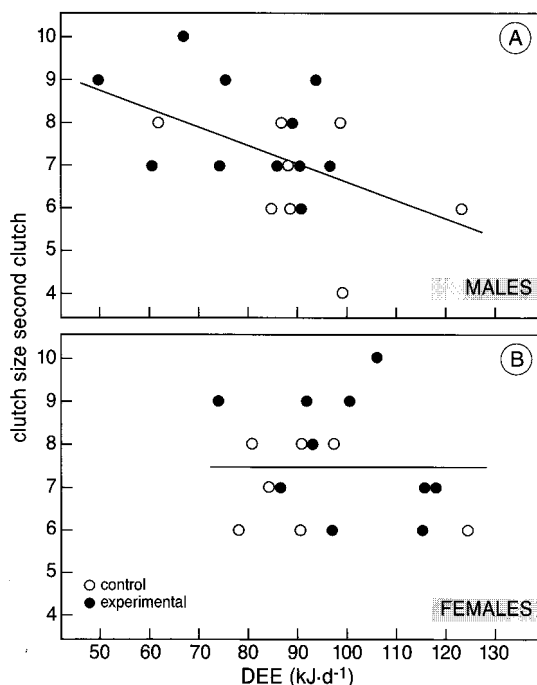
Fig. 5. Daily energy expenditure and clutch size of the second clutch of (A) ♂♂ and (B) ♀♀ rearing control or experimental clutches.
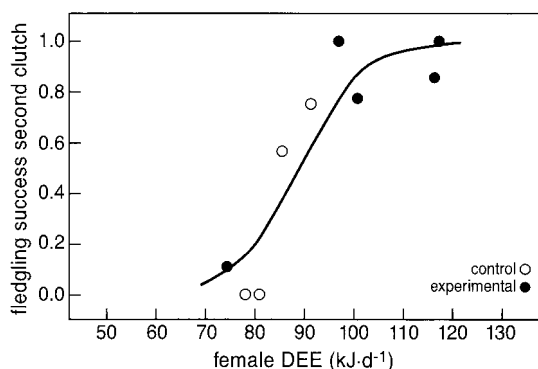
Fig. 6. Daily energy expenditure and fledging success (number of fledglings/number of eggs) of the second clutch of ♀♀ of which the first clutch was a control clutch or an experimental clutch. Line calculated with logistic regression.

[1] d^{-1}, SE = 0.02, n = 17, P = 0.4, controlling for year). The number of young that fledged from the second clutch was correlated with female DEE, but this correlation was *positive* (b = 0.16, SE = 0.04, R^2 = 0.69, n = 9, P < 0.01). This was due to a correlation between female DEE and fledging success (= number of fledglings/number of eggs, Fig. 6; analysed with arcsine transformed values, R^2 = 0.66, n = 9, P < 0.01), and not due to a relationship between female DEE and clutch size (Fig. 5B). There was no difference in DEE between surviving and non-surviving ♀♀ (t_{29} = 0.4, P = 0.7).

DISCUSSION

Young from experimental broods fledged at a younger age (as in Smith *et al.* 1987). Parental care continues after fledging, but in our study the duration of post-fledging care was not affected by the experiment (Verhulst & Hut 1996). The experimental effect on the duration of the nestling phase could largely be explained by enhanced development of flight-feathers in experimental broods (Verhulst 1995), in accordance with other studies that showed correlations between feather development and fledging age (e.g. Lack 1956,

Gibb & Betts 1963, Smith *et al.* 1987, Nilsson 1990, Viñuela & Bustamante 1992, Keller & van Noordwijk 1994). This relationship implies that birds can deliver food at a lower rate, in combination with a prolongation of the nestling phase: there is a trade-off between the duration and intensity of parental care. The optimal solution to this trade-off will depend on the fitness-consequences for the young of variation in growth rate, and the consequences for the residual reproductive value of the parents of different combinations of duration and intensity of parental care.

Division of labour

When the young were small the ♀ spent a large proportion of her time brooding (Verhulst 1995), and ♂♂ took a larger share in food provisioning. At higher ages the ♀ brooded the young only a small proportion of the day, and the difference in feeding rate between ♂♂ and ♀♀ disappeared. However, male DEE was 9% lower than female DEE, despite the fact that ♂♂ had higher mass. Only the female roosts with the young and it seems possible that the ♀ expends energy on brooding, which could explain the difference between the sexes in DEE. It must be noted that a difference between the sexes in DEE does not necessarily imply a difference in parental investment (Trivers 1972), because ♂♂ take more risk during brood defence (e.g. Curio & Onnebrink 1995).

In species with biparental care, the optimal level of investment depends on the level of investment of the partner (Chase 1980, Houston & Davies 1985, Winkler 1987). Theoretically, a negative correlation between the effort of two pair-members is expected. In contrast to this prediction, feeding rate of ♂♂ and ♀♀ was positively correlated, while there was no significant correlation between male and female DEE (Fig. 4). In retrospect, this is not surprising when we take into account that this concerns natural variation in feeding rate and DEE. Both members of a pair breed in the same environment (e.g. raise the same number of young) and therefore a positive correlation in feeding rate is not surprising. Due

to confounding variables, the relationship be-
tween level of investment of the two members of
a pair can only be investigated through manipula-
tion of the level of investment of one of the birds
(see e.g. Wright & Cuthill 1989).

Clutch size and energy expenditure

The clutch size manipulation did not have a
significant effect on male *DEE*, although *DEE* of
experimental ♂ ♂ was lower in each of the three
years (Table 3A). Males reduced their feeding
rate when clutch size was experimentally reduced
(Fig. 1A), and male *DEE* was significantly corre-
lated with feeding rate (Fig. 3A). The reduction in
DEE of experimental ♂ ♂ was in agreement with
what would have been expected on the basis of
the relationship between *DEE* and feeding rate,
but a power-analysis revealed that the probability
of significantly detecting such an effect was only
55%. This suggests that the experiment did have
an effect on male *DEE*, and that lack of statistical
significance can be attributed to the power of the
test applied.

Female *DEE* was not affected by the clutch
size manipulation, which is consistent with the
absence of a correlation between *DEE* and feed-
ing rate in ♀ ♀ (Fig. 3B). The absence of an ex-
perimental effect contrasts with the results of a
comparable study carried out with Great Tits
breeding in the Hoge Veluwe, The Netherlands,
where experimental reduction of brood size re-
sulted in a significant decrease in female *DEE*
(Tinbergen & Verhulst unpubl.). The absence of a
decrease in *DEE* could arise because ♀ ♀ with re-
duced broods are already investing in the second
clutch, but this does not seem likely because in
the Hoge Veluwe study the experimental effect on
second clutches was approximately the same as
on Vlieland, while clutch size reduction did result
in a decrease in female *DEE*. Furthermore, inter-
clutch interval was not correlated with *DEE*, and
there was no manipulation × interval interaction
(Verhulst unpubl.).

Why would Great Tits on Vlieland and the
Hoge Veluwe differ in their response to a clutch
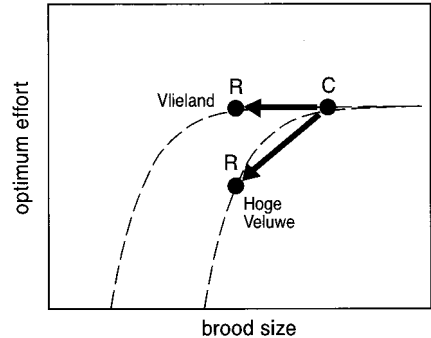size manipulation? Winkler & Wallin (1987) pre-

Fig. 7. Hypothetical relationship between brood size
and optimal parental effort on Vlieland and the Hoge
Veluwe (broken lines). Solid circles are a schematic
representation of *DEE* of ♀ ♀ with control broods (C)
and experimentally reduced broods (R) on Vlieland and
the Hoge Veluwe.

dicted on theoretical grounds that optimal effort
increases with increasing brood size, but at a de-
creasing rate. Thus at some point a substantial
change in brood size would lead to only a small
change in effort. A possible explanation of the ab-
sence of an experimental effect on female *DEE* is
that the optimal effort had already levelled off,
and the difference between this study and the re-
sults obtained on the Hoge Veluwe suggest the
curve representing optimal effort as a function of
brood size differs between Vlieland and the Hoge
Veluwe (Fig. 7). It is not clear what caused this
shift, but the brood size at which optimal effort
levels off is determined by the relationship be-
tween parental effort and the associated costs and
benefits, and either of these relationships could
differ between the two populations studied.

A differential response to brood size manipu-
lation between the sexes has previously been re-
corded in Pied Flycatchers *Ficedula hypoleuca*
(Moreno *et al.* 1995) and Collared Flycatchers *F.
albicollis* (Verhulst & Gustafsson unpubl.), but
studies of Great Tits (Smith *et al.* 1988) and Star-
lings *Sturnus vulgaris* (Wright & Cuthill 1990)
failed to find an effect of brood size manipulation
on the division of labour. In our study there was a
suggestion that only ♂ ♂ responded to the manip-
ulation with a reduction in *DEE*, but the sexes did

not differ significantly in this respect. This suggests that in great tits there is no large difference between the sexes in the costs associated with variation in feeding rate and/or *DEE*.

Mass

Parental mass may reflect aspects of their physiological state, parental effort prior to the measurement and/or a strategic decision to regulate flight costs (Freed 1981, Norberg 1981, Houston 1993). Birds rearing young often lose mass (Moreno 1989), and we found that ♀♀, but not ♂♂, lost mass during the nestling period. However, clutch size manipulation did not significantly affect female mass. This is in agreement with the results of the Great Tit brood size manipulation studies of Tinbergen (1987) and Smith *et al.* (1987), but not with the results of Lindén (1990), who found that ♀♀ with experimentally reduced broods had slightly higher mass. The absence of an experimental effect on female mass loss confirms that ♀♀ did not change their energy expenditure in response to the clutch size manipulation.

Parental effort, energy expenditure and the costs of reproduction

The experimental reduction in clutch size led to an increase in residual reproductive value (increased production of second clutches, Verhulst 1995), but not to a decrease in *DEE*, which shows that there are costs of reproduction which are apparently not contingent on increased rate of energy turnover. This is in agreement with the results of a comparative analysis, which also failed to reveal associations between metabolic rate and life history traits (Trevelyan *et al.* 1990). An alternative mechanism for the experimental effect on second clutches involves the effect of brood size on the trade-off between investment in successive clutches, and this is discussed elsewhere (Verhulst & Hut 1996, Verhulst *et al.* 1997).

It is a longstanding issue as to how reproductive effort should be defined (Hirshfield & Tinkle 1975, Kozlowski 1991). Some authors have advocated the use of daily energy expenditure, because

in this way various aspects of parental care can be integrated (e.g. Drent & Daan 1980). In our study there was no clear relationship between *DEE* and (components of) the residual reproductive value and this argues against this suggestion. However, the absence of such an effect does not necessarily imply that energy expenditure does not play a role in mediating the costs of reproduction. Since we found no clear experimental effects on *DEE* we studied in essence the effect of natural variation in *DEE* on fitness components, and natural variation in *DEE* may have been confounded with variation in environmental and/or phenotypic quality. This reasoning is supported by the study of Deerenberg *et al.* (1995) of the Kestrel *Falco tinnunculus*, in which birds adjusted their *DEE* to manipulated brood size, and hence a negative correlation between *DEE* and the residual reproductive value emerged.

An alternative explanation for the absence of a correlation between *DEE* and the residual reproductive value is compensation of hard work with energy-savings on other energy-consuming processes. Birds that feed their offspring at a high rate may for example compensate energy allocated to work with a reduction in, e.g. immunological defence (Sheldon & Verhulst 1996), physiological maintenance and repair (e.g. feather regrowth, White *et al.* 1991) or reducing body temperature at night (e.g. Reinertsen & Haftorn 1986, Daan *et al.* 1989). Compensation could also be achieved by altering behaviour, e.g. by foraging for cheaper prey (Tinbergen 1981). Variable results have been obtained in studies that have related *DEE* to feeding frequency (Bryant 1988, Pärt *et al.* 1992, Fig. 3 in this study), and compensation processes could potentially explain this variation (Wilson & Culik 1993).

Thus birds with equal *DEE* may differ in their residual reproductive value as a consequence of variation in energy allocation between reproductive effort and e.g. aspects of physiological maintenance and repair. This is in agreement with the proposition of Gadgil & Bossert (1970) that reproductive effort is the proportion of resources that is allocated to reproduction. This does not rule out

the possibility that the absolute level of energy-flow also plays a role (Drent & Daan 1980, Deerenberg et al. 1995), in which case ultimately combined knowledge of the absolute level of metabolism, and the energy-allocation may be required to understand the relationship between *DEE*, the concept of parental effort, and the costs of reproduction.

ACKNOWLEDGEMENTS

REFERENCES

Bryant D.M. 1988. Energy expenditure and body mass changes as measures of reproductive costs in birds. Funct. Ecol. 2: 23-34.

Charnov E.L. & J.R. Krebs 1974. On clutch size and fitness. Ibis 116: 217-219.

Chase I.D. 1980. Cooperative and noncooperative behaviour in animals. Am. Nat. 115: 827-857.

Clutton-Brock T.H. 1991. The evolution of parental care. Princeton University Press, Princeton.

Curio E. & H. Onnebrink 1995. Brood defence and brood size in the Great Tit (*Parus major*): a test of a model of unshared parental investment. Behav. Ecol. 6: 235-241.

Daan S., D. Masman, A.M. Strijkstra & S. Verhulst 1989. Intraspecific allometry of basal metabolic rate: relations with body size, temperature, composition, and circadian phase in the Kestrel *Falco tinnunculus*. J. Biol. Rhythms 4: 267-283.

Daan S., C. Dijkstra & J.M. Tinbergen 1990. Family planning in the Kestrel (*Falco tinnunculus*): the ultimate control of covariance of laying date and clutch size. Behaviour 114: 83-116.

Deerenberg C., I. Pen, C. Dijkstra, B-J. Arkies, G.H. Visser & S. Daan 1995. Parental energy expendi-ture in relation to manipulated brood size in the European Kestrel *Falco tinnunculus*. Zool. - Anal. Complex Syst. 99: 39-48.

Drent R.H. & S. Daan 1980. The prudent parent: energetic adjustments in avian breeding. Ardea 68: 225-252.

Freed L.A. 1981. Loss of mass in breeding Wrens; stress or adaptation? Ecology 62: 1179-1186.

Gadgil M. & W. Bossert 1970. Life history consequences of natural selection. Am. Nat. 104: 1-24.

Gibb J.A. & M.M. Betts 1963. Food and food supply of nestling tits (Paridae) in Breckland Pine. J. Anim. Ecol. 32: 489-533.

Grafen A. 1988. On the uses of data on lifetime reproductive success. In: T.H. Clutton-Brock (ed.) Reproductive success: 454-471. University of Chicago Press, Chicago.

Gustafsson L. & W.L. Sutherland 1988. The costs of reproduction in the Collared Flycatcher *Ficedula albicollis*. Nature 335: 813-817.

Hirshfield M.F. & D.W. Tinkle 1975. Natural selection and the evolution of reproductive effort. Proc. Nat. Acad. Sci. USA 72: 2227-2231.

Houston A.I. 1993. The efficiency of mass loss in breeding birds. Proc. R. Soc. Lond. B 254: 221-225.

Houston A.I. & N.B. Davies 1985. The evolution of cooperation and life history in the Dunnock, *Prunella modularis*. In: R. Sibly & R. Smith (eds) Behavioural ecology: the ecological consequences of adaptive behaviour: 471-487. Blackwell Scientific Publications, Oxford.

Kacelnik A. & I. Cuthill 1990. Central place foraging in Starlings (*Sturnus vulgaris*). II Food allocation to chicks. J. Anim. Ecol. 59: 655-674.

Keller L.F. & A.J. van Noordwijk 1994. Effects of local environmental conditions on nestling growth in the Great Tit *Parus major* L.. Ardea 82: 349-362.

Kluyver H.N. 1933. Bijdrage tot de biologie en de ecologie van den Spreeuw (*Sturnus vulgaris vulgaris* L.) gedurende zijn voortplantingstijd. Ph.D. thesis, University of Wageningen, Wageningen.

Kozlowski J. 1991. Optimal energy allocation models - an alternative to the concepts of reproductive effort and cost of reproduction. Acta Oec. 12: 11-33.

Lack D. 1956. Swifts in a tower. Methuen & Co., London.

Lazarus J. & I.R. Inglis 1986. Shared and unshared parental investment, parent-offspring conflict and brood size. Anim. Behav. 34: 1791-1804.

Lessells C.M. 1993. The cost of reproduction: do experimental manipulations measure the edge of the options set? Etología 3: 95-111.

Lifson N. & R. McClintock 1966. Theory of use of the turnover rates of body water for measuring energy and material balance. J. Theor. Biol. 12: 46-74.

Lifson N., G.B. Gordon & R. McClintock 1955. Measurement of total carbon dioxide by means of $D_2^{18}O$. J. Appl. Phys. 7: 704-710.

Lindén M. 1990. Reproductive investment and its fitness consequences in the Great Tit *Parus major*. Ph.D. thesis, University of Uppsala, Upsala.

Masman D. & M. Klaassen 1987. Energy expenditure for free flight in trained and free living Kestrels. Auk 104: 603-616.

Mertens J.A.L. 1987. The influence of temperature on the energy reserves of female Great Tits during the breeding season. Ardea 75: 73-80.

Moreno J. 1989. Strategies of mass change in breeding birds. Biol. J. Linn. Soc. 37: 297-310.

Moreno J., R.J. Cowie, J.J. Sanz & R.S.R. Williams 1995. Differential response by males and females to brood manipulations in the Pied Flycatcher *Ficedula hypoleuca*. J. Anim. Ecol. 64: 721-732.

Nilsson J-Å. 1990. What determines the timing and order of nest-leaving in the Marsh Tit (*Parus palustris*)? In: J. Blondel, A. Gosler, J-D. Lebreton & R. McCleery (eds) Population biology of passerine birds: an integrated approach. Nato series G 24: 369-379. Springer-Verlag, Berlin.

Norberg R.Å. 1981. Temporary weight decrease in breeding birds may result in more fledged young. Am. Nat. 118: 838-850.

Pärt T., L. Gustafsson & J. Moreno 1992. 'Terminal investment' and a sexual conflict in the Collared Flycatcher (*Ficedula albicollis*). Am. Nat. 140: 868-882.

Reinertsen R.E. & S. Haftorn 1986. Different metabolic strategies of northern birds for nocturnal survival. J. Comp. Physiol. B 156: 655-663.

Sheldon B.C. & S. Verhulst 1996. Ecological immunology: costly parasite defence and trade-offs in evolutionary ecology. Trends Ecol. Evol. 11: 317-321.

Smith H.G., H. Källander, K. Fontell & M. Ljungström 1988. Feeding frequency and parental division of labour in the double-brooded Great Tit *Parus major*: effects of manipulating brood size. Behav. Ecol. Sociobiol. 22: 447-453.

Smith H.G., H. Källander & J-Å. Nilsson 1987. Effect of experimentally altered brood size on frequency and timing of second clutches in the Great Tit. Auk 104: 700-706.

Tinbergen J.M. 1981. Foraging decisions in Starlings (*Sturnus vulgaris*). Ardea 69: 1-67.

Tinbergen J.M. 1987. Costs of reproduction in the Great Tit: intraseasonal costs associated with brood size. Ardea 75: 111-122.

Tinbergen J.M. & S. Daan 1990. Family planning in the Great Tit (*Parus major*): optimal clutch size as integration of parent and offspring fitness. Behaviour 114: 161-190.

Tinbergen J.M. & M.W. Dietz 1994. Parental energy expenditure during brood rearing in the Great Tit (*Parus major*) in relation to body mass, temperature, food availability and clutch size. Funct. Ecol. 8: 563-572.

Trevelyan R., P.H. Harvey & M.D. Pagel 1990. Metabolic rates and life histories in birds. Funct. Ecol. 4: 135-141.

Trivers R.L. 1972. Parental investment and sexual selection. In: B. Campbell (ed.) Sexual selection and the descent of man, 1871-1971: 136-179. Aldine-Atherton, Chicago.

Verboven N. & S. Verhulst 1996. Seasonal variation in the incidence of double broods: the date hypothesis fits better than the quality hypothesis. J. Anim. Ecol. 65: 264-273.

Verhulst S. 1995. Reproductive decisions in Great Tits: an optimality approach. Ph.D. thesis, University of Groningen, Groningen.

Verhulst S. & H.M. van Eck 1996. Gene flow and immigration in an island population of Great Tits. J. Evol. Biol. 9: 771-782..

Verhulst S. & R.A. Hut 1996. Post-fledging care, multiple breeding and the costs of reproduction in the Great Tit. Anim. Behav. 51: 597-966.

Verhulst S., J.M. Tinbergen & S. Daan 1997. Multiple breeding in the Great Tit; a trade-off between successive reproductive attempts? Funct. Ecol. 11 (in press).

Viñuela J. & J. Bustamante 1992. Effect of growth and hatching asynchrony on the fledging age of Black and Red Kites. Auk 109: 748-757.

White D.W., E.D. Kennedy & P.C. Stouffer 1991. Feather regrowth in female European Starlings rearing broods of different sizes. Auk 108: 889-895.

Williams G.C. 1966. Natural selection, the cost of reproduction, and a refinement of Lack's principle. Am. Nat. 100: 687-690.

Williams J.B. 1985. Validation of the doubly-labeled water technique for measuring energy metabolism in Starlings and Sparrows. Comp. Biochem. Physiol. 80A: 349-353.

Williams J.B. 1987. Field metabolism and food consumption of Savannah Sparrows during the breeding season. Auk 104: 277-289.

Williams J.B. & K.A. Nagy 1984. Validation of the doubly-labeled water technique for measuring energy in Savannah Sparrows. Phys. Zool. 57: 325-328.

Wilson R.P. & B.M. Culik 1993. Activity-specific metabolic rates from doubly labeled water studies: are activity costs underestimated? Ecology 74: 1285-87.

Winkler D.W. 1987. A general model for parental care. Am. Nat. 130: 526-546.

Winkler D.W. & K. Wallin 1987. Offspring size and

number: a life history model linking effort per off-
spring and total effort. Am. Nat. 129: 708-720.
Wright J. & I. Cuthill 1989. Manipulation of sex differ-
ences in parental care. Behav. Ecol. Sociobiol. 25:
171-181.
Wright J. & I. Cuthill 1990. Biparental care: short-term
manipulation of partner contribution and brood
size in the Starling, *Sturnus vulgaris*. Behav. Ecol.
1: 116-124.
Zar J.H. 1984. Biostatistical analysis, second edition.
Prentice-Hall, Eaglewood Cliffs.

SAMENVATTING

Door het aantal eieren kunstmatig te verminderen werd
de relatie tussen legselgrootte en de inspanning van de
ouders bij het verzorgen van de jongen bestudeerd bij
Koolmezen op Vlieland. Het aantal uren per dag dat de
jongen werden gevoerd veranderde niet wanneer er
minder jongen waren, maar verkleinde broedsels vlo-
gen wel op jongere leeftijd uit. Vrouwen voerden ver-
kleinde broedsels minder vaak, maar gaven niet minder
energie uit. Mannen voerden verkleinde broedels ook
minder vaak, en er was een tendens dat ze minder ener-
gie uitgaven. De manipulatie had geen effect op het ge-
wicht van de ouders. Paren met een verkleind broedsel
maakten vaker en sneller tweede broedsels, ondanks het
feit dat ze (in energie uitgedrukt) niet minder hard ge-
werkt hadden. Dit geeft aan dat de kosten van reproduk-
tie niet zonder meer worden veroorzaakt door verande-
ringen in het niveau van de dagelijkse energiebesteding.